CHEMOTHERAPY OF MALARIA WITH SPECIAL REFERENCE TO DRUG INTERACTIONS WITH CHLOROQUINE AND HERBAL MANAGEMENT OF MALARIA IN KENYA.

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A thesis submitted in fulfilment for the degree of DOCTOR OF PHILOSOPHY in the University of Nairobi.

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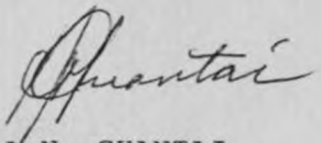
1990.

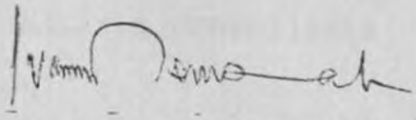
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.



A.N. GUANTAI

This thesis has been submitted for examination with our approval as University supervisors.



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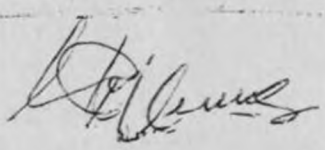

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ABSTRACT

The first part of this project deals with chloroquine and its interactions with other drugs.

A survey carried out in New Nyanza General Hospital (Kisumu, Kenya) a malaria endemic area, revealed that chloroquine was indeed the first line of management in the chemotherapy of malaria. The same survey indicated that chloroquine poisoning contributed to a total of 429 deaths in the above hospital within a span of seven years.

Out of a sample of 1,153 patients treated in the outpatient clinic during the survey period showed that 65.9% were adults. No sex variation in the treatment and incidence of malaria was noticed.

Chloroquine dosage varied from an underdose of 6 by 250mg tablets in 3 days to an overdose of 20 by 250mg tablet in 5 days. Out of the analysed population 3.4% was given prophylactic treatment with a dose of 2 by 250mg chloroquine tablets weekly for a period ranging from 10 weeks to 6 months.

Chloroquine drug combination therapy showed that the highest frequency was with aspirin at 23.12% followed by paracetamol with a frequency of 18.85%. This reveals that analgesic antipyretics are routinely given with chloroquine.

Experiments on rats revealed that chloroquine in

therapeutic doses caused a significant increase ($p<0.05$) in the urinary glucose excretion.

In rabbits, chloroquine at 25mg/kg daily dose for four days induced a significant decrease in non fasting blood sugar levels ranging from -19.7% after the first dose to -33.1% after the fourth dose. However there was significant difference in the hypoglycaemic potency of chlorpropamide and chloroquine with chlorpropamide registering a higher potency. Chloroquine potentiated the chlorpropamide induced hypoglycaemia significantly ($p<0.02$).

Three-day treatment with therapeutic doses of chloroquine was found to induce a significant increase in proteinuria in rats.

The chloroquine induced hyperproteinuria was antagonised by indomethacin significantly but less so by aspirin.

Chloroquine, chloroquine/aspirin and chloroquine/indomethacin combination caused a significant increase in erythrocyte sedimentation rate with chloroquine recording the fastest rate 2.6 mm/hr as compared to the untreated control in rabbits (1.71 mm/hr). In addition all chloroquine containing blood samples recorded marked *in vitro* haemolysis.

Chloroquine treatment caused mild ulcerogenic effect and significantly potentiated the ulcerogenic effect of aspirin and indomethacin.

On the cardiovascular system, apart from exhibiting potent myocardial depressant activity, chloroquine anatagonised adrenaline and isoprenaline induced cardiostimulation with the inotropic response being more sensitive than the chronotropic response.

Chloroquine also caused dose dependent inhibition of calcium and barium induced cardiostimulation. The magnitude or degree of chloroquine induced myocardial depression was found not only to be dependent on the dose, but also on the tissue drug contact time. Prolonged contact caused an almost irreversible myocardial conduction block. This is significant in terms of instituting a chloroquine poison control programme.

Atropine partially antagonised the chloroquine induced myocardial depression, implying a possible cholinergic activity of chloroquine especially on muscarinic receptors of the heart.

The sympathetic nervous system was found to be more sensitive than the parasympathetic with regards to myocardial activity and confirms the previous report that death from chloroquine poisoning is usully as a result of myocardial depression.

Chloroquine induced a dose dependent neuromuscular junction blockade that was not significantly antagonised by physostigmine. An increase in the dose of physostigmine in an effort to antagonise the

neuromuscular junction transmission blockade by chloroquine caused pronounced potentiation of the blockade. Chloroquine also potentiated the neuromuscular junction blockade induced by succinylcholine, gallamine and lignocaine. The calcium induced skeletal muscle contraction was also antagonised by chloroquine.

A 3.00% mean reduction in body weight was noted in rabbits fed on 25mg/kg chloroquine phosphate for two weeks with a drug free period of one week in between.

On the renal system chloroquine had a mild diuretic effect (14.9% increase) with respect to the control, but decreased the frusemide/chlorthiazide induced diuresis by 16.1% and 9.2% respectively.

The drug also significantly decreased the natriuretic properties of frusemide/chlorthiazide by 163.1% and 85.5% respectively. The sodium retention might explain the antagonism of chlorthiazide/frusemide induced diuresis by chloroquine.

Chloroquine induced mild increase in potassium loss (18.6%) as compared to frusemide 25.2% and chlorthiazide 58.9%. Combination of chloroquine with the above diuretics led to the potentiation of the kalliuretic effect of the two diuretics. Chloroquine induced hypokalemia might explain the muscle weakness experienced by some patients during prolonged

treatment with the agent. The decreased effectiveness of the diuretic activity of frusemide/chlorthiazide by chloroquine points at a potential drug interaction with clinical significance.

The tracheal smooth muscle relaxant properties of chloroquine were demonstrated. In addition, chloroquine was shown to antagonise acetylcholine, histamine, and barium induced tracheal muscle contractions with barium and acetylcholine activity being more sensitive to chloroquine.

The drug significantly potentiated the adrenaline, isoprenaline and salbutamol induced tracheal smooth muscle relaxation. The degree of antagonism and potentiation was not only dependent on dose of chloroquine but also on the tissue drug contact time. Prolonged tissue/chloroquine contact led to an overwhelming potentiation of the adrenaline, salbutamol and isoprenaline induced bronchial relaxation. The bronchial constrictors were unable to reverse the bronchial relaxation.

In vitro experiments performed on pregnant rat's uterus confirmed that chloroquine is a uterine relaxant. The drug antagonised the powerful uterotonic effects of oxytocin, PGF-2 alpha and carbachol. The carbachol induced contractions were more sensitive to chloroquine inhibition.

In vivo experiments in mice showed that chloroquine

exerted uterotonic effect and induced premature evacuation. All the chloroquine treated mice presented with blood stained birth canal with or without premature foetal evacuation. All foetuses were dead. Visual assessment revealed that there was increased cervical mucus secretion in the chloroquine treated mice. Chloroquine therefore may cause *in vivo* foetal death, cervical congestion and induce premature evacuation, hence the rationale behind its misuse as an arbotifacient.

In the gastrointestinal tract, chloroquine on its own enhanced *in vivo* gastrointestinal smooth muscle activity, but antagonised the laxative effect induced by senokot, bisacodyl, cascara and sodium sulphate while mildly enhancing the laxative effect of castor oil. Though the mode of action of chloroquine induced antagonism or enhancement was not clear, the interaction between chloroquine and laxatives was evident, therefore concomitant administration of the agents during or before routine bowel evacuation before surgery, radiological procedures or even before birth should be discouraged as there are chances of effecting incomplete bowel evacuation.

In the second part of this work the role of herbal medicine in management of malaria in Kenya was assessed. Five plants, *Ajuga remota*, *Caesalpinia volkensii*, *Schkuhria pinnata*, *Warburgia ugandensis*

Artemisia afra were assessed for in vitro antimalarial activity using chloroquine sensitive P. falciparum strain. Ethanolic extracts of all the plants were found to exhibit antimalarial activity, inducing a dose dependent reduction in parasitaemia and growth rate. Caesalpinia volkensii exhibited the least antimalarial activity while Warburgia ugandensis exhibited the highest antimalarial activity attaining 64.6 and 65.10 mean % reductions in parasitaemia and growth rate respectively at 1:80,000 dilution. At 1:800 dilution Warburgia ugandensis induced 90.17 and 90.10 mean % reductions in parasitaemia and growth rate respectively with a 100.0% activity being achieved at 1:200 dilution.

Ajuga remota berth showed an initial low antimalarial activity achieving only 21.73 and 22.12 mean % reduction in parasitaemia at 1:80,000 dilution a concentration that is 80 times higher than that of Warburgia ugandensis capable of inducing over 50% mean reduction in parasitaemia and growth rate. At dilution lower that 1:8,000 the activities of Ajuga remota and Warburgia ugandensis compared favourably at times even being equipotent.

Schkuhria pinnata exhibited moderate antimalarial activity whereas Artemisia afra had fairly high antimalarial activity achieving a 99.43 and 99.45 mean % reduction in parasitaemia and growth rate

respectively. Though the plant showed low activity at low concentrations the activity increased fairly rapidly with increase in dose and compared well with that of Warburgia ugandensis and Ajuga remota.

Phytochemical investigations of A. afra yielded artemisia oil (0.4%), from hydrodistillation of the dried leaves, scopoletin from the methanol extract long chain fatty esters, alpha-amyrin, beta-sitosterol, friedelin, and 7,4'-dimethoxy-5-hydroxy flavone from the petrol ether extract. All the above chemical compounds were characterised by using physicochemical and spectrophotometric methods and also by comparison with literature values.

The essential oil of A. afra did not inhibit the growth of Staphylococcus aureus and Bacillus cereus, at concentrations below 400ug/ml. High concentrations of 1800ug/ml inhibited the growth level of Escherichia coli, Pseudomonas and Klebsiella species.

7,4'-Dimethoxy-5-hydroxy flavone, the long chain fatty esters and alpha-amyrin and the ethanol soluble fraction of the petrol extract were also tested for antimicrobial activity and found to have variable degrees of activity with alpha-amyrin being the most active.

The mosquito larvicidal activity of the constituents of A. afra were investigated using Aedes aegypti species. AA-4 (unidentified) was the most

potent, achieving an LD-50 of 51.89 parts per million (ppm) as compared to 75.00ppm and 83.14ppm for the long chain esters and Artemisia oil respectively. No mosquito larvicidal activity was detected with the aqueous extract of A. afra.

Aqueous extract of A. afra induced dose dependent cardiovascular responses. The adrenaline induced positive inotropic and chronotropic effects were significantly decreased by concomitant administration with aqueous Artemisia extract.

Scopoletin exhibited cardiodepressant properties ranging from -22.2% to -33.3% for 1mg and 2.5mg doses respectively. The higher dose also induced negative chronotropic effect. The above property partly explains the hypotensive effect of scopoletin reported in literature.

Intramuscular doses of the long chain esters at 1.0, 1.5 and 3.0mg/kg induced a dose dependent reduction in systolic and diastolic pressures in anaesthetised rats.

The mean % reduction in systolic pressure ranged from 32.4% to 50.4% for 1mg/kg and 3mg/kg doses after three hours of treatment. The effect on diastolic pressure was even more pronounced with 44.6% and 61.9% reductions in diastolic pressures being recorded for 1mg/kg and 3mg/kg doses respectively. The aqueous A. afra extract also exhibited significant mean %

decrease in both the systolic and diastolic pressures.

On the skeletal muscle contractile activity the aqueous A. afra extract induced a mild (29.0%) neuromuscular junction blocking effect. The effect was not significantly altered by administration of 5ug/ml chloroquine. However gallamine and succinylcholine induced significant potentiation of the neuromuscular block.

The long chain esters had hypoglycaemic effect of delayed onset but long duration after oral administration. The intramuscular route caused a faster onset and a shorter duration of hypoglycaemic activity with signs of recovery after 3 hours.

The aqueous A. afra extract also exhibited hypoglyceamic properties qualitatively similar to that of the long chain esters. Booster doses of the aqueous extract administered at the onset of recovery from the effects of the first dose, arrested the recovery. But the hypoglycaemic effect induced by the booter dose was similar to that induced by the initial dose and there was no evidence of accumulation of drugs. Similar activity was recorded for the standard oral hypoglycaemic agent chlorpropamide.

CHAPTER ONE

1 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

1.1.1 BIOLOGY OF MALARIA

Primate malaria or more specifically human malaria is caused by four species of obligate intracellular protozoa of the genus Plasmodium, namely Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae. These protozoa reproduce asexually in man, but sexually in female mosquitoes of the genus Anopheles. The type of malaria caused by the four different protozoal organisms is as distinct as their morphological features. For example:

(a) Plasmodium falciparum causes malignant tertian malaria which is characterised by headaches and high fevers that recur after every 48 hours. It is the most dangerous form of malaria that requires immediate and complete treatment otherwise any delay may lead to brain involvement characterised by manic states followed by irreversible state of shock that may lead to rapid death. Early and adequate treatment affords complete cure without relapses. Inadequate treatment may be followed by a state of recrudescence of infection from the multiplication of parasites that persist in the blood.

(b) Plasmodium vivax. This species causes benign

tertian malaria, which is milder than malaria due to P. falciparum with low mortality rate in untreated adults. It has an erythrocytic cycle of 48 hours and is characterised by relapses that occur even as long as two years after the primary infection. This is due to exo-erythrocytic forms that persist in the body.

(c) Plasmodium ovale. This is a rarely encountered species that causes even a milder form of malaria than that produced by Plasmodium vivax. It has 48 hours cycle with a periodicity and relapses similar to those of Plasmodium vivax. Although the clinical attack is readily cured, the exo-erythrocytic stage of the parasite may persist and this is the cause of the relapses.

(d) Plasmodium malariae. This causes quartan malaria which has a cycle of 72 hours. It has no exo-erythrocytic stage and the clinical attack may occur years after infection. The infections are rarer than those of Plasmodium vivax.

The term "quartan" and "tertian" refer to the frequency of bouts of fever. In "tertian" the periodicity is after every two days whilst "quartan" refers to a periodicity of fever every four days with a 72 hour gap between attacks.

"Benign" and "malignant" refer to the severity of attacks. "Benign" is a milder attack with a low mortality rate whereas "malignant" refers to the

severe clinical attack characterised by a high incidence of brain involvement and other organ complications. It is mainly associated with P. falciparum which is the most pathogenic species.

Life Cycle of Malaria Parasite

Although there have been cases of transmission of malaria by transfusion with infected blood (Bruce-Chwatt, 1982; Rawlings and Beall, 1982; Camazine, 1985), and through transplacental transfer, (De Silvar et al, 1982; Virgilio, 1982), the natural transmission method is through the bite of an infected Anopheles mosquito. Man is the natural reservoir of the malaria parasites and transmission to another person is through the bite of mosquito during a blood meal. The malaria cycle has therefore two stages, a sexual stage in the female anopheline mosquito and an asexual stage in man.

The infective form of malaria parasites are the sporozoites which are spindle-shaped uninucleate cells that are injected into the blood stream through the bite of an infected mosquito. They quickly clear from the circulation and localise in the liver where they invade the hepatic parenchymal cells, multiply and develop into tissue schizonts within 5-16 days depending on the species of the plasmodium. This asymptomatic pre-erythrocytic or exo-erythrocytic stage, is followed by rapture of the tissue schizonts

releasing thousands of merozoites (small uninucleated organisms) into the blood stream. These enter the erythrocytes and initiate the erythrocytic stage also referred to as tissue stage of cycle of infection. They then undergo an asexual transformation from the young ring forms into motile intracellular parasites called trophozoites. These feed on protein content of the haemoglobin (haem portion is not digested) by process of phagocytosis, undergo nuclear or mitotic fission to form a multinucleate schizont. This subsequently divides into uninucleate merozoites. The erythrocyte now containing the mature schizont ruptures releasing at least 6-24 merozoites. Also released are pyrogens and the undigested haem from the haemoglobin. The attacks of fever coincide with the release of pyrogens and merozoites into the plasma. This is referred to as the febrile clinical attack. In tertian malaria the erythrocytic stage lasts for 48 hours whereas in quartan malaria the stage lasts 72 hours.

Some of the released merozoites enter new erythrocytes and the cycle is repeated giving the characteristic bouts of fever. The infected erythrocytes easily agglutinate (especially for P. falciparum) and may cause capillary obstruction in the brain and kidneys, a condition associated with advanced and complicated falciparum malaria (Chianta

et al, 1981).

Some of the erythrocytic parasites do not develop into schizonts but instead they undergo sexual differentiation to form haploid male or female gametocytes (micro and macrogametocytes respectively). Though these can survive in the erythrocytes for many days, they cannot differentiate or develop further until they are ingested by a female anopheles mosquito. If this happens, then the male gametocytes undergo exflagellation in the mosquito followed by male gametogenesis to yield microgametes. These swim in the stomach of a mosquito until one of them fuse with a female macrogametocyte to produce a zygote. This motile zygote also referred to as ookinete penetrates the stomach wall of the mosquito to form a spherical oocyst.

Within the oocyst multiple fission or sporogony takes place to yield thousands of the infective sporozoites. When the oocyst finally ruptures it releases the sporozoites which quickly invade the salivary glands of the mosquito. This mosquito is now capable of transmitting malaria.

It should be noted that in P. falciparum and sometimes P. malariae infections, the tissue schizonts burst more or less spontaneously leaving no forms of the parasite in the liver. Clinical cure in these infections is therefore usually followed by complete

cure unless the parasites are resistant to the antimalarial drug used. In P. vivax and P. ovale however some tissue parasites remain dormant (latent forms) before they start proliferating and produce relapses of erythrocytic infections months or years later. It is also possible when the tissue schizonts burst some of the released merozoites with the exemption of those of P. falciparum, may remain in the liver and re-enter the hepatocytes to begin a similar cycle now known as paraerythrocytic stage. This is another cause of relapses associated mainly with P. vivax, P. ovale and occasionally with P. malariae.

It therefore seems that in any human host, malaria is self propagating unless death intervenes or the infection is eventually terminated by immune mechanisms.

1.1.2 CHEMOTHERAPY OF MALARIA

The drugs used in the management of malaria can be classified in three ways:-

(a) according to the action they have on different stages of the life cycle

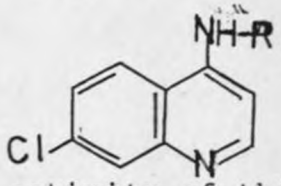
(b) according to the stage of parasite that they affect or

(c) according to their chemical group.

For ease of discussion, the chemical classification will be used.

4-aminoquinolines

This group of compounds share the following basic structure.



The antimalarial activity of this series of compounds was first described by Russian workers and later by German and French workers (Surrey, 1946).

During World War II when the Japanese cut off the supply of cinchona bark from Java (Indonesia), several synthetic antimalarials were developed including the revival of research on the antimalarial activity of the 4-aminoquinolines in the United States of America (USA). Though thousands of compounds of this series were synthesised, only three members have found clinical application based on their efficacy and relatively minimal mammalian toxicity when used in therapeutic doses. These members namely chloroquine (1), hydroxychloroquine (2) and amodiaquine (3) share the same pharmacological actions. They have rapid blood schizonticidal action against the erythrocytic forms of all types of malaria except some P. falciparum resistant strains. They are also effective against the gametocytes of P. vivax, P. ovale and P. malariae but not those of P. falciparum.

However even in massive doses these drugs have been found to exert no significant action on the exo-erythrocytic tissue stages of plasmodia and therefore

cannot prevent the establishment of the disease. They are consequently not casual prophylactic agents (Bowman and Rand, 1980). With the exception of drug resistant plasmodium strains, the 4-aminoquinolines completely cure falciparum malaria and lengthen the interval between relapses of P. vivax malaria.

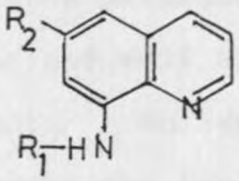
The drugs are well absorbed from the gastrointestinal tract, well tolerated, highly effective and have a long duration of action. This is because they are highly and strongly bound to serum proteins, to nucleic acid, to bone and even to melanin and soft tissues such as liver, spleen, kidney and lung, thus necessitating the administration of a loading dose to saturate these binding sites before giving the maintenance dose (Martindale, 1989).

The drugs are slowly metabolised in the liver by the microsomal enzymes and slowly excreted through the kidneys, thus exhibiting a long half-life.

After absorption the drugs are rapidly concentrated in the parasitised erythrocyte to the extent of being one hundred times higher than in the plasma. Here the drugs become intercalated between the stacked base pairs of the DNA double helix by interacting with the purine bases (adenine and quanine (Allison et al, 1964; Cohen and Yielding, 1965; Allison et al, 1966).

8-aminoquinolines

Many members of this group of compounds were screened for antimalarial activity before and during World War II in a large scale cooperative antimalarial programme conducted in the USA. They have the following basic structure:-



Pamaquine (4) was the first member to be introduced into medicine (Muhlens, 1926). However its low potency and high toxicity prompted the search for more potent and less toxic members. From this, three members namely pentaquine (5), isopentaquine (6) and primaquine (7) were selected for further research. Primaquine received greater clinical field trials with the United Nations forces in Korea and was found to be the least toxic. Another compound similar in structure to primaquine and also having low toxicity is quinocide (8).

The 8-aminoquinolines have no activity on the asexual blood forms of malaria (erythrocytic schizonts), but are effective against the sexual forms in the blood. They exert the gametocidal effect on all forms of plasmodia species especially P. falciparum. The drugs are also effective against the pre-erythrocytic and para-erythrocytic forms in the liver and spleen. For this reason, the drugs can effect

radical cure even for those forms of malaria in which the parasites have a dormant stage in the liver (P. ovale and P. vivax). Primaquine is the prototype of the 8-aminoquinolines. It is commonly combined with chloroquine so as to eliminate the asexual erythrocytic forms of the malaria parasites.

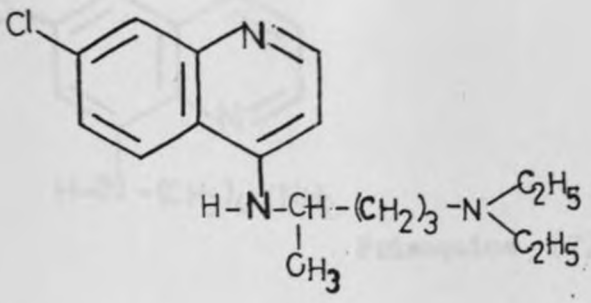
Primaquine is orally potent and well absorbed from the gastrointestinal tract. Unlike the 4-aminoquinolines, primaquine does not bind to tissues. The drug is rapidly metabolised and only a small portion of the parent drug is excreted unchanged.

Except for those patients with glucose-6-phosphate dehydrogenase deficiency, most people tolerate primaquine with mild gastrointestinal tract disturbances which cease after withdrawal of the drug.

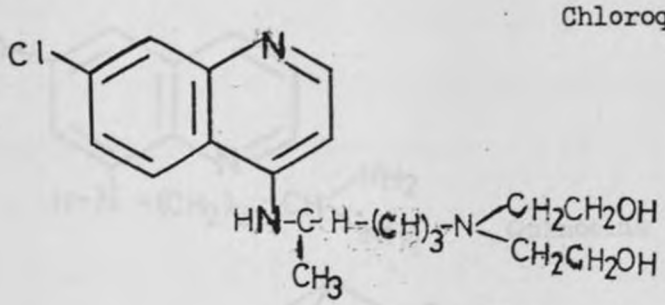
The mechanism of action of the 8-aminoquinolines is not well understood. Though they are known to bind to DNA (Bowman and Rand, 1980; Whichard et al, 1968) they do not affect DNA transcription or replication (Rang and Dale, 1987). They do not inhibit the incorporation of P labelled phosphate into DNA or RNA by mouse plasmodia species (P. gallinaceum and P. berghei) (Schellenberg and Coatney, 1960).

4-quinoline methanols

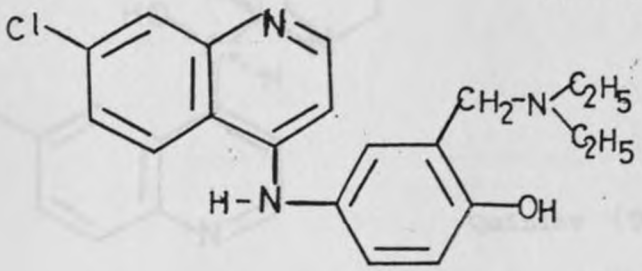
The main members in this group are quinine (9) and mefloquine (10). Quinine is the chief alkaloid of the various species of Cinchona



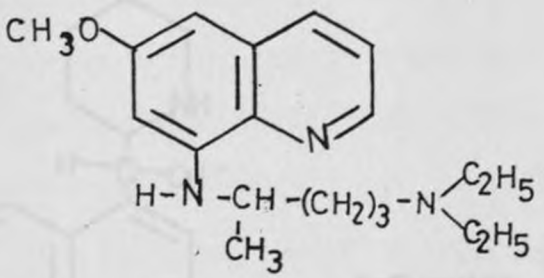
Chloroquine (1)



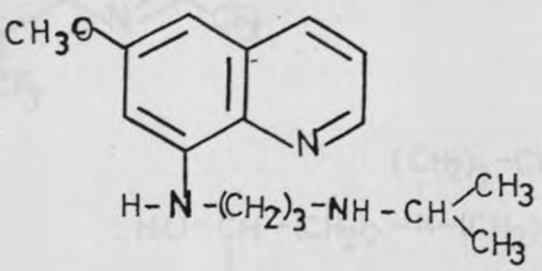
Hydroxychloroquine (2)



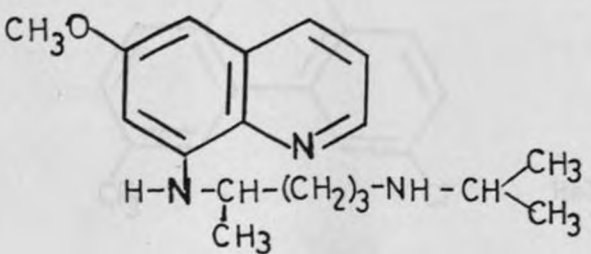
Amodiaquine (3)



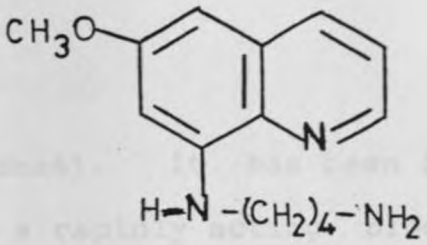
Pamaquine (4)



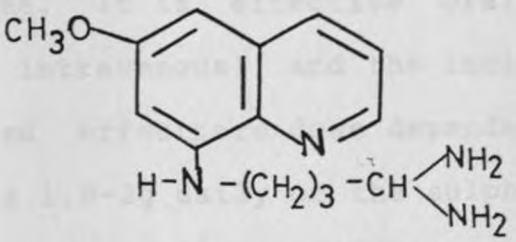
Pentaquine (5)



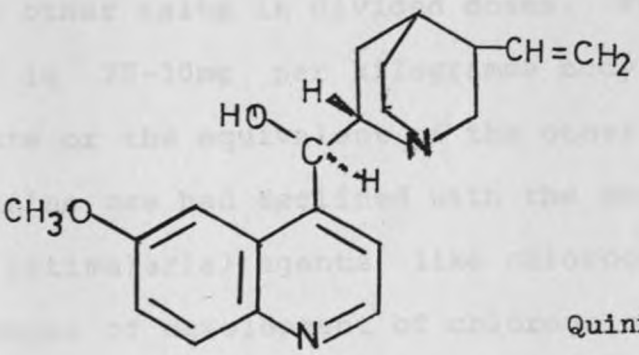
Isopentaquine (6)



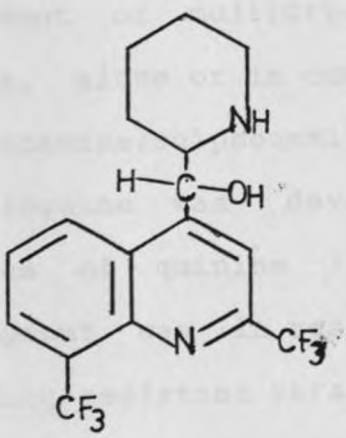
Primaquine (7)



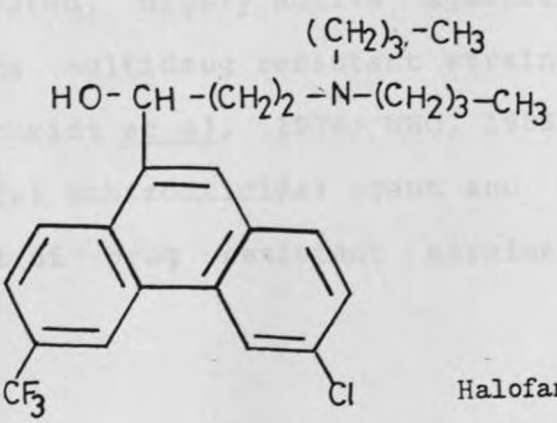
Quinocide (8)



Quinine (9)



Mefloquine (10)



Halofantrine (11)

(Rubiaceae). It has been formulated into many salts and is a rapidly acting blood schizonticide effective against P. falciparum, P. vivax, P. ovale and P. malariae. It is effective orally and parenterally (slow intravenous) and the incidence and severity of unwanted effectsare dose dependent. The usual adult dose is 1.8-2g daily of the sulphate or the equivalent of the other salts in divided doses. Paediatric dose range is 25-30mg per kilogramme body weight (of the sulphate or the equivalent of the other salts).

Quinine use had declined with the emergence of less toxic antimalarial agents like chloroquine but recent incidences of development of chloroquine and multidrug resistant strains of P. falciparum have seen quinine back into clinical use. It is mainly used in the management of multidrug resistant P. falciparum malaria, alone or in combination with tetracycline or pyrimethamine/sulphonamide combination.

Mefloquine was developed using the structural features of quinine (Schmidt et al, 1978). Its development was in response to the proliferation of multidrug resistant strains of P. falciparum. The drug is well tolerated, highly active against both the usual and the multidrug resistant strains of P. falciparum (Schmidt et al, 1978; WHO, 1983 and 1984). It is a powerful schizonticidal agent and is reserved for treatment of drug resistant strains of P.

falciparum.

Promising alternative to mefloquine include the amino-alcohol halofantrine (11) (Canfield et al, 1980).

This drug has been subjected to clinical trials (Cosgriff et al, 1982; Watkins et al, 1988; Wirima et al, 1988) and has been recommended for use as an alternative to mefloquine in chloroquine resistant malaria using an adult dose range between 1.0-1.5g in divided doses (Martindale, 1989).

Anti-Folates

(Folate antagonists and Inhibitors of Folate Synthesis).

These compounds were developed in Britain during World War II with the aim of producing substances that would affect pyrimidine metabolism in the malaria parasites.

Folates are essential in the biosynthesis of purines and pyrimidines which are necessary for the synthesis of DNA and RNA and certain amino acids such as thymine, methionine and serine. It therefore follows that folates are essential for growth and multiplication of body cells.

Mammals do not synthesise folates. They derive their folates from foods as essential dietary constituents which after ingestion are absorbed in the

proximal part of the intestines (jejunum and duodenum).

Bacteria and certain protozoa for example plasmodia cannot absorb folate from the medium around them. They therefore have to endogenously synthesise them intracellularly using para-amino benzoic acid (PABA) as one of the starting materials. Hence, plasmodial and bacterial growth and multiplication can be inhibited by substances that block folate synthesis. The anti-folates act by causing selective inhibition of plasmodial dihydrofolate reductase enzyme at concentrations much lower than that required to produce comparable inhibition of the mammalian enzyme (Ferone et al, 1969). Dihydrofolate reductase is the enzyme that catalyses the reduction of dihydrofolate (folic acid) to tetrahydrofolate (folinic acid) which is the active form of folates used in DNA and RNA synthesis. This is the site of action of cycloguanil (12) a metabolite of proguanil also known as chloroguanil. Cycloguanil is a triazine which is a potent blood schizonticide mainly used for long term prophylaxis and suppression of chloroquine sensitive strains of P. falciparum. It is orally potent and well tolerated. However development of resistant strains of P. falciparum to cycloguanil as a result of over exposure to the drug is common.

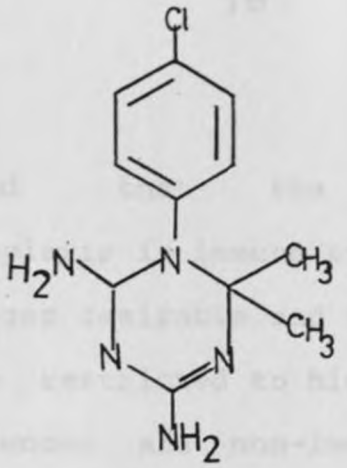
Synthesis of proguanil opened a way to the

synthesis of other compound with similar modes of action. These include substances such as pyrimethamine (13) and trimethoprim (14) which are very potent inhibitors of dihydrofolate reductase enzyme. The potency of these substances can further be enhanced by combination with compounds that block the first step of folate synthesis. These agents are the structural analogues of PABA possessing the para-amino substituent. They include sulphonamides and sulphones. Major examples of sulphonamides are sulphamethoxazole (15), sulphadoxine (16), sulphametopyrazine (17) and sulphalene (18). Dapsone (19) is the only representative of the sulphones and it is mainly used in treatment of leprosy.

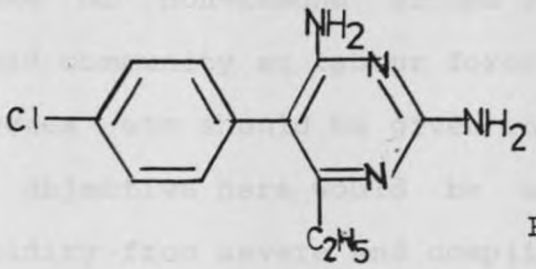
These compounds act as competitive antagonists of PABA, competing for the enzyme dihydropteroate synthetase.

1.1.3 Principles of malaria prophylaxis and suppression

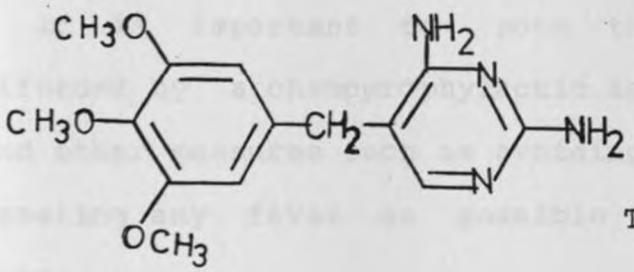
The rapid spread of malaria and the increased prevalence of drug resistant strains have made recommendations for malaria prophylaxis difficult. Though drug resistance has been observed with all the species of plasmodia that infect man it is the resitance of P. falciparum that is of major concern due to the severity of malaria infection associated with this species. The World Health Organisation has



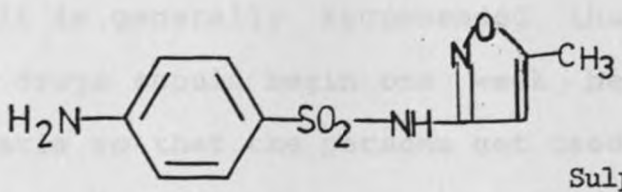
Cycloguanil (12)



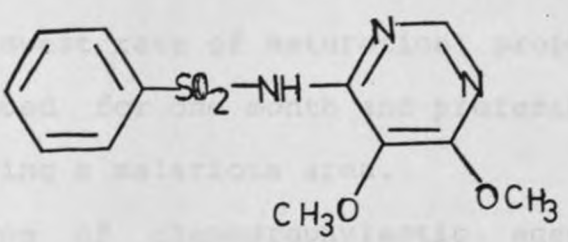
Pyrimethamine (13)



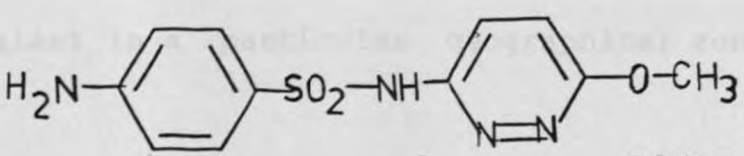
Trimethoprim (14)



Sulphamethoxazole (15)



Sulphadoxine (16)



Sulphametopyrazine (17)

recommended that the widespread use of chemoprophylaxis in immune and semi-immune population is no longer desirable and that the chemoprophylaxis should be restricted to high risk groups such as pregnant women and non-immune visitors. Also semi-immune or non-immune groups living temporarily in a closed community eg labour forces, police, army units, refugees etc should be given chemoprophylactic drugs. The objective here would be mainly to reduce the morbidity from severe and complicated malaria.

It is important to note that the protection afforded by a chemoprophylactic agent is not absolute and other measures such as avoiding mosquito bites and treating any fever as possible malaria should be taken.

It is generally recommended that prophylaxis with all drugs should begin one week before exposure to malaria so that the persons get used to the habit of taking drugs and hence decrease the chances of forgetting and also to allow for change to another drug preparation if adverse effects occur. To allow for the slowest rate of maturation, prophylaxis should be continued for one month and preferably 6-8 weeks after leaving a malarious area.

The type of chemoprophylactic agent used will depend on the degree and extent of drug resistance prevalent in a particular geographical zone and also

toxicity of the drugs.

In areas where chloroquine resistant P. falciparum has not been detected, a once weekly dose of chloroquine phosphate 500mg is given. In places where there is low grade chloroquine resistance, proguanil and chloroquine can be used to enhance prophylaxis. However a therapeutic dose of fansidar, quinine or mefloquine should be kept in readiness in case of clinical malaria attack. In areas with P. ovale, P. vivax endemicity, primaquine is a useful prophylactic agent.

In areas with high grade chloroquine resistance, chloroquine/pyrimethamine/dapsone combination is effective but the potential toxicity hinders its widespread use. Less effective, but less toxic agents such as mefloquine can be tried if available.

Pregnant women should as far as possible avoid travelling to malarious areas, but if malaria infection develops chloroquine and even quinine may be used with caution. Antifolates, tetracycline and primaquine should be avoided except sulfadoxine /pyrimethamine combination. This has been used safely for prophylaxis over long periods (Martindale, 1989).

1.1.4 Resistance to Antimalarials

Drug resistance is defined as the ability of a parasite strain to multiply or to survive in the presence of concentrations of a drug that normally

destroy parasite of the same species or prevent their multiplication (Martindale, 1989).

The mechanism of development of resistance to antimalarial drugs is not very clear. However chloroquine resistant Plasmodium falciparum strains have been shown to accumulate less chloroquine than sensitive strains. In addition the resistant strains tend to release chloroquine at rates 40-50 times faster than the sensitive strains. The higher rate of release might explain the lower rate of accumulation and hence resistance (Krogstand et al, 1987).

It has also been shown that erythrocytes infected with chloroquine sensitive P. berghei possess proteases to degrade haemoglobin (Cook et al, 1961; Levy and Chou, 1973) and they accumulate abundant amounts of malaria pigment (Peters, 1964; Ladda and Sprinz, 1969) which contain ferriprotoporphyrin IX (Fulton and Remington, 1953). Degradation of haemoglobin is diminished or totally absent in chloroquine resistant P. berghei (Eckman et al, 1977), and the malaria pigment does not accumulate (Peters,1964, Ladda and Sprinz,1969 but the process reverts to normal degradation and accumulation when the chloroquine susceptibility is restored (Peters, 1964; Ladda and Sprinz, 1969).

Ferriprotoporphyrin IX, probably in the form of an aggregate, is the receptor involved in the

accumulation of chloroquine with high affinity (Chou et al, 1980) and its absence in chloroquine resistant malaria parasites may explain the mechanism of resistance.

Ferriprotoporphyrin IX-chloroquine complex may be directly toxic to malaria parasites (Chou et al, 1980; Banyal and Fitch, 1982) by possibly being involved in pigment clumping which is the earliest morphological event after exposure of malaria parasites to chloroquine (Macomber et al, 1967) or by causing lysis of malaria parasite (Fitch et al, 1982).

Antimalarial drugs may bring about their antimalarial activity by inducing oxidant stress on the malaria parasites (Clark and Hunt, 1983; Clark et al, 1983; Pfaller and Krogstad, 1983) through generation of free oxygen radicals (Clark et al, 1983; Dockrell and Playfair, 1983; Allison and Eugui, 1982). The asexual form of the malarial parasites in the erythrocytes are very sensitive to oxidant stress (Allison and Eugui, 1982) and resistance to chloroquine may be acquired by a switch in the respiratory pathway (Howell et al, 1970).

Pyrimethamine resistance has been associated with a 2-fold increase in synthesis of dihydrofolate reductase enzyme by mutant strains of P. falciparum (Inselburg et al, 1987).

The resistant parasites were found to accumulate

pyrimethamine effectively (Inselburg et al, 1987). However, though the resistant parasites had greater than normal enzyme content, the affinity of the enzyme was much less than that of the sensitive strains (Bowman and Rand, 1980).

Whatever the mechanism of development of resistance to antimalarial agent is, a knowledge of the extent of the resistance in terms of geographical distribution and degree of resistance is important for the selection of appropriate measures and for the development of policies for the rational use of drugs.

Sensitivity to malarial agents is defined as clearance of asexual parasitaemia within 7 days of the treatment without subsequent recrudescence. Based on this, antimalarial resistance can be grouped into three categories:- (Martindale, 1989).

RI resistance; which is defined as clearance of asexual parasitaemia as in sensitivity followed by recrudescence.

RII resistance; is defined as marked reduction of asexual parasitaemia but not clearance.

RIII resistance; defined as no marked reduction in parasitaemia.

Some of the acknowledged factors that have precipitated the development of resistance of malaria parasites to the conventional antimalarial agents are the use of subcurative doses of drugs, and the

overusage of some antimalarial drugs during mass drug administration for the suppression of clinical malaria or for prophylaxis against the same (Thaithong et al, 1983).

Due to its availability and its presumed lack of side effects, chloroquine has been overused as the drug of choice for malaria eradication programmes and is the first drug to be tried in the treatment of any fever suspected to be due to malaria. All was well with chloroquine use until 1959 and 1960 when the first reports of resistance of malaria parasites to chloroquine came from Colombia (Martindale, 1977). Since then reports have come from very many countries both in the tropics and in the temperate regions. It has been reported in Thailand (Cadigan, 1968; Johnson et al, 1982; Hall et al, 1975; Boudreau et al, 1982 and Thaithong et al, 1983), Malaysia (Fung, 1971 and Fung, 1972), Brazil (Reyes, 1981), Burma (Tin et al, 1982), Vanuatu (Bowden et al, 1982), Madagascar (Aronsson et al, 1981), Tanzania (Peters, 1982; Schwartz et al, 1983), Kenya (Oloo et al, 1986; Turner, 1984; Brandling-Bennett et al, 1988). Upto about 1978, resistance to chloroquine had only been found in P. faciparum strains isolated from non-immune visitors to East Africa (Peters, 1982; Charmot et al, 1983; Weniger et al, 1982) and was rare among the indigenous Africans (Spencer et al, 1983). Now the

pattern has changed to show the predominant resistance (Weniger et al, 1982; Sixsmith et al, 1983) and even found in semi-immune populations (Onori, 1982).

Due to structural similarity, a degree of cross resistance was expected among the 4-aminoquinolines (Hall et al, 1975). However, Geary and Jensen (1983) observed no cross-resistance among the 4-aminoquinolines and chloroquine resistant strains of P. falciparum have been treated with amodiaquine (Schmidt et al, 1977; Spencer et al, 1983; Watkins et al, 1984; Thaithong et al, 1983) though resistance to this agent has also been observed (Hall et al, 1975).

Not only has resistance developed to chloroquine, but strains of plasmodium resistant to other antimalarials have also been reported. For example, cases of quinine resistant P. falciparum have been reported (Duriyananda and Noey-Patimanond, 1982; Thaithong et al, 1983). Resistance to pyrimethamine (Lucas et al, 1969; WHO, 1973; Thaithong, 1983) and even pyrimethamine/sulfadoxine combination (Eichenlaub et al, 1983; De Geus et al, 1982; Vleugels et al, 1982; Dixon et al, 1982; Cook and McGregor, 1982; Reacher et al, 1981) have been reported.

Partial pyrimethamine resistance can sometimes be effectively treated with pyrimethamine/dapsone combination though resistance to this combination is also developing (Cook and McGregor, 1982). Mefloquine,

a 4-quinoline-methanol derivative has been reserved for treatment of high degree of chloroquine resistant and multidrug resistant strains of P. falciparum (Danis et al, 1982; Thaithong et al, 1983; Reacher, 1981; Tin et al, 1982).

Unfortunately P. falciparum strains resistant to mefloquine are emerging and even type II mefloquine resistance has been reported in Thailand (Boudreau et al, 1982; Bygbjerg et al, 1983).

Drugs that belong to different chemical classes are recommended especially combinations of drugs with similar half lives may be preferred in that no single drug would be left in the body in sublethal doses to enhance selection of resistance. Also radical curative treatment is probably the most reliable way of avoiding selective parasite survival.

Some of the drug combinations that have been tried include antibiotic/antimalarial regimen (Puri and Dutta, 1982) for example quinine/tetracycline (Reacher et al, 1981), quinine/clindamycin; trimethoprin/sulphamethoxazole (La, 1982; Fasan, 1971), chloroquine/minocycline (Willerson et al, 1972) and quinine/sulfadoxine/pyrimethamine (Poncin et al, 1983). Also a short course of quinine, followed by a single dose of mefloquine is very effective for the very severe chloroquine resistant P. falciparum strains. Other effective combinations include

mefloquine/tetracycline and mefloquine/antifolates.

The danger of using antibiotic/ antimalarial combination lies in the possibility of not only producing drug resistant plasmodium strains but also resistance of pathogenic bacteria to antimicrobial agent may develop (WHO, 1973).

The discovery of newer agents such as artemisinin and its derivatives, halofantrine, mefloquine etc place some hope of counteracting the multi-drug resistance menace. But research into newer agents with antimalarial activity ought to keep pace with the development of parasite resistance to the currently available drugs.

In children and pregnant women prophylaxis and treatment of malaria present a major problem. The transplacental transfer of drugs puts the growing foetus at risk and the degree of teratogenicity will depend on the stage of trimester, with the first trimester being the most prone and the third trimester least prone.

In growing children especially very young ones the drawback is the underdevelopment of the metabolic enzymes making the metabolism of the drugs difficult and the incidence of side/toxic effects high.

However with appropriate dosage adjustments the treatment in children can be effected but the antifolates and tetracyclines should be avoided.

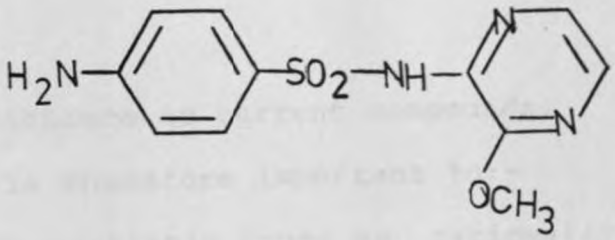
Quinine is thought to be better tolerated by children than chloroquine (Martindale, 1989).

1.1.5 Socio-economic Implications of Resistance To Antimalarials

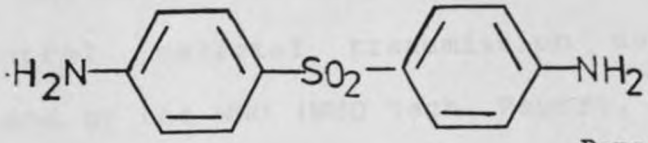
Resistance of Plasmodium falciparum to most of the current antimalarial drugs is now the main technical problem in malaria control. The emergence of resistant parasites may necessitate the deployment of other drugs of which there is a limited range. Use of chloroquine base is the least expensive. The cost of the first and second line alternative drugs is considerably higher and this puts financial strain on individual and the public economy (Government). Compared with chloroquine (1500mg) treatment, the cost of curative treatment with amodiaquine (1500mg of base) is 2-3 times higher, sulfadoxine/pyrimethamine (1500mg) 4 times higher, quinine alone for 7 days 15.5 times higher, quinine for 7 days plus tetracycline for 7 days 19.8 times higher and mefloquine (750mg) plus sulfadoxine/pyrimethamine (1500mg) 33.3 times higher (WHO, 1986).

Introduction of these alternative drugs brings with it additional problems of toxicity side effects and, lengthy treatment regimens that often result in poor patient compliance eg quinine/tetracycline regimen.

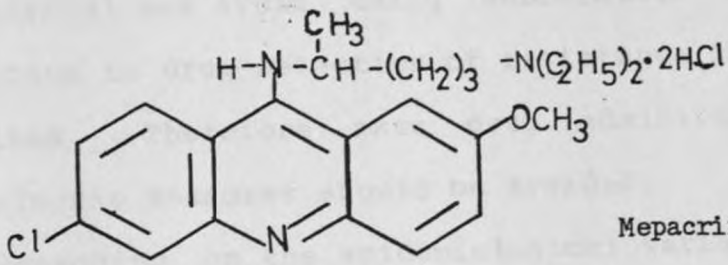
Another problem is the slow development of new antimalarials that cannot keep ahead of the evolution



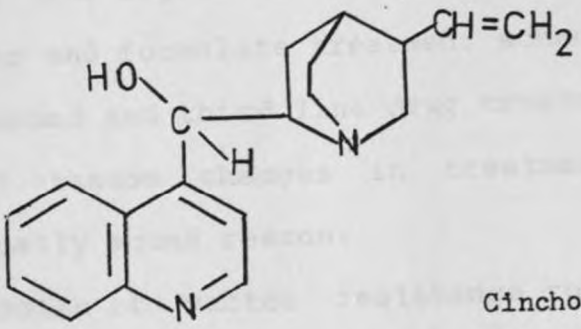
Sulphalene (18)



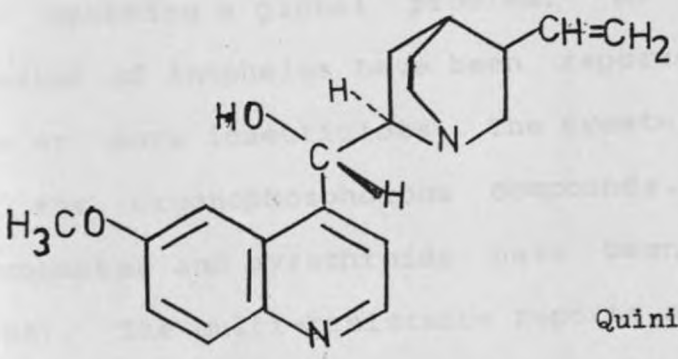
Dapsone (19)



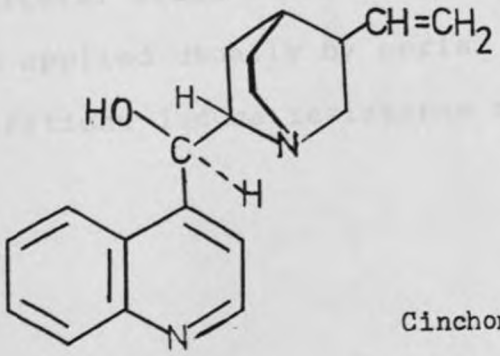
Mepacrine (20)



Cinchonine (21)



Quinidine (22)



Cinchonidine (23)

of resistance to current compounds.

It is therefore important to:-

1. Use available drugs as rationally as possible to minimise the risk of development of resistance.

2. Control malarial transmission using methods recommended by the WHO (WHO Tech. Report, 1984).

3. Use the appropriate curative dose of an antimalarial and avoid using subcurative doses that contribute to drug-selection of resistance in malarial parasites. Therefore mass drug administration as prophylactic measures should be avoided.

4. Depending on the epidemiological variables, the frequency, and degree of resistance, it is essential to consider and formulate treatment schedule based on first, second and third line drug treatment schedules and avoid random changes in treatment without a scientifically sound reason.

The problem of vector resistance to pesticides is now becoming a global problem. At the moment 57 species of Anopheles have been reported resistant to one or more insecticides. The greatest resistance is to the organophosphorous compounds. Resistance to carbamates and pyrethroids have been reported (WHO, 1980). The multi-resistance reports are mainly from highly agricultural areas where large quantities of pesticides are applied usually by aerial sprays. These repeated applications induce resistance since they

contaminate all the stages of mosquito population but are in sublethal doses.

Continued use of pesticides on these resistant strains is therefore a waste of money and also a global concern due to increase in environmental pollution.

The World Health Organization suggested some guidelines in malaria treatment and control as summarised below.

Treatment

Chloroquine is the drug of choice for treatment of suspected or confirmed cases of malaria where there is no resistance or in areas where RI type resistance is present. The recommended dose is 25mg chloroquine base/kg body weight.

Amodiaquine can replace chloroquine even where RI or RII type resistance to chloroquine is known to be present.

Sulphadoxine/pyrimethamine should be used as second line treatment in areas with high level chloroquine resistance of suspected or confirmed cases of malaria if there is no established resistance to it or resistance is infrequent or of low grade. It is known that *P. vivax* is intrinsically less sensitive to sulphonamides and rapidly develops resistance to pyrimethamine. It is therefore advisable to determine the sensitivity of the local plasmodium strain to the

drug combination before deploying it.

Where there is established resistance to both chloroquine and sulphadoxine/pyrimethamine combination, mefloquine can be used. This drug has been shown to be safe and efficacious against chloroquine resistant P. falciparum.

A few instances of resistance (Bygberg et al, 1983) or decreased sensitivity to the drug have been reported. The resistance is sometimes intrinsic and not related to previous exposure to mefloquine (Bygberg et al, 1983). A combination of mefloquine/pyrimethamine has been shown to delay development of resistance (WHO, 1986).

Quinine is recommended for use in instances where P. falciparum resistant to the 4-aminoquinolines and to the sulfadoxine/pyrimethamine combination exists and in countries where mefloquine has not been registered. Quinine is expensive and cannot be distributed to all the peripheral rural health centres. The drug is also relatively toxic and requires to be used for a long time (7 days) and therefore its use is accompanied by high incidence of patient non-compliance. It can be combined with tetracycline to shorten the time duration of treatment to 3 days but tetracycline should be used for 7 days to prevent development of bacterial resistance to the drug.

Primaquine, a drug with both schizonticidal and

gametocytocidal properties is reserved for use in multi-drug resistant strains and also where it is important to reduce the chances of transmission since its use is followed by complete eradication of malaria parasite and the patient can no longer infect the mosquitoes. It can also be used to prevent P. vivax relapse. The drug is also highly toxic and should be used with caution and is contraindicated in patients with glucose-6-phosphate dehydrogenase deficiency who usually develop fatal haemolytic episode upon using it.

Chemoprophylaxis

Though mass drug administration for prophylactic measures improves community health by decreasing incidence of anaemia and splenomegaly due to malaria (Delmon et al, 1981; Garfield and Vermund, 1983) and also greatly reduces morbidity, it is unfortunately accompanied by a high degree of development of resistance to the drug in use. This poses a big problem in the eradication of malaria. As pointed out earlier, chemoprophylaxis should therefore be restricted to the high risk groups earlier specified, and it should be accompanied by vector control measures aimed at reducing mosquito bite.

1.1.6 Vector Control

The main objective of mosquito vector control is to

reduce man-mosquito contact. There are many ways in which this can be achieved. For example:-

(i) Individual protection by use of bed nets impregnated with safe, long lasting and repellent insecticide eg permethrin emulsifiable concentrate (0.08-0.2g/100ml) or by use of mosquito coils and wall mats or use of protective clothing (WHO, 1984).

(ii) The choice of residential sites should be away from swamps, stagnant pools of water and thick bushes which are good breeding areas for mosquitoes.

(iii) The mosquito population in residential houses should be reduced by use of insect sprays preferably with residual insecticides and even the outdoor space spraying can be done to augment the vector control. The main limitations in this control method are the cost and the consequent environmental pollution.

(iv) Programmes aimed at controlling mosquito breeding are useful in urban areas, irrigation schemes and arid rural areas. The programmes include environmental manipulations of breeding grounds through drainage and the use of biological and chemical larvicidal agents to eradicate the mosquitoes.

The limitation of these programmes are that they require considerable resources and technical expertise when applied in large scale.

1.1.7 Immunity to Malaria

Resistance developed by immune system in response

to malaria is complex in nature and despite many years of investigations the mechanism of its development still remains controversial. It occurs especially in those individuals living in highly malaria endemic areas. It protects them against severe clinical attacks of malaria and it is lost if the individual is absent from the endemic area for more than six months.

The immunity is thymus dependent and is probably mediated by the binding of effector cell macrophages on the natural killer cells with concomitant production of superoxide anion and induces oxygen stress (Allison and Eugui, 1982). Asexual forms of malaria parasite in erythrocytes are sensitive to oxidant stress, and degenerate under these conditions (Clark and Hunt, 1983). P. falciparum is more sensitive to oxidant stress in human erythrocytes with abnormal haemoglobin (Hb) such as sickle cell haemoglobin (Hbs) (Friedman, 1978; Pasvol, 1978; Allison, 1964) or in glucose-6-phosphate dehydrogenase enzyme (G6-P-D) deficiency and thalassaemic erythrocytes than in normal erythrocytes (Friedman, 1979).

The sera from patients recovered from malaria is known to possess the capacity to inhibit the growth of P. falciparum in vitro (Golenser et al, 1983) and even to protect mice infected with P. berghei against early death from cerebral malaria, hence providing passive immunity (Finley et al, 1983. The protective factors

are believed to be protein in nature presumably immunoglobulin G (IgG) (Golenser et al, 1983) or plasma alpha-1 acid glycoprotein (AGP) (Finley et al, 1983) as these have been found to increase considerably during a malaria attack. AGP acts by inhibiting parasite erythrocyte interaction during invasive phase and apart from providing natural protection to malaria it might perhaps protect against other infectious diseases (Friedman, 1983).

Antibodies can facilitate the binding of the effector cell macrophages or natural killer cells to the surface of the parasitised cell (Allison and Eugui, 1982) and re-inforce the effects of the cell mediated immunity.

To facilitate the body to manufacture antibodies against malaria, the role of malaria vaccines can be very important in the prevention of malaria attacks.

1.1.8 Development of Malaria Vaccine

With the increased resistance of the malaria parasite to drugs and the failure of mosquito control methods the need for a vaccine against this killer disease is critical.

Many obstacles have hindered the smooth development of a malaria vaccine. For example different species of malaria parasites vary in their ability to elicit natural immunity in infected humans, and also vary in their susceptibility to the natural immune factors if

already established. The life cycle of the malaria parasite goes through many stages; the sporozoites, liver stages, merozoites and intra-erythrocytic stages all of which possess largely unique repertoires of antigen (Cox, 1988). Attempts to develop vaccines against all the stages of the malaria parasite have concentrated on the identification and synthesis of dominant surface proteins (WHO, 1987) but results have been largely disappointing.

In 1985, the "Immunology of Malaria Scientific Working Group" after working for many years on the antigenic material from P. falciparum and other strains of malaria parasite, made a dramatic discovery by isolating the P. falciparum sporozoite surface antigen which they named "Circumsporozoite" (CS) protein (Hockmeyer and Dame, 1985).

Similar surface antigens have recently been identified in all other malaria parasites affecting humans (Nussenzweig and Nussenzweig, 1989).

The isolation of these sporozoite surface antigens has assisted in the preparation of sporozoite vaccine (Howard, 1989; Moreno, 1989). However, clinical trials on the sporozoite vaccine has had a few drawbacks. One is that only one sporozoite needs to escape to the liver to initiate an infection. Two, that in malaria endemic areas people acquire high levels of anti-sporozoite antibodies and still become infected (Cox,

1988). Also the vaccine does not provide protection in all the immunised people (Nussenzweig and Nussenzweig, 1989).

Current research is focussed on the continuous evaluation of asexual blood stage antigens and on improvement of the immunogenicity of candidate antigens through the stimulation of more effective cellular and humoral responses (Cattani, 1989).

Vaccines for the merozoites which mature in the liver and later re-infect red blood cells have been synthesised (Peltola et al, 1984) but they also have their own limitations in that although they may ameliorate the clinical disease, they cannot prevent infection (Cox, 1988). Also efforts are underway to develop transmission-blocking vaccines based on antigens of sexual stages of the malaria parasite (Carter and Kaushall, 1984; Carter et al, 1984; Cattani, 1989). If this is achieved it would prevent maturation of gametocytes from red blood cells, so breaking the cycle of infection via the mosquito (Carter et al, 1984).

An enzyme based vaccine is also being considered. This is based on the parasitic enzyme aldolase which is 60% homologous with the mammalian aldolase. This enzyme catalyses the splitting of fructose 1,6-diphosphate into glyceraldehyde-3-phosphate and dihydroxy acetone phosphate thereby acting as a rate

limiting step in glycolysis. The blood stage of the malaria parasite lacks the citric acid cycle and so uses vast amounts of glucose. The inhibition of aldolase activity would therefore totally inhibit the maturation of the parasite and subsequent invasion of fresh red blood cells (Cox, 1988). Unlike the other antigenic materials, aldolase does not exhibit allelic polymorphism which is thought to confer species or strain specificity and thus there is a real possibility that aldolase could be a basis for a vaccine against many if not all types of malaria (Cox, 1988).

Remarkable progress has been made in the search for an ideal malaria vaccine. We are now closer than ever before to getting a true and effective malaria vaccine. However the battle is not yet won and more concerted effort is required.

An effort should also be continuously made to look for new drugs preferably from plant origin to counter the ever growing incidence of resistance development of malaria parasites to the currently available drugs.

1.1.9 TOXICITY AND SIDE EFFECTS OF CHLOROQUINE AND OTHER ANTIMALARIALS

Chloroquine is the official first line treatment in management, treatment and prophylaxis of malaria in Kenya (Watkins *et al*, 1984) and generally in the tropics (Olatunde, 1972). When used in therapeutic

doses as in malaria, chloroquine and the other 4-aminoquinolines are claimed to be less toxic than quinine and mepacrine (quinacrine) (20). However some common adverse effects such as dizziness, headache, difficulty in eye accommodation, itching, vomiting and skin reactions have been associated with the use of therapeutic doses of chloroquine.

When used in management of other diseases such as amoebiasis, rheumatoid arthritis and systemic/discoid lupus erythematosus (S.L.E) higher doses than those used in management of malaria are required. With these, a number of toxic effects have been reported; for example skin eruptions, photosensitivity, alopecia, bleaching of hair, leucopenia, blurring of vision which can progress to severe retinopathy and blindness (Martindale, 1989).

Chapman et al (1969) reported a case of generalised muscle weakness in a 64 year old woman who was on 400mg chloroquine phosphate daily for seven years for treatment of a non-specific rash on the face.

Chloroquine was implicated in the muscular myopathy and injury which was probably due to inhibition of glycolysis, since glucose was found to have accumulated in the cell vacuoles (Chapman et al, 1969). Four patients were also reported to have developed peripheral neuropathy following prolonged treatment with chloroquine phosphate (Loftus, 1963).

This was manifested as bilateral weakness of quadriceps muscles with loss of patellar and achilles tendon reflex, paraesthesias of the thighs and loss of vibratory sensation in both legs. All four were being treated for rheumatoid arthritis.

Foetal abnormalities comprising of congenital deafness with instability of the gait, chorioenteritis, hemihypertrophy and Wilms tumor have also been observed in children whose mother had been given chloroquine during four of her eight pregnancies (Hart and Naunton, 1964).

Complete cochlear damage (Matz and Naunton, 1968) and embryonic death and eye abnormalities (Udalova, 1967) both in humans and animals have been recorded after chloroquine use during the period of gestation.

Cardiovascular toxicity manifested as hypotension and death has been reported. This has occurred with overdose of chloroquine and also sometimes with therapeutic doses (Michael and Aiwazzadeh, 1970; Aviado et al, 1970; Charles and Bertrand, 1982).

In Kenya chloroquine has acquired a reputation as a common toxicological agent (Maitai et al, 1981). This is probably due to easy access to the drug which is freely sold in 'kioski' and 'dukas' all over the country. The drug is used as a self medicament for undiagnosed malaria (Maitai et al, 1981) and also abused as an arbotifacient. The doses commonly

employed are above the therapeutic range and toxic effects such as hypotension and cardiac arrest culminating in death have occurred (Michael and Aiwazzadeh, 1970).

In the eyes chroroquine causes ultrastructural changes (Smith et al, 1971; Lagros and Jean, 1971 and Ramsey et al, 1972). These effects have been found to be related to the daily dosage and frequency of drug administration and not the total quantity of the drug administered.

These electroretinographic changes have been attributed to the direct action of the drug on the neuroretina and not to the binding of chloroquine to the melanin which on the contrary could have a protective role (Lagros and Jean, 1971; Lagros and Rosner, 1971). Gregory et al (1970) found that the primary site of the retinotoxic effects of chloroquine is the ganglion of the ganglion cell layer whereas Ramsey et al (1970) observed the presence of membranous cytoplasmic bodies in the enlarged ganglion cell and also in the other cells of the retina though to a lesser extent.

It should be noted that ophthalmological changes can occur even with therapeutic doses of chloroquine especially in nephrotic patients (Zubarev and Polyantseva, 1972). This is because of decreased excretion of chloroquine through the kidneys.

Excretion of chloroquine by the kidneys is not dependent on the amount of chloroquine ingested (Grundmann et al, 1972) but on the integrity of the excretory tissues in the kidney. Even low doses of chloroquine as in prophylaxis have been associated with eye damage. For example eye damage has been reported in people living in malaria endemic areas who are on long term prophylaxis with chloroquine 300-500mg base once weekly, and it has been suggested that people living in high malarious areas should undergo regular ophthalmologic checkup and the drug withdrawn immediately signs of damage show up (Trojan, 1982). Unfortunately the retinopathy can sometimes progress even after withdrawal of the drug (Sassani et al, 1983).

The other antimalarials are also not devoid of side effects and toxicity even with therapeutic doses. Therapeutic doses of pyrimethamine if used for a long time has been associated with haemopoetic depression due to interference with folic acid metabolism. Other side effects include megaloblastic anemia of the bone marrow which receded on withdrawal of the drug (Herbert, 1969), leucopenia (Regab, 1973), photosensitivity (Craven, 1974), atrophic glossitis, pancytopenia, thrombocytopenia, convulsions, vomiting, respiratory failure and death. Blindness, deafness and ataxia occur but are rare. Pyrimethamine is sometimes

combined with a sulphonamide to enhance inhibition of folinic acid synthesis. This preparation commonly known as Fansidar has been known to induce serious reactions (Esten et al, 1982), including Steven-Johnson Syndrome in some people (Hornstein and Ruprecht, 1982; Phillips-Howard et al, 1989) as well as the above mentioned side effects associated with pyrimethamine.

Chlorproguanil, cycloguanil, proguanil and trimethoprim all of which are dihydofolate reductase inhibitors exhibit pyrimethamine-like side effects.

Quinine the first antimalarial agent to find clinical application in treatment of clinical malaria attack was and still is a very potent schizonticidal agent, but its regular use has declined due to a number of disturbing side effects. In therapeutic doses, quinine induces a chain of disturbing symptoms referred to as cinchonism. These are characterised by tinnitus (ringing in the ears), headaches, nausea and vomiting, abdominal pain, skin rashes, disturbed vision (diplopia), photophobia and paralysis of extraocullar muscles leading to mydriasis. When taken in excess of therapeutic dose quinine causes toxic effects that manifest as headache, fever, vomiting, deafness, blindness, excitement, confusion, feeble pulse, decreased myocardial excitability, occasional renal failure and death. Death can be sudden or

delayed for 1-2 days (Martin, 1974). Hypersensitivity in a man has been reported after the partner had used a pessary containing quinine or even after drinking bitter lemon soda containing 3mg quinine in 100ml or taking an analgesic tablet containing quinine. The hypersensitivity reaction is mainly of dermatological nature characterised by generalised skin rashes (Cundall, 1964; Savin, 1970) acne-form exanthematic or purpuric skin reactions (Verbov, 1968).

Thrombocytopenia has also been reported after prolonged use of quinine (Bottiger and Westerholm, 1973).

Primaquine sensitivity in glucose-6-phosphate dehydrogenase deficient patients characterised by heamolytic anemia is a well documented side effect (Reilly, 1982).

1.1.10. Management of Chloroquine Toxicity

Various techniques and methods have been employed in the management of moderate and severe chloroquine poisoning. Induction of emesis or aspiration and lavage to get rid of the unabsorbed drug, accompanied by support respiration has been used and advocated (Rothermich (1967). Administration of ammonium chloride in doses of up to 12g by mouth has been used (Rothermich, 1967; Michael et al, 1970; Bruno et al, 1988) in conjunction with sodium lactate injection (iv) to counter the cardiodepressant effects of

chloroquine (Rothermich, 1967). In very severe cases electrical pacing of the heart may be required (Rothermich, 1967).

Haemoperfusion (Garnier et al, 1985), peritoneal dialysis (McCann et al, 1975) and dialysis of drug against activated charcoal (Decker et al, 1971) have all been tried and found to be useful.

Diazepam and chlorpromazine have been used to counteract the agitation and the psychotic symptoms attendant to chloroquine poisoning (Bruno et al, 1988; Kabir, 1969).

Other successful treatment of chloroquine poisoning has also been achieved in three patients by washing out the intestinal contents after laporatomy.

However, regardless of the methods applied in the management of chloroquine poisoning, the success wholly depends on the interval between the ingestion of poison and the commencement of the management procedures. The message is "THE EARLIER THE BETTER".

1.1.11. Drug Interactions With Chloroquine

Literature on chloroquine induced drug interaction is scanty. However reports on reduced ampicillin bioavailability have been recorded after oral co-administration with chloroquine in healthy male volunteers (Ali, 1985). The effect has been attributed to slowing of gastric emptying (Varga, 1966; Adjepon-Yamoah, 1985) and increased gut motility (Rollo,

1975). However chloroquine does not impair the bioavailability of ampicillin from the pro-drug bacampicillin capsules (Ali, 1981).

It has also been reported that chloroquine contributes significantly to the risk of serious adverse reactions (Stevens-Johnson Syndrome) to fansidar (Rombo 1985; Bamber 1986). It also interferes with the disposition and kinetics of paracetamol (Adjepon-Yamoah 1986).

Absorption of chloroquine is delayed in presence of aspirin, but the distribution and elimination are not significantly affected (Adelusi, 1984).

The influence of various diets on chloroquine bioavailability has been assessed (Lagrave, 1985). Chloroquine has also been shown to potentiate the phenobarbitone sleeping time in rats by decreasing the liver metabolism of phenobarbitone (Ayitey-Smith, 1976). Similar inhibitory effects in rats have been observed with other drugs that are metabolised by the liver (Back, 1983).

Chloroquine and other antimalarials such as primaquine have been shown to increase the rate of conjugation of oral contraceptives following concurrent administration (Back, 1984).

Myaesthenic-like crisis has been induced by concomitant administration of chloroquine and penicillamine for management of rheumatoid arthritis

(Hearn 1986).

1.2. THE PRESENT PROJECT

From the literature survey a number of observations can be made with regard to the incidence, severity and management of malaria.

1. Malaria is no longer a disease of only the warm tropical regions. Incidences of "imported malaria" in the temperate climatic regions is increasing. This phenomenon can be attributed to the fast and advanced modes of travel.

2. Malaria is still a scourge despite great strides in vector control and chemotherapeutic management and prophylaxis of the disease.

3. Resistance to antimalarial agents is now a global problem inspite of the effort to curb the spread. Resistance has developed to monotherapy especially to chloroquine and also to multitherapy regime.

4. Chloroquine is still the drug of choice in the management of uncomplicated malaria due to chloroquine sensitive strains of Plasmodium. The drug has a central role to play in malaria chemotherapy especially in the third world countries where the availability of more effective, but expensive drugs is limited. For this reason the research on chloroquine needs to be continously updated.

5. There seems to be adequate coverage of short and long term toxic/adverse effects of chloroquine

especially on the eye. However the work done on other organs and organ systems is far from being exhaustive.

6. As regards drug interactions with chloroquine, there are very few reports and these cover a small range and class of chemotherapeutic agents. A lot more needs to be done in this field.

7. There is general abuse and misuse of antimalarial agents especially the 4-aminoquinolines, chloroquine and amodiaquine. This can be blamed on the free sales and promotions of these agents without the authorities (drug companies, medical professionals) providing adequate and up to date information on these drugs. This trend has to a large extent encouraged the overuse of antimalarials for poorly diagnosed disease conditions claimed to present with symptoms similar to malaria and hence assumed to be malaria. But worse still, is the indiscriminate use of antimalarial drugs in subtherapeutic doses either through self-medication or non-compliance by the ill informed public hence encouraging the selection of resistant strains of the Plasmodia that grow and multiply in presence of sublethal drug concentrations. All in all, there is promotion of growth and spread of antimalarial resistance with its accompanying socio-economic implications.

8. Though great advancement has been made in the field of malaria vaccine development, the possibility of getting an ideal vaccine that can provide immunity for all the stages of malaria parasite cycle including the

notorious liver and sexual stages is doubtful at this moment.

9. There is a dire need to develop cheaper and easily available antimalarial agents that can either be used for the management of chloroquine resistant malaria or as substitute for chloroquine so as to minimise the overuse of this drug (chloroquine). This would curb the rate of development of parasite resistance to chloroquine. Development of a herbal preparation even in its crude form would fill the above gap since it would be within reach of the rural community, cheaper and discourage the overuse of synthetic antimalarials to which resistance easily develops.

1.2.1. Objective of The Current Research

Based on the above mentioned observations the following are the two main objectives of this project.

a. To carry out experiments aimed at detecting likely drug interactions that would occur during routine use of chloroquine in the treatment/prophylaxis of malaria. As the basis for setting up these experiments a field survey on purchase and utilisation of chloroquine was deemed necessary. This would provide among other things:-

(i) The prescribing patterns for chloroquine, in malaria endemic area where chloroquine is required for both treatment and prophylaxis of the disease.

(ii) Access to prescriptions, which when analysed

would reveal the type of drugs that are commonly combined with chloroquine in the management of malaria and other malaria-related symptoms such as fever and headaches. The procedure would also reveal other drugs that are used in combination with chloroquine for the management of other disease conditions unrelated to malaria.

Though the frequency of a particular combination therapy was used as one factor of selecting drugs that are likely to interact with chloroquine, other factors were also taken into account. These include any documented physiological or pharmacological activity of a drug that may lead to potentiation or antagonism by chloroquine during concurent therapy. Chronic disease conditions such as asthma, diabetes and hypertension whose treatment has to continue during antimalarial treatment, in case of malaria attack. Surgical operations requiring the use of general anaesthetics and muscle relaxants. This procedure if carried out without due regard to prior or current drug therapy, may lead to interference with cardiovascular, respiratory and skeletal muscle contractile mechanisms hence affecting the smooth surgical procedure and even the full and quick recovery of the patient from anaesthesia.

Using these guidelines experiments were carried out on the cadiovascular system, carbohydrate and protein

metabolism, smooth and skeletal muscles, urinary system and blood rheology. Any other supplementary experiments were carried out as and when necessary.

Experimental details, results and discussions to the above objective are given in PART 1 of this thesis.

b. To assess the role of herbal medicine in management of malaria particularly in Kenya. To achieve this objective it was found necessary to analyse any available information published or folkloric so as to come up with plants that are commonly used for management of malaria in Kenya.

From that information, selection of at least five plants to be tested for antimalarial activity was made. The selection criteria are specified in PART II of this thesis. One of the plants which was found to possess potent antimalarial activity but with little or no previous phytochemical and pharmacological work was selected for further investigations. Details are presented in PART II of this thesis.

c. Conclusions and recommendations arrived at as a result of previous and mainly the present work are presented.

CHAPTER TWO

2. MATERIALS AND METHODS

2.1. SURVEY OF CHLOROQUINE PURCHASE AND UTILISATION IN A MALARIA ENDEMIC AREA

The choice of the survey area was influenced by geographical distribution of malaria which in turn is infuenced by climatic and environmental factors that favour rapid multiplication of malaria spreading vectors.

In Kenya malaria is predominantly found in Western Kenya around the shores of Lake Victoria and also in the costal region although cases of malaria attacks have also been reported from the highland areas. This is mainly due to easy means of transport, as well as deterioration in environmental management, especially in urban areas where untreated open drains and refuse dumps are found.

The World Health Organisation's major emphasis on malarial control has been on vector control eg swamp drainage, oiling of the surface of stagnant water to asphyxiate mosquito larvae, spraying houses and nearby bushes with stable insecticides, use of mosquito coils and other mosquito repellants, and also personal protection by use of mosquito nets at night. The Organisation however still recognises the significant contribution of drug therapy in management of malaria.

Chloroquine is the first line of management of malaria in Kenya (Sixmith et al, 1983; Watkins et al, 1988) and in Africa (Sowunmi, 1989). Having therefore recognised the widespread use of chloroquine in the country (professionally and unprofessionally) the present survey was carried out, to monitor the purchase and rational utilisation of chloroquine in an endemic area in Kenya. New Nyanza General Hospital in Kisumu, near the shores of Lake Victoria was therefore chosen as the study centre based on the above-mentioned basis.

2.1.1. Method

A total of 1,153 prescriptions were collected from the outpatient Pharmacy of the New Nyanza Hospital. The presence of chloroquine was the main criterion for prescription selection. Any other drugs prescribed with chloroquine were noted. The collection period was from 26th to 30th August 1983, a total of 6 days. The period was limited to a week in order to avoid the chance of hospital revisit by the patients.

The outpatient Pharmacy was chosen because of its wide spread services covering a cross-section of the outpatient clinics.

The prescription analysis was based on:-

1. Sex (male or female) 2. Age (paediatric or adult)
3. Drug combination
4. Dose

5. Duration of therapy

2.1.2. Drug Combination

The information contained in this data was used to design the experiments on drug interactions with chloroquine. The choice of the drug was not only based on the frequency of combination with chloroquine, but also on physical, chemical, pharmacokinetic and pharmacological characteristics of the drug.

2.1.3. Age and Sex

These data would provide information on distribution of malaria in children, adults (males and females). Peadiatric (P) stands for age in days to 13 years and adult (A) for 14 years and above. No reference was made to the weight of the patients, occupation or drug resistance or even the ailment that had made him or her seek medical treatment. Any prescription bearing chloroquine was assumed to be for either prophylaxis or treatment of malaria.

In addition to prescription collection, records showing chloroquine purchased by the hospital in form of tablets and syrup were assessed. This was a complete one year purchase record of 1982. Information obtained from these purchase records was supposed to reflect on chloroquine consumption hence the incidence of malaria cases.

Records showing outpatients treated for malaria and

2.2 Physiological Solutions*

	TYPE OF PHYSIOLOGICAL SOLUTION			
	LOCKE	TYRODE	KREBS HENSELETT	DeJALON
NaCl	9.0	8.0	6.87	9.0
KCl	0.42	0.2	0.4	0.42
$CaCl_2$	0.24	0.2	0.28	0.06
$MgCl_2$	-	0.1	-	-
$MgSO_2$	-	-	0.14	-
NaH_2PO_4	-	0.05	-	-
KH_2PO_4	-	-	0.16	-
$NaHCO_3$	0.2	1.0	2.1	0.5
Glucose	1.0	1.0	2.0	0.5

*The above quantities are grams per litre.

discharged from July-December 1982 (5 months period) and the inpatients admitted in the same year (January-December 1982) suffering from malaria were assessed.

The number of deaths from malaria recorded in 1982 were also noted.

A record of fatal chloroquine poisoning covering a span of 7 years (1973-1979 inclusive) was also obtained from the hospital. No details of the circumstances leading to the poisoning could be obtained. But three possibilities are likely. Chloroquine is commonly abused as an abortifacient when used in high doses. Poisoning can also occur accidentally or out of ignorance of therapeutic doses or even ignorance of different trade names that refer to the same product and hence overdosing of oneself.

2.3. EFFECTS OF CHLOROQUINE ON CARBOHYDRATE METABOLISM

The investigation of the influence of chloroquine on carbohydrate metabolism was carried out in two stages:

(i) Effect of chloroquine on carbohydrate metabolism and the influence of co-administered aspirin and indomethacin.

(ii) Influence of chloroquine on the hypoglyceamic properties of chlorpropamide.

2.3.1. Preparation of Test Reagents

(i) Tungstic Acid

2 g of analytical sodium tungstate was weighed and dissolved in 800 ml of distilled water in a 1000 ml volumetric flask that had previously been thoroughly cleaned and rinsed with distilled water. To this solution was added with shaking 20 ml 0.67 N sulphuric acid. The contents were made to volume with distilled water.

(ii) Ferricyanide

1 g analytical potassium ferricyanide was accurately weighed and dissolved in distilled water and diluted to 500 ml. The solution was then stored in the dark.

(iii) Cyanide Carbonate

10 g analytical anhydrous sodium carbonate was weighed and dissolved in about 50 ml distilled water contained in a 500 ml flask. 200 ml of freshly prepared 1% potassium cyanide solution was added, and the contents diluted to volume.

(iv) Ferric iron

1 g analytical ferric ammonium sulphate was carefully weighed and dissolved in about 500 ml distilled water contained in a 1000 ml flask. To this was added 50 ml of 85% phosphoric acid and contents made to volume.

2.3.2. Preparation of Standard Glucose Solutions

1 g of analytical glucose was weighed and dissolved in distilled water and the solution diluted to 1000 ml. This solution formed a 0.1% stock solution. From this 5, 10, 15, 20 and 25 mg percent standard glucose solutions were made by diluting 5, 10, 15, 20 and 25 ml of stock solution to 100 ml with distilled water.

2.3.3. Preparation of Drug Samples

(i) Indomethacin

Indomethacin being insoluble in water was triturated in 1% carboxymethylcellulose to make a 20% w/v stock suspension which was stored in the refrigerator at 4 degrees C for not more than one week.

(ii) Aspirin

Acetylsalicylic acid was triturated in 2% acacia to make a 200% w/v stock suspension.

(iii) Chloroquine

100% w/v aqueous stock solution of chloroquine phosphate was prepared and kept as stock solution.

(iv) Chlorpropamide

250 mg chlorpropamide tablets were weighed and finely powdered. 84.5 mg of powder was dissolved in 10 ml of water to give 6 mg/ml chlorpropamide base.

The stock drug solutions were subsequently diluted to give the required concentrations for the pharmacological investigations.

2.3.4 Experimental Animal Classification

Forty two Wistar rats were weighed and appropriately marked. They were then divided into seven groups of six rats and put in metabolic cages. The treatment was as follows:-

Group 1 The control group.

The animals in this group were orally hydrated with 50 ml of warm water/kg body weight once a day for three days. Urine samples were collected overnight for the three days.

Group 2: The chloroquine treated group.

The rats in this group were hydrated as in group 1 and also orally fed with single daily doses of chloroquine 50 mg/kg for three consequtive days. Urine samples were collected overnight for three days.

Group 3: The aspirin treated group.

After being hydrated as in the control group, the rats were orally fed with aspirin 51.4 mg/kg body weight once a day for three days and urine collected overnight for three days.

Group 4: The indomethacin treated group.

The animals were orally hydrated as in the control group. In addition they were intragastrically fed with indomethacin 4.3 mg/kg body weight once daily for

three consecutive days. Twenty four hour urine samples were collected for three days.

Group 5: Chloroquine + Aspirin treated group.

The rats in this group were concomitantly treated with 50 mg/kg chloroquine phosphate and 51.4 mg/kg aspirin orally once daily for three consecutive days. The animals were then allowed free access to food and water.

Group 6: Chloroquine + Indomethacin treated group.

In this group the rats were orally fed on combination of chloroquine phosphate 50 mg/kg and indomethacin 4.3 mg/kg body weight orally as single daily dose. No food or water restriction was imposed on the animals.

Group 7: Aspirin + indomethacin treated group.

The rats in this group were orally fed on aspirin/indomethacin combination. The drug doses were as mentioned above. As in all other groups no food or water restriction was imposed on these animals.

2.3.5. Analysis of Sugar Levels

The analysis of sugar levels was carried out using modified Landgrebe Munday Method (1954). The principle of the method is that after precipitation of urine proteins using tungstic acid, addition of ferricyanide and alkaline cyanide to the supernatant, boiling

causes the glucose in the urine to reduce some of the ferricyanide to ferrocyanide. Addition of acid ferric solution plus further heating results in the development of a Prussian blue colour. The depth of the colour is dependent on the amount of glucose present in the biological specimen. Differences in colour intensity are compared photometrically by use of an absorptiometer.

Since this method estimates the total reducing substances, care was taken to ensure that all apparatus was scrupulously clean and that it remained uncontaminated during the course of the experiment. The details of the analytical method are as follows:-

Using an accurate 0.02 ml pipette, 0.02 ml of urine was transferred to 5 ml tungstic acid in a centrifuge tube rinsing the pipette by sucking and blowing into the pipette. This was repeated for all the urine samples. After thorough mixing, the contents were centrifuged and 3 ml of clear supernatant was pipetted into a 25 ml graduated test tube.

Standard glucose solutions of 5, 10, 15, 20 and 25 mg% w/v were also prepared. 0.2 ml of each of the standard glucose solutions were transferred into test tubes containing 5 ml tungstic acid. After thorough mixing, 3 ml of each of these solutions were pipetted into 25 ml graduated test tubes. A blank, consisting of 3 ml tungstic acid was also prepared in a 25 ml

test tube.

The test tubes were all properly labelled and treated as follows, paying careful attention to timing:

To each tube was added 1 ml ferricyanide solution and immediately placed in vigorously boiling water. After 30 seconds 1 ml of cyanide carbonate solution was added and the test tubes covered with cotton wool to avoid excessive evaporation. The contents were then left for a further 10 minutes in the boiling water bath, after which 5 ml of the ferric solution was added. After further heating for 2 minutes, the test tube was quickly removed from the water bath and the contents diluted to 25 ml mark with cold distilled water. To arrest further development of colour, the test tubes were placed in a bath of cold water. If many samples were run together a procedure that strictly adhered to the times mentioned above was set up. All the solutions used in this experiment had been freshly prepared using analytical grade reagents and any water required was freshly distilled.

Colour intensities were measured at 630 nm wavelength using absorptiometer (Spectronic 20, Bausch and Lomb, Arthur, Thomas Co. Scientific apparatus) using the control of tungstic acid as a reference. A calibration curve using readings obtained from the standard glucose solutions was prepared by plotting

absorbance versus glucose concentration in mg percent. A straight line was obtained. Using the calibration curve glucose concentration in the urine samples was estimated.

2.4. The Influence of Chloroquine on the Hypoglyceamic Properties of Chlorpropamide

The effect of chloroquine on blood sugar levels and its influence on the hypoglyceamic properties of chlorpropamide were assessed.

Normotensive white New Zealand rabbits of both sexes were used. The animals were divided into four groups of six and treated as follows:-

GR1 Control group.

The animals were fed on normal rabbit pellets, vegetables and water ad libitum.

GR2 Chloroquine treated group.

Single oral chloroquine dose(25 mg/kg) was given, plus normal diet.

GR3 Chlorpropamide treated group.

Single oral chlorpropamide dose(4 mg/kg) was given plus normal diet.

GR4 Chloroquine/chlorpropamide group.

Animals were treated with drugs as above plus normal diet.

Before drug administration a zero hour blood sample was collected from all the animals and this was used

as a control sample for the particular animal. Blood samples were drawn from the marginal ear vein. All subsequent blood samples were collected after every 24 hours for 4 days. Samples from the control groups were also collected to determine the normal diurnal variation of the blood sugar levels.

Chlorpropamide and chloroquine are drugs with relatively long half lives. Due to this factor cumulation is likely to occur and influence the effect of subsequent single daily doses. To assess this, the collected blood samples were designated 0, 24, 48, 72 and 96 hour samples respectively. Since the experiment was carried out over a long period, it was not possible to starve the animals. Therefore the blood sugar levels were the non-fasting levels (NFBS) unlike the conventional fasting blood sugar levels (FBS). Analysis of the blood samples was carried out as detailed under section (2.3.5.).

2.5. Effect of Chloroquine, Aspirin, Indomethacin and Their Combinations on Urinary Protein Excretion

Protein Assay

The Biuret test for total protein and protein like materials was used. Biuret reagent which is an alkaline copper solution reacts with peptide bonds in the protein molecule to give an intense violet coloured complex which absorbs at a wavelength of 555

nm. Since the colour intensity is proportional to the amount of proteins present in the specimen, the test is quantitative.

(i) Preparation of reagents and solutions

(a) Bovine albumin

1.0 g bovine albumin was dissolved in a little distilled water in a 100 ml volumetric flask. The contents were then diluted to volume with water. This formed the stock solution. Then 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml were pipetted into clean test tubes and the volume adjusted to 1.0 ml with distilled water. This gave standard samples of 2 mg, 4 mg, 6 mg, 8 mg and 10 mg/ml.

(b) Biuret reagent

9.0 g sodium potassium tartarate was weighed and dissolved in 400 ml of 0.2 M sodium hydroxide. 3.0 g hydrated copper sulphate was added followed by 5.0 g potassium iodide and the solution made up to 1 litre with 0.2 M carbon dioxide free sodium hydroxide.

(ii) Preparation of protein calibration curve.

To 1 ml of each protein standard sample, 1.5 ml biuret reagent was added. A blank was prepared by adding 1.5 ml biuret reagent to 1 ml distilled water and the tubes incubated in a water bath at 37 degrees C for 30 minutes. They were then removed and 10 ml distilled water added and absorbance read at 555 nm

using an absorptiometer (Spectronic 20, Bausch and Lomb, Arthur H. Thomas Co. Scientific Apparatus). The results obtained were used to plot the protein calibration curve.

(iii) Collection and Analysis of Urine Samples

Winstar albino rats were grouped and treated as in section 2.3.4. The urine samples collected were divided into two portions. One portion was used for the analysis of urinary glucose levels, and the other portion was used for urinary protein analysis.

To analyse the test samples, 1ml of urine samples in duplicate were pipetted into different test tubes and treated in the same way as the standard protein samples (ii above).

Using the absorbance readings of the test samples, the corresponding protein concentrations were read off from the standard protein calibration curves.

2.6. THE EFFECT OF CHLOROQUINE, ASPIRIN, INDOMETHACIN AND THEIR COMBINATIONS ON THE ERYTHROCYTE SEDIMENTATION RATE (ESR) OF RABBIT BLOOD

There are two standard methods for ESR determination. These are:-

(a) Westergren's method

(b) Wintrobe's method

The first method is more sensitive and has normal values ranging from 0-20 mm/hr for human blood. The

second method, although less sensitive has the advantage that packed cell volume (PCV) can be determined using the same tubes. It is also simpler and less cumbersome. It has normal value of 3-7 mm/hr for human blood. The values are smaller because the blood is not diluted and the rate of sedimentation is inversely proportional to the plasma viscosity and directly proportional to the size of the red blood cells.

Materials and Method

The effect of chloroquine, aspirin, indomethacin and their combinations on the erythrocyte sedimentation rate was investigated using the Wintrobe's method.

Stock drug solutions were prepared as in experiment 2.3. Groups of 7 rabbits were orally fed with the following drugs:-

Aspirin (AA)	50 mmg/kg
Indomethacin (IM)	25 mg/kg
Chloroquine (CQ)	20 mg/kg and then 10 mg/kg, after 15 hours.

Combination of aspirin/chloroquine and chloroquine/indomethacin were also investigated.

After intubation the animals were left overnight (15 hours) after which a small dose of heparin was administered intravenously. This was necessary so as to allow good bleeding and ease of withdrawing the

blood by lowering the rate of coagulation.

For the groups on chloroquine treatment, the initial chloroquine dose was followed by 10 mg/kg after 15 hours and 4 hours later by intravenous heparin. Blood was drawn from the marginal ear vein into a syringe that had been rinsed with heparin. The drawn blood was immediately mixed together and put into Wintrobe tubes (2x100 mm) that had been sealed at the bottom. The test tubes were then left undisturbed for 2 hours. Readings were taken every 30 minutes.

The final erythrocyte sedimentation rate was calculated in mm/Hr after 2 hours. For each blood sample, triplicate determinations were made.

2.7. EFFECT OF CHLOROQUINE, ASPIRIN, INDOMETHACIN AND THEIR COMBINATIONS ON GASTROINTESTINAL MUCOSA

The degree of gastrointestinal damage can be considered in terms of the gastrointestinal mucosal barrier damage eg erosion, microbleeding and whether peptic ulcers are formed. This can be assessed using macroscopical and microscopical examinations. Measurement of gastrointestinal blood loss (Occult blood test) is also widely used.

Cobden *et al* (1980); Nakamura *et al* (1983) have shown that the permeability of a normally poorly absorbed compound through the damaged mucosa is proportional to the degree of gastrointestinal mucosal damage. Using phenol red as as the marker compound,

the above mentioned investigators were able to assess the ulcerogenic effects of indomethacin. The method is simple and noninvasive and consequently was chosen as the test method in the following experiment.

1. Materials

High quality phenol red, indomethacin, chloroquine and aspirin (from B.D.H., Sigma Chemicals, and E.T. Monks respectively) were used. The acacia and carboxymethylcellulose used as suspending agents were also of the highest quality. All the other reagents were of the finest grade available.

The stock drug solutions were made as outlined below and used as required.

(i) Indomethacin

2% w/v carboxymethylcellulose in water was used as a vehicle to prepare indomethacin (pure powder) 0.125% suspension.

(ii) Aspirin

0.25% w/v aspirin powder suspension was prepared by triturating and homogenising the aspirin powder in 2% w/v acacia suspension.

(iii) Chloroquine

0.1% w/v chloroquine phosphate was prepared by dissolving 0.1 g chloroquine phosphate powder in 100 ml distilled water.

(iv) Animals

Male Wistar albino rats were used.

2. Method

Seventy male Wistar albino rats weighing between 200-250 g were divided into seven groups of 10. The animals were fasted for a minimum of 18 hours after which they were orally fed with the test drugs as per the following schedule.

GROUP	TREATMENT
1	Control no drug treatment (C)
2	Chloroquine 20 mg/kg (CQ)
3	Aspirin 50 mg/kg (AA)
4	Indomethacin 25 mg/kg (IM)
5	Chloroquine + Aspirin 20 mg/kg + 50 mg/kg (CQAA)
6	Chloroquine + Indomethacin 20 mg/kg + 25 mg/kg (CQIM)
7	Aspirin + Indomethacin 50 mg/kg + 25 mg/kg (AAIM).

The animals were allowed free access to water but were denied solid food. Fifteen hours after the drug treatment the animals were orally given phenol red (2 µmol in 2 ml of normal saline) after which they were placed in metabolic cages in groups as specified above. Urine samples were collected after 4, 8 and 24 hours.

3 Treatment of Urine Samples

The voided urine was made up to 10 ml with distilled water and centrifuged for 10 minutes at 2500 revolution per minute. A one ml sample solution was made alkaline with 5 ml of 1.0 M sodium hydroxide solution. The absorbance was determined spectrophotometrically at 560 nm using spectronic 21 (Bausch and Lomb, Scientific Apparatus, Philadelphia, PA. USA).

Calculation of Ulceration Index

The ulceration index of the various drugs and their combinations were determined as follows. Aspirin, a known ulcerogenic agent was taken as standard. Since the optical density or absorbance of the urine samples was directly proportional to the amount of the marker compound (phenol red), which was directly proportional to the extent of gastrointestinal ulceration with respect to aspirin, then

$$\text{Ulceration index} = \frac{\text{absorbance of urine from drug treated animals}}{\text{absorbance of urine from aspirin treated animals.}}$$

For example after 4 hours the ulceration index of indomethacin with respect to aspirin was found to be

$$\frac{0.022}{0.015} = 1.47$$

2.8. EFFECT OF CHLOROQUINE ON THE CONTRACTILE PROPERTIES OF AN ISOLATED RABBIT HEART

Mature New Zealand white rabbits were used. A rabbit was killed by a blow at the back of the head and the neck cut to bleed the animal as much as possible. After quickly opening the chest cavity, the heart was removed with at least 1cm of the aorta attached to it. It was immediately placed in a dish containing warm Ringer Locke solution of the following molar concentrations:-

Sodium chloride 0.15; potassium chloride 5.6×10^{-3}; sodium bicarbonate 2×10^{-3}; calcium chloride 2.2 and glucose 5.6×10^{-3}. It was washed several times squeezing out any blood that may otherwise clot inside. The excess extraneous matter was removed and the heart attached through the aorta onto a glass cannula at the base of the Langendorff apparatus ensuring that no air bubbles were trapped inside. The tissue was then perfused with the Ringer Locke physiological solution maintained at 37 degrees C and aerated with carbogen (95% oxygen and 5% carbon dioxide).

The rate of perfusion and aeration were adjusted until the heart was beating satisfactorily and then hooked and attached via a string to a recorder (Harvard recorder, Bioscience, USA).

After recording the normal contractions, a bolus

dose of the test drug was injected into the heart via a plastic rubber tubing connecting the Langendorff's perfusion apparatus to the glass cannular supporting the heart. The effects of the standard drug alone were recorded after which the heart was challenged with specified doses of the standard drug in the presence of increasing doses of chloroquine and the responses also recorded. The effect of varying doses of chloroquine on the myocardial contractility were also recorded and these were used as another set of control. The specific contractile parameters investigated were changes in (a) inotropic and (b) chronotropic responses.

2.9. THE EFFECT OF CHLOROQUINE ON SKELETAL MUSCLE CONTRACTION

The rat's phrenic nerve-diaphragm muscle preparation was used to assess the effect of chloroquine on skeletal muscle contraction. Modification of chloroquine effect by other drugs was also investigated.

White Wistar rats weighing 130-175 g were used. The rats were sacrificed. The skin over the chest and around the pectoral muscle was removed. The thorax and the rib cage were opened along one side to expose the phrenic nerve which was tied with the thread and cut some distance away from the diaphragm. A section of the hemidiaphragm was carefully dissected out and

placed in a petri-dish containing modified Krebs' Henselleit physiological solution prepared as indicated on section 2.2.

The nerve was carefully attached to an electrode and placed in a double walled organ bath containing Krebs' Henselleit solution gassed with carbogen (95% oxygen and 5% carbon dioxide) and thermostatically maintained at 37 degrees C.

The tissue was allowed to stabilise for 20 minutes after which the nerve was electrically stimulated at one pulse/second and at a voltage of 5 volts.

The effect of fixed doses of standard drugs alone and in combination with increasing doses of chloroquine were investigated.

Though the normal tissue drug contact time was 2 minutes, it was observed that after chloroquine treatment, the tissue took at least 30-40 minutes to recover with 4-5 rinsings using fresh physiological solution before another test could be done.

Changes in contractile activity of the nerve diaphragm muscle were recorded via a kymograph (Bioscience, USA, 400).

2.10. EFFECT OF CHLOROQUINE ON BODY WEIGHT

In this experiment, the effect of chloroquine on body mass was investigated.

Eight adult New Zealand white rabbits of both sexes

were used. They were obtained from National Public Health Laboratories, Nairobi where they were bred for research purposes.

The treatment of the animals was as follows:-

(i) Day 0-7

The rabbits were weighed individually and the initial weights (W1) were recorded. The animals were then orally fed with 25 mg/kg body weight chloroquine phosphate equivalent to 15.62 mg/kg chloroquine base. The frequency of treatment was a single daily dose for seven days.

After feeding, the animals were allowed free access to food and water and left to freely mix with the other animals. After seven days of treatment the animals were weighed again and the weights recorded as (W2).

(ii) Day 8-17

During this 10 day wash out period, the animals were kept drug free but were given warm water 3 ml/100g body weight daily.

The aim of this stage of experiment was to ascertain that the effects observed in (i) above were due to chloroquine treatment and not due to mechanical trauma caused by daily intubation of the rabbits. At the end of day 17, the rabbits were weighed and the weight recorded as (W3).

(iii) Day 18-24

During this period, the rabbits were exposed to a second course of chloroquine phosphate. The dose was maintained at 25 mg/kg chloroquine phosphate as in (i).

This step was designed to assess whether the results obtained in (i) could be reproduced.

At the end of 24th day the final weights of the rabbits were recorded (W4).

Data Analysis

Percentage change in body weight were calculated in respect to the initial pretreatment weight (W1). To arrive at the final overall effect, the final weights (W4) were expressed as percentage of the second pretreatment weight (W3). This was aimed at removing any mask that may have appeared due to the effect of stage (ii).

2.11. EFFECT OF CHLOROQUINE ON THE DIURETIC PROPERTIES OF FRUSEMIDE AND CHLORTHIAZIDE

In the survey carried out in New Nyanza General Hospital on the prescription pattern of chloroquine, it was noticed that out of 1,153 chloroquine drug combinations 18 (1.33%) and 7 (0.52%) consisted of chloroquine/frusemide and chloroquine/hydroflumethiazide combinations. Usually the duration of the diuretic therapy per prescription

order ranged from 2-3 months.

Diuretics are chronically used in management of chronic hypertensive conditions. In view of the above it was decided that investigations into the effect of chloroquine on the diuretic properties of some commonly prescribed diuretics be carried out. However in the present study hydroflumethiazide was replaced by chlorthiazide which is also a thiazide diuretic with similar pharmacological profile because it was more readily available.

Materials and Method

Thirty rats were fasted for 24 hours. They were then weighed individually and water-loaded by stomach tube with warm water 5 ml/100g body weight. The animals were then divided into six groups, put in metabolic cages and left for 40-60 minutes before drug administration. The groups were later treated as follows:-

GR1 - The control group that was fed on normal saline

GR2 - This group was given chloroquine phosphate 50 mg/kg

GR3 - This group was treated with both chloroquine phosphate 50 mg/kg and chlorthiazide 35 mg/kg

GR4 - Was given only chlorthiazide 35 mg/kg

GR5 - This group was fed on frusemide 20 mg/kg and chloroquine phosphate 50 mg/kg

GR6 - This group was given frusemide 20 mg/kg.

The drugs were all adminstered orally through an oral feeding tube.

Urine samples were collected at one hour intervals via a plastic funnel packed with some glass wool to trap feacal material. The urine was collected into graduated measuring cylinder with a thin layer of paraffin oil to minimise evaporation and also to assist in reading off urine meniscus easily and correctly.

After every one hour the volume and pH of the urine were determined. The pH (approximate) was determined using Whatman full range (1-14) pH Universal indicator. The urine was pooled in one hour samples per group and the urinary sodium and potassium excretion determined using the flame photometer (Corning-EEL Scientific Instruments). The experiment was repeated six times per group.

Urinary Analysis

First the calibration curves for potassium and sodium ions were prepared as follows:-Standard solutions of potassium and sodium ions were prepared from analytical grade potassium chloride and sodium chloride. Serial dilutions were made using distilled water and the respective ionisation intensities of the solutions determined using potassium and sodium ions filter cells fitted into a flame photometer (Corning-

EEL Scientific Instruments). Using that data, standard calibration curves of ion concentration versus ionisation intensity were drawn for potassium and sodium ions.

Secondly the urine samples were diluted (2 ml urine in 50 ml distilled water) and the readings for ionisation intensity of the corresponding solutions determined as described above for the standard solutions. Sometimes further dilutions were required in cases where the ionisation intensities of the 1:25 dilution exceeded the photometric full scale detection range.

Using the standard calibration curves, the corresponding ion concentrations of the diluted samples was read off and the final urinary concentration found by multiplying the calibration curve reading with the dilution factor.

2.12. EFFECT OF CHLOROQUINE ON DRUG INDUCED CONSTRICTION OF INTACT GUINEA PIG TRACHEA

The effect of challenging doses of chloroquine on standard doses of broncho-constrictor agents was investigated. In addition the effect of chloroquine was compared to that of standard bronchodilator agents, adrenaline and salbutamol.

Adult guinea pigs of both sexes weighing more than 300 g were killed by a blow on the back of the head. The throat was cut as near to the head as possible.

The trachea was dissected out into a petri-dish containing Tyrode solution (section 2.2), and freed from extraneous matter. The preparation was set up in a double walled 20 ml organ bath containing Tyrode solution aerated with carbogen (95% oxygen and 5% carbon dioxide), and thermostatically maintained at 37 degrees C.

After ten minutes of tissue stabilisation, the volume of the physiological solution in the graduated pipette was adjusted to the starting level reading. The tissue was then challenged with the standard broncho-constrictor agents acetylcholine, histamine and barium chloride with a tissue-drug contact time of 5 minutes followed by four washings and a recovery time of 10-15 minutes. Any change in the constrictor tone of the bronchial muscle was indicated by a change in the level of the fluid in the graduated pipette. Broncho-constriction was manifested as a rise in level of he fluid whereas a fall in the fluid level signified broncho-relaxation.

The effect of standard broncho-relaxants adrenaline, isoprenaline and salbutamol was also assessed. Chloroquine influence on the effect of the chosen broncho-constrictors and broncho-relaxants on the bronchial smooth muscle was investigated after (a) simultaneous addition of the standard and chloroquine and (b) chloroquine pretreatment with a contact time

of 15 minutes.

2.13. EFFECT OF CHLOROQUINE ON DRUG INDUCED UTERINE ACTIVITY

To investigate the interaction between chloroquine and uterotonic agents, the primed rat uterus was used. Young virgin female rats weighing between 120-150 g were intraperitoneally injected with 0.1 mg/kg stilboestrol to sensitise the uterus. The animals were left for 24-48 hours for maximum uterine sensitivity to be attained after which they were sacrificed and the uterine horns carefully dissected into a petri-dish containing de Jalon's physiological solution (section 2.2). The tissue was then carefully cleaned of extraneous matter and about 2.5 cm was cut and set up in a 20 ml double walled organ bath containing de Jalon's solution thermostatically maintained at 32 degrees C and gassed with carbogen (95% oxygen and 5% carbon dioxide).

The tissue was allowed 20-30 minutes to stabilise after which it was challenged with fixed doses of standard uterotonic agents carbachol, prostaglandin F-2 alpha (PGF-2 alpha) and oxytocin and the response recorded via an isotonic lever system using a student kymograph.

After obtaining reproducible results with the standards, the effect of increasing doses of chloroquine on the uterotonic effect induced by a

fixed dose of the above standard was investigated.

A minimum of ten determinations were done with tissues from different rats.

2.14 INTERACTION BETWEEN CHLOROQUINE AND LAXATIVES

Twelve groups of eight mice of both sexes weighing approximately 20 g were treated as follows:-

Group 1(a) or the control was given 0.2 ml water and 0.2 ml indigo carmine as the indicator.

Grop 1(b) was given 0.2 ml indigo carmine and chloroquine 25 mg/kg base.

Group 2(a) was treated with pure castor oil 0.2 ml plus indicator.

Group 2(b) was given 0.2 ml castor oil + chloroquine base 25 mg/kg plus indicator.

Group 3(a) was treated with sodium sulphate 10 mg per mouse plus indicator.

Group 3(b) was treated as in 3(a) plus 25 mg/kg chloroquine base plus indicator.

Group 4(a) was treated with cascara 0.6 mg/mouse plus indicator.

Group 4(b) was treated as in 4(a) plus chloroquine base 25 mg/kg plus indicator.

Group 5(a) was treated with bisacodyl 0.2 mg/mouse plus indicator.

Group 5(b) was treated as in 5(a) plus chloroquine base 25 mg/kg plus indicator.

Group 6(a) was treated with senokot 2 mg/mouse plus indicator.

Group 6(b) was treated as in 6(a) plus 25 mg/kg chloroquine base.

The mice were sacrificed one hour after the treatment and the extent to which the dye had travelled down the alimentary tract was used to indicate the effectiveness or the potency of the drug(s). The results were expressed as the percentage of the total length of the gut, that is from the stomach to the rectum.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 SURVEY OF CHLOROQUINE PURCHASE AND UTILISATION

3.1.1 DEATHS FROM CHLOROQUINE POISONING AS COMPARED TO DEATHS FROM MALARIA

From 1973 to 1979, a total of 429 cases of fatal chloroquine poisoning received at the New Nyanza General Hospital were recorded, giving an average of 60 deaths per year.

It was not possible to get informatiom on deaths due to clinical attacks of malaria in the same period of 7 years. But in 1982 the same Hospital admitted a total of 824 patients confirmed to be having malaria. Out of these 45 deaths (8.24%) were recorded (Table 1).

Chloroquine poisoning can arise from deliberate overdosing with intent to commit suicide, or to procure abortion in cases of unwanted pregnancies, or excessive intake of chloroquine due to the ignorance of the normal doses for adult and children. The main objective in the last case would be treatment of malaria. The ignorance could also be on the different brands of pharmaceutical preparations containing chloroquine base as an active ingredient. Failure of one brand to treat malaria attack may compel the

patient to take a dose of another brand with these same active ingredients leading to overdose and hence poisoning. This problem could be avoided if chloroquine is only dispensed by qualified personnel and not freely sold in kiosks and "dukas" without any restriction.

Deaths from malaria usually are as a result of delays in seeking proper treatment, delayed diagnosis, undertreatment and failure of treatment due to development of resistance to the particular agent. These fatalities can be overcome by seeking early treatment from qualified and efficient laboratory technicians who can quickly diagnose malaria and even detect and keep tract of the development of drug resistance to the available antimalarials.

The data on annual deaths from chloroquine poisoning shows an average figure of 60 as compared to 45 for malaria. The figure of 60 is a significant one, considering the fact these data are obtained from one provincial hospital and not a national figure. Since most of the poisoning cases are as a result of ignorance and suicidal tendencies, the free availability of chloroquine in the market, should be restricted and this could at least reduce if not completely abolish the incidence of poisoning.

3.1.2 Prescription Analysis

A total of 1,153 prescriptions representing the

same number of patients were collected in 6 working days in New Nyanza General Hospital. This was an average of 192 patients per day seen at the outpatient clinics and diagnosed to be suffering from malaria and consequently treated with chloroquine alone or combination with other drugs. The number also included those people who required prophylactic chloroquine for one reason or another.

The Period of survey was 25th to 30th August 1983 inclusive.

Hospital records revealed that in the previous year (1982) the number of outpatients seen from 27th July to 31st December and diagnosed as suffering from malaria were 12,865. This is a period of 158 days and the daily outpatient malaria cases works out to 81.4 patients per day. This figure is less than half of the patients recorded per day in August 1983. The difference could be accounted for in terms of climatic changes that favour mosquito breeding at specific times of the year. For example the month of August is characterised by hot and humid weather favourable for mosquito breeding. The increased incident of malaria should be reflected by an increase in the purchasing of chloroquine preparation by the hospital.

Data on purchase of chloroquine preparations by the New Nyanza General Hospital in 1982 (Table 2) shows that the highest purchases were made from January to

August inclusive and then November to December inclusive. The months of September and October however had the lowest purchase figures.

Assuming that the hospital was not stocking chloroquine against use, and that the purchasing was not influenced by the availability of funds at specific months of the year, the months of September and October should therefore have had the lowest malaria cases and hence chloroquine consumption. The inclusion of these two months in the above mentioned period of 27th July to 31st December 1982 might have had an effect of lowering the mean of daily outpatient malaria cases in that period as compared to the figures obtained for August 1983.

3.1.2.1 Age Distribution

The data on distribution of chloroquine prescriptions with respect to age (Table 3) indicates that the highest incidence of malaria was found in adults. In fact out of 1,153 prescriptions, 760 (65.9%) were for adults (above 14 years) whereas 393 (34.1%) were for children aged from days to 13 years. The implications of these findings are that, the adult population is more exposed to malaria causative factors than the paediatric population.

The exposure may be in the form of lack of protective mosquito nets during sleep, night and evening activities such as farm produce marketing,

fishing, beer drinking, night watching, factory night shifts etc. The children population is however usually protected from this exposure by early indoor retirement and also by use of mosquito nets over and around their beds.

3.1.2.2. Sex Distribution

The distribution of chloroquine prescriptions does not vary greatly among males and females implying that the incidence of malaria is not very much influenced by sex. Out of 1,153 prescriptions, 590 (51.2%) were for males and 563 (48.8%) were for females. This gives a difference of 27 (2.4%) (Table 4). This slight difference could be due to differences in sex activities for example most outdoor night duties are usually performed by the males.

The difference is slight probably because even though females may not be involved in long night duties, the adult female population is exposed in the early evenings and mornings in duties such as milking, tending the fields, marketing of farm products, cooking in the kitchen etc. These activities are performed away from the bedroom where mosquito protective systems eg nets and mosquito proofing of windows are found. It should be noted that mosquitoes are most active in the evenings, throughout the night to early mornings. During the day the insects retreat to their hiding places.

3.1.2.3 Combination Therapy

All the 1,153 prescriptions bearing chloroquine were assessed for the other combination drugs. The highest percentage combination was found to be with aspirin (acetysalicylic acid) 23.12% followed by paracetamol 18.85% (Table 5).

The rationale here is to use an analgesic-antipyretic agent to counteract the fever that is usally associated with a clinical attack of malaria.

However aspirin and chloroquine share a number of side effects for example nausea and vomiting, dizziness and mental confusion, hypersensitivity reactions, blood dyscrasias, muscular weakness, auditory and vision disturbance, neurological disorders (in high doses), disturbances of carbohydrate metabolism (high doses for prolonged periods), increase in bleeding time (decreased platelet aggregation) and also increased prothrombin time (Martindale, 1989). A combination of chloroquine with antipyretic agents may therefore lead to antagonism.

A number of drug interactions of chloroquine with aspirin were therefore investigated as will be discussed in later chapters.

Prolonged treatment with chloroquine can cause damage to the insulin secreting cells (beta-cell) of the pancreas and lead to decreased insulin production

(Andersson et al, 1980).

To investigate the degree of anatagonistic effect, experiments were carried out to investigate the blood sugar as explained later

3.1.2.4 Combination Therapy With Chloroquine where Drug-interactions are likely to occur

The groups of drugs in table 6 were found prescribed together with chloroquine during a survey carried out in a malaria endemic area (New Nyanza General Hospital). Chloroquine was mainly prescribed for treatment of malarial infection and occasionally for prophylaxis.

The probability of drug interaction occuring with the combination therapy was based on the literature findings involving the pharmacological, adverse and toxic effects of chloroquine as related to the effects of the other drugs combined with it (Martindale, 1989).

3.1.2.5 Dose And Duration of Therapy With Chloroquine

(i) Pediatric

The Pediatric dose varied as follows:-

50mg injection then 75mg orally once daily for 3 days

60mg injection for 3 days

100mg injection for 3 days

75mg orally once a day for 3 days

200mg start then 100mg once daily for 3 days
250mg once daily for 3 days.

(ii) Adult Dose

There was also a variation in the dose administered to adults as shown below:-

160mg injection then 2 tablets once daily for 3 days
200mg injection then 2 tablets once daily for 3 days
4 tablets start, then 2 after 6 hours and then 2 tablets daily for 2 days
4 tablets once daily for 5 days
2 tablets once daily for 3 days.

Duration of Clinical Treatment

This varied from two days to five days. The two day period was noticed with pediatric therapy.

Prophylactic Treatment

Forty patients out of 1,153 analysed in this study had prophylactic treatment. This accounts for 3.4% of the analysed population.

The prophylactic dose was 2 tablets once weekly for as long as the individual was in a malaria endemic area. In this study group the period ranged from 10 weeks to 6 months.

TABLE 1 FATAL CHLOROQUINE POISONING OVER A PERIOD OF SEVEN YEARS IN NEW NYANZA GENERAL HOSPITAL, KISUMU, KENYA.

YEAR	DEATHS RECORDED
1973	35
1974	52
1975	55
1976	59
1977	69
1978	82
1979	77
TOTAL 7	429

This gives an average of 60 deaths per year. The cause of death was most probaly determined by pathologists report after postmortem.

DEATHS FROM MALARIA

In January to December 1982 a total of 824 patients were admitted into New Nyanza General Hospital confirmed to be malaria cases. Out of these inpatients 45 deaths (8.24%) were recorded.

3.2 EFFECTS OF CHLOROQUINE, ASPIRIN, INDOMETHACIN AND THEIR COMBINATION ON CARBOHYDRATE AND PROTEIN METABOLISM.

In the above *in vivo* metabolic studies in rats, urinary excretion of glucose and total proteins were used as indices of carbohydrate and protein

TABLES 2 PURCHASE OF CHLOROQUINE BY NEW NYANZA GENERAL HOSPITAL 1982 (JANUARY - DECEMBER).

MONTH	TABLET	SYRUP (LITRES)
January	370,000	175
February	314,000	335
March	328,000	293
April	228,000	265
May	296,000	260
June	293,000	175
July	147,000	235
August	249,000	145
September	57,000	175
October	29,000	210
November	354,000	460
December	408,000	45
Total	3,667,000	27,773

Average monthly purchase of chloroquine preparations by the New Nyanza General Hospital.

Tablets = 305,584

Syrup (litres) = 231

TABLE 3 AGE DISTRIBUTION OF THE POPULATION ASSESSED

AGE	NO	% DISTRIBUTION
adult (a)	760	65.9
Paediatric (P)	393	34.1
Total Population	1,153	100

Table 4 SEX DISTRIBUTION OF THE POPULATION ASSESSED

Sex (M)	590	51.2
Females (F)	563	48.8
Total Population	1,153	100

metabolism. The treatment period was limited to 3 days which is the accepted treatment period for clinical malaria with chloroquine.

Combination of therapeutic doses of chloroquine with aspirin and indomethacin were used in the study to assess the influence or otherwise of the chloroquine on carbohydrate and protein metabolism induced by these non-steroidal anti-inflammatory agents. Similar combination is encountered clinically in management of malaria and the accompanied pyrexia, or in the management of collagen and arthritic disease conditions.

It was assumed in this study that an increase in the urinary excretion of any of the above substances indicated a rise in the plasma concentration above renal threshold for absorption of the substance to occur. It also signified an increase in proteolysis, decrease in tissue glucose utilisation or increase in glycogenolysis.

3.2.1 Carbohydrate Metabolism

Previous *in vitro* studies have demonstrated that chloroquine is highly and persistently taken up by the islet of Langerhans and causes reversible inhibition of glucose stimulated pro-insulin biosynthesis in isolated mouse pancreatic islets (Andersson *et al*, 1980).

The *in vitro* uptake of chloroquine by the

percentage increase in glucosuria compared to the first day (Table 10). The most pertinent question regarding the chloroquine inhibition of insulin production by the pancreatic islet is its significance or implications in a diabetic especially the non-insulin dependent type who are normally controlled on oral hypoglycaemic agents. Experiments on the interaction between chloroquine and oral hypoglyceamic agents should probably provide an insight into this problem (see section 3.2.3). However suffice it to say that the present results indicate that chloroquine even in therapeutic dose range is likely to interfere with the oral hypoglycaemic agents which work by stimulating the beta-cells of the pancreas to produce insulin leading to decreasing insulin production as a result of damage of the beta-cells by chloroquine (Anderson et al, 1980).

Aspirin and paracetamol were the two common analgesic antipyretics encountered during a prescription survey on the drugs used in combination with chloroquine (Table 5).

This is probably due to their ready availability and low cost and also due to the fact that they are perhaps the best and relatively safe antipyretics.

It was found that aspirin caused decreased glucosuria (-0.23%). Aspirin has been shown to cause hypoglycaemia (Cotton and Fahlberg, 1964) probably by

decreasing hepatic glycogenolysis and increase glucose utilisation.

Aspirin/chloroquine combination induced an overall increase in glucosuria (9.5%) after a three day treatment with 20mg/kg chloroquine and 50mg/kg aspirin. This implies that the hyperglucosuria induced by chloroquine is to some extent antagonised by the aspirin induced hypoglucosuria (Table 10 and 11).

Chloroquine/aspirin combination therefore looks more favourable for a diabetic patient who has been stabilised on antidiabetic drugs than monotherapy with chloroquine or aspirin. The combination is unlikely to interfere with the antidiabetic therapy.

Indomethacin 25mg/kg induced a significant ($p<0.05$) decrease in mean percentage glucosuria (-19.48%) with respect to control. Decreased glucosuria and hypoglyceamia have been observed with indole acetic acid (Campbell, 1968). Indomethacin is an indole acetic acid derivative and one of its metabolic products is indole acetic acid. Therefore similar results to those obtained with indole acetic acid were expected with indomethacin and indeed this was demonstrated.

Chloroquine/indomethacin combination gave a picture similar to that of chloroquine-aspirin combination. However the degree of antagonism of the chloroquine induced hyperglucosuria by the indomethacin

TABLE 5 COMBINATION THERAPY WITH CHLOROQUINE

Chloroquine Plus	Active Principle	Pharmacological Activity	Prescription No	% Combination
Aspirin	Acetylsalicylic acid	Analgesic/ Antipyretic	314	23.12
Panadol	Paracetamol	Analgesic/ Antipyretic	256	18.85
Ketrax	Levamisole	Antihelmintic	129	9.50
Septrin	Co-trimoxazole	Anti-microbial	102	7.51
Piriton	Chlorphreniramine	Antihistamine	48	3.53
Seclopen		Anti-microbial	48	3.53
Diazepam	Diazepam	Sedative	45	3.31
Penicillin-V	Penicillin-V	Antibiotic	38	2.80
Ferrous Sulphate	Iron	Haematinic	33	2.43
Butazolidine	Phenylbutazone	Antiinflammatory	31	2.28
Antacid	Mgtricillicate	Anti-ulcer	30	2.21
Largactil	Chlorpromazine	Tranquiliser	27	1.99
Phenobarbitone	Phenobarbitone	Sedative/hypnotic Anticonvulsant	20	1.47
Ampicillin	Ampicillin	Antibiotic	20	1.47
Lasix	Frusemide	Diuretic	18	1.33
Aldomet	alpha-Methyldopa	Antihypertensive	17	1.25
Epanutin	Phenytoin sodium	Anticonvulsant	15	1.10
Kaolin Sed.	Kaolin	Antidiarrhoea	15	1.10

TABLE 5 CONT.

Diabenese	Chlorpropamide	Anti-diabetic	14	1.03
Paludrine	Proquanil	Antimalarial	14	1.03
Triplopen	Benethamine	Antibiotic	13	0.96
Tetracycline	Tetracycline	Antibiotic	13	0.81
Senokot	Sennosides A&B	Laxative	11	0.74
Folic acid	Folic acid	Vitamin	10	0.74
Naclex	Hydroflumethiazide	Diuretic	7	0.52
Propranalol	Propranalol	Antihypertensive	6	0.44
Tegretol	Carbamazepine	Anticonvulsant/ Antiarrythmic	6	0.44
Digoxin	Digoxin	Cardiostimulant	5	0.37
Franol	Ephedrine+ Phenobarbitone	Bronchodilator	5	0.37
Artane	Benzhexol	Anti-parkinsonism	5	0.37
Erythromycin	Erythromycin	Antibiotic	5	0.37
Stemetil	Prochlorperazine	Anti-emetic	5	0.37
Flagyl	Metronidazole	Antiprotozoal	5	0.37
Amoxil	Amoxycillin	Antibiotic	5	0.37
Ventolin	Salbutamol	Bronchodilator	5	0.37
S/Thalazole	Sulphathalazole	Antimicrobial	4	0.29
B/Complex	Vitamin B Group	Vitamin	4	0.29
Lidaprim	Trimethoprin 80mg Sulphametrole 400mg	Antibiotic	4	0.29
Ampiclox	Ampicillin& Cloxacillin	Antibiotic	4	0.29

TABLE 5 CONT.

Buscopan	Hyoscine	Antispasmodic	4	0.29
Slow-K	Potassium	Mineral	3	0.22
Antepar	Piperazine	Antihelmintic	3	0.22
Alcopar	Biphenium	Antihelmintic	3	0.22
Ceporex	Cephalexin	Antibiotic	3	0.22
Laroxyl	Amitriptyline	Antidepressant	3	0.22
Anafranil	Clomipramine	Antidepressant	3	0.22
Periactin	cyproheptadine	Antihistamine	3	0.22
Plasil	Metoclopramide	Anti-emetic	2	0.15
S/Dimidine	Sulphadimidine	Antimicrobial	2	0.15
Ambilhar	Niridazole	Schistosomicide/ Antiprotozoal	2	0.15
Serenace	Haloperidol	Tranguilizer	2	0.15
Dalacin-C	Clindamycin	Antibiotic	2	0.15
Hydrallazine	Hydrallazine	Vasodilator	1	0.07

TABLE 6 SAMPLE PRESCRIPTIONS SHOWING COMBINATION THERAPY WITH CHLOROQUINE

	DRUGS PLUS CHLOROQUINE	DAILY DOSE	DURATION OF TREATMENT
1.	Feffol tablets	1 bd	One week
	Aldomet tablets	500 mg tds	Seven weeks
	Inderal tablets	120 mg bd	Seven weeks
	Naclex tablet	50 mg od	Seven weeks
2.	Largactil tablets	50 mg bd	Three months
	Butazolidine tablets	200 mg bd	Three months
3.	Aldomet tablet	50 mg tds	Two months
	Lasix tablets	20 mg od	Two months
	Digoxin tablets	0.25 mg od	Two months
4.	Diabenese tablets	500 mg od	Three months
5.	Valium tablets	2 mg bd	One week
	Largactil tablets	25 mg bd	Three months
	B-Complex	1 od	Three months
6.	Aspirin tablets	2 tds	One week
	Caps Ampicillin	500 mg tds	One week
7.	Valium	5 mg bd	Three months
	Mist Antacid belladona	20 ml qid	Three months
	Buscopan tablets	2 tds	Seven days

Legend to table 6

bd - Twice daily

tds - Three times daily

od - Once daily

qid - Four times daily

(2.16%) was much more significant than that obtained with aspirin/chloroquine combination (9.5%).

Therefore the chloroquine/indomethacin combination seems a better alternative to aspirin/chloroquine combination for a properly stabilised diabetic.

The minimal glucosuria induced by chloroquine/aspirin and chloroquine/indomethacin combination may not be a serious side effect in a normal healthy individual, but it should be a notable side effect in a diabetic having a poorly controlled diabetes.

3.2.2 Protein Metabolism

Chloroquine and other antimalarials such as quinine, primaquine and quinacrine have been reported to inhibit or block *in vivo* amino acid uptake into the cells and therefore inhibit cellular protein synthesis (Conklin and Chou, 1970). The uptake of protein precursors such as thymidine, uridine is inhibited (Conklin and Chou, 1972) leading to inhibition of DNA and RNA synthesis (Greilling *et al*, 1969).

Other reports have cited chloroquine inhibition of protein synthesis (Bertinin *et al*, 1970; Roskoski and Robert, 1972) and inhibition of collagen metabolism (Wibo and Poole,1974; Etherington and Evans, 1977; Haataja *et al*, 1978; Poole *et al*, 1978 and Pietras *et al*, 1978). There is also evidence that lysosomotropic

agents such as chloroquine inhibit both the synthesis and secretion of pro-collagen protein without interfering with non-collagen protein synthesis (Neblock and Richard, 1982).

However cell-free protein synthesis is less affected (Cocklin and Chou,1970).

Results of the present in vivo studies in rats have shown that three-day treatment with antimalarial doses of chloroquine can induce moderately significant increase in proteinuria (Table 8 and 9) with respect to the control. There was significant decrease in proteinuria after aspirin treatment and also after co-treatment with chloroquine and aspirin (Table 9) implying that the mild chloroquine induced hyperproteinuria was antagonised by the aspirin induced hypoproteinuria (Table 8).

Indomethacin on its own induced a very mild decrease in proteinuria (Table 9) with respect to the control. A similar pattern of effect was observed with chloroquine plus indomethacin combination suggesting anatagonistic effect of chloroquine induced hyperproteinuria.

However the antagonism by indomethacin was not as pronounced as with aspirin.

The expected potentiation can be explained by the findings that aspirin decreased the bioavailability of indomethacin by either decreasing its gastrointestinal

Table 8 MEAN % CHANGE IN PROTEINURIA INDUCED BY CHLOROQUINE (CQ), ASPIRIN (AA), INDOMETHACIN (IM) AND THEIR COMBINATIONS IN RATS

n = 6 mean (SEM)

DRUGS	DOSE(mg/kg)	Mean % CHANGE IN PROTEINURIA		
		DAY 1	DAY 2	DAY3
Control	Normal Saline 20.7(Day 1)	-1.8(0.29)	+2.5(0.5)	0.0(0.0)
CQ	6.9(Day 2&3)	+28.6(1.3)	+37.1(1.2)	+40.0(4.9)
AA	50.0	-27.9(1.6)	-31.3(0.4)	-45.8(1.9)
Im	4.3	-11.9(1.9)	-17.4(2.9)	-25.6(2.8)
CQAA	50+51.4	-9.3(2.6)	-14.9(2.3)	-23.9(5.6)
CQIM	50+4.3	-8.3(3.8)	-19.0(4.8)	-20.2(2.9)
AAIM	51.4+4.3	-20.9(4.2)	-25.9(1.5)	-27.9(3.6)

CQAA - Chloroquine + Aspirin

CQIM - Chloroquine + Indomethacin

AAIM - Aspirin + Indomethacin

TABLE 9 MEAN % CHANGE IN PROTEINURIA AFTER A 3 DAY TREATMENT WITH CHLOROQUINE, ASPIRIN, INDOMETHACIN AND THEIR COMBINATIONS

Drug	Total mean* % proteinuria with respect to control after 3 days
Chloroquine	+9.0
Aspirin	-25.99
Indomethacin	-9.04
Chloroquine + Aspirin	-16.95
Chloroquine + Indomethacin	-14.69
Aspirin + Indomethacin	-11.86

* = Total mean was obtained after adding the mean values for the 3 day treatment period.

Total mean % change was obtained by dividing the mean proteinuria induced by drug or drug combination by the normal mean values x 100.

(-) Value indicates a decrease in proteinuria with respect to normal value.

(+) Value indicates an increase in proteinuria with respect to normal values.

FIGURE 1 THREE DAY TOTAL PROTEIN EXCRETION IN RATS AFTER ORAL TREATMENT WITH CHLOROQUINE (CQ) ASPIRIN (AA) INDOMETHACIN (IM) AND THEIR COMBINATIONS

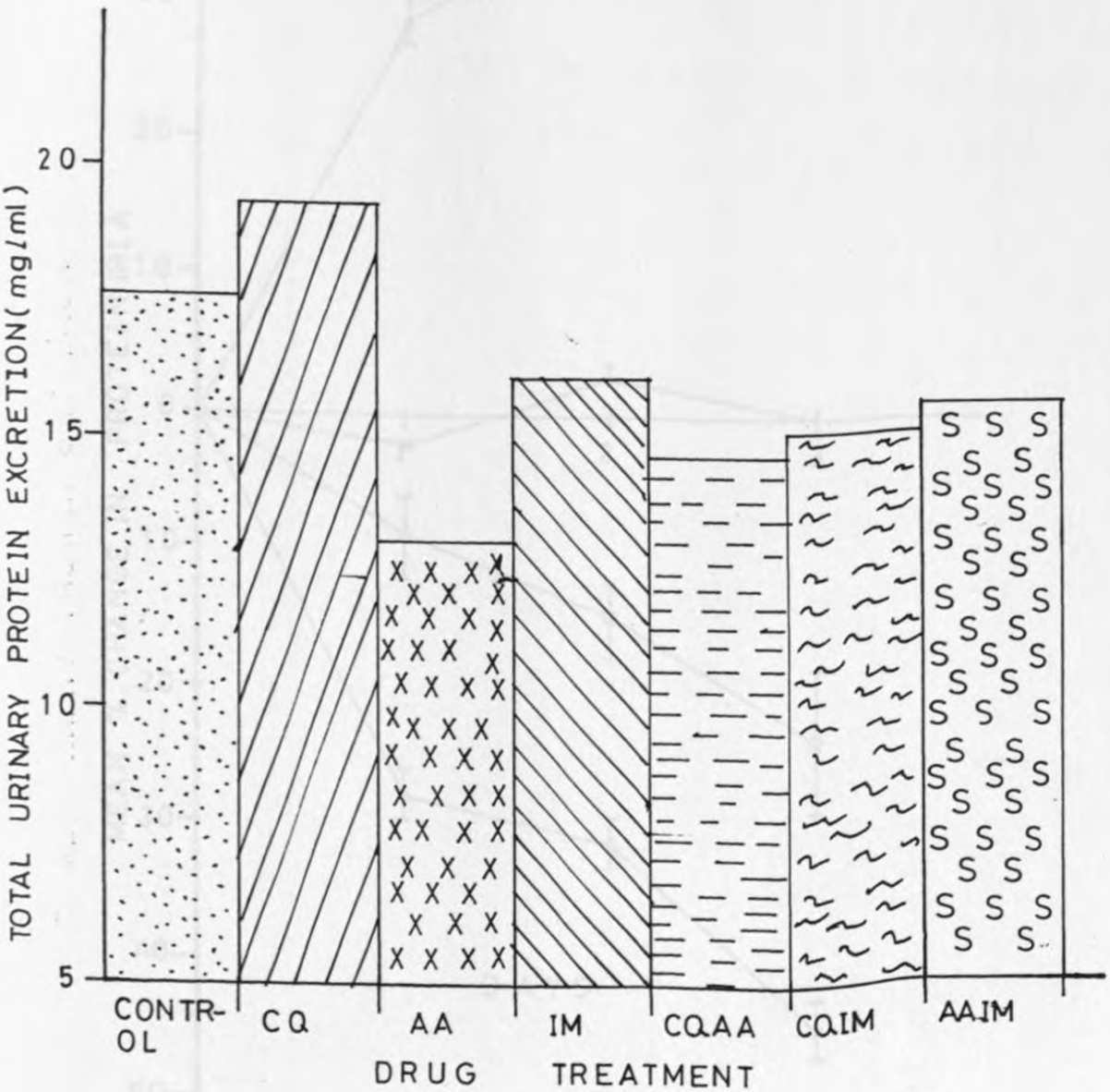


FIGURE 2 MEAN % CHANGE IN PROTEINURIA INDUCED BY CHLOROQUINE, ASPIRIN AND THEIR COMBINATIONS n=6, Error bars=SEM

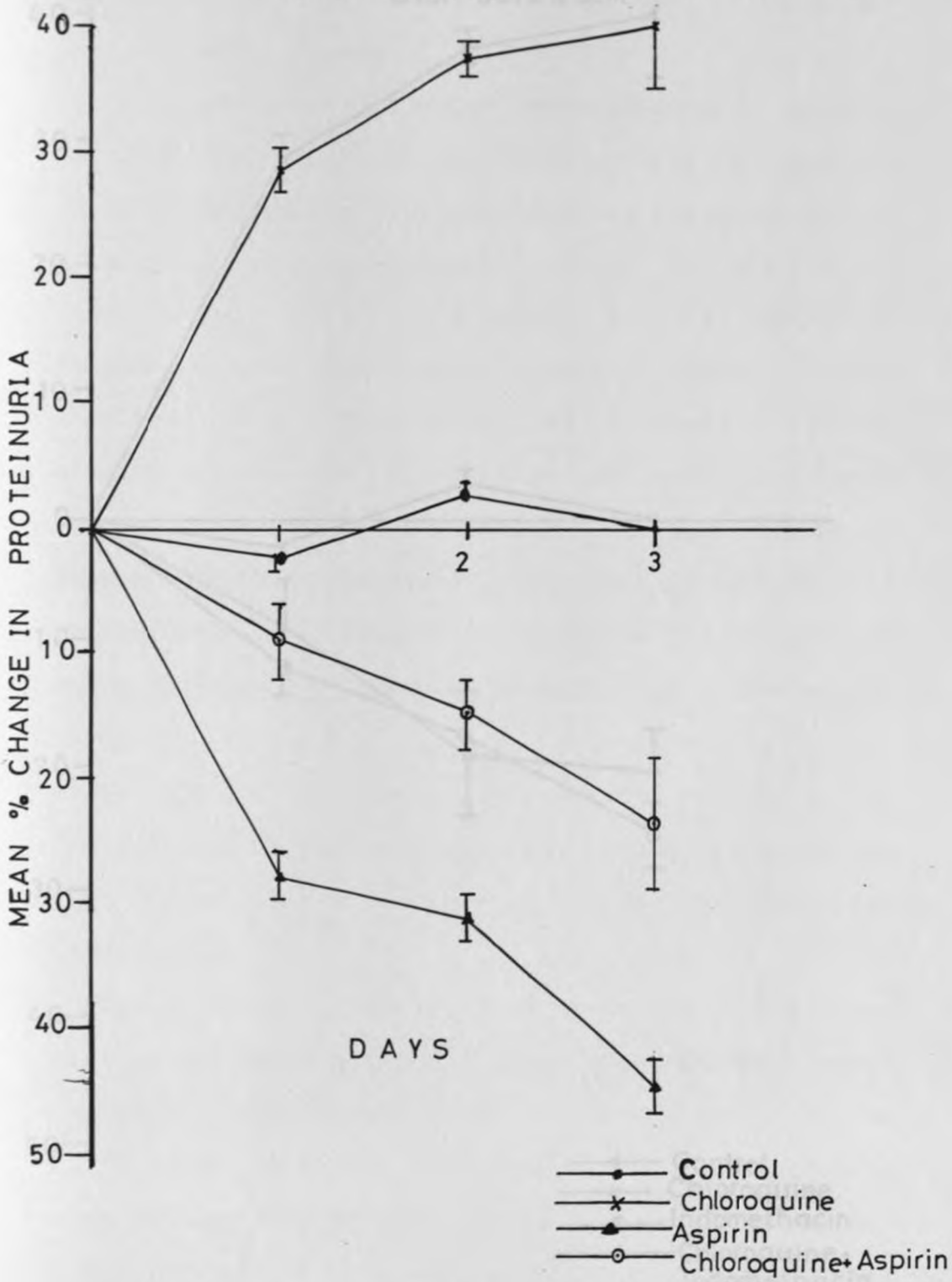
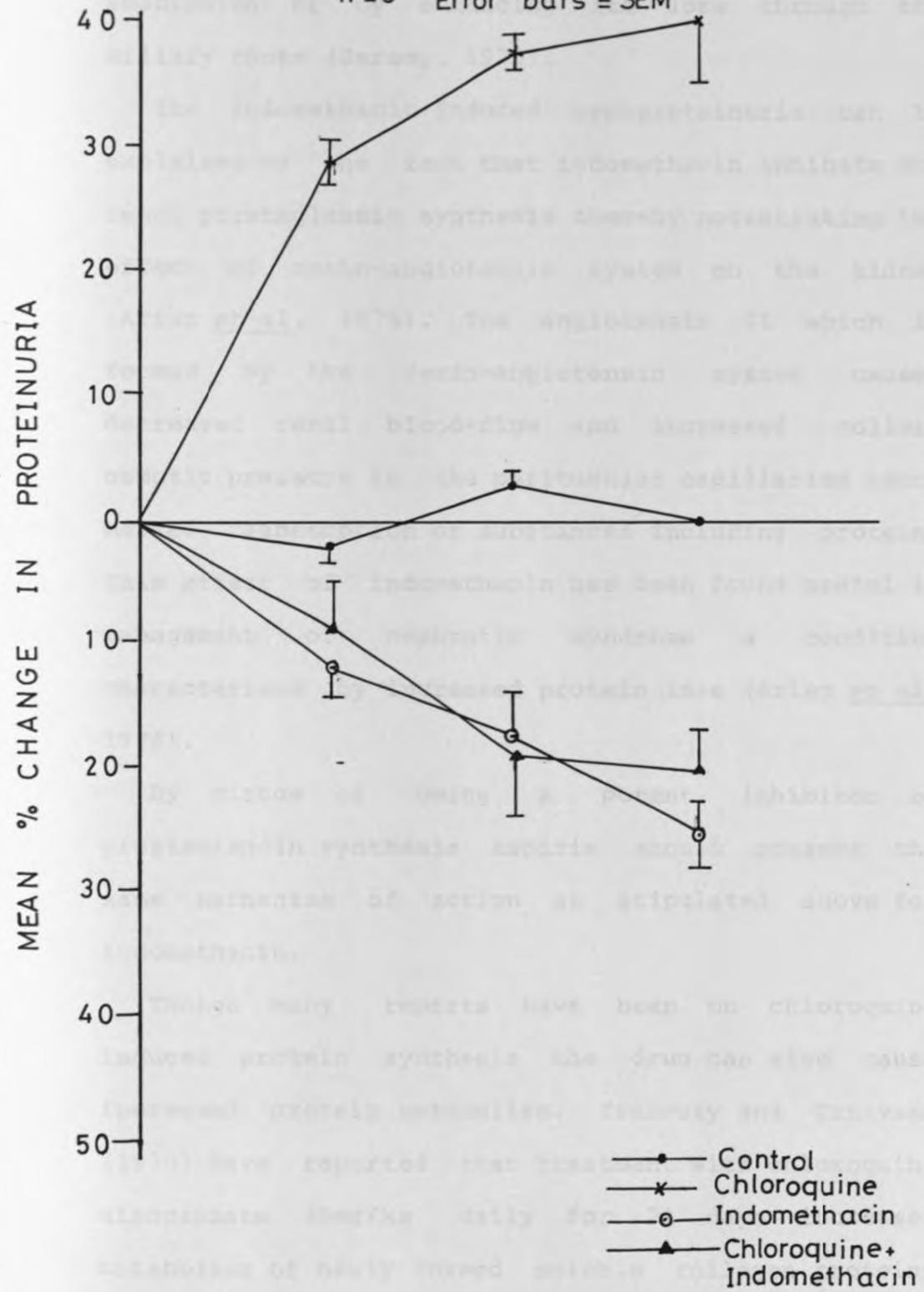


FIGURE 3 MEAN % CHANGE IN PROTEINURIA INDUCED BY CHLOROQUINE, INDOMETHACIN AND THEIR COMBINATIONS

n =6 Error bars = SEM



absorption or by enhancing its loss through the biliary route (Jeremy, 1970).

The indomethacin induced hypoproteinuria can be explained by the fact that indomethacin inhibits the renal prostaglandin synthesis thereby potentiating the effect of renin-angiotensin system on the kidney (Arisz et al, 1976). The angiotensin II which is formed by the renin-angiotensin system caused decreased renal blood-flow and increased colloid osmotic pressure in the peritubular capillaries hence marked reabsorption of substances including protein. This effect of indomethacin has been found useful in management of nephrotic syndrome a condition characterised by increased protein loss (Arisz et al, 1976).

By virtue of being a potent inhibitor of prostaglandin synthesis aspirin should possess the same mechanism of action as stipulated above for indomethacin.

Though many reports have been on chloroquine induced protein synthesis the drug can also cause increased protein metabolism. Trnavsky and Trnavska (1970) have reported that treatment with chloroquine diphosphate 30mg/kg daily for 21 days increased catabolism of newly formed soluble collagen proteins without significantly affecting the catabolism of insoluble collagen. Chloroquine has also been reported

to increase the rate of proteolysis *in vitro* within the liver lysosomes by activating the intraparticulate hydrolysis (Bertini and Daniel, 1970). These findings are in agreement with those obtained from the present study indicating a slight increase in chloroquine induced proteinuria.

The results of the present study have important clinical implications in that they indicate a possible contraindication of chloroquine in nephrotic patients suffering from chloroquine sensitive malarial infection. But the results go further to show that a combination of chloroquine and aspirin or chloroquine and indomethacin would be beneficial to nephrotic patients since in the course of treating the malarial attack with the accompanying pyrexia, the excessive urinary protein loss characteristic of nephrotic syndrome will be counteracted by the drug combination.

3.2.3 EFFECT OF CHLOROQUINE ON BLOOD SUGAR LEVELS AND INTERACTION WITH CHLORPROPAMIDE

Results of the present study have indicated that chloroquine increases urinary glucose excretion in rats (Table 10 and 11) implying nephrotic damage by the drug. Treatment of rabbits with single daily doses of chloroquine, 25mg/kg orally for 4 days caused progressive decrease in non fasting blood sugar levels ranging from -19.7 (2.8)% after the first dose to -33.1 (3.4)% after the fourth dose (Table 12). There is a significant difference ($p<0.05$) to ($p<0.02$) between the hypoglycaemic potency of chlorpropamide and that of chloroquine. After the first dose of 4mg/kg, chlorpropamide induced a mean % change in glycaemia of -36.95 (3.45)% as compared to -19.7 (2.8)% induced by 25mg/kg chloroquine.

The figure after the fourth dose of chlorpropamide was -43.3 (2.8)% as compared to that of chloroquine, -33.1 (3.4)%. The results of the present study clearly show that chloroquine has hypoglycaemic properties even when used in anti-malarial doses and for short periods of time.

Contrary to the above findings White et al (1986) have reported that though chloroquine may cause inhibition of insulin degradation (Blazar et al, 1984; Smith et al, 1937) there is no evidence that the drug causes hypoglycaemia in malaria. The authors (White et

al, 1987b) attributed any hypoglycaemia experienced during a malaria attack to the disease rather than the drug. Similar results have been reported by Brueton and Greenwood (1987) who while working with Nigerian children found that severe hypoglycaemia sometimes with poor prognosis can occur during acute malaria attack and this could be the cause of the irreversible cerebral damage that occurs in untreated malaria. Fisher (1983) has also reported similar findings whereas Ogbuokiri (1987) found no change in blood glucose levels after intramuscular 10mg/kg chloroquine injection in healthy men.

In good agreement with the findings of the present research, Bamber and Rodpath (1987) gave a detailed account of a case of chloroquine suicide characterised by severe hypoglycaemia (1mmol/l) convulsions, unconsciousness and bradycardia and despite careful fluid monitoring drug therapy and intermittent positive pressure ventilation the patient's blood pressure fell progressively and he died 18 hours later. Liver chloroquine concentration was 57.2mg/100g liver which is within the range associated with chloroquine poisoning (Kiel, 1964). The hypoglycaemia could not be attributed to anything else except chloroquine.

In addition to inducing hypoglycaemia chloroquine was found to enhance chlorpropamide induced

hypoglycaemia when the two drugs were administered together. The potentiation was highly significant ($p<0.02$) after two days treatment when the mean percentage decrease in glycaemia stood at -46.05 (5.0)% for the drug combination as compared to -38.8 (1.35)% for chlorpropamide alone (Table 12 and Figure 5). Figure 5 shows a plot of mean % reduction in non-fasting blood sugar levels against time.

The influence of chloroquine on glucose homeostasis has previously been recognised. Some authors have suggested that chloroquine can be useful in treating Type II diabetes that is resistant to insulin therapy (Rees and Smith, 1987). Its mode of action has been attributed to its ability to cause in vivo inhibition of insulin degradation (Blazer et al, 1984). This concurs with the present findings obtained using rabbits.

Although chloroquine can improve glucose homeostasis in diabetics (White et al, 1987(a)) the interaction between this drug and chlorpropamide and other oral hypoglycaemic agents should be borne in mind. The interaction is of clinical significance in view of the chronic nature of diabetes, requiring life long therapy with hypoglycaemic agents and also the availability of chloroquine encouraging an increase in self medication for any symptoms associated with malarial attack.

TABLE 10 INFLUENCE OF CHLOROQUINE (CQ), ASPIRIN (AA), INDOMETHACIN (IM) AND THEIR COMBINATION ON URINARY GLUCOSE EXCRETION IN RATS

mg % n = 12 mean (SEM)

DAY	CONTROL	CQ	AA	IM	CQAA	CQIM	AAIM
0	11.6	11.4	12.9	10.8	12.6	11.5	11.7
	(0.73)	(0.86)	(1.06)	(0.41)	(0.72)	(0.53)	(0.41)
1	11.4	12.9	11.9	9.9	12.9	11.9	12.2
	(0.64)	(0.66)	(1.11)	(0.47)	(0.59)	(0.49)	(0.41)
2	11.7	14.3	11.5	8.60	12.5	11.6	11.7
	(0.73)	(0.67)	(0.92)	(0.66)	(0.76)	(0.40)	(0.58)
3	11.5	14.9	9.80	7.90	12.6	12.1	11.3
	(0.80)	(0.91)	(1.18)	(0.43)	(0.74)	(0.54)	(0.42)
Total	46.2	53.5	46.1	37.2	50.6	47.1	46.9
	(2.90)	(3.10)	(4.30)	(1.97)	(2.81)	(1.96)	(1.82)

CQAA - Chloroquine + Aspirin

CQIM - Chloroquine + Indomethacin

AAIM - Aspirin + Indomethacin

TABLE 11 % MEAN TOTAL CHANGE IN GLUCOSURIA IN RATS AFTER 3 DAY TREATMENT WITH CHLOROQUINE ASPIRIN, INDOMETHACIN AND THEIR COMBINATIONS.

Drug	Mean Total* % change in glucosuria in rats after 3 days treatment period
Chloroquine	+15.80
Aspirin	-0.23
Indomethacin	-19.48
Chloroquine + Aspirin	+9.50
Chloroquine + Indomethacin	+2.16
Aspirin + Indomethacin	+1.5

* = Mean total % change was calculated with respect to total mean control values.

TABLE 12 THE INFLUENCE OF CHLOROQUINE ON THE CHLORPROPAMIDE INDUCED HYPOGLYCAEMIA. n = 6 mean (SEM)

MEAN % CHANGE IN GLYCAEMIA ON ORALLY TREATED RABBITS WITH

Time (hrs)	Control saline	Chloroquine 25mg/kg	Chlorpropamide 4mg/kg	Chloroquine+ Chlorpropamide 25mg+4mg/kg
0	0.0	0.0	0.0	0.0
24	2.5(1.25)	-19.7(2.8)	-36.95(3.45)	-33.02(4.0)
48	1.65(0.24)	-20.7(3.0)	-32.87(1.80)	-37.22(3.1)
72	5.00(0.28)	-30.2(2.3)	-38.86(1.35)	-46.05(5.1)
96	2.45(1.65)	-33.1(3.4)	-43.30(2.80)	-53.1(2.0)

Experiment was carried out in non-fasting state of the rabbits

There was statistically significant difference ($p<0.05$ to $p<0.02$) between the different groups

FIGURE 4 THREE DAY TOTAL URINARY GLUCOSE EXCRETION IN RATS AFTER ORAL TREATMENT WITH CHLOROQUINE, ASPIRIN, INDOMETHACIN AND THEIR COMBINATIONS.

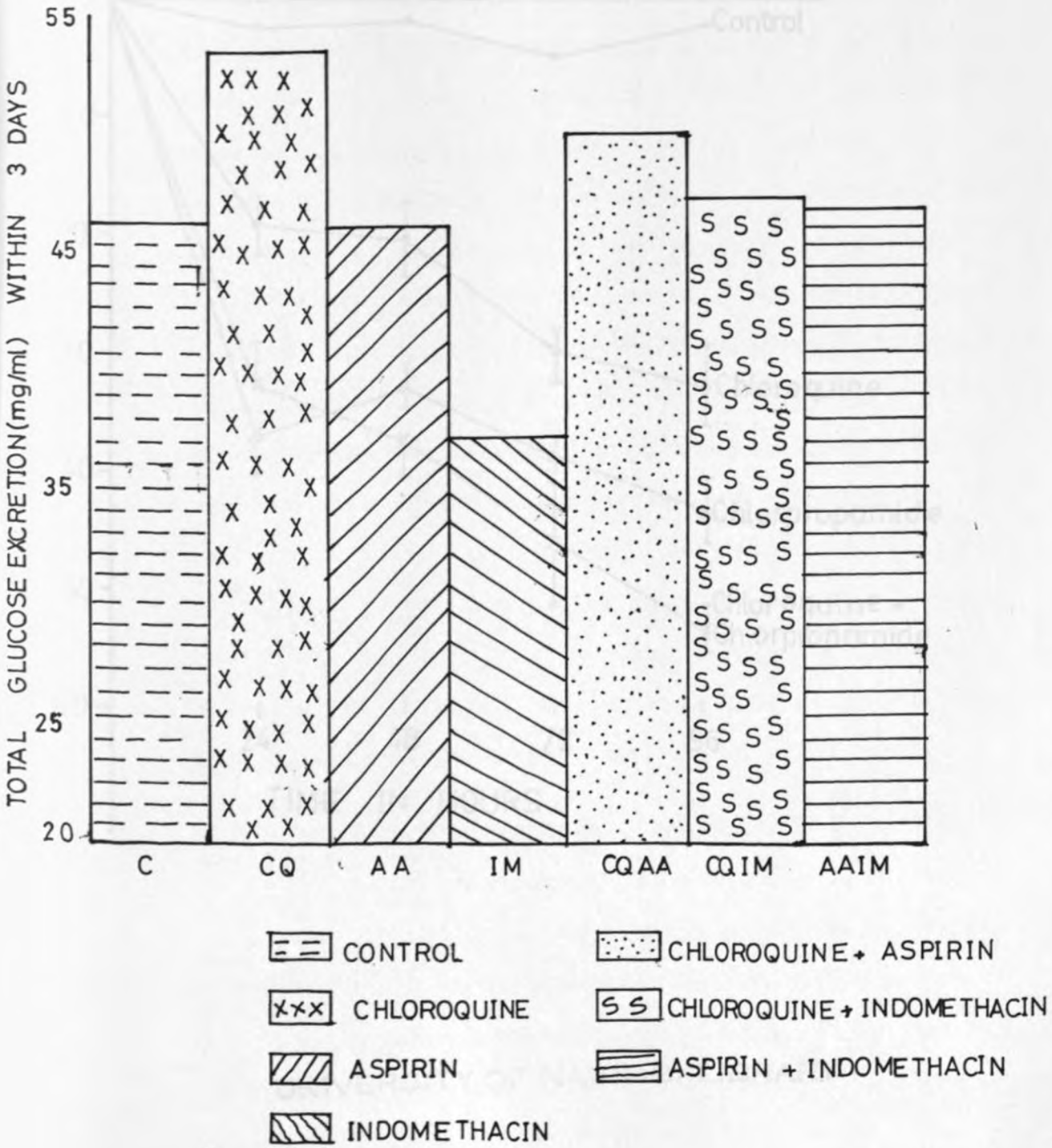
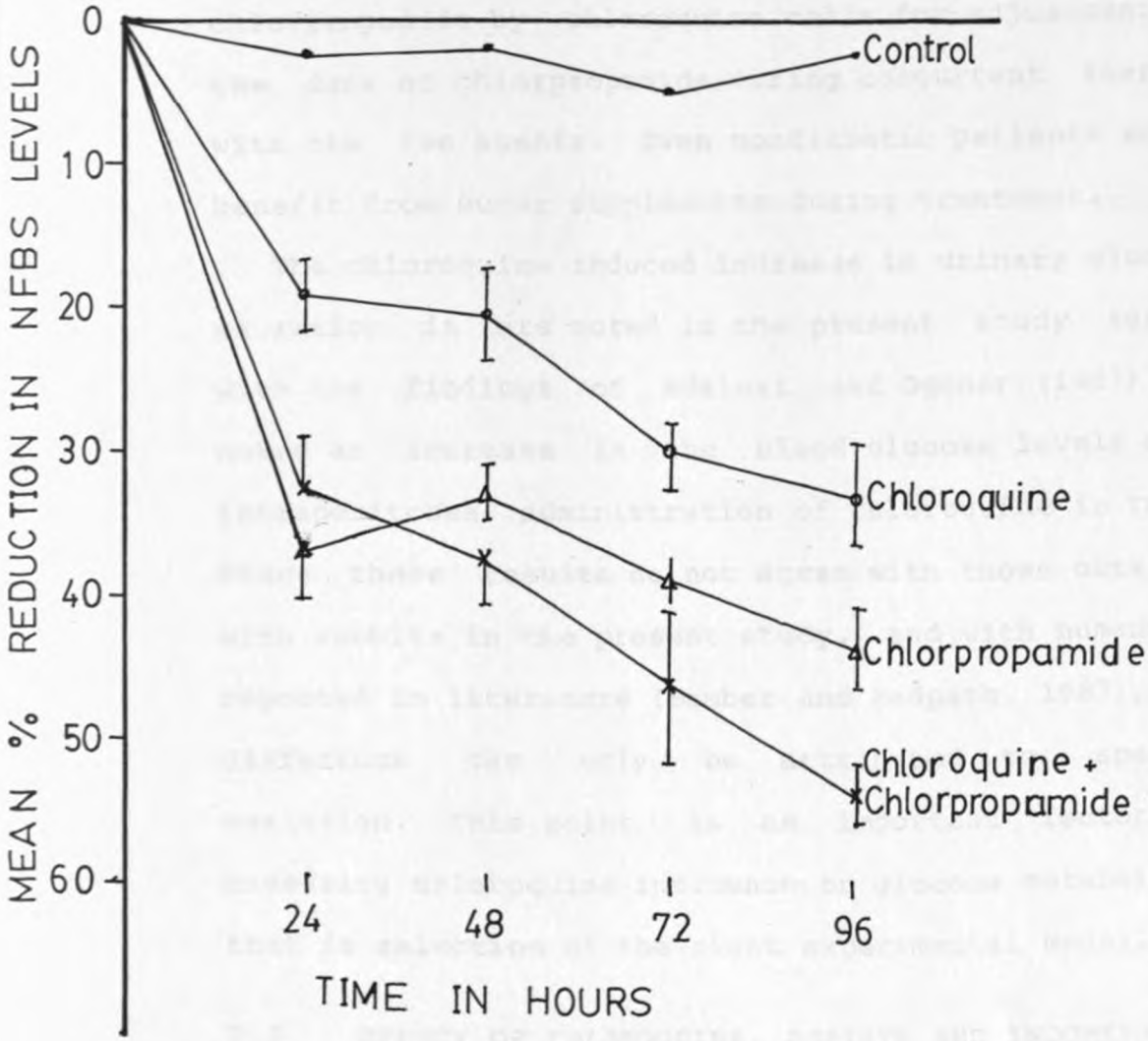


FIGURE 5 THE INFLUENCE OF CHLOROQUINE ON CHLORPROPAMIDE - INDUCED HYPOGLYCAEMIA IN RABBITS



The prescribing and dispensing of chloroquine to patients without due regard to the type of medication that the patient is currently on should be discouraged.

The potentiation of the hypoglycaemic effect of chlorpropamide by chloroquine calls for adjustment of the dose of chlorpropamide during concurrent therapy with the two agents. Even nondiabetic patients would benefit from sugar supplements during treatment.

The chloroquine induced increase in urinary glucose excretion in rats noted in the present study agrees with the findings of Adelusi and Ogonor (1987) who noted an increase in the blood glucose levels upon intraperitoneal administration of chloroquine in rats. Since these results do not agree with those obtained with rabbits in the present study, and with humans as reported in literature (Bamber and Redpath, 1987), the difference can only be attributed to species variation. This point, is an important factor in assessing chloroquine influence on glucose metabolism, that is selection of the right experimental model.

3.3 EFFECT OF CHLOROQUINE, ASPIRIN AND INDOMETHACIN ON ERYTHROCYTE SEDIMENTATION RATE (ESR)

Results obtained in the present experiment indicate that when compared to the control, chloroquine caused the highest rate of erythrocyte sedimentation of 2.6 (0.038)mm/hr equivalent to 52.63% increase over the

control. This was followed by chloroquine/aspirin combination 2.17 (0.087)mm/hr equivalent to 26.9% increase), indomethacin 2.0 (0.102)mm/hr equivalent to 17.0% increase) and chloroquine/indomethacin combination 1.83 (0.079)mm/hr equivalent to 17.0% increase). The lowest ESR was recorded for aspirin 1.44 (0.038)mm/hr equivalent to 12.28% decrease), whereas the aspirin/indomethacin combination induced an ESR comparable to the control value 1.71 0.038)mm/hr). The results are summarised in Table 13.

In addition to recording raised ESR for the chloroquine and chloroquine combination groups, there was marked haemolysis in all the blood samples obtained from these groups.

However aspirin, indomethacin and their combination did not induce any in vitro haemolysis.

Some disease conditions are characterised by raised blood and plasma viscosity. This interferes with blood flow, causing a decrease in the rate of blood flow especially through the smaller blood vessels (lower limbs, fingers and toes are commonly affected). There is increased fibrinogen levels, digital ischaemia (McGrath et al, 1973; Friedman et al, 1969; McGrath and Penny, 1974).

Diseases associated with the above pathological factors include Raynauds syndrome (Lottenbach, 1969; Pringle et al, 1965), various inflammatory, malignant,

necrotic conditions and malaria (Rigdon, 1950).

Malaria is a disease condition characterised by increased rigidity of fragmented red cells and decreased ability of smaller but more rigid cells to pass through small blood vessels. There is aggregation of the parasite infected red cells with possible occlusion of small blood vessels (Dintenfass, 1976). This can lead to decreased renal glomerular filtration rate (Sitprija et al, 1967); Wells and Schmid-Schonbein, 1968) and possible renal failure (Sitprija et al, 1967). Sludging of blood has been shown to occur in malaria (Rigdon, 1950) and improvement of renal function has been demonstrated after antimalarial treatment (Madow, 1960).

Results of this study have shown that chloroquine causes a significant increase in the ESR (Table 13). It is therefore possible that the drug acts by decreasing the viscosity of the blood or decreasing the aggregation of red blood cells, hence increasing the rate of blood flow through small blood vessels including those of the kidneys and the brain.

Indeed chloroquine is very useful in management of cerebral malaria caused by chloroquine sensitive strains of Plasmodium falciparum.

Antimalarial agents have effectively been used as "desludging" agents in vascular disease process (Madow, 1960).

TABLE 13 EFFECT OF CHLOROQUINE (CQ), ASPIRIN (AA), INDOMETHACIN (IM) AND THEIR COMBINATIONS ON THE ERYTHROCYTE SEDIMENTATION RATE (ESR) in mm/hr

n = 7 mean (SEM)

Rabbit	Control	CQ*	AA	IM	CQAA*	CQIM	AAIM
1	1.70	2.60	1.50	1.93	2.00	2.00	1.50
2	1.85	2.50	1.47	1.50	2.30	2.00	1.90
3	1.60	2.60	1.40	2.00	2.50	2.18	1.50
4	1.70	2.50	1.13	2.00	2.00	1.93	2.15
5	1.85	2.80	1.50	1.91	1.90	1.50	1.95
6	1.65	2.64	1.50	2.00	2.50	1.90	2.00
7	1.60	2.71	1.40	2.50	2.00	1.50	1.15
mean	1.71	2.62	1.41	1.98	2.17	1.86	1.74
(SEM)	(0.04)	(0.04)	(0.05)	(0.11)	(0.10)	(0.10)	(0.14)

Each mean is the mean of three determinations obtained from a particular rabbit.

* - These samples were characterised by marked haemolysis

Aspirin has the ability to inhibit platelet aggregation (Joseph et al, 1987) and to interfere with synthesis of vitamin K dependent clotting factors VII, IX and X, thus prolonging prothrombin time (Stuart et al, 1979). The drug has mild anticoagulant effects (James and Walsh, 1985). It is for these reasons that aspirin finds clinical application in management of Raynaud's disease (Fitzgerald and Butterfield, 1969), ischaemic heart disease and other conditions with possible incidence of thrombosis (Zekert et al, 1975; Vreeken and Aken, 1971; Preston et al, 1974).

Since aspirin is commonly used with chloroquine in management of pyrexia resulting from a clinical malarial attack (Table 5), it was found necessary to investigate the effect of aspirin/chloroquine therapy on the physical flow properties of the blood.

The results obtained in the present study indicate that aspirin/chloroquine combination significantly raised the ESR (26.9% increase) as compared to aspirin alone (-12.28%).

The decreased ESR after aspirin treatment could be due to the ability of the drug to inhibit platelet aggregation through inhibition of cyclo-oxygenase enzyme, hence decreased formation of thromboxane A2 (Szczeklik, 1980).

The increase in ESR after chloroquine treatment

could possibly be due to the ability of the drug to decrease plasma viscosity and hence allow free fall of the erythrocytes.

Both of these properties could be useful in management of vascular disorders characterised by tissue ischaemia.

The drug interaction between aspirin and chloroquine may be useful in management of cerebral malaria with chloroquine susceptible strains of the malaria parasite.

The haemolysis noted in all the blood samples obtained from chloroquine treated rabbits is of clinical significance in that it points at a likely haemolytic side effects of chloroquine in humans. In fact intravascular haemolysis has been noted in humans with glucose-6-phosphate dehydrogenase deficiency following antimalarial therapy with chloroquine (Choudry et al, 1980).

3.4 THE INFLUENCE OF CHLOROQUINE ON THE ULCEROGENIC EFFECTS OF ASPIRIN AND INDOMETHACIN

The permeability of phenol red, a poorly absorbed drug was used as index of assessment of gastrointestinal mucosal damage in vivo in rats (Nakamura et al, 1983). It was chosen as a marker compound due to its poor absorbability at any physiological pH of the gastrointestinal tract, rapid renal tubular secretion and ease of assay. It is

completely ionised at pH above 1 and hence has poor lipoid solubility and consequently very low affinity of the intestinal mucosa. Nakamura et al (1976 a,b) and Schanker et al (1957, 1958) have shown that phenol red is poorly absorbed from the stomach and small intestines of the rats.

The effects of oral pretreatment with chloroquine, aspirin, indomethacin and their combinations on the gastric mucosa in rats, were assessed using the spectrophotometric method of Nakamura et al (1983).

The results indicate that the urinary recovery of phenol red and hence the ulceration index were significantly increased after oral treatment with aspirin, indomethacin, aspirin-indomethacin, aspirin-chloroquine and chloroquine-aspirin combinations.

Results obtained with chloroquine may suggest that on its own, the drug is not ulcerogenic (Table 15). However the dose for chloroquine was 2.5 times less than that of aspirin. Considered on equal dose levels, and assuming that the ulcerogenic potential of the drugs is proportional to the drug concentration, chloroquine was as effective as aspirin as an ulcerogenic agent (Table 16).

In addition to having ulcerogenic properties of its own, chloroquine was also found to potentiate the ulcerogenic effect of aspirin and indomethacin (Table 16). Though the present short term experiments were

TABLE 14 MEAN OPTICAL DENSITIES OF URINE COLLECTED FROM RATS FED ON SPECIFIED DRUGS n = 6 MEAN (SEM)

DRUGS	mg/kg DOSE	4Hrs	8Hrs	24Hrs
CQ	20	0.0069	0.0104	0.0076
		(0.0018)	(0.0027)	(0.0017)
IM	25	0.0220	0.0422	0.0168
		(0.0081)	(0.0092)	(0.0021)
AA	50	0.0150	0.0262	0.0165
		(0.0019)	(0.0051)	(0.0044)
AAIM	50/25	0.0240	0.0410	0.0341
		(0.0079)	(0.0070)	(0.0038)
AACQ	50/20	0.0166	0.0301	0.0248
		(0.0019)	(0.0049)	(0.0037)
CQIM	20/25	0.0138	0.0262	0.0203
		(0.0028)	(0.0052)	(0.0037)

AAIM - Aspirin + Indomethacin

AACQ - Aspirin + chloroquine

CQIM - Chloroquine + Indomethacin

TABLE 15 ULCERATION INDEX OF CHLOROQUINE (CQ), INDOMETHACIN (IM), ASPIRIN (AA) AND THEIR COMBINATIONS WITH RESPECT TO ASPIRIN

n = 6 mean (SEM)

DRUG	DOSE mg/kg	4Hrs	8Hrs	24Hrs
CQ	20	0.46	0.40	0.46
AA	50	1.00	1.00	1.00
IM	25	1.47	1.61	1.02
AAIM	50/25	1.60	1.56	2.07
CQAA	50/20	1.11	1.15	1.50
CQIM	20/25	0.92	1.00	1.23

AAIM - Aspirin + Indomethacin

AACQ - Aspirin + Chloroquine

CQIM - Chloroquine + Indomethacin

Ulceration index was calculated using the mean optical densities (see Table 14)

TABLE 16 COMPARATIVE ULCERATION INDICES OF CHLOROQUINE AND INDOMETHACIN

DRUG	Doses mg/kg	4Hrs	8Hrs	24Hrs
AA	50	1.00	1.00	1.00
CQ	25	1.15	1.00	1.15
IM	50	2.94	3.22	2.04
IMCQ	50+50	1.84	2.00	2.46

Aspirin was taken as standard

AA - Aspirin

CQ - Chloroquine

IM - Indomethacin

IMCQ - Indomethacin + Choroquine

carried out in animals, they give a reflection of the likely drug interaction that might occur in human. The possibility of inducing ulcer formation during long term therapy with high doses of chloroquine for example in treatment of systemic lupus erythematosus, rheumatic or arthritic conditions or in management of porphyria should be borne in mind. There is even a higher possibility when chloroquine, aspirin and indomethacin are used in combination. These have the ability to exacerbate an already existing ulcer, even reactivate a quiescent or a partially healed one.

The combination of chloroquine and aspirin or chloroquine/indomethacin were found to be highly ulcerogenic in rats even after short term treatment (Table 15). Caution should therefore be exercised during combination therapy with these agents during treatment of clinical malaria and the attendant pyrexia in people with a history of peptic or gastric ulcers.

3.5 INFLUENCE OF CHLOROQUINE ON THE RESPONSE OF AN ISOLATED RABBIT HEART TO DRUGS

Death from chloroquine intoxication is invariably due to cardiac toxic effects of chloroquine that are characterised by decreased cardiac output, disturbances of impulse condition, bradycardia, arrhythmias and electrocardiogram changes (Michael et al, 1970).

The present results show that chloroquine exerts a dose dependent negative inotropic and chronotropic effects on the heart ranging from 0.0 to -36.8% and 0.0 to -46.8% at 2µg and 20µg dose ranges respectively (Table 17). The mean percentage decrease in heart rate is more pronounced than the mean percentage change in contractile force (Figure 6 and Table 17).

These effects of chloroquine have been attributed by some authors to direct action of the drug on the heart (Michael et al, 1970) or via enhanced parasympathetic activity (Agarwal and Arora, 1956). The drug has also been shown to possess beta-receptor blocking effects (Cao et al, 1970) and even adrenergic neurone blocking effects (Jindal, 1970).

Present results show that chloroquine antagonised the positive inotropic effect of adrenaline and isoprenaline. In addition it antagonised barium, calcium and potassium induced cardiostimulation (Table 17). The drug also significantly decreased the positive chronotropic effects induced by the above mentioned myocardial stimulants.

Adrenaline is an alpha and beta adrenergic receptor agonist, whereas isoprenaline is a potent beta-receptor agonist with minimal or no effect on the alpha-adrenergic receptors. The antagonism of the cardiostimulant effects of adrenaline and isoprenaline by chloroquine suggests that this drug has some alpha

and beta-receptor blocking effects. This drug interaction could be put into some clinical application whereby adrenaline could be used to antagonise the cardiodepressant effects induced by chloroquine poisoning. In actual fact adrenaline in conjuction with diazepam have been tried as antidotes to chloroquine poisoning (Bruno et al, 1988; Michael et al, 1970).

However it should be realised that the antagonism of adrenaline and chloroquine is dose dependent. Preliminary results have indicated that the duration and the magnitude of chloroquine induced myocardial depression is crucial if adrenaline has to be an effective antidote, implying that the sooner the treatment of poisoning is started the better the prognosis.

The interaction between chloroquine and isoprenaline could also be of clinical significance. Isoprenaline is clinically used in management of heart block (Stokes-Adams) syndrome with syncope (Goodman and Gilman, 1985; Koda-Kimble et al, 1980). Administration of myocardial depressants is contraindicated in patients with high degree of heart block as this would increase the atrial-ventricular blockade and suppress the ventricular pacemakers leading to cardiac standstill (Koda-Kimble, 1980). The present results indicate that chloroquine, a potent

TABLE 17 INFLUENCE OF CHLOROQUINE(CQ) ON THE RESPONSE OF AN ISOLATED RABBIT HEART TO DRUGS

mean % change n = 8

Drug	Dose	Drug		Drug+CQ			Myocar Response	
		Inot	Chrono	Dose	Inot.	Chrono.	Inot.	Chrono.
CQ	2ug	0.0	0.0					
	4ug	-5.0	-22.6					
	8ug	-30.6	-44.4					
	10ug	-32.3	-44.8					
	20ug	-36.8	-46.8					
Atropine sulphate	20ug	+17.6	0.0	20ug	-33.3	0.0	-50.9	0.0
Potassium chloride	2mg	+33.3	0.0	10ug	+20.0	0.0	-13.3	0.0
				20ug	-56.8	0.0	-90.1	
Adrenaline	0.2ug	156.9	50.5	2ug	151.3	47.5	-5.6	-3.0
				4ug	143.4	25.5	-13.5	-25.0
				6ug	112.7	15.5	-44.2	-35.0
				8ug	102.5	8.2	-54.4	42.3
				10ug	58.9	0.0	-98.0	-50.3
Isoprenaline	50ug	146.1	40.6	2ug	143.8	32.8	-2.3	-7.8
				4ug	132.4	26.7	-13.7	-13.9
				6ug	95.8	25.8	50.3	-14.8
				8ug	86.7	20.2	-59.4	-20.4
				10ug	45.3	8.3	-100.8	-32.3

TABLE 17 CONT.

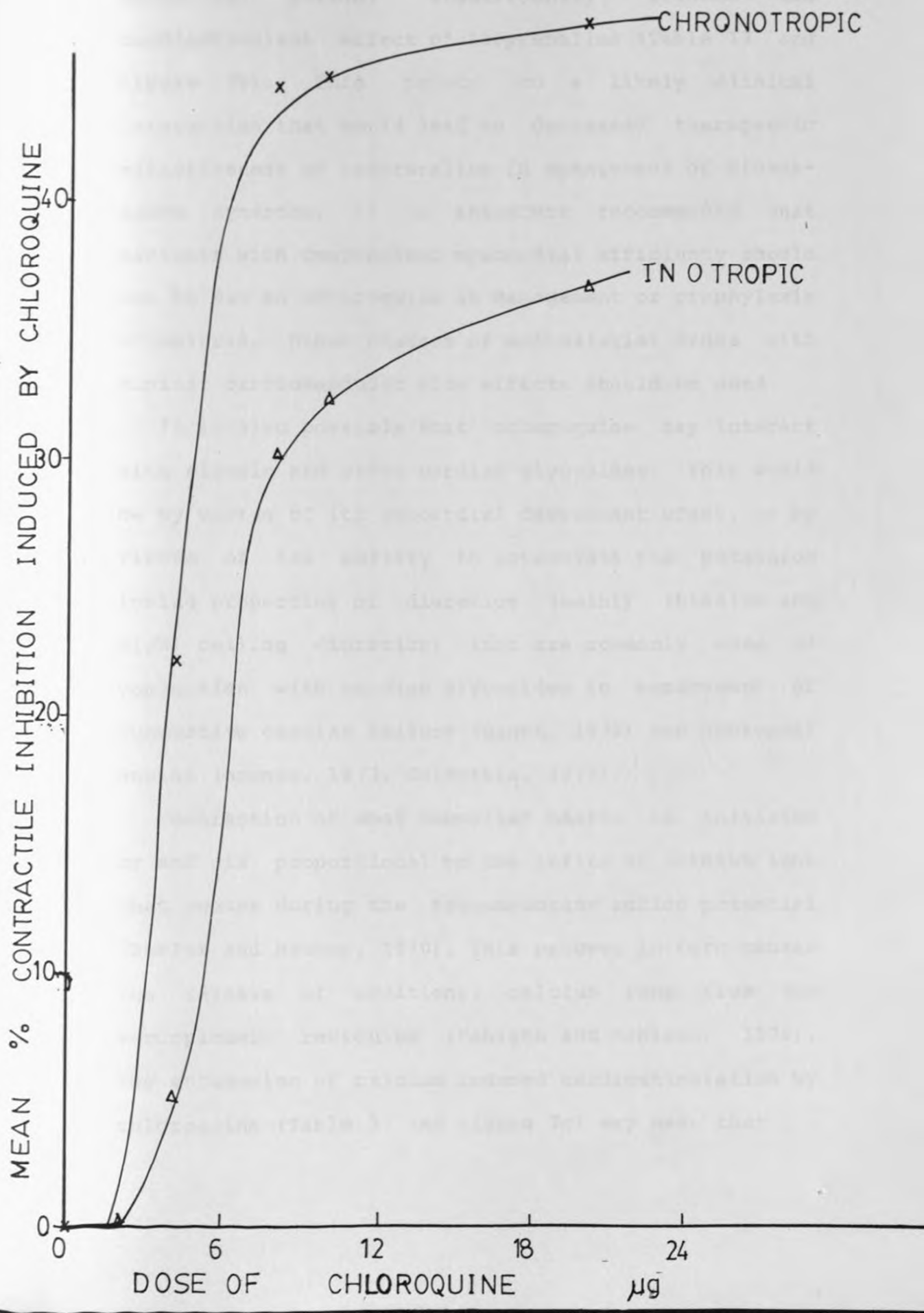
Barium chloride	1mg	94.7	10.6	2ug	85.0	10.6	-9.7	0.0
				4ug	70.6	6.9	-24.1	-3.7
				6ug	40.0	2.4	-54.7	-8.2
				8ug	10.3	0.0	-84.3	-10.6
				10ug	-22.9		-115.6	
				20ug	-55.3		-150.0	
Calcium sulphate	5.0mg	129.2	0.0	4ug	130.3	0.0	+1.0	0.0

Myocar - myocardial

Inot - Inotropic

Chrono - Chronotropic

FIGURE 6 INFLUENCE OF CHLOROQUINE ON INOTROPIC AND CHRONOTROPIC RESPONSES



myocardial poison, significantly reduced the cardiostimulant effect of isoprenaline (Table 17 and Figure 7b). This points to a likely clinical interaction that would lead to decreased therapeutic effectiveness of isoprenaline in management of Stokes-Adams syndrome. It is therefore recommended that patients with compromised myocardial efficiency should not be put on chloroquine in management or prophylaxis of malaria. Other classes of antimalarial drugs with minimal cardiovascular side effects should be used.

It is also possible that chloroquine may interact with digoxin and other cardiac glycosides. This would be by virtue of its myocardial depressant efect, or by virtue of its ability to potentiate the potassium losing properties of diuretics (mainly thiazide and high ceiling diuretics) that are commonly used in conjuction with cardiac glycosides in management of congestive cardiac failure (Gantt, 1972) and nocturnal angina (Aronow, 1973; Goldstein, 1972).

Contraction of most mammalian hearts is initiated by and is proportional to the influx of calcium ions that occurs during the transmembrane action potential (Beeler and Reuter, 1970). This process in turn causes the release of additional calcium ions from the sarcoplasmic reticulum (Fabiato and Fabiato, 1970). The antagonism of calcium induced cardiostimulation by chloroquine (Table 17 and Figure 7c) may mean that

chloroquine has the ability to interfere with the calcium induced stimulus secretion of neurotransmitters at sympathetic nerve synaptic sites or it may directly block the entry of calcium into the myocardial cells by blocking the slow calcium channels which are essential for the generation and propagation of the action potential. In this way the calcium dependent slow inward current that occurs during the plateau of the action potential is blocked.

This finding points at a likely interaction between chloroquine and calcium channel blockers in which concomitant administration of the two groups of drugs would lead to potentiation of myocardial depression. However this is contrary to the findings of Cao et al (1970) who found that chloroquine has no effect on calcium induced cardiostimulation.

The myocardium is parasympathetically innervated by the vagus nerve. Cholinergic agents acting via the parasympathetic nerve cause both negative inotropic and chronotropic responses. These effects are antagonised by anti-cholinergic (antimuscarinic) agents such as atropine (Martindale, 1989).

Table 17 and Figure 7e show that 20ug atropine sulphate induced a mild cardiostimulation characterised by positive inotropic response with no change in the chronotropic effet. The mild atropine induced cardiostimulant effect was not neutralised by

20ug chloroquine phosphate but the positive inotropic effect was converted into negative response (Figure 7e). This is contrary to the findings of Salako and Sangoday (1976) who found that atropine did not reverse the cardiodepressant effects of chloroquine on isolated guinea pig atria.

Pharmacologically, the above findings would suggest that chloroquine exerts cholinergic and specifically muscarinic effects on the isolated rabbit heart.

In agreement with this hypothesis, Agarwal and Arora (1956) suggest that chloroquine probably induced myocardial depression by enhancing vagal inhibition through an increase in parasympathetic activity.

It is of clinical significance that lower doses of chloroquine (2-10ug) were required to antagonise the strong cardiostimulant effects induced by adrenaline (0.2ug) and isoprenaline (50ug) than were required for atropine (20ug). This is probably a manifestation of the sensitivity of the sympathetically innervated organs to chloroquine and explains why the first sign of chloroquine toxicity are manifested as changes on the ECG pattern. These changes are mainly observed after voluntary or accidental chloroquine intoxication or in case of normal doses in patients with renal insufficiency or in hypokalaemia (Charles and Bertrand, 1982).

Normal therapeutic doses of chloroquine have been

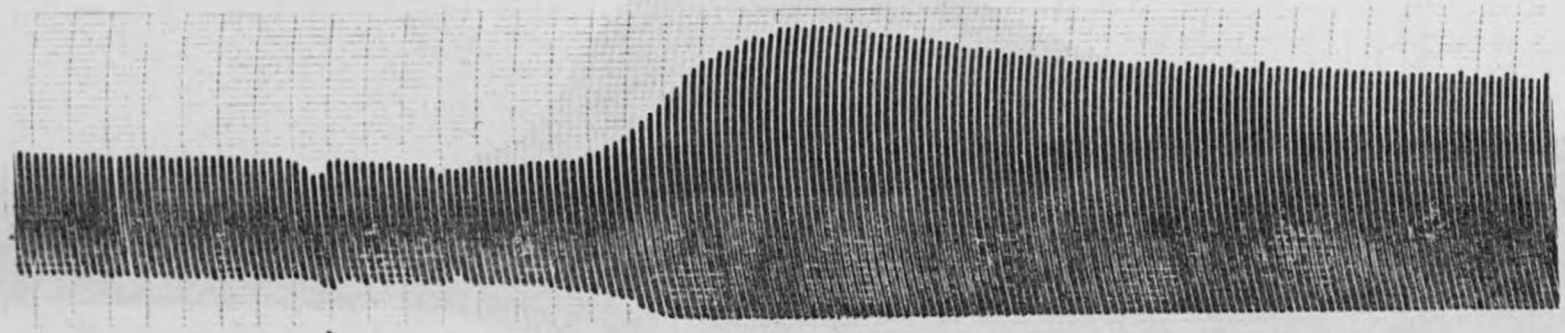
reported to cause death (Michael et al, 1970). This can be considered as an idiosyncratic response related to decreased metabolism of chloroquine as in liver disease and also decreased chloroquine excretion as occurs in renal insufficiency (Bennett et al, 1973). In addition the possibility of patients with glucose-6-phosphate dehydrogenase deficiency who respond to chloroquine therapy with severe haemolytic anaemia (Pannaciuli et al, 1969; Girdwood, 1973; Beutler, 1973) should be borne in mind.

Apart from the above proposed mechanisms of chloroquine induced myocardial depression, chloroquine has also been reputed to possess local anaesthetic effect (Jindal et al, 1960). By virtue of this activity, chloroquine might potentiate the myocardial depression induced by some of the general anaesthetics especially if the patient was on antimalarial therapy with chloroquine before surgery.

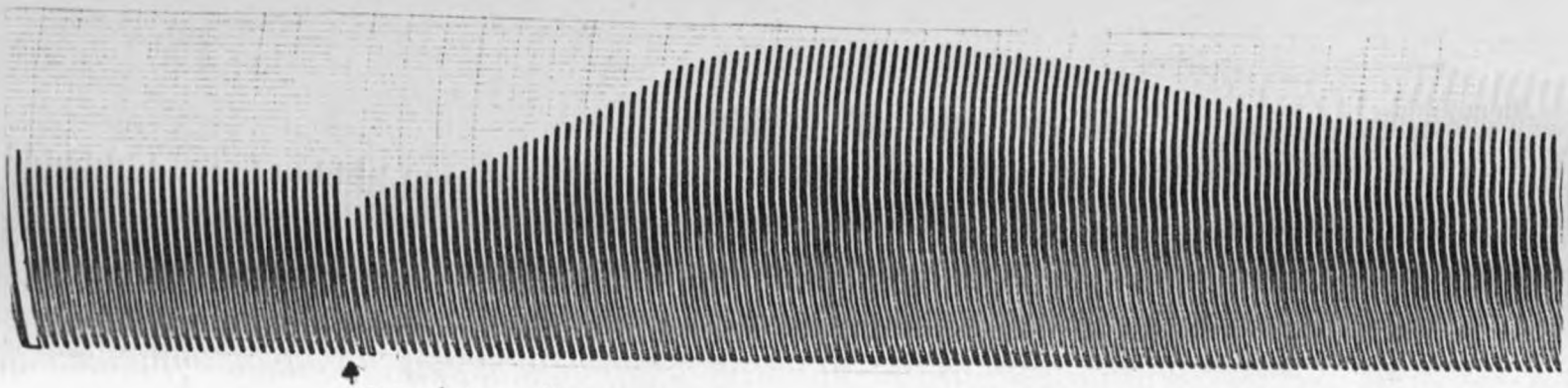
All these serious and sometimes fatal reactions associated with chloroquine call for a closer monitoring of the drug instead of the current situation where the drug is available in ordinary shops as an over the counter drug accessible to all people regardless of their pathological state or current chemotherapy, chronic or otherwise.

FIGURE 7 EFFECT OF CHLOROQUINE ON DRUG INDUCED CARDIAC ACTIVITY

(a) Adrenaline and Chloroquine



0.2µg Adrenaline



0.2µg Adrenaline
+4µg Chloroquine

FIGURE 7(Cont) (b) Isoprenaline and Chloroquine

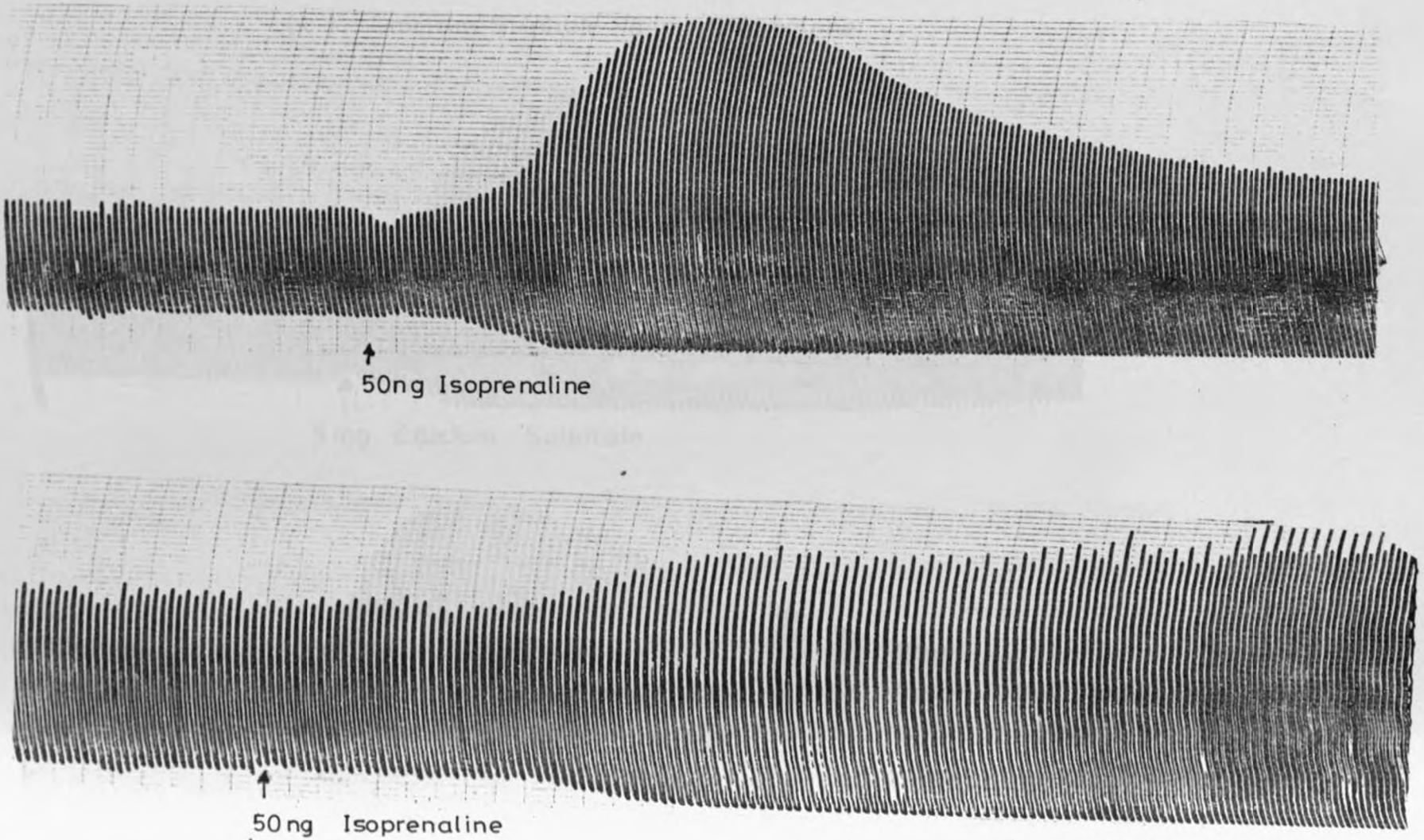
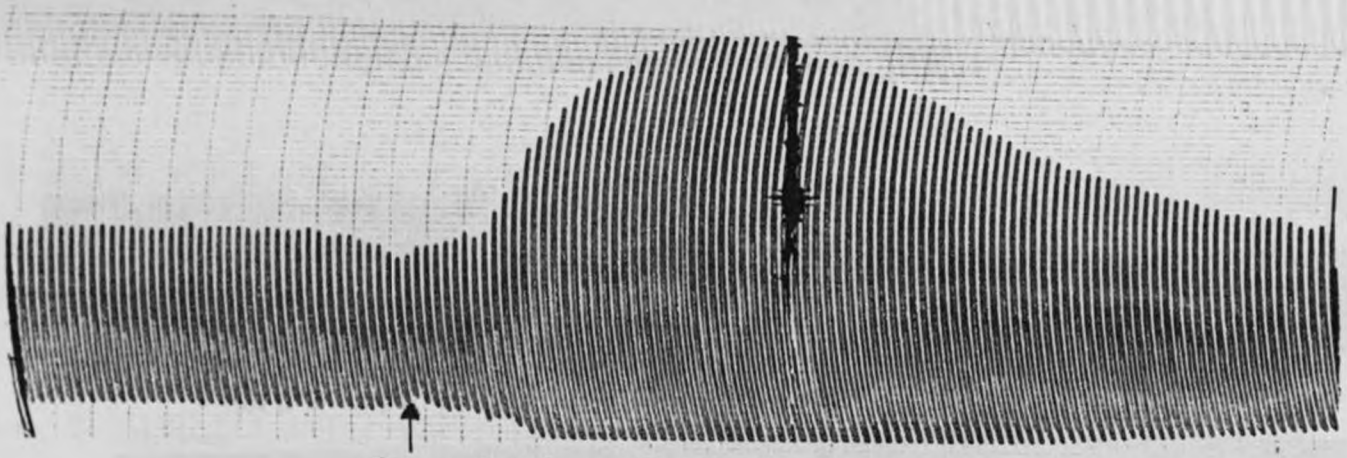


FIGURE 7(Cont.)

(c) Calcium Sulphate and Chloroquine



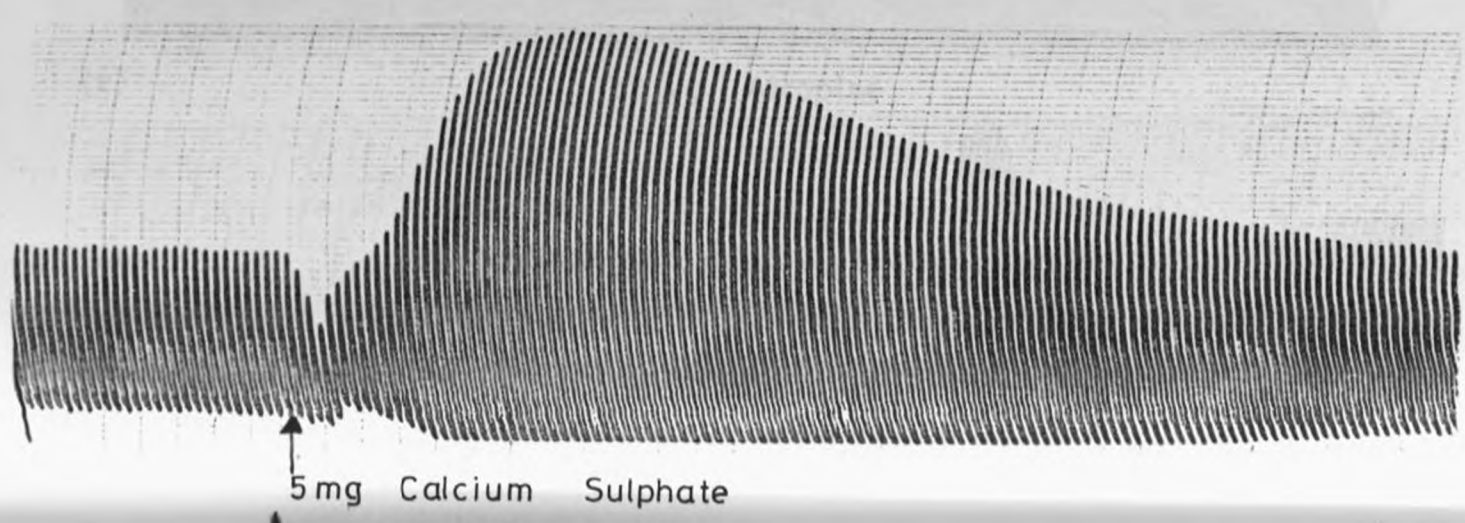


FIGURE 7 (Cont)

(d) (d) Atropine and Chloroquine
(e) Barium Chloride and Chloroquine

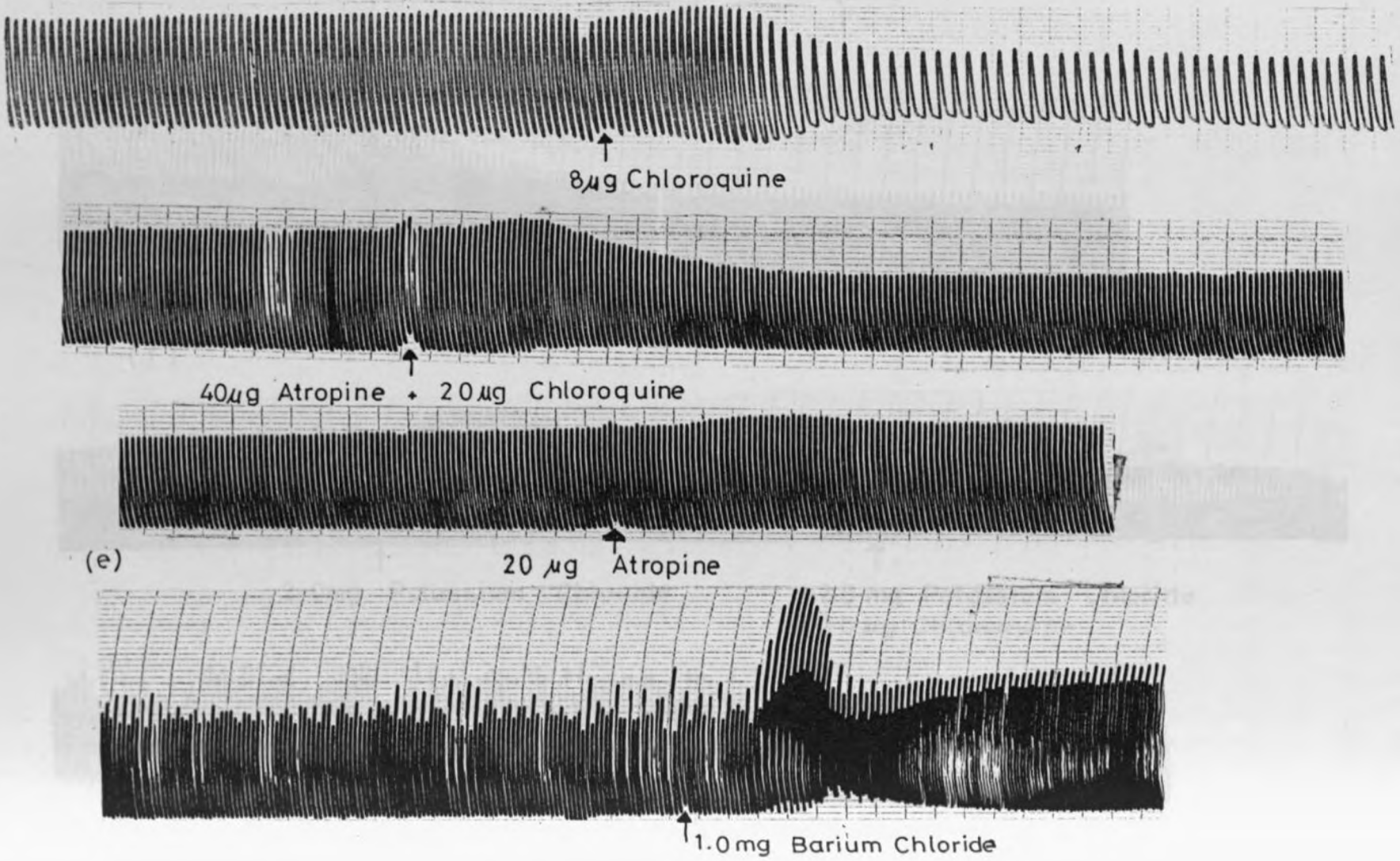
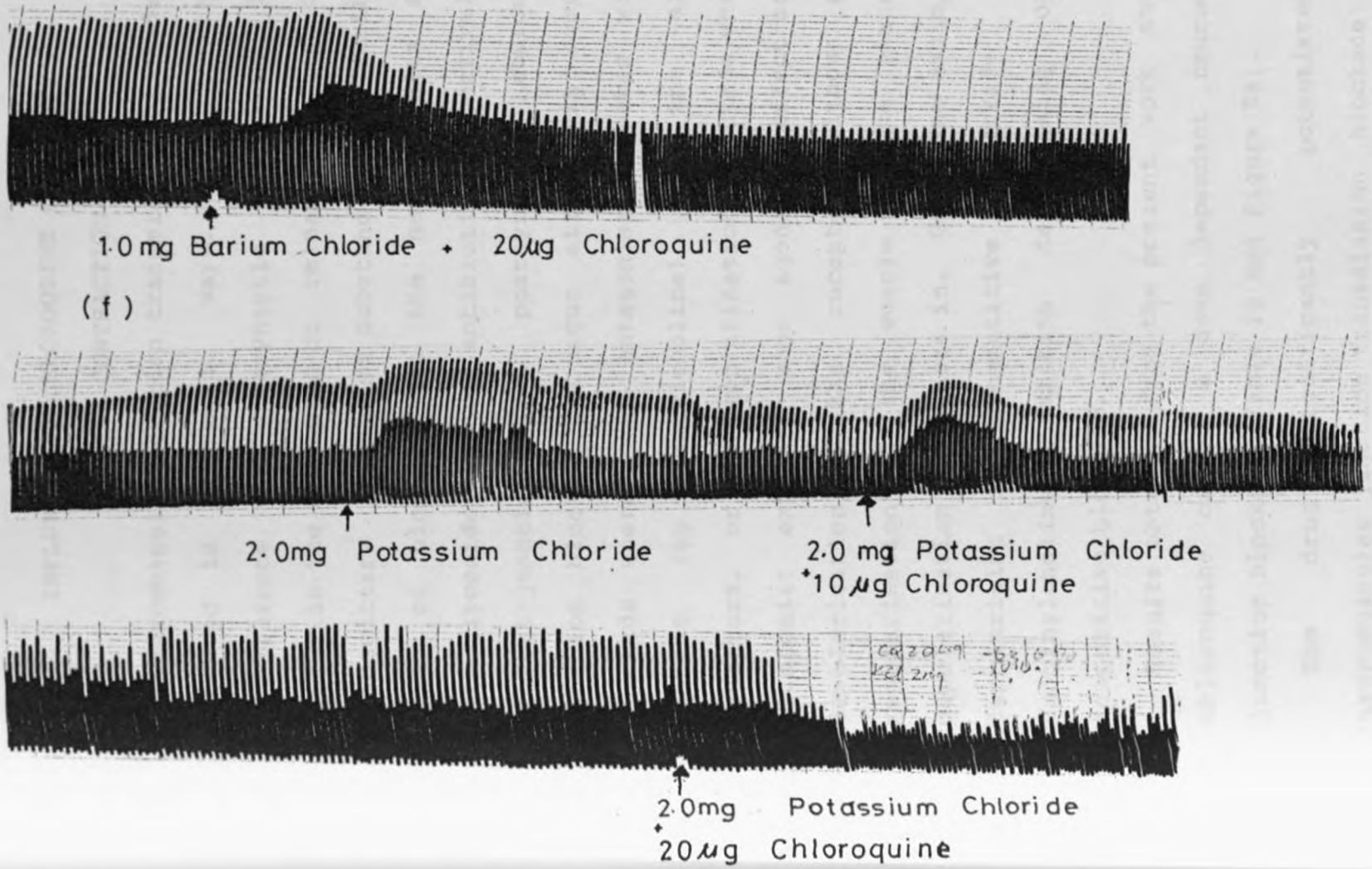


FIGURE 7 (Cont)

(f) Potassium Chloride and Chloroquine



3.6 INFLUENCE OF CHLOROQUINE ON SKELETAL MUSCLE CONTRACTION

Neuromuscular junction transmission blockade can be effected in different ways. One of these is interference with presynaptic mechanisms involved either in the synthesis or release of the chemical transmitter. Example of substances that exhibit this type of blockade are the hemicholinium and the aminoglycoside class of antibiotics respectively.

Post-junctional or postsynaptic neuromuscular junction blockade can occur either via competitive blockade whereby an antagonist competes with the agonist (eg acetylcholine) for the nicotinic receptors, or via depolarisation blockade whereby an antagonist exhibits some agonistic effect and cause depolarisation of the receptors without allowing repolarisation of the muscle to take place hence depolarisation block sets in. Gallamine exhibits non-depolarising or competitive blockade whereas succinylcholine exhibits depolarising or non-competitive blockade.

Results obtained from the present work show that chloroquine caused a dose dependent neuromuscular junction blockade (Table 18 and Figure 8a).

The drug significantly potentiated the neuromuscular junction transmission blockade induced by succinylcholine, gallamine and lignocaine. These

are pharmacologically different agents effecting their neuromuscular blockade by different mechanisms. Since chloroquine potentiated the effect of all these substances, then it is possible that the drug affects or alters a step in the overall mechanism that is common to all the three drugs. It could be exerting a non-specific neuromuscular blockade by affecting the sodium conductance mechanisms (Chinyanga et al, 1972).

The neuromuscular junction transmission blockade induced by chloroquine could not be antagonised by the indirect acting cholinergic agent physostigmine (Figure 8u). Physostigmine at a dose of 0.075ug/ml bath concentration caused stimulation of neuromuscular junction transmission 33.3 (1.5)% whereas a dose of chloroquine 0.125ug/ml bath concentration caused inhibition of neuromuscular transmission of -45.5 (9.1)%. The effect of the two drugs combined in the same dose range caused -45.5 (1.8)% inhibition of neuromuscular junction transmission. This is equivalent to the effect of chloroquine alone, implying that the effect of physostigmine was not manifested. Increasing the dose of physostigmine 0.15ug/ml bath concentration in presence of 0.125ug/ml chloroquine caused pronounced potentiation of the neuromuscular blocking effect of chloroquine.

This fact coupled with the observation that chloroquine potentiated the neuromuscular blocking

effect of succinylcholine implies that chloroquine could be causing desensitisation blockade or depolarisation blockade. This could only happen if chloroquine caused release of acetylcholine or if it had partial agonistic effect at the nicotinic receptor sites of the neuromuscular post-synaptic site.

Vartanian and Chinyanga (1972) found that chloroquine in therapeutic doses induced muscle paralysis characterised by decreased excitability of electrically excitable membranes of the axon and muscle fibre, decreased action potential, decreased transmitter release at the end plate, decreased firing index and also decreased amplitude of action potential of muscle fibre itself. Chinyanga et al (1972) found that chloroquine depressed the action potential without altering membrane potential. The authors likened the mechanism of neuromuscular junction blockade induced by chloroquine to that induced by local anaesthetics. When tested for local anaesthetic activity chloroquine was found to possess mild local anaesthetic effect (Jindal et al, 1980). It is therefore not surprising to note that chloroquine enhanced the neuromuscular blocking effects of lignocaine from 56.2 (1.9)% for lignocaine 0.125mg/ml to -69.6 (1.3)% for lignocaine 0.125mg/ml plus 0.125µg/ml chloroquine.

Abnormalities associated with neuromuscular

junction transmission can primarily be associated with hypokalemia. These include impaired neuromuscular function varying from minimal weakness to frank paralysis (Perez-Stable and Caralis, 1983).

Potassium is the principal cation of the intracellular fluid, but it is also a very important constituent of the extracellular fluid where it acts in conjunction with sodium/potassium pump to regulate muscle activity (Harper, 1976).

Present investigations have revealed that in vivo chloroquine causes increased urinary excretion of potassium and potentiates kalliuretic effect of diuretics (Table 21 and Figure 11 & 12). It is therefore possible that the neuromuscular blocking property of chloroquine may be related to its ability to cause depletion of potassium making it unavailable for the contractile mechanisms. In addition chloroquine induced myopathy after prologed use as in arthritic conditions (Chapman et al, 1969; Hughes et al, 1971) could be attributed to the inhibition of glycolysis evidenced by accumulation of glucose (Chapman et al, 1969) and degeneration of muscle fibres (Hughes et al, 1971). All these effects culminate in muscle paralysis and inhibition of contractile mechanisms.

Calcium ion is another cation that plays a role in muscle contraction. It is involved in the coupling of the nerve impulse to the release mechanism (excitation-

release coupling) (Bowman and Rand, 1980; Goodman and Gilman, 1985) and transmitter release cannot take place in absence of an adequate calcium ions concentration (threshold at the neuromuscular junction is 0.1mmol/litre). Usually the amount of acetylcholine released is proportional to the amount of extracellular calcium (Bowman and Rand, 1980; Goodman and Gilman, 1985). Physiologically barium ions can carry current through the calcium channels in place of calcium and to some extent can substitute for calcium or make more calcium available for contractile mechanisms (Bowman and Rand, 1980).

Present findings show that chloroquine antagonised the neuromuscular stimulant effect induced by calcium or barium on the isolated rat phrenic nerve diaphragm preparation. The antagonism is dose dependent. These findings imply that chloroquine has anti-calcium effects at the neuromuscular junction, probably blocking the calcium mediated excitation-release coupling and hence inhibits transmitter release. This conclusion concurs with the findings of Vartanian and Chinyanga (1972).

Due to the synergistic efect of chloroquine with succinylcholine and gallamine, concurrent administration of this drug with any of the two muscle relaxants may prolong the recovery of muscular

activity after general anaesthesia and increase the risk of death of patients from respiratory paralysis.

Myasthenic patients are also another likely high risk group as the neuromuscular blocking effect of chloroquine may precipitate myasthenic crisis in this group of patients. Actually chloroquine induced myasthenic syndrome has been reported (Pichon et al, 1984) as a symptom of poisoning with chloroquine and also after prolonged use of chloroquine in arthritis (Hearn et al, 1986)

The risk is made worse by the fact that anticholinesterases, a commonly used therapy for myasthenia does not seem to be an effective antidote for chloroquine induced neuromuscular junction blockade (Table 18). Increasing the dose of physostigmine in an effort to counteract the chloroquine induced neuromuscular junction blockade may precipitate a cholinergic crisis leading to desensitisation block. Ayitey-Smith and Vartanian (1975) have demonstrated that high concentrations of chloroquine 5×10^{-4} g/ml and 2×10^{-3} g/ml caused competitive antagonism to acetylcholine, carbachol, caffeine and potassium on isolated frog skeletal muscle. The authors concluded that chloroquine induced neuromuscular blockade was due to local anaesthetic effect and probably interference with intracellular calcium movement.

TABLE 18 INFLUENCE OF CHLOROQUINE ON THE RESPONSE OF SKELETAL MUSCLE TO DRUGS

n = 8 means (SEM)

DRUG AND DRUG COMBINATION	BATH CONC. Units/ml	MEAN % CONTRACTILE RESPONSE
Chloroquine	0.025µg	-12.4 (6.5)
	0.025µg	-15.0 (3.1)
	0.125µg	-45.5 (2.1)
	0.25µg	-50.3 (0.7)
	0.375µg	-64.5 (1.6)
Succinylcholine	0.15mg	-33.3 (1.3)
Lignocaine	0.125mg	-56.2 (1.9)
Barium chloride	0.25mg	+30.6 (2.3)
Calcium chloride	0.05mg	+16.6 (2.1)
Physostigmine	0.075mg	+33.3 (1.5)
Gallamine	0.2mg	-48.5 (1.7)
Gallamine+Chloroquine	0.2mg+0.125mg	-66.0 (1.4)
Succinylcholine+Chloroquine	0.125mg+0.125mg	-56.1 (2.4)
Lignocaine+Chloroquine	0.125mg+0.125mg	-61.1 (2.1)
Barium chloride+Chloroquine	0.125mg+0.25mg	-39.3 (1.8)
	0.25mg+0.25mg	-51.9 to +33.3*
	0.25mg+0.37mg	-78.6 to +23*
Calcium chloride+Chloroquine	0.05mg+0.125mg	-20.0 (1.9)
Physostigmine+Chloroquine	0.075ug+0.125mg	-55.5 (1.8)

TABLE 18 CONT.

Acetylcholine+Chloroquine	0.25µg+0.125mg	+54.5 (2.7)
Calcium chloride+gallamine	0.05mg+0.2mg	-50.0 (2.4)
Succinylcholine+gallamine	0.125mg+0.2mg	-62.5 (3.6)
Succinylcholine+lignocaine	0.125mg+0.125mg	-69.6 (1.3)

- means contractile inhibition

+ means contractile stimulation

* - The neuromuscular junction blockade induced by chloroquine was effectively antagonised and reversed in the dose of barium chloride.

FIGURE 8 INFLUENCE OF CHLOROQUINE ON SKELETAL MUSCLE CONTRACTION

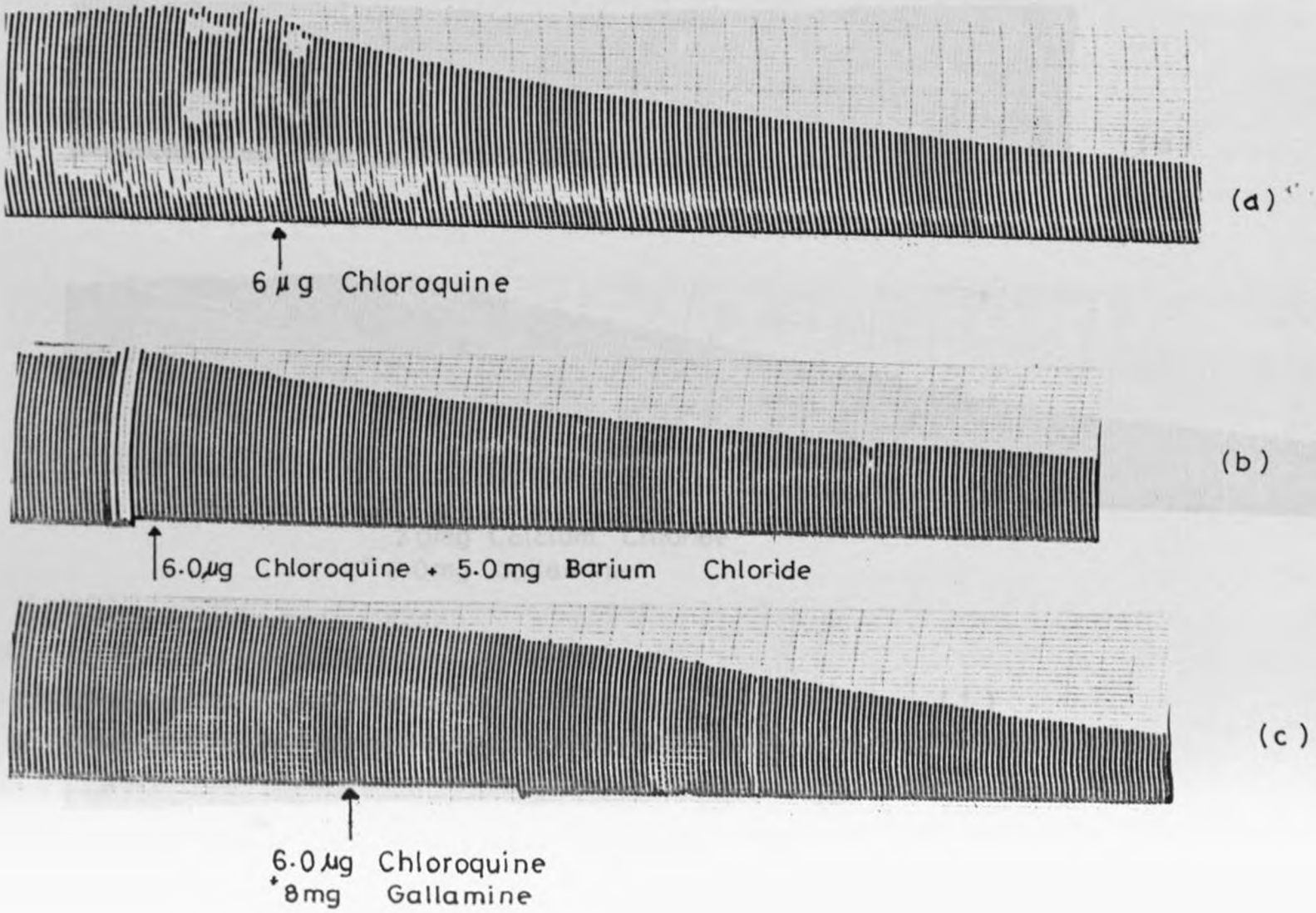
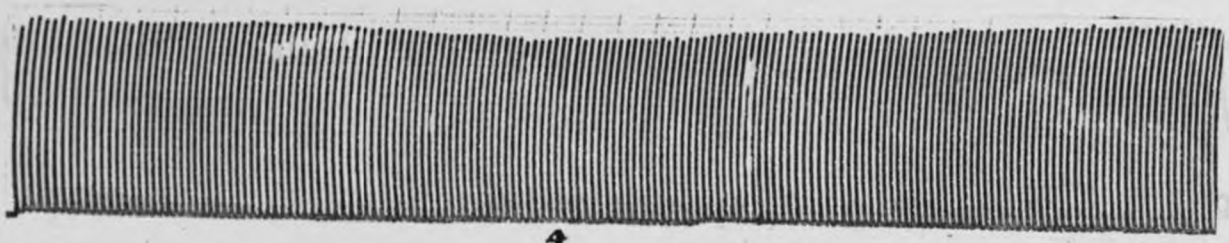
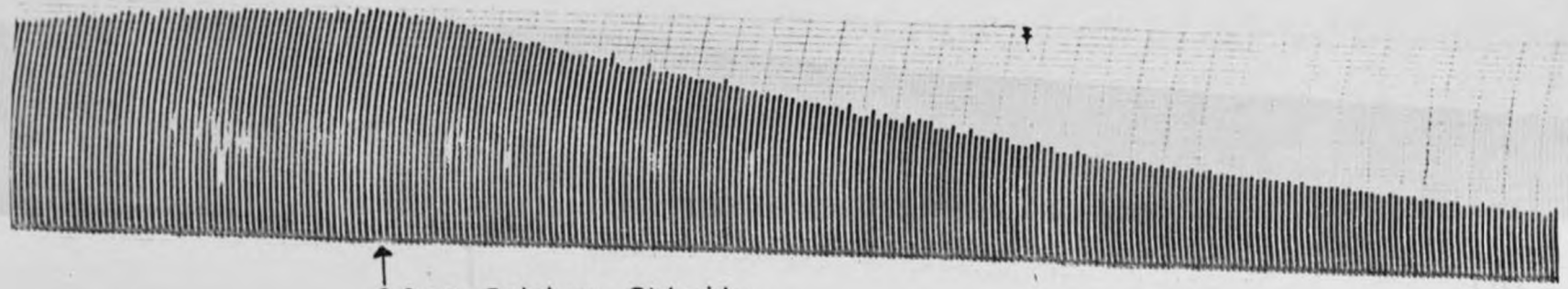


FIGURE 8 (Cont)



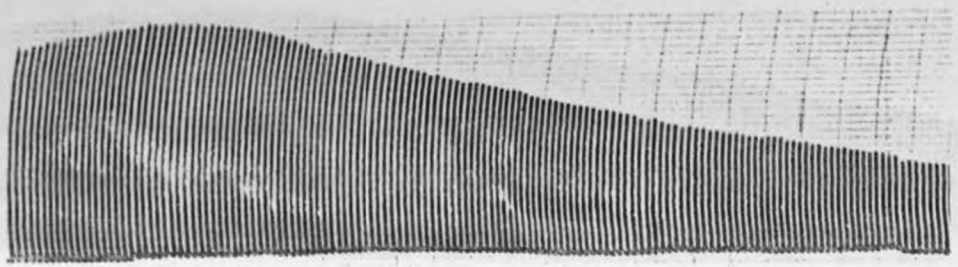
(d)

2·0mg Calcium Chloride



(e)

2·0mg Calcium Chloride
+ 8·0mg Gallamine



(f)

5·0 mg Succinylcholine
8·0 mg Gallamine

FIGURE 8 (Cont)

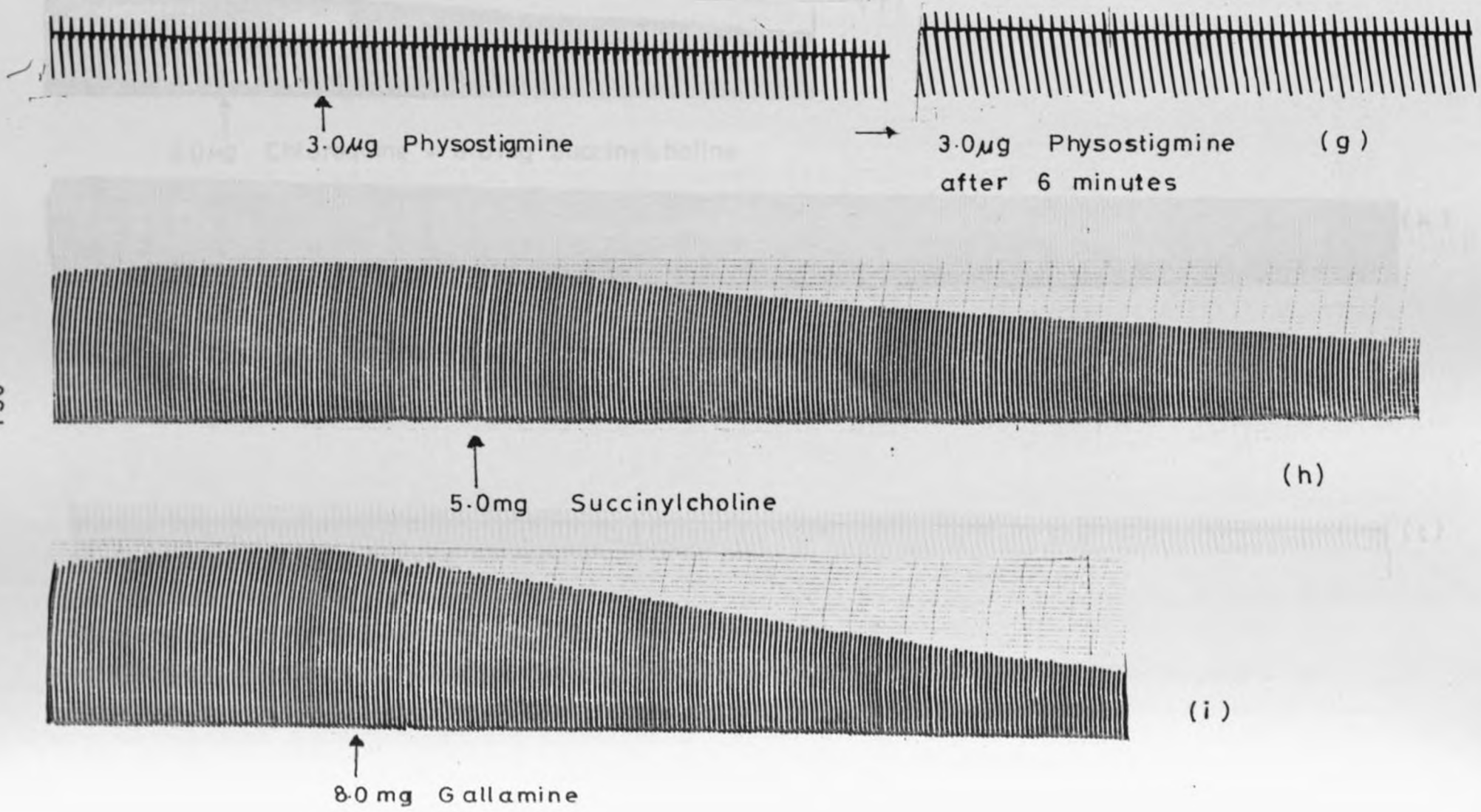


FIGURE 8 (Cont.)

(j)

6.0μg Chloroquine + 8.0 mg Succinylcholine

(k)

6.0μg Chloroquine + .0 mg Calcium chloride

(l)

3.0μg Physostigmine
+6.0μg Chloroquine

FIGURE 8 (Cont.)

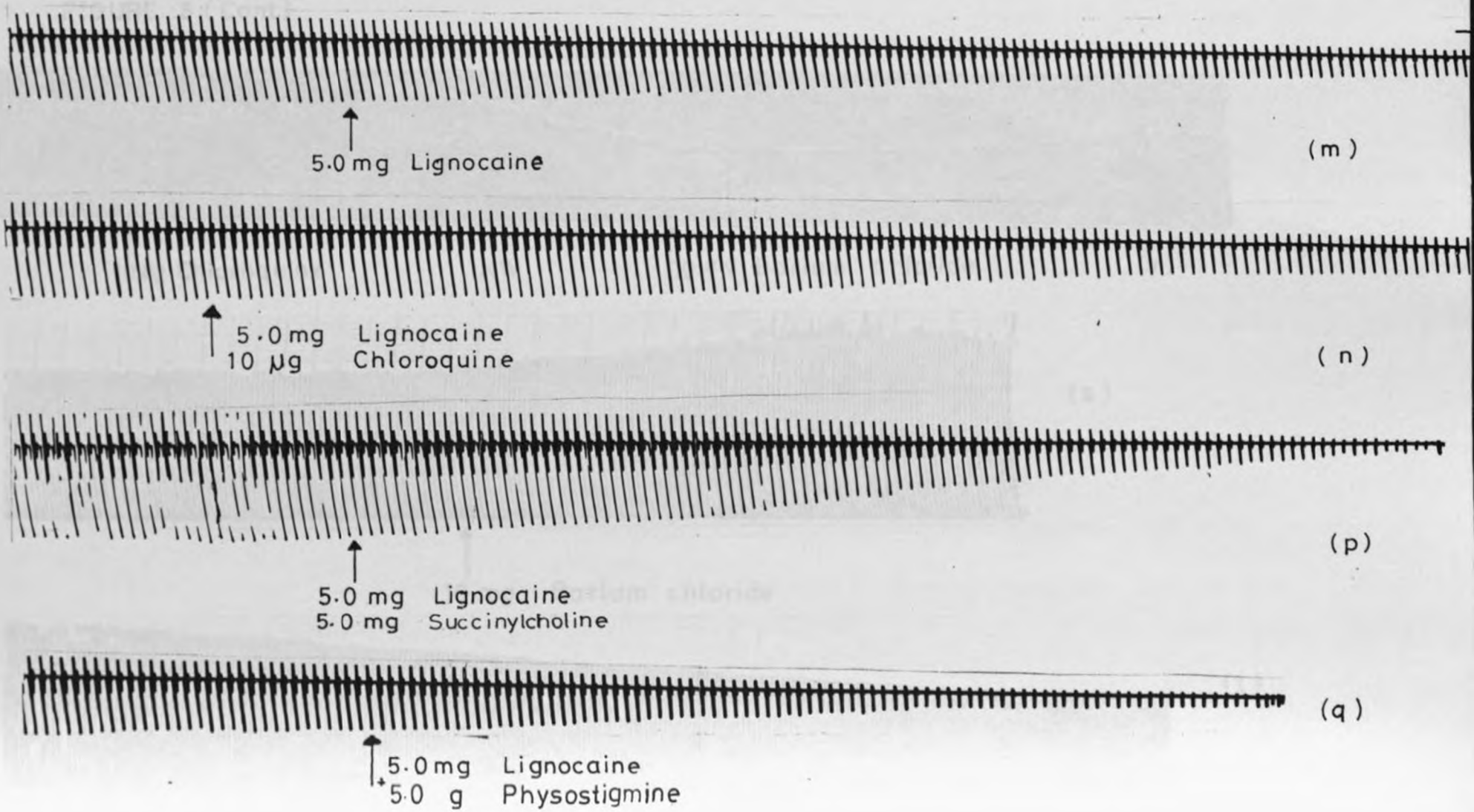


FIGURE 8 (Cont.)

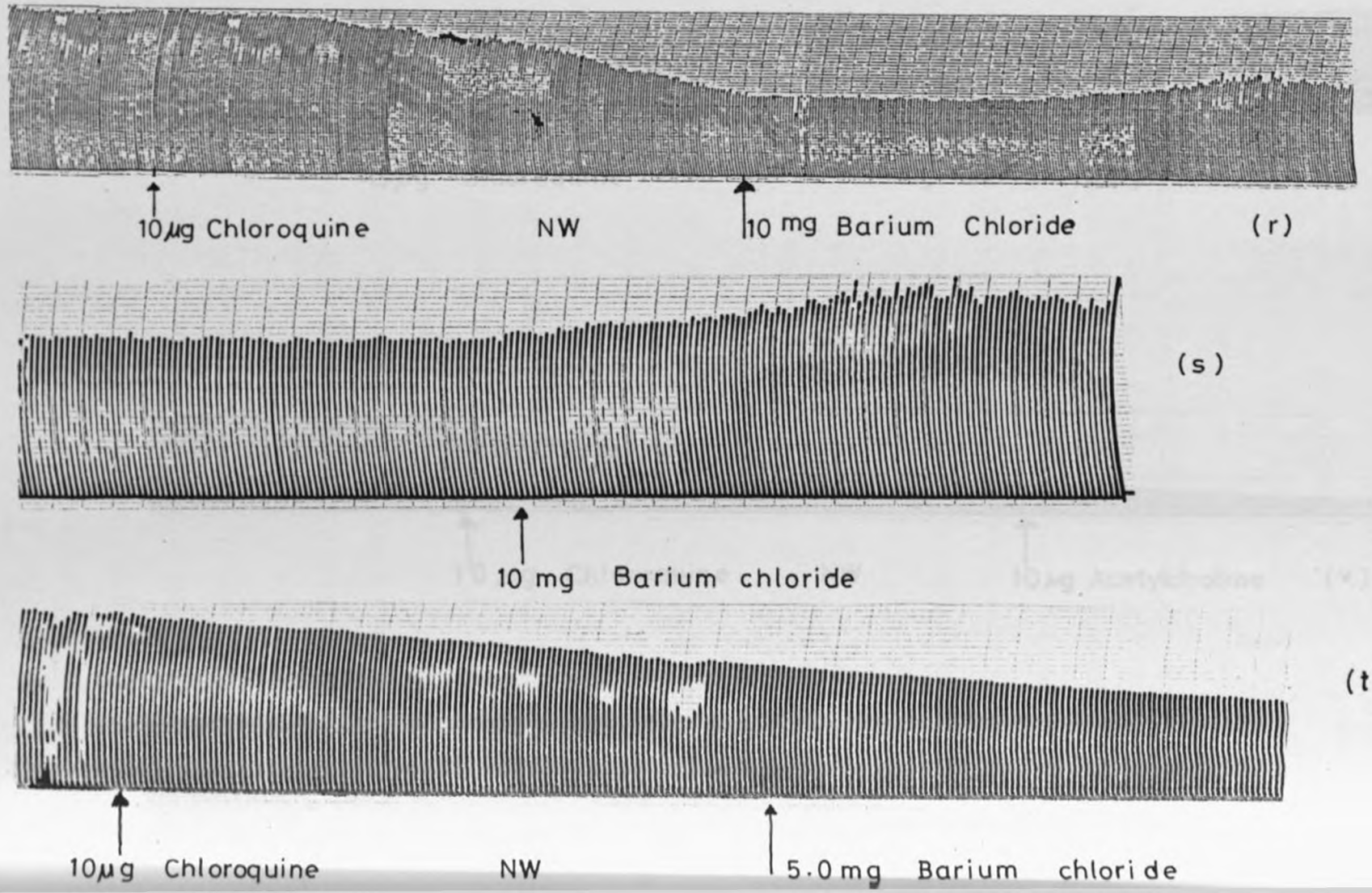
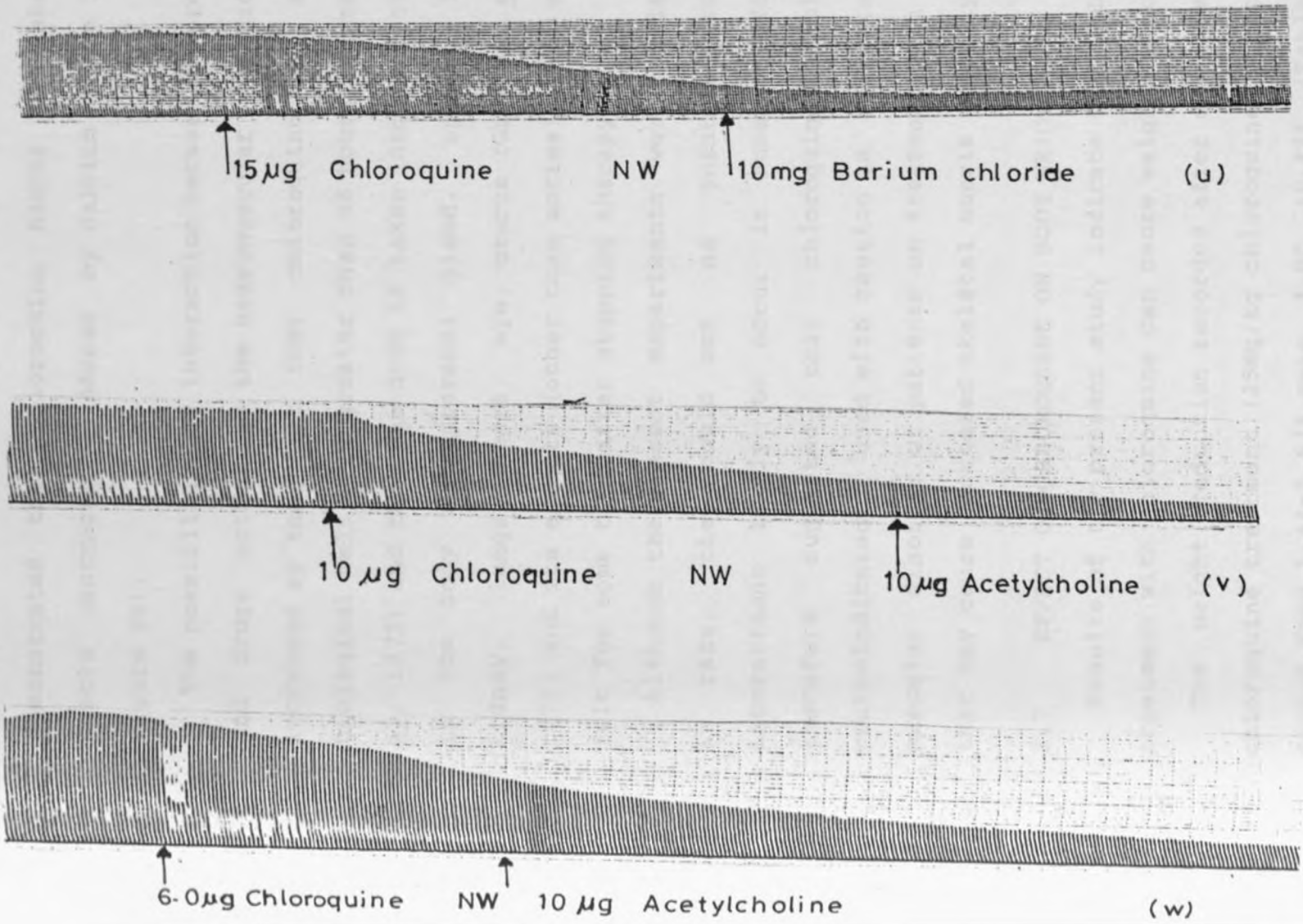


FIGURE 8 (Cont)



The probability of calcium involvement has been confirmed in the present findings which has demonstrated that chloroquine blocks the skeletal muscle contraction induced by calcium (Table 18 and Figure 8k).

The possibility of interaction between chloroquine and drugs acting at the neuromuscular junction is increased by the fact that chloroquine has a long biological half life greater than 48 hours (Bennett et al, 1973) and that the drug is taken into many tissues in the body eg suprarenal gland, spleen, liver, kidney, lungs, heart, eye, uterus (Grundman et al, 1972) and its effects (other than antimalarial) may be felt for some time after stopping therapy.

Although the present experiments were carried out in rats, they could act as pointers to the interactions likely to occur in humans. It is therefore suggested that chloroquine should be contraindicated or used with caution in patients with muscular disorder or patients on treatment with drugs that may cause decreased skeletal muscle activity.

3.7 EFFECT OF CHLOROQUINE ON BODY WEIGHT

Results of the present study indicate that chronic treatment with chloroquine can cause weight reduction.

The weight reduction recorded after one week of chloroquine treatment (25mg/kg chloroquine phosphate) ranged from 1.48-5.67% mean 3.00 (0.42) (Table 19).

After a ten day drug free period, recovery was recorded as a gain in weight in all the animals. Some animals recorded weights above the initial pretreatment meaning a recovery of over 100% (Table 19). The minimum recovery recorded after the wash out period was 98.40% whereas the maximum percentage recovery stood at 103.45%.

Since the rabbits were subjected to daily intubation even during this drug free period, it can be said that the loss in weight recorded during the chloroquine treatment period was attributable to chloroquine. This observation was even further confirmed by the fact that the pattern of weight loss was reproduced when chloroquine administration was resumed after the wash out period (Table 19, see Δ_3 in relation to Δ_2). The final loss in weight was calculated as a percentage of the initial pretreatment weight (Δ_3%) and also as a percentage of the weight after the wash out period (Δ_4 %). Both sets of results confirm the loss of body weight induced by chloroquine.

In vitro experiments have shown that chloroquine causes myopathy (Chapman et al, 1969; Hughes et al, 1971) characterised by frequent splitting of muscle fibre, and appearance within them of innumerable course granules. These ultrastructural changes were seen within the first 5 days of treatment (Macdonald

and Andrew, 1970).

The chloroquine induced myopathy could be attributed to inhibition of glycolysis evidenced by accumulation of glucose (Chapman et al, 1969).

After one week's culture of mouse pancreatic islets in the presence of chloroquine (10^{-5} M) Andersson et al (1980) found that there was decreased production of labelled carbon dioxide from ^{14}C-glucose indicating that there is a reduction in energy source from the decreased rate of glucose oxidation. It is possible that the supply of energy for cell activity comes from breakdown of protein or fats.

Chloroquine has been shown to enhance proteolysis in vitro (Bertini and Daniel, 1970) and to increase the rate of catabolism of newly formed collagen but has little effect on the conversion of soluble collagen to insoluble collagen at doses of 30mg/kg daily for 21 days (Trnavsky and Trnavska, 1970). In addition the drug also decreases protein biosynthesis in vitro (Andersson et al, 1980; Roskoski and Jaskunsas, 1972) probably by interacting with free polynucleotide and preventing the subsequent formation of an active polynucleotide ribose complex. The drug has been shown to inhibit protein biosynthesis in bovine (Gonasum and Potts, 1974) and rabbit (Giuffrida et al, 1976) retinal preparations and also to inhibit polypeptide synthesis in subcellular rat liver systems

TABLE 19 PERCENTAGE CHANGE IN BODY WEIGHT AFTER CHLOROQUINE TREATMENT

mean (SEM)

W2/W1%	$\Delta_{1\%}$	W3/W1	$\Delta_{2\%}$	W4/W1%	$\Delta_{3\%}$	W4/W3	$\Delta_{4\%}$
98.00	-2.00	102.33	+2.33	98.44	-1.56	96.20	-3.80
98.52	-1.48	103.45	+3.45	100.49	+0.49	97.49	-2.51
97.19	-2.81	98.40	-1.60	98.79	-3.21	98.37	-1.63
94.33	-5.67	100.94	+0.94	95.00	-5.00	97.20	-2.80
96.34	-3.66	99.09	-0.01	97.26	-2.74	98.16	-1.84
96.84	-3.16	98.81	-1.19	96.05	-3.95	97.20	-2.80
97.45	-2.55	98.90	-1.10	96.70	-3.30	97.79	-2.21
97.34	-2.66	98.86	-1.14	95.06	-4.94	96.15	-3.85
mean	-3.00		0.21		3.02		-2.68
(SEM)	(0.42)		(0.00)		(0.002)		(0.77)

$\Delta_{1\%}$ - Percentage change in body weight after the first one week course of chloroquine treatment.

$\Delta_{2\%}$ - Percentage weight change in body weight after 10 days of drug free period

$\Delta_{3\%}$ - Percentage change in body weight after the second course of chloroquine treatment with respect to W1

$\Delta_{4\%}$ - Percentage change in body weight after the second course of chloroquine treatment with respect to W3.

(Lefler et al, 1973), probably by blocking the amino acid uptake by the cells (Conklin and Chou, 1970; Conklin and Chou, 1972).

The results obtained in the present work suggest that chloroquine is basically a catabolic agent causing decrease in body weight. Since this experiment was carried out for only 24 days, it can appropriately be concluded that prolonged use of chloroquine as in the treatment of rheumatoid arthitis, or in sarcodiasis could lead to a significant loss of body weight. This is further supported by Zubarev and Polyantseva (1972) who found that though there was increase in the weight of adrenal gland during 6-20 weeks of treatment with 30, 40 and 80mg chloroquine base/kg body weight there was significant loss in total body weight. Similar results have been obtained with healthy volunteers given high doses of chloroquine (Alving et al, 1948).

The above findings indicate that prolonged use of chloroquine for prophylaxis or otherwise in patients with muscle wasting diseases such as the acquired immune deficiency syndrome (AIDS) is inadvisable.

3.8 MODIFICATION OF THE DIURETIC PROPERTIES OF FRUSEMIDE AND CHLORTHIAZIDE BY CHLOROQUINE

Chloroquine and diuretics are found in concurrent therapy of malaria complicated by oedematous conditions such as congestive cardiac failure, and

hypertension. Assessment of the influence of each of these agents on the therapeutic value of the other is therefore of clinical significance.

Using rats, the results of the present study indicate that chloroquine decreased frusemide/chlorthiazide induced diuresis. The drug caused 9.2% and 16.1% decrease in the chlorthiazide and frusemide induced diuresis respectively (Table 20 and Figures 9 & 10).

However on its own, chloroquine exhibited a mild diuretic effect (14.9%) with respect to control.

On electrolyte excretion chloroquine exhibited relatively mild natriuretic (82.4%) effect whereas frusemide and chlorthiazide caused 337.7% and 282.3% sodium loss with respect to the control values.

Concurrent administration of chloroquine with frusemide or chlorthiazide led to a decrease in the natriuretic properties of these diuretics. There was 263.1% and 85.5% decrease in the sodium losing activity of frusemide and chlorthiazide respectively (Table 22).

Sodium is the major cation associated with oedematous conditions and facilitation of its loss is an important property of an effective diuretic. Therefore the chloroquine induced inhibition of the natriuretic property of frusemide and chlorthiazide (Figure 15) is a big drawback to the diuretic

effectiveness of the substances. This coupled with the chloroquine induced inhibition of urine output by frusemide and chlorthiazide (Figure 9 and Table 20) makes the drug interaction between these agents of clinical significance.

With regard to potassium excretion chloroquine phosphate caused a mild increase in potassium loss (18.6%) as compared to frusemide (25.2%) and chlorthiazide (58.9%) (Table 21). Hypokalemia is one of the major side effects associated with the use of potent diuretics such as the thiazide diuretics eg (Chlorthiazide).

Combination of chloroquine with frusemide or chlorthiazide led to potentiation of the kalliuretic effect of the two diuretics (Table 21 and Figures 11 & 12). There was a 39.0% and 4.2% increase in the kalliuretic effect of frusemide and chlorthiazide respectively. This is a serious drug interaction that might lead to very severe and even fatal consequences especially on those patients who in addition to the above combination therapy are also put on cardiac glycosides. This is because the decreased plasma potassium concentrations causes increased sensitivity of the myocardium to cardiac glycosides. To counteract the above side effect potassium supplimentation is needed.

As noted earlier (Table 5) aspirin is a drug that

is commonly used with chloroquine to alleviate the pyrexia that accompanies a clinical malarial attack. Just like chloroquine, aspirin has been reported (Oyekan and Laniyonu, 1986) to decrease diuresis and natriuresis induced by bendrofluazide a thiazide diuretic with similar pharmacological effects as chlorthiazide used in the present study.

Concomitant use of aspirin, chloroquine and a thiazide diuretic (chlorthiazide or bendrofluazide) would greatly compromise the diuretic potency of the thiazide diuretic. Other nonsteroidal anti-inflammatory agents such as indomethacin have also been reported to antagonise the diuretic effect of frusemide by decreasing urine volume, decreasing sodium excretion, decreasing glomerular filtration rate (Kahles and Riegger, 1987) and antagonising the intratubular effects of frusemide (Kent, 1987).

The mechanism of the antidiuretic action of chloroquine is not yet known. However it could be due to inhibition of prostaglandin synthesis and release (especially renal prostaglandin E) just like indomethacin (Kahles and Riegger, 1987).

Chloroquine has prostaglandin synthesis inhibitory properties (Bailey and Chakrin, 1981; Bresloff, 1977) and this has been claimed to be a possible mechanism of action for the antirheumatic action of chloroquine and other 4-aminoquinolines (Bresloff, 1977; Shem,

TABLE 20 EFFECT OF CHLOROQUINE ON THE DIURESIS* INDUCED BY FRUSEMIDE AND CHLORTHIAZIDE

n=6, mean (SEM)

GROUP t	URINE VOLUME (CUMULATIVE) (mls/kg body weight)				
	1Hr	2Hr	3Hr	4Hr	%CHANGE
1	10.3(1.3)	27.1(5.2)	31.8(4.0)	33.6(3.5)	0.0
2	7.7(1.2)*	26.52(5.2)	35.5(7.2)	38.6(6.6)++	14.9
3	13.0(2.3)*	30.7(4.4)*	36.6(4.9)*	40.3(4.5)*	20.0
4	11.9(1.5)b	31.4(3.0)*	38.0(3.1)*	43.4(2.4)+	29.2
5	16.2(5.7)+	36.7(9.4)++	44.5(9.2)*	47.1(8.7)+	40.2
6	15.44(2.5)++	35.4(2.9)++	45.6(2.3)+	52.5(0.9)+	56.3

*Diuresis was assessed cumulatively and not per unit hour

Statistical significance are expressed as the difference in diuresis after drug treatment.

Group 1, the untreated group is used as the base

+ ($p<0.001$) ++($p<0.01$) b ($p<0.02$) * ($p<0.05$)

t 1 - Control (normal saline)

2 - Chloroquine phosphate 50mg/kg

3 - Chloroquine 50mg/kg + Chlorthiazide 35mg/kg

4 - Chlorthiazide 35mg/kg

5 - Frusemide 20mg/kg and Chloroquine 50mg/kg

6 - Frusemide 20mg/kg

TABLE 21 MODIFICATION OF CHLORTHIAZIDE/FRUSEMIDE INDUCED KALLIURESIS BY CHLOROQUINE

n = 6 mean (SEM)

TIME(HRS)	MEAN URINARY POTASSIUM EXCRETION IN RATS µg/ml/kg					
	GR1*	GR2	GR3	GR4	GR5	GR6
0	288.5	340.6	360.4	325.3	318.5	323.1
	(25.6)	(30.7)	(20.3)	(27.4)	(18.7)	(15.9)
1	340.7	455.0	480.3	556.9	472.4	544.6
	(49.8)	(73.0)	(28.0)	(61.3)	(27.3)	(13.0)
2	279.3	400.0	440.2	506.0	499.8	476.3
	(23.0)	(36.4)	(42.3)	(56.7)	(35.1)	(36.0)
3	301.7	442.9	616.2	527.6	652.2	450.9
	(38.8)	(31.8)	(38.9)	(61.2)	(55.5)	(43.6)
4	312.1	471.9	702.9	573.6	680.7	430.5
	(38.4)	(61.6)	(36.2)	(68.3)	(49.5)	(42.2)
**	1233.8	1769.8	2239.6	2164.1	2305.1	1902.3
	(150.0)	(202.8)	(145.5)	(247.5)	(167.4)	(134.8)
***	0.0	43.4	81.5	75.4	86.8	54.2

* - See table 20 (footnote) for the treatment of the animal groups

** - Total potassium loss after 4 hours excluding 0hr reading

*** - %loss with respect to control (GR1)

TABLE 22 MODIFICATION OF FRUSEMIDE/CHLOROQUINE INDUCED NATRIURESIS BY CHLOROQUINE

n = 6 mean (SEM)

MEAN URINARY SODIUM EXCRETION (µg/ml/kg) OBTAINED FROM DRUG TREATED RATS

TIME(HRS)	GR1	GR2	GR3	GR4	GR5	GR6
0	35.8	40.6	43.9	39.8	46.8	50.8
	(3.6)	(4.8)	(5.4)	(2.7)	(5.3)	(3.1)
1	37.4	62.7	82.9	115.9	100.2	124.0
	(6.5)	(8.2)	(4.2)	(11.4)	(8.4)	(6.5)
2	28.11	62.6	76.1	118.3	95.2	148.2
	(4.3)	(8.5)	(7.5)	(5.4)	(6.4)	(8.5)
3	37.4	58.9	102.0	142.0	97.3	145.7
	(6.8)	(7.3)	(8.0)	(10.6)	(9.7)	(7.9)
4	36.5	70.1	152.8	155.7	63.1	192.3
	(8.6)	(6.7)	(8.4)	(11.3)	(7.0)	(4.6)
*	139.4	254.3	413.8	531.9	355.8	610
	(26.0)	(30.7)	(28.1)	(38.7)	(31.5)	(27.5)
**	0.0	82.4	196.8	281.3	155.2	337.7

* - Total sodium loss after 4 hours excluding 0hr reading

** - % Change with respect to control

GR1 - Control group (Normal saline)

GR2 - Chloroquine group 50mg/kg

GR3 - Chloroquine 50mg/kg + Chlorthiazide 35mg/kg

GR4 - Chlorthiazide 35mg/kg

GR6 - Frusemide 20mg/kg

FIGURE 9 INFLUENCE OF CHLOROQUINE (CQ) ON THE DIURETIC PROPERTIES OF FRUSEMIDE (F) AND CHLORTHIAZIDE (CTZ)

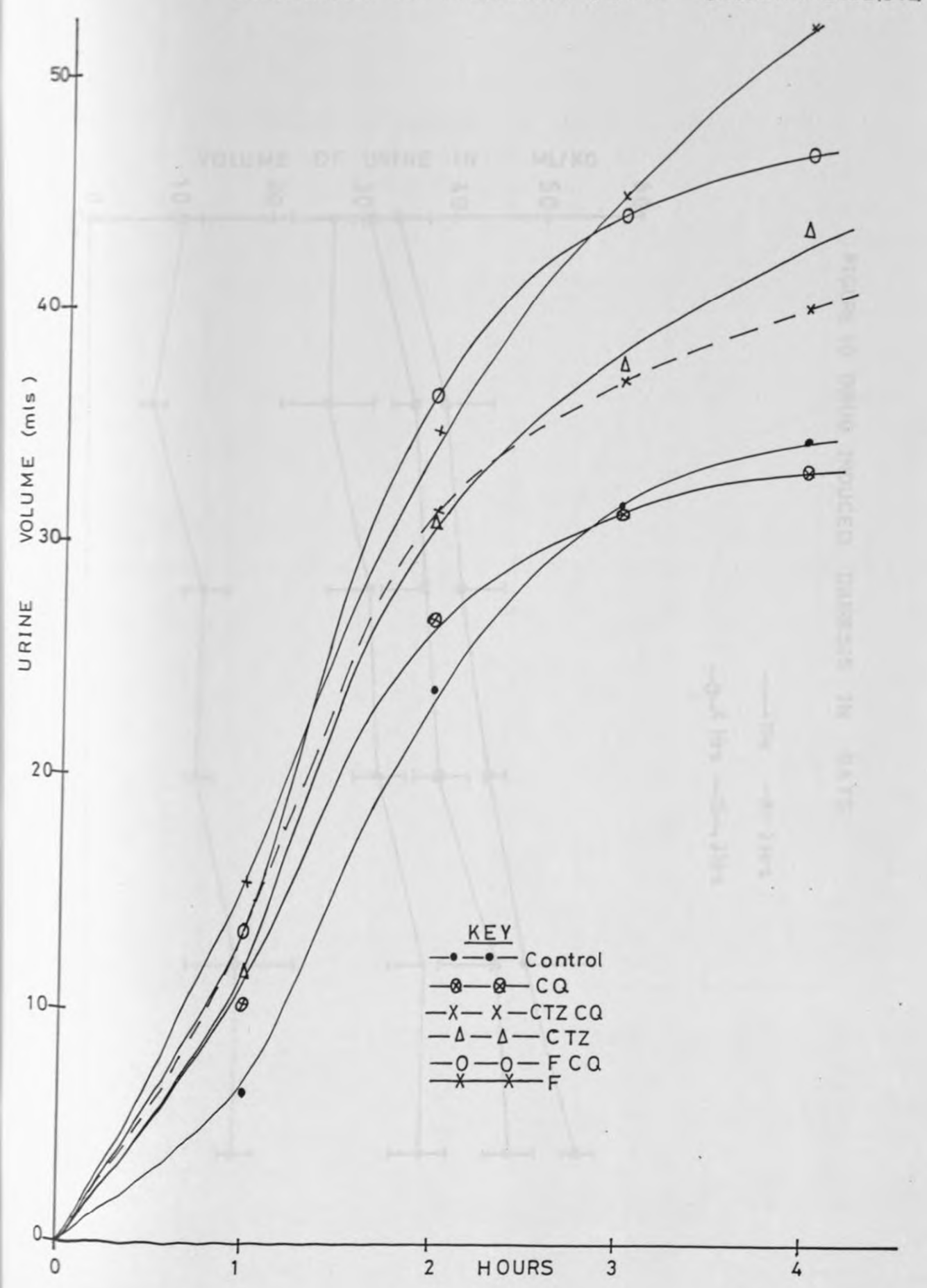


FIGURE 10 DRUG INDUCED DIURESIS IN RATS

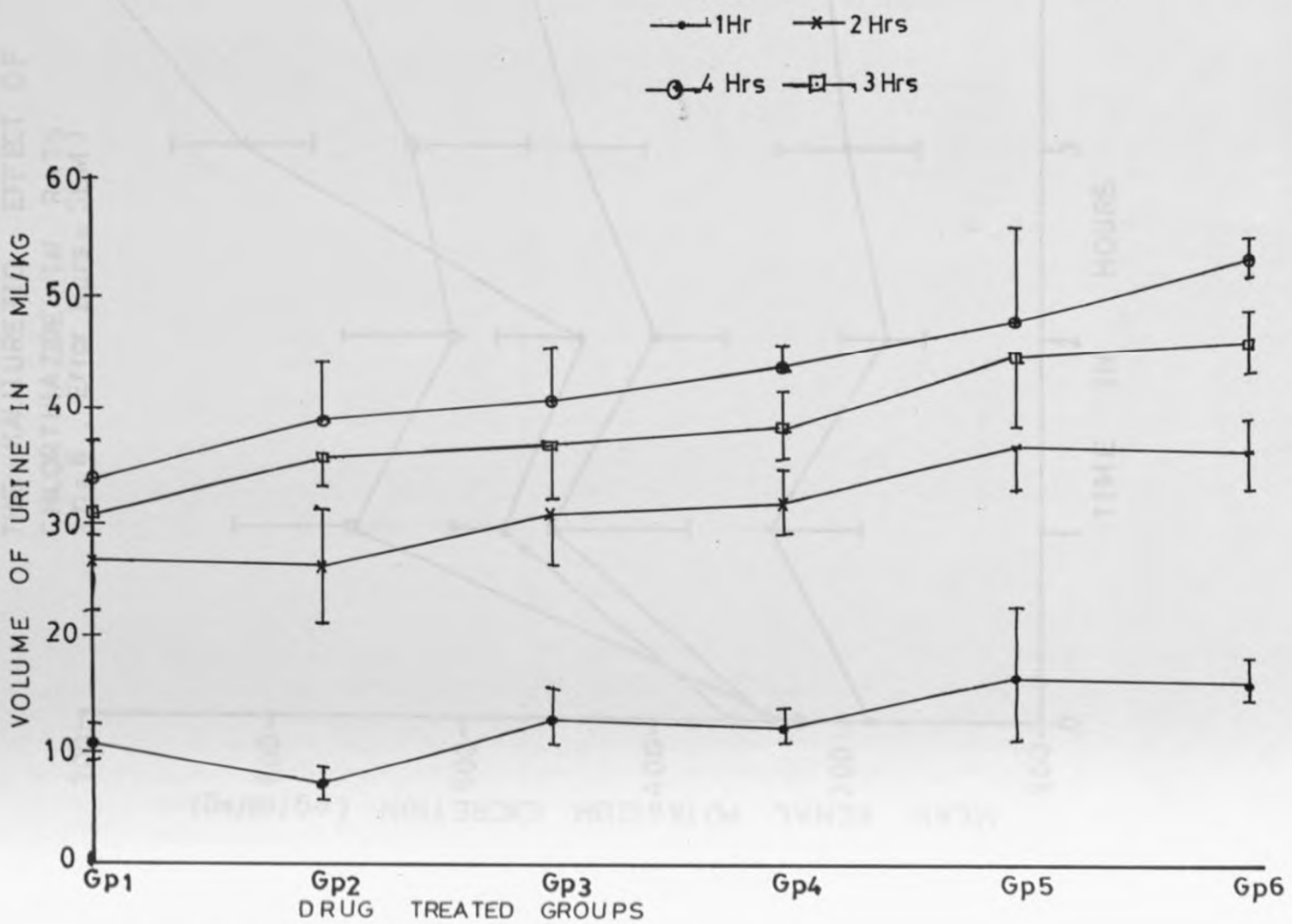


FIGURE 11 INFLUENCE OF CHLOROQUINE ON THE KALLIURETIC EFFECT OF CHLORTHIAZIDE IN RATS
(n = 6 , Error bars = SEM)

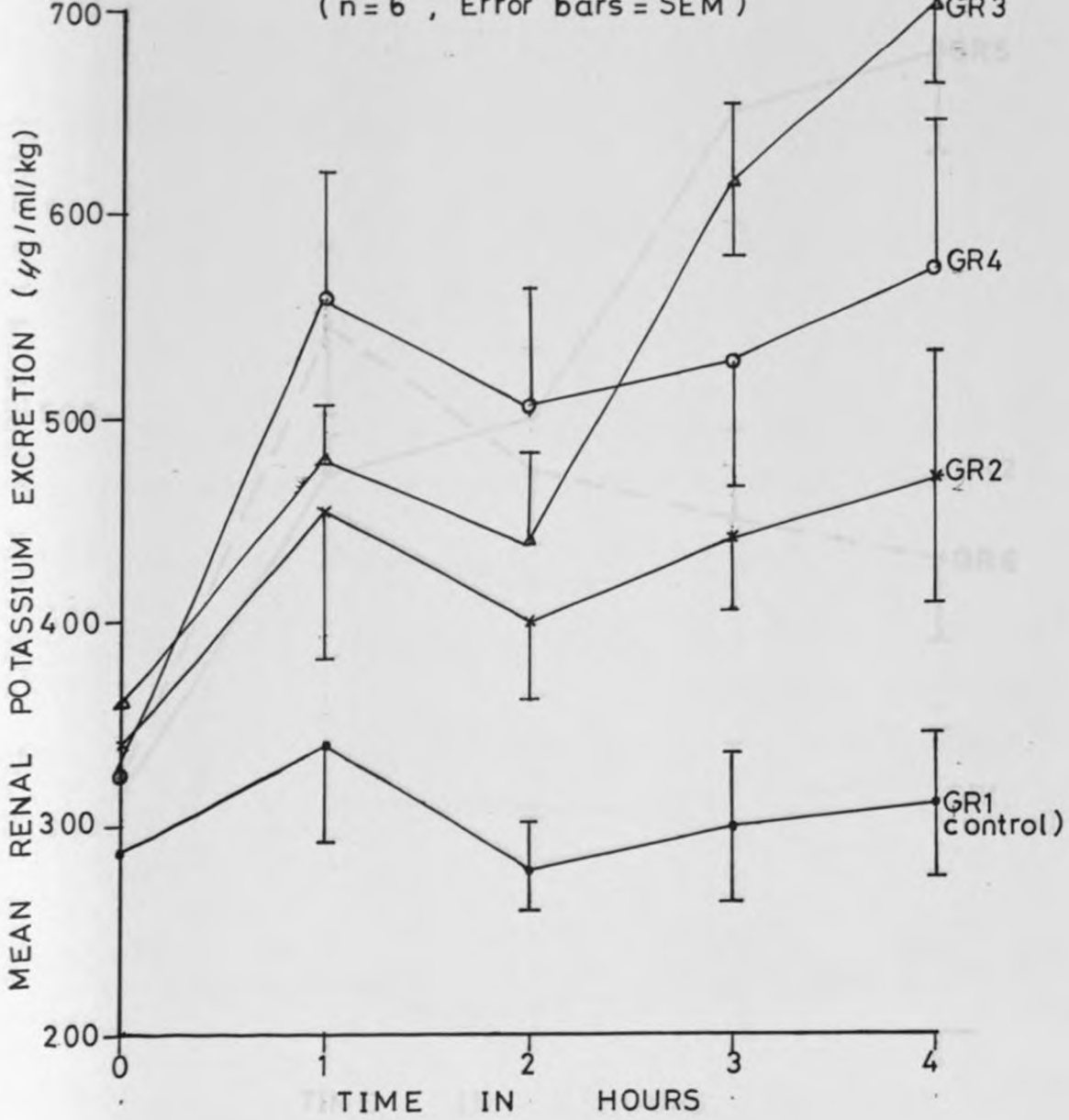


FIGURE 12 EFFECT OF CHLOROQUINE ON THE KALLIURETIC PROPERTY OF FRUSEMIDE IN RATS
(n=6, Error bars = SEM)

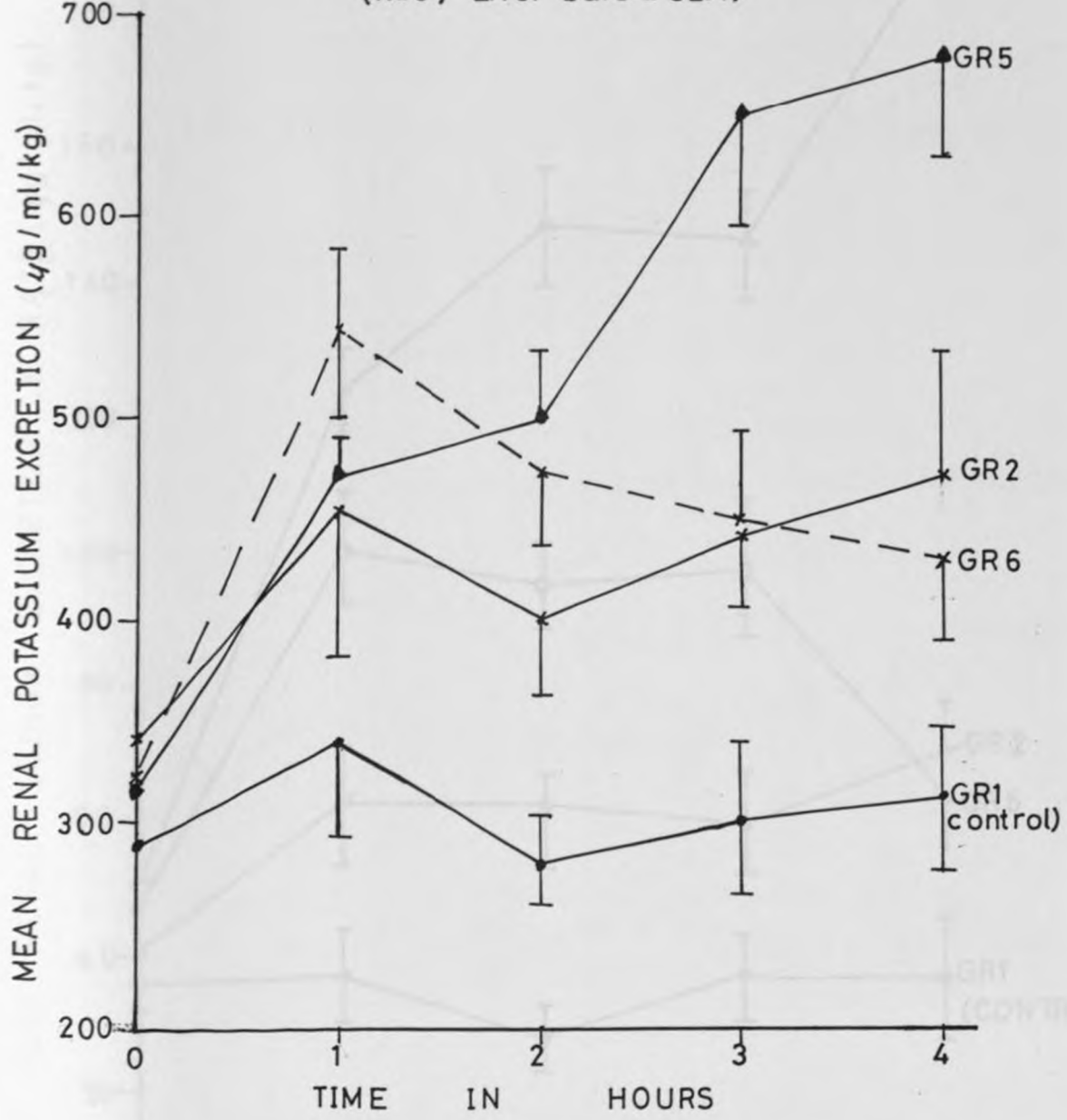


FIGURE 13 MODIFICATION OF FRUSEMIDE INDUCED NATRIURESIS BY CHLOROQUINE
(n=6, Error bars = SEM)

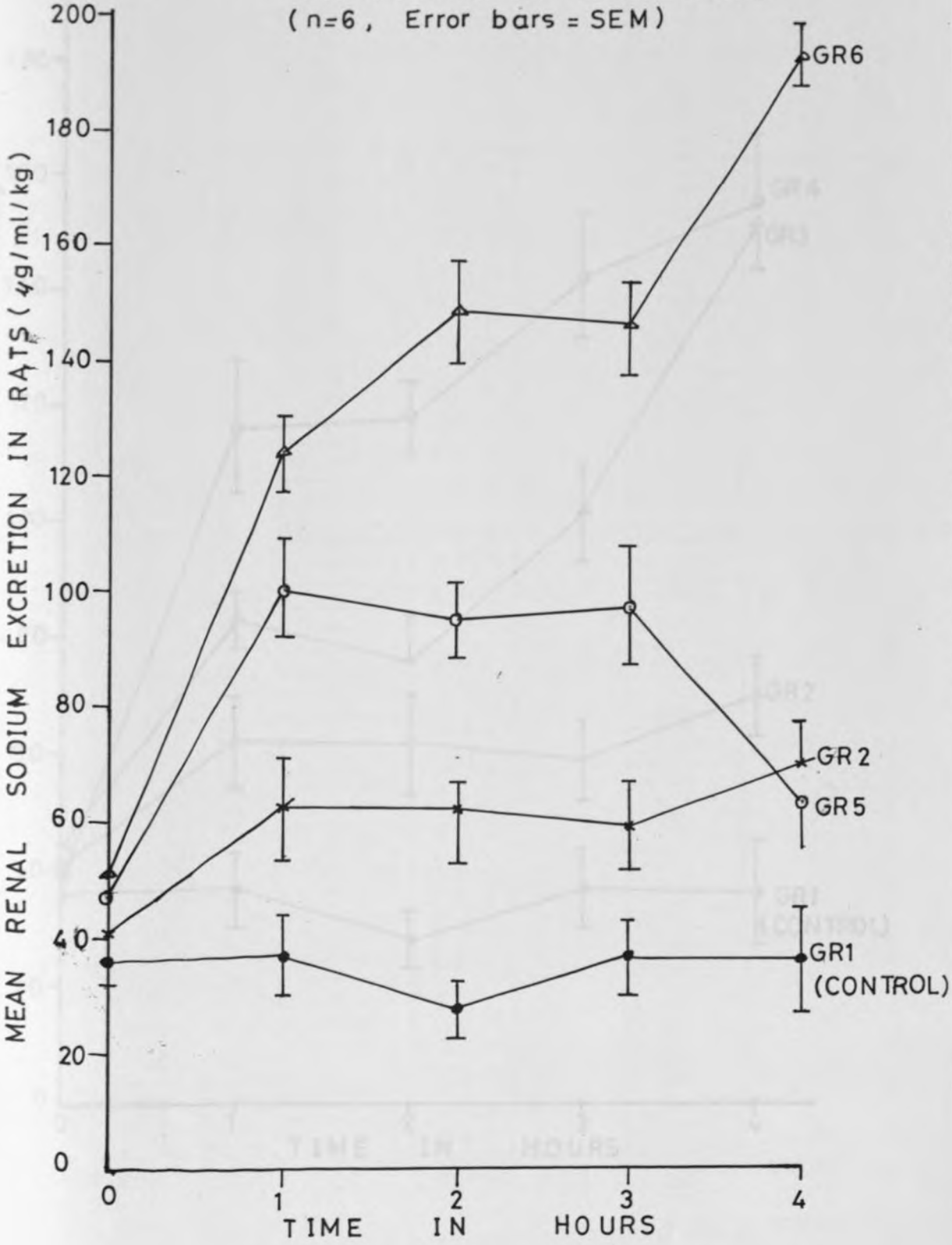


FIGURE 14 MODIFICATION OF CHLORTHIAZIDE INDUCED NATRIURESIS BY CHLOROQUINE
n = 6 , Error bars = SEM

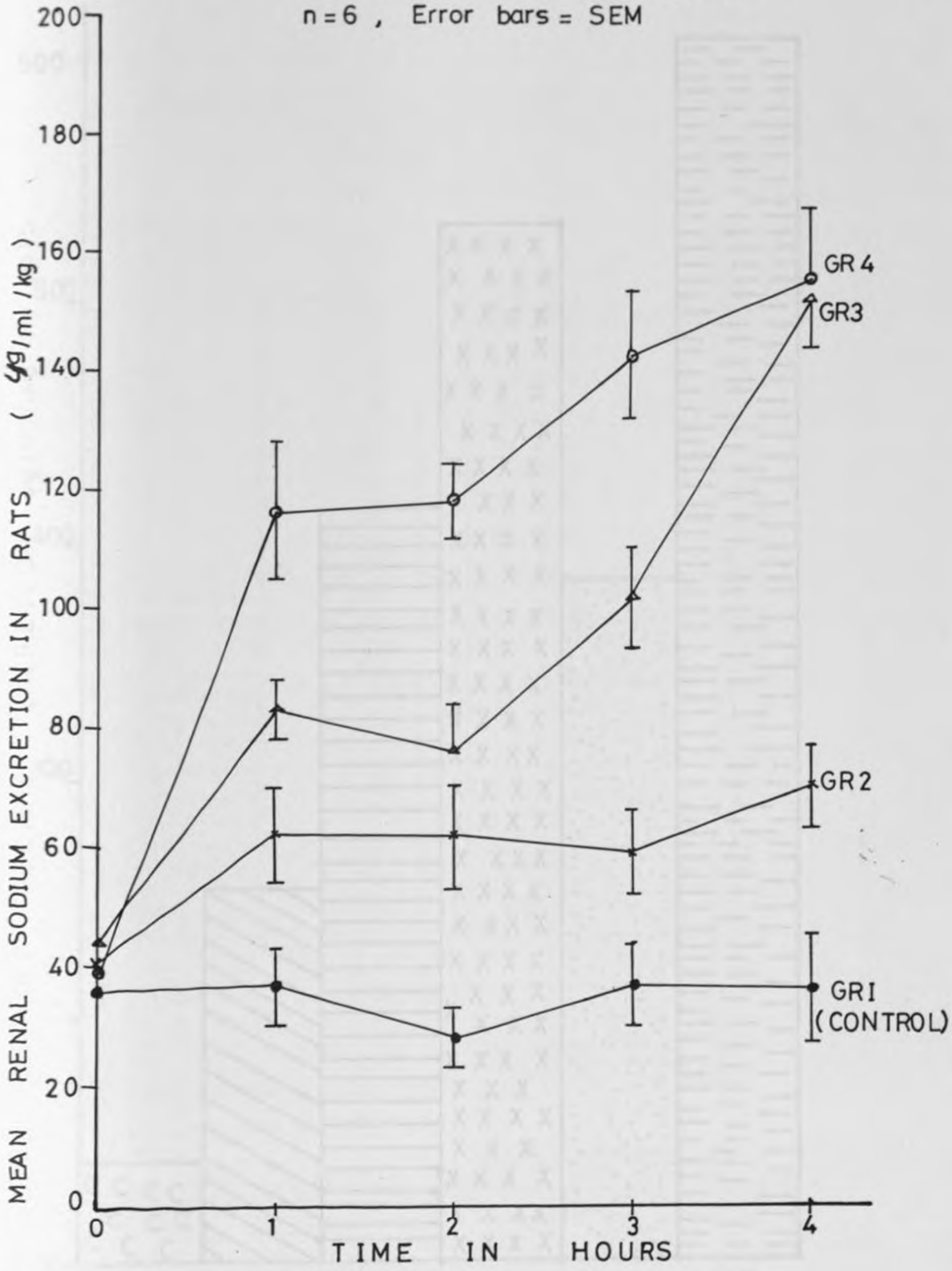


FIGURE 15 TOTAL URINARY SODIUM EXCRETION FOR 4 HRS INDUCED BY ORAL ADMINISTRATION OF CHLOROQUINE (CQ) FRUSEMIDE (F) CHLORTHIAZIDE (CTZ) AND THEIR COMBINATIONS TO RATS

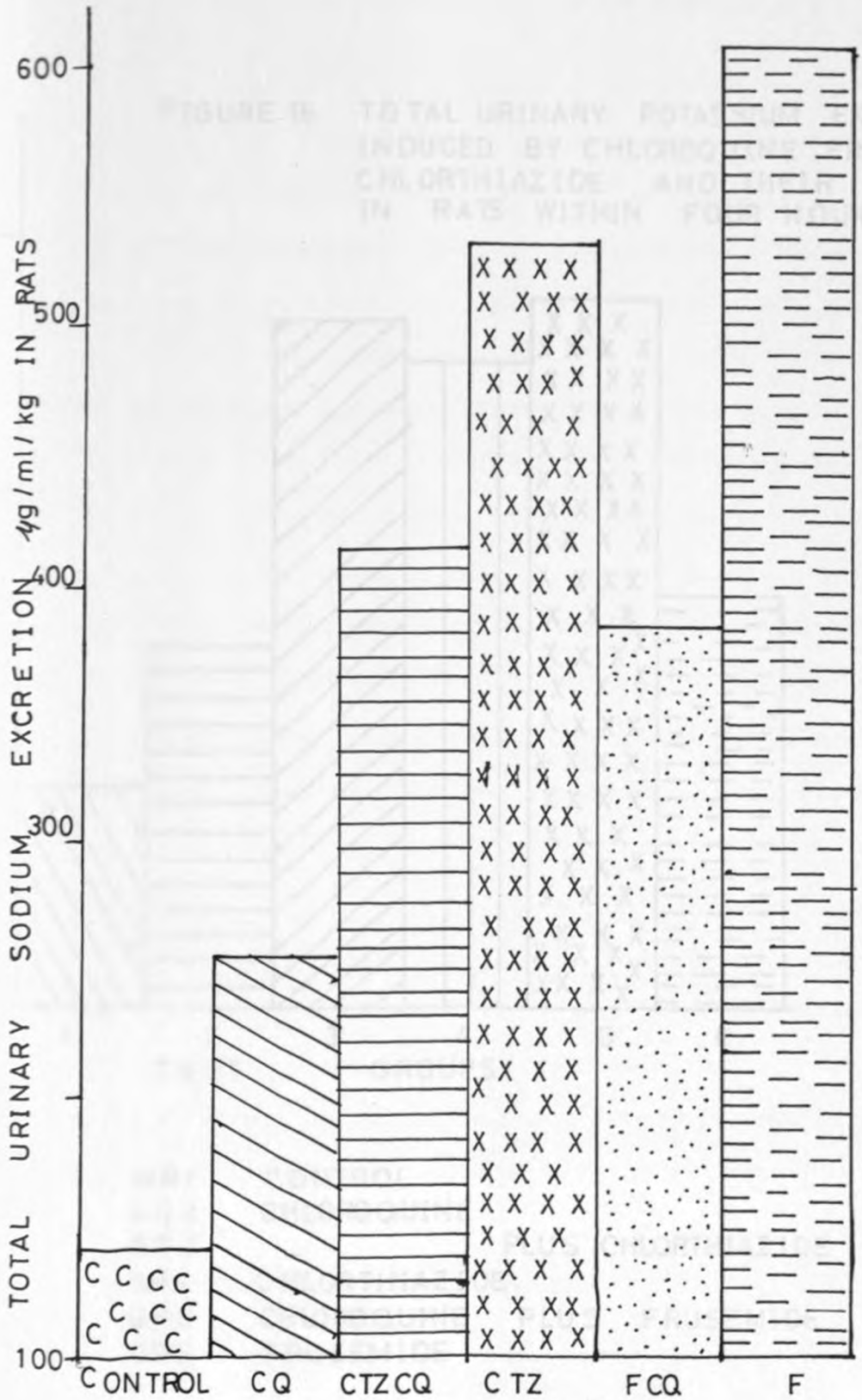
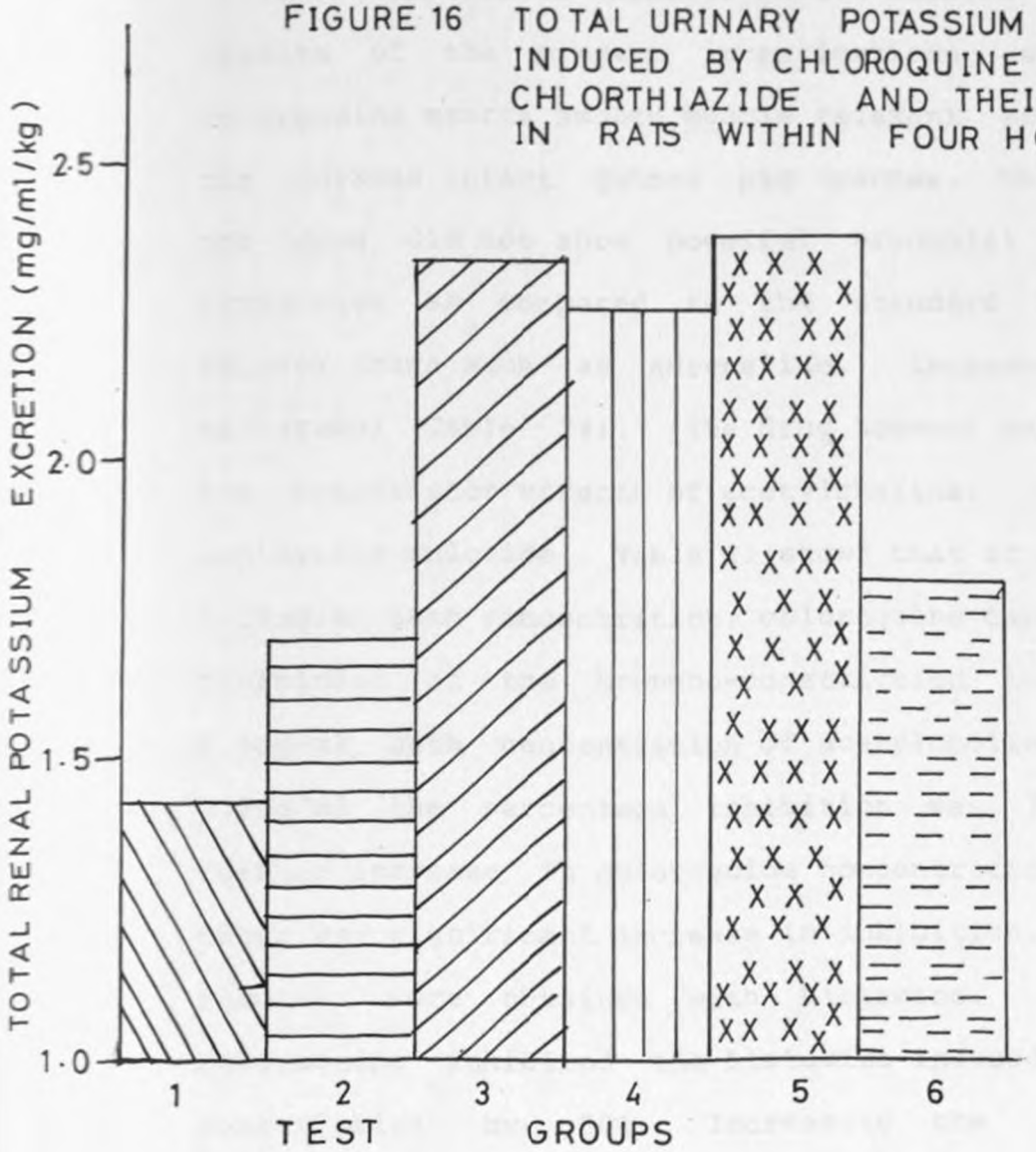


FIGURE 16 TOTAL URINARY POTASSIUM EXCRETION INDUCED BY CHLOROQUINE FRUSEMIDE CHLORTHIAZIDE AND THEIR COMBINATIONS IN RATS WITHIN FOUR HOURS



GR1 CONTROL
GR2 CHLOROQUINE
GR3 " PLUS CHLORTHIAZIDE
GR4 CHLORTHIAZIDE
GR5 CHLOROQUINE PLUS FRUSEMIDE
GR6 FRUSEMIDE

1980).

3.9 EFFECT OF CHLOROQUINE ON SMOOTH MUSCLES

3.9.1 Effects on Tracheal Smooth Muscles

Results of the present investigations show that chloroquine exerts smooth muscle relaxant effects on the isolated intact quinea pig trachea. On its own, the drug did not show powerful bronchial relaxant properties as compared to the standard bronchial dilator drugs such as adrenaline, isoprenaline and salbutamol (Table 24). The drug however antagonised the constrictor effects of acetylcholine, histamine and barium chloride. Table 23 shows that at a dose of 0.10ug/ml bath concentration, chloroquine caused a 50% inhibition of the broncho-constriction induced by 0.5ug/ml bath concentration of acetylcholine and at 0.2ug/ml the percentage inhibition was 71.4. Any further increase in chloroquine concentration did not cause any significant increase in inhibition. Similar results were obtained with histamine. 0.2ug/ml chloroquine inhibited the histamine induced tracheal constriction by 50%. Increasing the dose of chloroquine did not effect any further inhibition. It was also noted that the constrictor effects induced by barium chloride were more sensitive to inhibition by chloroquine than those induced by histamine and acetylcholine (Table 23). For example a dose of

chloroquine 0.3ug/ml that caused 71.4% and 32.1% inhibition of acetylcholine and histamine induced bronchoconstriction respectively, caused a 130% inhibition of barium induced bronchoconstriction. This in essence means that not only was there a 100% inhibition of the barium induced bronchoconstriction, but the constriction was completely reversed to a relaxant effect by 30%.

It is known that barium ions can carry ionic currents in the same way as calcium ions and also make more calcium available for the contractile processes. Barium ions can subtitute for calcium ions in muscle activity (Bowman and Rand, 1980). Antagonism of barium induced muscular constrictions is equivalent to antagonism of calcium effects. Chloroquine therefore might bring about bronchorelaxation by inhibiting or blocking calcium induced muscular activity. It is possible that even the antimalarial effect of chloroquine has some relationship to the ability of this agent to block calcium modulated cellular activity. This hypothesis is supported by the finding that calcium channel blockers have been shown to possess antimalarial activity (Jutamaad, 1987).

Chloroquine antagonised the bronchoconstrictor effect of acetylcholine and histamine by antagonising the effects of those agents on the muscarinic (M2) and histaminic (H1) receptors respectively. Chloroquine is known to possess antihistaminic effects (Gavend et al, 1971; Szilagyl and Kaval, 1970).

The effect of chloroquine on the tracheal smooth muscle has been compared to that of steroidal and non-steroidal antiinflammatory agents which reduce isometric contractions induced by prostaglandins E_1 and $F_{2\alpha}$ (Famaey et al, 1975), acetylcholine (Agarwal et al, 1956) and by coaxial stimulation of the guinea pig trachea (Famaey et al, 1975).

The bronchial relaxant effects of chloroquine have long been recognised and it has even been used in the treatment of asthma (Engeset, 1957; Sylvio de Carmargo, 1958). However no literature is available showing the type of interaction, if any, that would occur if chloroquine was used concomitantly with clinically accepted antiasthmatic agents.

Results from the present study show that chloroquine potentiates the bronchial relaxant effects of adrenaline, isoprenaline and salbutamol. These are agents that cause relaxation of the bronchial muscles by interacting agonistically with the B_2 receptors located in the smooth muscles of the bronchioles. Table 24 shows the percentage potentiation induced by varying doses of chloroquine on fixed doses of adrenaline, isoprenaline and salbutamol. However the degree of potentiation varies even at a fixed dose of chloroquine. For example at 0.3ug/ml chloroquine induced 23.1%, 50% and 66.7% potentiation of the bronchial relaxation induced by adrenaline,

isoprenaline and salbutamol respectively. The results imply that adrenaline is the least sensitive to the potentiating effect of chloroquine whereas salbutamol is the most sensitive. These differences could probably be related to the relative selectivities of the three agents to the adrenergic receptors. Of these three, salbutamol is the most selective B receptor agonist. However the theory of relative selectivity does not adequately explain why adrenaline is much more potent than isoprenaline and salbutamol. The difference in activity is probably related to the receptor affinities of the compounds.

Another major revelation from the present work is that pretreatment of the bronchiole muscle with chloroquine sensitises the tissue to the bronchodilator effects of adrenaline, isoprenaline and salbutamol. It was found that after allowing the guinea pig tracheal muscle to be in contact with chloroquine (0.3ug/ml for 15 minutes, introduction of even a small amount of salbutamol (1ug/ml) caused profound potentiation of salbutamol-induced bronchial relaxation (16.7% in 2 minutes and 50% in 4 minutes). There was no recovery even after washing off the drugs. The relaxant effect continued steadily reaching 150% in 50 minutes and 216% after 65 minutes. An effort to reverse the excessive bronchial relaxation with acetylcholine or histamine was fruitless.

Similar results were observed when adrenaline or isoprenaline were substituted for salbutamol.

These results are of clinical significance in that if they are extrapolated to human beings, a serious and possible fatal drug interaction is likely to result from a combination of chloroquine and antiasthmatic drug whereby hyperventilation can occur due to excessive and uncontrolled bronchial relaxation and the patient can die from increased oxygen tension.

Further studies are needed in humans to assess the severity of the above likely interaction and the kind of dosage adjustments that need to be made to avoid serious consequences.

It should be noted here that if any drug adjustments have to be made, they will have to be on the antiasthmatic agent and not on chloroquine because unlike the potentiation of the antiasthmatic effect by chloroquine, there is no potentiation of the antimalarial effect of chloroquine by the bronchodilator agent. Therefore to get the full antimalarial effet of chloroquine, the drug must be given in the full recommended therapeutic dose.

However it is also important to point out that chloroquine <u>per se</u> is not contraindicated in asthmatics or in asthmatic prone patients as the drug does not aggravate or precipitate an asthmatic attack. It might actually relieve some of the asthmatic

TABLE 23 EFFECT OF CHLOROQUINE ON DRUG INDUCED CONSTRICTION OF THE ISOLATED GUINEA PIG TRACHEA

n = 6

DRUG	FINAL BATH CONCENTRATION UNITS/ML	MEAN RESPONSE	MEAN % INHIBITION
Acetylcholine	5.0µg	+7.0	0.0
Histamine	1.0µg	+14.0	0.0
Barium chloride	0.2µg	+5.0	0.0
Chloroquine(CQ)	0.1µg	-2.0	
Acetylcholine+CQ	0.5µg+0.1µg	+3.5	50.0
	0.5µg+0.2µg	+2.0	71.4
	0.5µg+0.3µg	+2.0	71.4
	0.5µg+0.3µg	+2.0	71.4
	0.5µg+0.4µg	+2.0	71.4
Histamine	1.0µg+0.1µg	+8.0	42.9
	1.0µg+0.2µg	+7.0	50.0
	1.0µg+0.3µg	+9.5	32.1
Barium chloride+CQ	0.2mg+0.1µg	+2.5	50.0
	0.2mg+0.2µg	+2.0	60.0
	0.2mg+0.3µg	-1.5	130.0

TABLE 24 POTENTIATION OF BRONCHIAL RELAXATION INDUCED BY ADRENALINE ISOPRENALINE AND SALBUTAMOL BY CHLOROQUINE

n = 6

DRUG	FINAL BATH CONCENTRATION UNITS/ML	MEAN RESPONSE AFTER 5 MIN CONTACT	PERCENTAGE POTENTIATION
Adrenaline	0.1µg	-13.0	0.0
Isoprenaline	0.4µg	-5.0	0.0
Salbutamol	4.0µg	-6.0	0.0
Chloroquine(CQ)	0.1µg	-2.0	
Adrenaline+CQ	0.1µg+0.1µg	-13.0	0.0
	0.1µg+0.2µg	-14.5	11.5
	0.1µg+0.3µg	-16.0	23.1
Isoprenaline+CQ	0.4µg+0.1µg	-6.0	20.0
	0.4µg+0.2µg	-6.5	30.0
	0.4µg+0.3µg	-7.5	50.0
Salbutamol+CQ	4.0µg+o.1µg	-7.5	25.0
	4.0µg+0.2µg	-8.0	33.3
	4.0µg+0.3µg	-10.0	66.7

symptoms. But as pointed out earlier, the other drugs used together need a re-evaluation.

3.9.2 Smooth Muscle of the Uterus

Increased uterine activity can be attributed to endogenous and exogenous factors. The endogenous factors diverse as they may be, can all be hinged on increased hormonal levels, increased prostaglandin release or increased parasympathetic activity.

The exogenous factors can be ingestion of uterotonic agents such as drugs or poisons or introduction of foreign substances into the uterus. Usually these are done to therapeutically induce labour after full term pregnancy or to procure abortion either therapeutically or otherwise.

Chloroquine has been commonly abused as an abortifacient especially among the young girls in Kenya. However the mechanism of its abortifacient effect has not been fully elucidated.

Results from the present study showed that at the dose range used, chloroquine has no uterotonic effect of its own. It was actually behaving as an uterine relaxant. However when used in conjunction with the uterotonic agents carbachol, oxytocin and PGF-2alpha , chloroquine effectively antagonised the increased uterine activity normally induced by these agents (Table 25 and Figure 17a-i). Uterine relaxant effects have also been observed on guinea pig uterus

(Chinyanga et al, 1974).

The level of oxytocin are also known to rise during the full term stage of gestation (Bowman and Rand, 1980). It is therefore possible that by virtue of its ability to inhibit the uterotonic actions of oxytocin and PGF2alpha, chloroquine might delay labour or prolong it if used at the time of parturition. However this is not what happens in vivo as evidenced by Chatterjee et al (1985) who noticed that chloroquine could cause premature evacuation of the uterine conceptus in rats.

Experiments carried out in vivo using one week pregnant rats showed that after one week of chloroquine treatment (25mg/kg chloroquine) not only did the animals present with blood stained birth canals, but on dissection all the concepti were found to be dead and lacked the defined anatomical organ boundaries as seen in untreated animals. The concepti were fluid-like as if the mother's bodies were in the process of absorbing the foetal contents.

The cervix of the chloroquine treated animals was found to have comparatively more mucus than that of the untreated rats. Some rats presented with incomplete premature evacuation of the foetus evidenced by the finding of evacuated foetuses and still some of the conceptus retained in the uterus as seen after dissecting the mothers.

Carbachol induced contractions were found to be more sensitive to chloroquine inhibition than PGF-2alpha , and oxytocin with the order of sensitivity being carbachol, PGF-2alpha , oxytocin (Table 25).

This is supported by the finding that to induce 80% or more percentage contractile inhibition 30, 60 and 200µg/ml chloroquine concentration is required for cabachol, PGF-2alpha , and oxytocin respectively.

Though the inhibition of uterine contractions induced by PGF-2alpha showed a dose dependent pattern, no complete or total blockade was achieved (Figures 17g-i).

The inhibition of carbachol induced contractions by chloroquine (Figures 17a-c) implies that the chloroquine possesses some anticholinergic effects. This has been previously reported (Habara et al, 1986; Ebeigbe et al, 1986). It is most likely then that the abortifacient effect of chloroquine is not due to an increase in the parasympathetic activity.

With regard to prostaglandins, it is well documented that these agents especially those of the F_2 series are potent uterotonic agents (Bygdeman, 1964 and 1967; Karim, 1968) with the highest concentration appearing in the venous circulation just before uterine contraction (Karim, 1968; Karim et al, 1968). This indicates that these agents have a physiological role in parturition (Embrey, 1969).

TABLE 25 PERCENTAGE INHIBITION OF DRUG INDUCED RAT UTERINE CONTRACTIONS BY CHLOROQUINE

Dose of Chloroquine µg/ml bath concentration	%Contractile inhibition induced by chloroquine in presence of		
	Carbachol 0.3µg/ml	PGF2∝ 0.01µg/ml	Oxytocin 0.005IU/ml
2	28.6(1.5)	8.2(2.0)	
4	32.2(0.4)	32.6(0.8)	
6	35.8(0.5)	37.2(0.5)	
8	39.5(2.2)	37.5(0.5)	
10	50.1(0.3)	50.1(2.3)	
20	73.1(0.8)	64.1(0.8)	
30	82.5(0.9)	67.2(1.3)	
40	100.0(0.4)	72.3(0.9)	9.8(1.9)
50		81.1(1.1)	9.8(1.9)
80		87.4(1.1)	13.1(0.7)
100		90.5(2.8)	18.4(1.2)
150		85.0(1.2)	21.8(2.4)
200			30.7(1.2)
300			35.2(2.0)
400			50.5(0.9)
600			61.2(1.4)
1000			64.5(1.1)
2000			83.1(0.7)
3000			89.0(0.7)

FIGURE17

EFFECT OF CHLOROQUINE ON DRUG INDUCED UTERINE CONTRACTIONS

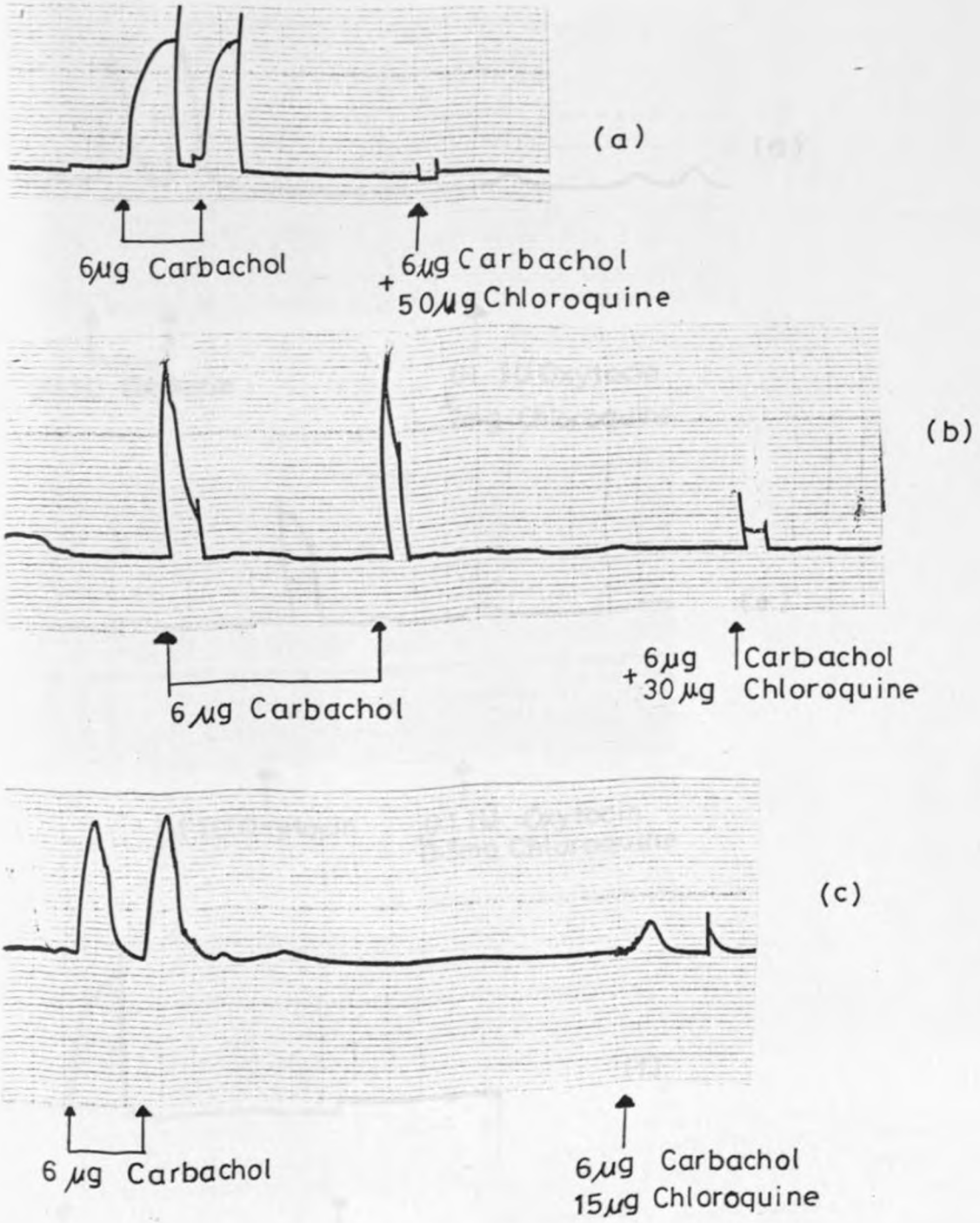
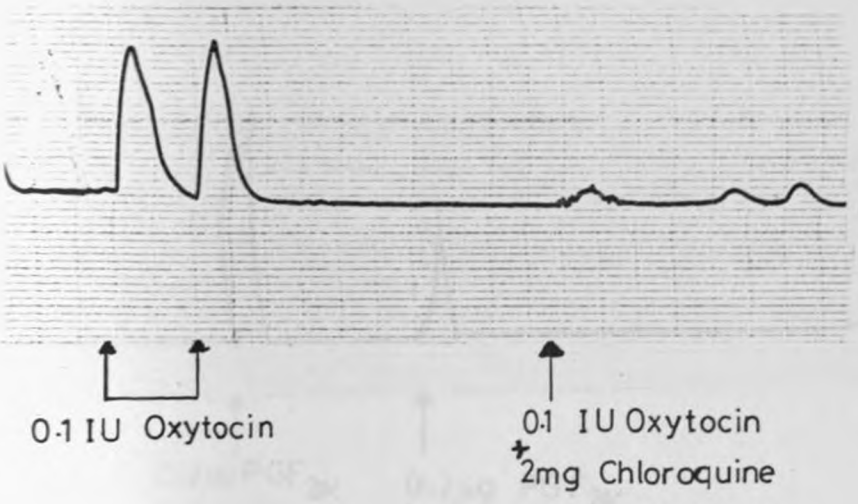
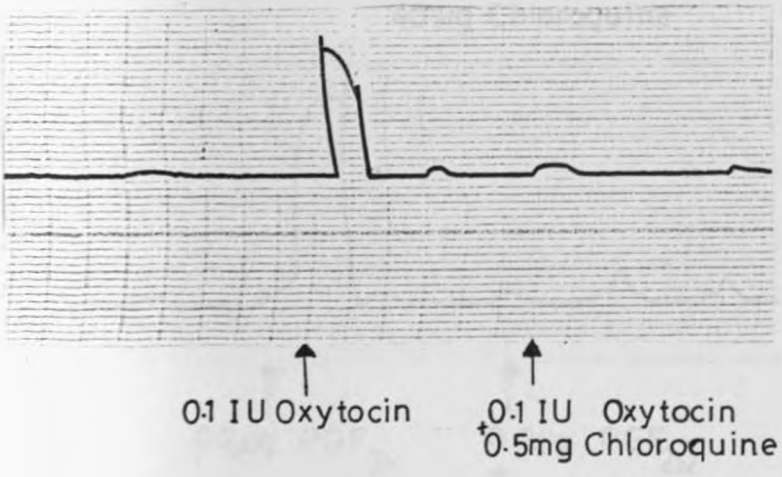
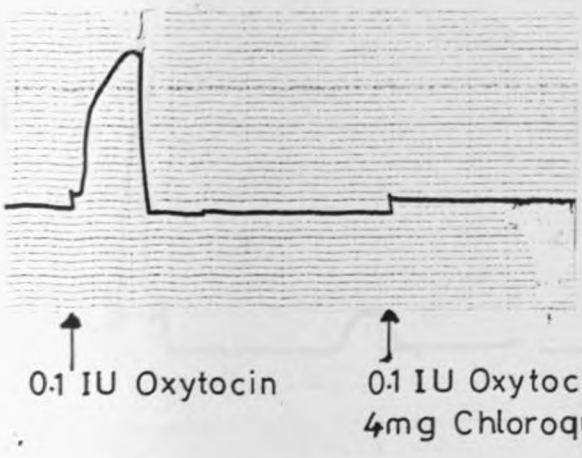


FIGURE 17 (Cont)







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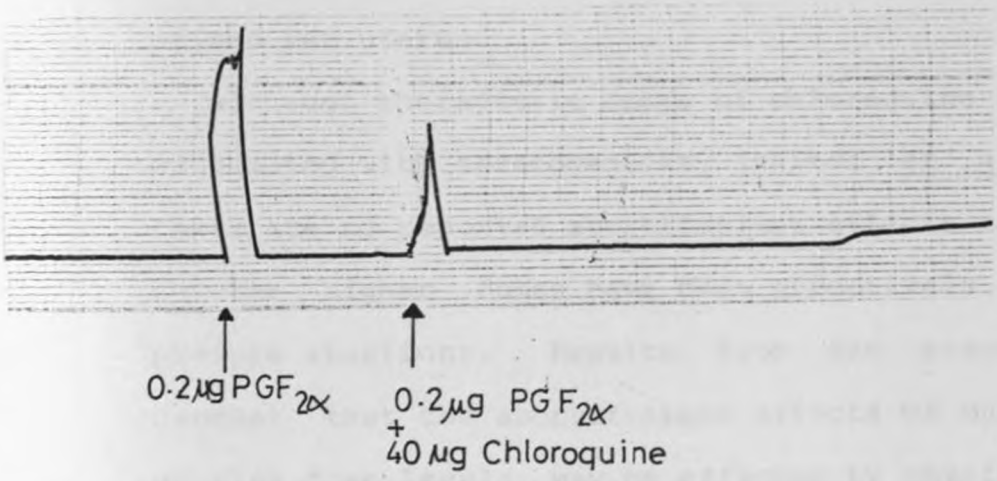
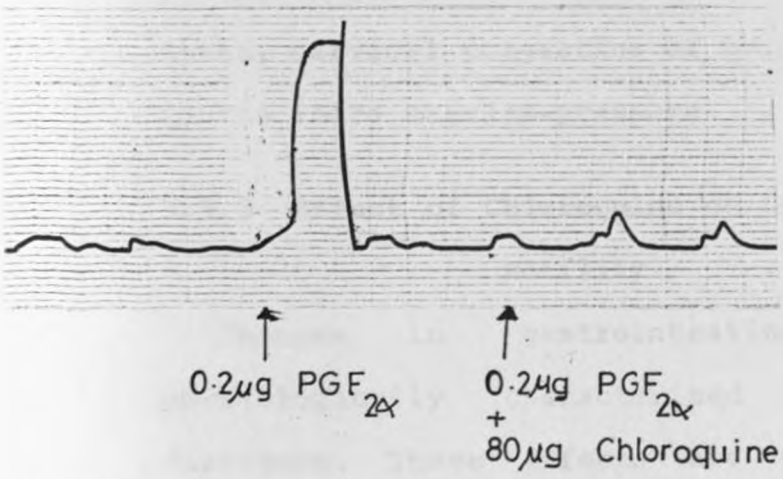
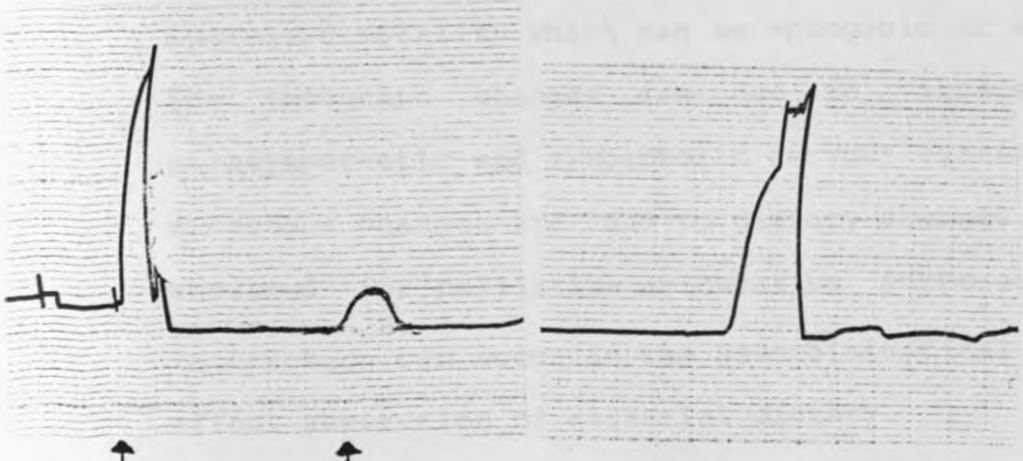






In conclusion, it is evident that chloroquine has varying physiological and pharmacological effects on the smooth muscle of the uterus. When tested *in vitro* chloroquine was found to exhibit anti-oxytocin, anti-cholinergic and anti-prostaglandin effect on the primed rat uterus.

Although therapeutic doses of chloroquine have been associated with teratogenicity (Walker *et al*, 1974) there are no reported abortifacient effects in humans. However higher doses have been effectively used to procure abortions. Results from the present study suggest that the abortifacient effects of chloroquine at high dose levels may be effected by causing foetal death, cervical congestion or by causing rapid changes in the intra uterine-pressure.

3.9.3 Effect of Chloroquine on Gastrointestinal Motility

Changes in gastrointestinal activity are physiologically characterised by constipation or diarrhoea. These effects are due to decreased or increased motility which can be endogenic or exogenic. The endogenic changes are due to imbalance of parasympathetic and sympathetic nervous systems. The exogenous changes are due to dietary changes or drugs consumed for palliative or curative purposes. Drug interaction can occur in the gastrointestinal tract to affect absorption of essential dietary

constituents or merely to increase or decrease the gastrointestinal movements.

Chloroquine has been reported to decrease gastrointestinal passage time (Adjepon-Yamoah, 1985) implying that the drug facilitates the passage of the intestinal contents through the gastrointestinal tract. However other authors claim that chloroquine has anti-muscarinic effects (Habara et al, 1986), inhibiting electrically induced contractions of the guinea pig ileum (Famaey et al, 1975) and other smooth muscles (Ebeigbe et al, 1986). Also the intestinal absorption of chloroquine can be influenced by the gastric emptying rate (Varga, 1966 and 1975).

The results of the present study show that chloroquine has variable influence on the activity of the smooth muscles of the alimentary tract in vitro (Figure 17ii) and in vivo (Table 26). In vitro, the drug causes initial smooth muscle stimulation followed by prolonged smooth muscle relaxation (Figure 17ii). Administration of chloroquine, followed by administration of acetylcholine without washing, caused a brief and unsustained stimulation, implying that, the cholinergic muscarinic receptors are not directly affected and could respond to exogenous muscarinic agents. Hence the anti-muscarinic effects of chloroquine reported by Habara et al, 1986, and demonstrated in the present findings as smooth muscle

relaxant could be due to physiological antagonism of chloroquine on the parasympathetic system, or could be due to an indirect action of the drug on the cholinergic system causing displacement and depletion of acetylcholine from the cholinergic vesicles. Although this type of pharmacological response has not been reported in the parasympathetic system, it is well documented in the sympathetic system especially with the adrenergic neurone blockers.

In vivo chloroquine caused mild laxative effect in mice by 20.3% increase in gastric motility as expressed by the distance travelled by the intragastrically administered dye with respect to untreated control (Table 26). A similar effect has been observed in humans (Martindale, 1989) and could be explained by the initial smooth muscle stimulation observed in vitro (Figure 17ii). However when used concomitantly with the clinically established laxatives such as senokot, bisacodyl, cascara, sodium sulphate and castor oil, chloroquine was found to have variable effect. It decreased the laxative effect of senokot, bisacodyl, cascara and sodium sulphate by a factor ranging from -3.3% for sodium sulphate to -20.9% for bisacodyl. The findings for senokot and cascara stood at -7.9% and -12.8% respectively. When used in combination with castor oil; chloroquine enhanced the laxative effect of castor oil by 10.0%.

Except for sodium sulphate, which is an osmotic purgative, all the other laxative tested act by a stimulant or irritant action, probably by inhibiting the intestinal sodium — potassium ATPase (Goodman and Gilman, 1985) or by enhancing the synthesis of prostaglandins and cyclic AMP hence increasing the secretion of the water and electrolytes into the intestinal lumen (Symposium, 1983).

While senokot and bisacodyl action is limited to the large intestine with delayed onset of action (6-12 hours), castor oil acts within the small intestine to induce a prompt and thorough bowel evacuation (Goodman and Gilman, 1985). The mild enhancement of the carthatic effect of castor oil by chloroquine may be due to initial smooth muscle stimulation, whereas, the antagonism of the effect of cascara, senokot and bisacodyl may be due to their delayed onset of action which coincides with the delayed smooth muscle relaxant effect induced by chloroquine.

To predict the type of interaction that is likely to occur between chloroquine and a specific laxative is difficult, the best advice is to avoid concomitant administration of the two types of drugs in those cases where laxative is indicated for bowel evacuation as in surgery, radiological procedures or before child delivery, as this effect cannot be guaranteed.

FIGURE 17(ii) EFFECT OF CHLOROQUINE ON THE ISOLATED RABBIT ILEUM

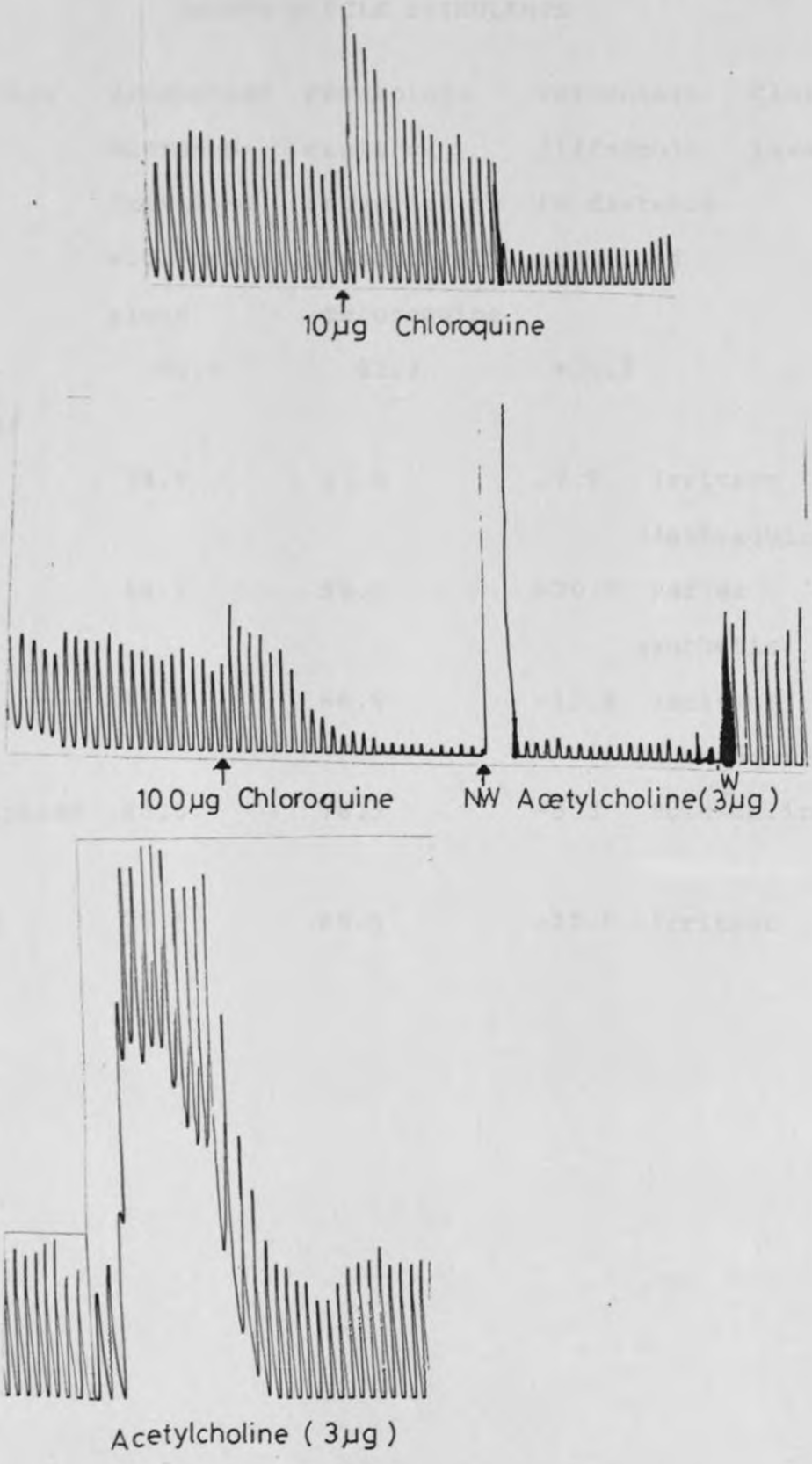


TABLE 26 THE INFLUENCE OF CHLOROQUINE ON THE LAXATIVE PROPERTIES OF COMMON INTESTINAL SMOOTH MUSCLE STIMULANTS

Laxative Drug	Percentage distance travelled with drug alone	Percentage distance travelled in presence of chloroquine	Percentage difference in distance travelled	Class of laxative
Control (water+dye)	62.5	82.8	+20.3	
Senokot	74.9	67.0	-7.9	Irritant (Anthraquinone)
Bisacodyl	80.1	59.2	-20.9	Reflex synthetic stimulant
Cascara	79.6	66.8	-12.8	Irritant (Anthraquinone)
Sodium sulphate	80.0	76.7	-3.3	Bulk/saline
Castor oil	75.5	85.5	+10.0	Irritant

PART II

CHAPTER ONE

INTRODUCTION

1.1 Plants as Sources of Drugs

Diseases and man have been in existence since the beginning of life. For fear of illness and death man has resorted to anything that nature can offer in terms of health protection. Animal and plants were the early man's close associates and he relied on them for food, drink, shelter, clothing and health needs.

The use of plants for medical purposes has transended social, ethnic and religious boundaries. It is part and parcel of human needs.

Three ways in which plants can act as sources of drugs (Elliot and Brimacombe, 1986) are:-

(i) They can be used unmodified as therapeutic agents.

(ii) They can provide raw materials for partial synthesis of drugs.

(iii) They can be used as molecular models by synthetic pharmacists and chemists to synthesise new drugs.

2.1 Traditional preparations

Approximately 70-90% of the rural population in developing countries depend on traditional plant remedies for health care. The percentage dependence

was even higher approaching 100% before the introduction of orthodox medicine (Elliot and Brimacombe, 1986).

With the introduction of orthodox or scientific medicine, the traditional healer tended to be pushed into the background and sometimes treated with suspicion. The suspicion sometimes had a basis. For example:-

1. Traditional medicine is not a unified practice.
2. Techniques and apprenticeships differ from country to country and from community to community within the same country. In most cases it is an individualised practice evolved and developed in different ways. The source of medical knowledge is exclusively from practical experience and observations inherited or handed down from generation to generation. This implies that there is no standard institution of learning and as such no standardised practice. This is true mainly in developing countries. China, India and some West African countries have taken traditional medicine a step further and started schools and centres where homeopathic medicine is taught thus trying to unify and educate the traditional healers. Traditional medicines are also being formulated in easily dispensable forms.
3. Traditional healers in general lack extensive diagnostic skills. They are not conversant with

diagnostic instruments, chemicals and pathological specimen analysis etc. The traditional healer relies on nature and his own intuition to provide him with the clue to the aetiology of the disease. Some types of traditional healers rely on divine or spiritual powers, natural senses mainly sight, touch and taste. Sometimes astrology and dream analysis may play a part in diagnosis. Due to these major differences in approach, the traditional healer is likely to confuse disease conditions especially those that present with similar symptoms. Hence the treatment could easily be the wrong one.

4. Lack of aetiological and pathophysiological understanding of diseases may lead a traditional healer to treat symptoms of disease rather than the disease itself. This implies that it is not easy to quantify the results of herbal treatment in terms of total cure eg a herbal remedy claimed to have antimalarial effect may only be acting as an antipyretic. Even though this is a beneficial effect it cannot be regarded as a cure, but due to his limited knowledge the herbalist equates the relief of symptoms to disease cure.

5. In many African countries there are no professional ethics, or laws governing the practice of traditional medicine, and this makes the traditional healer non-accountable or not answerable in case of death or

damage suffered during the course of treatment. Moreover, traditional healing usually has so many taboos, a patients is expected to observe or follow, that in case of death or damage, the healer is usually able to put the blame on the patient breaking one taboo or the other.

6. Most herbal remedies lack standard formulation and for this reason the dosage varies from herbalist to herbalist and even from batch to batch decocted by the same herbalist.

7. The hygiene standard involved in the herbal medicine preparations are on the whole very low.There are many reasons for this. Herbalists usually have no general education or sometimes have managed only the basic minimum primary education. The surroundings are also not conducive to proper hygiene eg lack of portable water, poor sanitation, uneducated community members etc.

8. On the whole there has been lack of acceptable scientific and rational analysis of data obtained from traditional medicine. It is only in recent times that scientific evidence is accumulating all over the world in support of some of the claims of traditional medicine. In spite of all these shortcomings of traditional medicine the glaring facts cannot be ignored that:-

(a) There is gross shortage of orthodox medical

manpower to deliver health care country-wide in many developing countries and particularly to rural areas where some places are inaccessible.

(b) There is great economical constraint to orthodox medical manpower training especially in the poor developing countries.

(c) Orthodox medicine has no absolute cure for all ailments including hypertension, broken bones, diabetes, complicated maternity cases, sickle cell anaemia, impotence, arthritis, asthma, the new scourge, acquired immune deficiency syndrome (AIDS), psychiatric disorders, infertility etc. The traditional healers have claimed and at times demonstrated clearly that they can handle many of these conditions better. For this reason there is an increase in the numbers of the population that are seeking traditional medical care. At times the urban health consumers commute freely between homes of herbalists and offices of orthodox doctors.

(d) In rural areas the herbalist has an added advantage over orthodox doctors, in that having grown up within his community, the herbalist has a closer relationship with his patient, giving him both physical and psychological satisfaction and confidence as opposed to the detached attitude of orthodox doctors.

(e) There is a rapidly growing interest and curiosity

amongst scientists to confirm claims that traditional medicine provides a cure to some diseases or disease conditions that had defeated modern medicine. These claims usually come either from the beneficiary (patient) or from the records of the herbalist sometimes supported by documentary evidence from modern hospitals that the patient was originally considered beyond medical help but is now scientifically confirmed "cured".

(f) About 25% of the world's pharmaceutical products used in orthodox medicine have their origin from plants and other natural sources (Elliot and Brimacombe, 1986).

Some few typical examples include:-

Antimalarials like	-quinine, cinchonine
Skeletal muscle relaxants	-d-tubocurarine
Local anaesthetics	-cocaine
Antihypertensives	-reserpine, forscolin
Analgesics	-morphine, codeine
Anticancer agents	-Vinca alkaloids (vincristine vinblastine)
Cardiac drugs	-Digoxin, Digitoxin
Laxatives	-Anthraquinones
Antidiabetic	-Insulin
Sex hormones	-Testosterone

(g) Plants have also provided raw materials for partial synthesis of orthodox drugs. The best examples

are the steroidal saponins that are used as the raw materials for synthesis of steroidal drugs that form a multimillion dollar industry. Diosgenin from Dioscorea species is used in the synthesis of oral contraceptives that have formed the basis for population growth control.

(h) Plant products can be used as molecular models or provide 'lead' compounds that are used as 'blueprints' in the chemical synthesis of wide range of drugs. For example the first naturally occuring local anaesthetic, cocaine, was found to be very potent, but due to its wide systemic effects, eg induction of mental and physical dependence and limitations on its routes of administration its use has declined. However it acted as a model or basis for manufacture of many synthetic local anaesthetics. Another stricking example is salicylic acid which has been utilised as a 'blueprint' for the synthesis of a wide range of non-steroidal anti-inflammatory analgesics such as aspirin.

In consideration of the shortcomings of both traditional medicine and orthodox medicine, it is now recognised that the two health care systems are complimentary.

The controversy on whether to integrate traditional medical care or some aspects of it with orthodox medicine is slowly coming to an end. The main question

now is how and when the integration can be effected? To achieve this integration traditional medical practice should be uplifted in terms of hygiene, standardisation of dosage etc. The World Health Organisation having recognised the important community health role of the traditional healers including traditional birth attendants, bone setters and acupuncturists has embarked on a major campaign to:-

(a) Promote the population awareness of traditional medicine

(b) Encourage scientific research into herbal medicine

(c) Train and educate these traditional community health workers to improve their health services.

1.3 ANTIMALARIALS FROM NATURAL ORIGIN

Cinchona (Family Rubiaceae) is native to the South America Andes (mainly Equador and Peru). It was introduced as a crop in Java (Indonesia) by the Dutch in 1854 and in India by the British in 1860. By 1900 the South American production was nil and the Dutch in Netherlands and East Indies took monopoly. Just before World War II Indonesia used to supply 90% of world demand of Cinchona (Evans, 1989).

There are 36 species of Cinchona mainly obtained from cross-breeding of three main species, Cinchona succirubra (Pavlon et Klotzsch) or red Cinchona, Cinchona ledgerian (Howard, moens et Trimen) named after Charles Ledger who introduced Cinchona into East

Indies and Cinchona calisaya (Weddell) commercially known as Calisaya Bark or yellow cinchona. The hybrids of Cinchona ledgeriana and Cinchona calisaya produce the highest yield of active constituents (Evans, 1989).

The first written record of the use of Cinchona bark (also known as Peruvian, Jesuits or Cardinal's Bark) is in a religious book written in 1633 and published in Spain in 1639. It was authored by Calancha of Lima (Peru) who was an Augustinian Monk (Evans, 1989).

By 1640 Cinchona was being employed in management of fevers in Britain and Europe. It gained its first official recognition in 1643 when it was mentioned in medical literature by a Belgian, Herman van der Heydan. It was included in London pharmacopoeia in 1677 as "Cortex Pernanus".

For two centuries the Cinchona bark was used medicinally in form of powder, extract or infusion. In 1820 two alkaloids quinine (9) and cinchonine (21) were isolated by two French chemists Pierie Pelletier and Joseph Caventon (Black, 1986). It is now recognised that there are 25 closely related cinchona alkaloids, the percentage quantities varying with the Cinchona species. The most important alkaloids are quinine, quinidine (22), cinchonine and cinchonidine (23). The total alkaloidal content ranges from 6-7% of

crude plant material of which 0.5-0.67 is quinine in the yellow bark. Cinchonidine exists in greater amounts in the red bark (Evans, 1989).

The antimalarial effect of the Cinchona bark is related to the quinine content. Commercial laboratory or chemical synthesis of quinine has been tried but the synthetic method has been found to be very complex and expensive. Therefore the commercial source of quinine still remains the plant source (Evans, 1989). For this reason the Cinchona plant has been cultivated in commercial scale in many countries including Kenya (around Kericho area). However the technology and expertise of commercial extraction of quinine is lacking in Kenya and the bark is exported and quinine imported for medicinal use.

Quinine is the oldest antimalarial compound and is virtually important even today. The drug is rapidly absorbed from the upper part of the small intestine and even in cases of diarrhoea the absorption is always complete. Quinine is concentrated into the intra-erythrocytic form of the malaria parasite, where it forms a tight complex with haem and prevents it being sequestered as haemozoin (malaria pigment) which is an inert crystalline haemin/protein complex formed after the digestion of haemoglobin by the malaria parasites and causes denaturing of the parasite DNA (Goodman and Gilman, 1985)).

Quinine affects a variety of biological systems and has been referred to as a "General Protoplasmic Poison" (Bowman and Rand, 1980).

It affects sensory nerves inducing local anaesthesia lasting for hours to days. It exerts curare-like effects on skeletal muscles and can induce respiratory distress and dysphagia in a myasthenic patient. Quinine inhibits the vagal mediated gastric secretion and causes local irritation leading to gastric pain, nausea vomiting and sometimes diarrhoea. Its emetic effect is both locally and centrally mediated through its effect on the medulla. Local injections of quinine (subcutaneous or intramuscular) are painful and may cause sterile abscesses. Intravenous injection can cause thrombosis, hence occassional use of quinine solution as a sclerosing agent for varicose veins.

Centrally, quinine exerts mild analgesic and antipyretic effect which are beneficial in malaria fever but may not be useful in other forms of pyrexia.

Quinine causes release of insulin through B-cell stimulation (Henguin et al, 1975) and may induce severe hypoglycaemia when used parenterally to treat cerebral malaria or in pregnancy (White et al, 1983).

The clinical use of quinine is associated with side effects that may arise after clinical overdose or due to hypersensitivity. The chain of events clinically

referred to a "Cinchonism" are characterised by visual and auditory disturbances. There is increased visual acuity, blurred vision, photophobia, disturbed colour perception, constricted visual field, mydriasis, diplopia and night blindness. In severe cases optic atrophy may occur characterised by ischaemic and oedematous retina. The patient complains of ringing in the ears and vertigo (Martindale, 1989). The skin becomes hot, flushed and rashy with accompanying angioedema of the face (Martindale, 1989).

Central nervous system toxicity is only noted in very severe poisoning. Noticeable changes include headache, fever, excitement and apprehension, confusion, delirium and finally syncope. Respiration is initially stimulated and later depressed and shallow. Finally the skin becomes cold and cyanotic, the blood pressure falls, the pulse becomes feeble, coma and finally death results from respiratory paralysis (Bowman and Rand, 1980).

Recovery may be accompanied by varying degrees of residual optic and auditory damage. Renal damage with anuria and uremia may occur. Haemolysis, thrombocytopenia, haemoglobinaemia, agranulocytosis, haemoglobinuria, transient ventricular tachycardia and asthmatic like attacks have been reported (Martindale, 1989).

Inspite of these side effects quinine is still

useful, alone or in combination with tetracycline in management of malaria due to chloroquine resistant P. falciparum strains (Reacher et al, 1981).

1.3.2 ARTEMISIA

Another plant that has been exploited for antimalarial activity is the herb Artemisia annua (Compositae). This plant has been used for many centuries in Chinese folk medicine for many ailments. In the years 340 A.D and 1596 Ge Hong and Li shizhen respectively recommended its use to alleviate the chills of malaria. In 1972, Chinese chemists isolated a new compound from the plant. The compound was known as "Qinghaosin" meaning the active principle of qinghao, the Chinese name for Artemisia annua (China, Co-operative research group, 1982).

In 1979 the structure of qinghaosin was reported (Liu et al, 1979) and the compound now is commonly referred to as artemisinin (24) (Peters, 1980).

Artemisinin is a sequiterpene lactone with an endoperoxide group which is essential for antimalarial activity (Peters, 1980).

Some derivatives of artemisinin have been synthesised and studied for antimalarial activity. It is known that reduction of the keto group of artemisinin enhances in vitro activity (Li et al, 1983). Also ring cleavage causes loss of antimalarial activity.

Artemisinin has been used to cure more than 2000 malaria patients infected with P. vivax and P. falciparum (Klayman et al, 1984) and has been found to be effective in strains of malaria parasite resistant to all known antimalarials (Klayman et al, 1984; Li et al, 1983). It has a structure completely different from the existing antimalarials and its mode of action must be unique.

This compound has been shown to concentrate only in infected red blood cells (Gu et al, 1984) and interferes with the integrity of the parasite membrane. Probably the rigid chemical structure with the polar oxygen groups is ideal for sitting on the parasite membrane-lipid bilayer.

Animal experiments and also clinical trials have shown that artemisinin is effective against the erythrocytic parasites, with low toxicity. Though it has a marked effect on even the chloroquine resistant P. falciparum malaria the average recrudescence rate of malaria is about 10% which is higher than the treatment with chloroquine.

Synthetic analogues of artemisinin for example the methyl ether (artemether) (25) and the water soluble ester (sodium artesunate) (26) derived from the reduction product of artemisinin are both more active than the parent compound (Li, 1987).

The genus Artemisia consists of very many species.

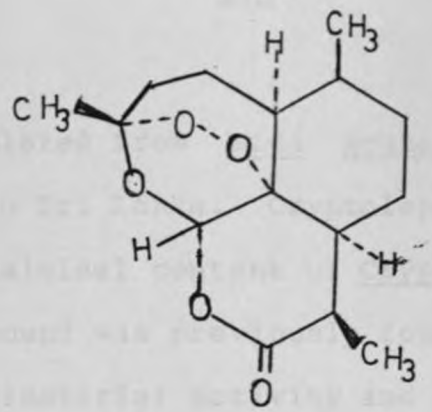
Although many have been screened for antimalarial activity, so far no other species of Artemisia has of now yielded artemisinin or any other antimalarial compound (Klayman et al, 1984). The research however continues. Laboratory chemical synthesis of artemisinin has been described (Schmidt and Hofheinz, 1983).

Artemisia afra found growing in South and East Africa including Kenya is cited as being used by Europeans, the Southern Sotho and the Zulu for malaria, other fevers and a wide range of diseases (Watt and Breyer-Brandwijk, 1962). However no other literature could be obtained to refute or confirm this folklore claim.

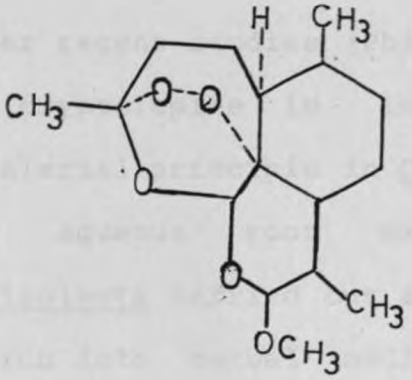
1.3.3 CRYPTOLEPIS

Cryptolepis sanguinolenta (Lindl Schr). (Family-Periplocaceae, Asclepiadaceae) is a plant found growing in West and Central Africa. The root from this plant has been used by Ghanaian traditional healers for many years to treat malaria, venereal diseases and wound infections (Bamgbose and Noamesi, 1981).

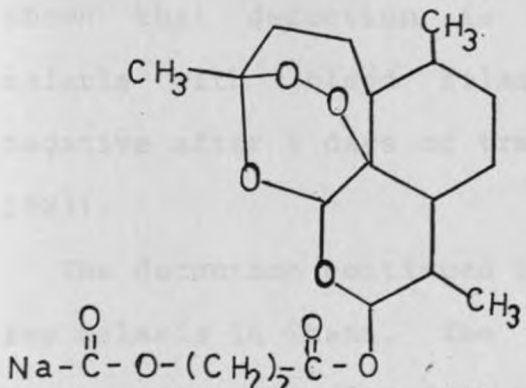
Cryptolepine (27) an indoquinoline was isolated from Cryptolepis triangularis by Cliquart (1929) and by Delvaux (1931). It was isolated from Cryptolepis sanguinolenta (Gellert et al, 1951) and also by Boakye-Yiadom and Dwuma-Bady, 1977. The compound has also



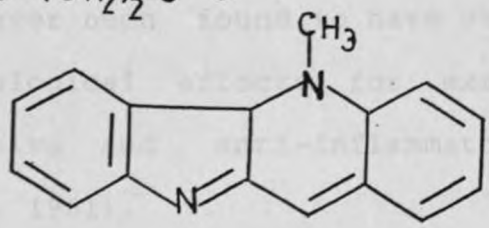
Artemisinin (24)



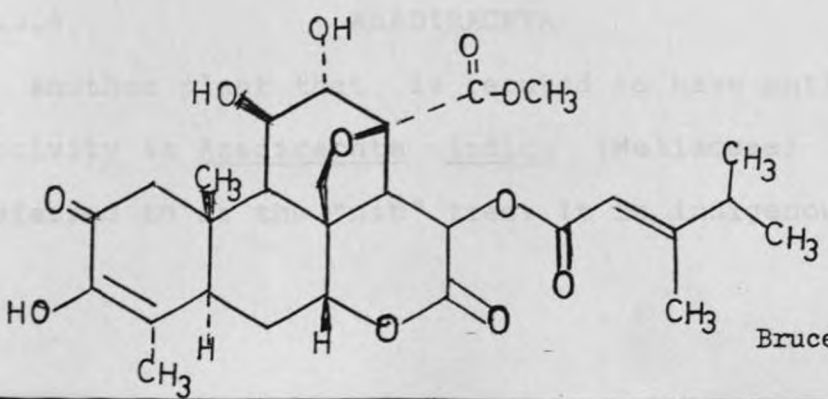
Artemether (25)



Sodium artesunate (26)



Cryptolepine (27)



Bruceantin (28)

been isolated from Sida acuta Burn F (Malvaceae) growing in Sri Lanka. Cryptolepine forms 90% of the total alkaloidal content of Cryptolepis sanguinolenta. The compound was previously found to be devoid of in vitro antimalarial activity and it was presumed that its antimalarial activity if any could be due to an in vivo metabolite, cryptolepine acting as a pro-drug. However recent studies (Phillipson, 1990) have shown that cryptolepine is in no doubt the active antimalarial principle in Cryptolepis. Clinical trials using aqueous root extracts of Cryptolepis sanguinolenta carried out at the centre for Scientific Research into herbal medicine (Ghana) in 1974 have shown that decoction is effective in treament of malaria with blood films being malaria parasite negative after 5 days of treatment (Boye and Ampofo, 1983).

The decoction continues to be a favourite remedy for malaria in Ghana. The compound cryptolepine (27) has however been found to have other potential useful pharmacological effects for example anti-pyretic, hypotensive and anti-inflammatory (Bamgbose and Noamesi, 1981).

1.3.4 AZADIRACHTA

Another plant that is reputed to have antimalarial activity is Azadirachta indica (Meliaceae) commonly referred to as the "nim" tree. It is indigenous to the

Indo-Pakistan subcontinent but has been introduced to some other countries eg Kenya, Ghana, Nigeria, Tanzania etc. The Kiswahili name for this plant is "Marubaine", meaning the plant with the ability to cure forty ailments.

Aqueous extracts of Azadirachta indica have been used in India for management of wounds, ulcers, skin diseases (including leprosy), rheumatic diseases, infective diseases such as hepatitis and syphilis and also feverish conditions including malaria (Nadkarni, 1954; Jayaweera, 1982; Chopra et al, 1956). There is a lot of controversy over the activity or otherwise of decoctions of this plant. For example when tested for in vitro antimalarial activity aqueous and alcoholic extracts of Azadirachta indica did not show any positive suppressive effects in Plasmodium berghei (mouse) and Plasmodium gallinaceum (chicks) (Odetola and Bassir, 1986; Spencer et al, 1947).

Some of the compounds isolated from the leaves, including Nimbin, Nimbidinin, Nombolidine and hydrocarbons were reported to be devoid of in vitro antimalarial activity (Odetola, 1975). On the contrary, Ekanem (1978) found that a leaf decoction of Azadirachta indica caused a decrease in parasitaemia in mice innoculated with chloroquine sensitive Plasmodium berghei. The growth inhibiting effect was half that of chloroquine (Ade-Serrano, 1982). Iwu et

al (1986) found that Azadirachta indica inhibits NADPH Cytochrome C (P450) reductase activity in rats, enhances oxidation of haemoglobin (Hb) to methaemoglobin (MetHb) and increases the metabolism of phenobarbitone presumably by inducing liver microsomal enzymes. By increasing the metabolic rate, the oxygen demand is increased above the normal and with time an oxygen debt sets in. The above authors proposed that Azadirachta indica, by its biochemical actions could impose oxidant stress on malaria parasites and hence inhibit their growth.

The difference in the results of Ekanem (1978) and National Institute for Medical Research and those of Odetola and Bassir (1986) could be due to differences in parasite strains, route of drug administration (parenteral as compared to oral). Other factors such as frequency and duration of treatment, diet and the presence of other infections may contribute.

1.3.5 MORINDA GENUS

Odetola and Bassir (1986) also noticed that extracts of Morinda lucida another plant claimed to have antimalarial activity, suppressed the growth of Plasmodium gallinaceum in chicks but not of Plasmodium berghei in mice. This indicates that there may be species differences in response to the antimalarial activity of the extracts. This observation is supported by Schneider (1954) who reported that

pamaquine and proquanil were relatively ineffective in Plasmodium berghei (mouse mode) and could have been missed as antimalarial.

1.3.6 BRUCEA

Brucea javanica (L) Merr (Simaroubacea) a plant widely distributed in South China is a Chinese herbal remedy for human cancer, amoebiasis and malaria (Lee et al,1987; O'Neill et al, 1987).The water extract and the powdered fruits are well known antimalarial drugs and clinically proven (Huo, 1953; Chiang su et al, 1962; O'Neill, 1987). The extract if taken in high doses is toxic, with headaches, nausea, vomiting and diarrhoea as the symptoms of toxicity. Its antimalarial activity is comparable to that of quinine (Lee et al, 1987).

A number of compounds mainly quassinoids have been isolated from B. javanica. These include bruceatin (28), bruceatinol, brucine and brusatol. Bruceatin has been found to exhibit potent antitumor (Cassady and Suffness, 1980), antimalarial (O'Neill et al, 1986; Gillin et al, 1982; Keen et al, 1986) and anti-parasitic activity against Entamoeba histolytica (Guru et al, 1983).

Due to high toxicity of quassinoids to mammalian cells (Fandeur et al, 1985) their use as antimalarial agents is limited.

Another plant worth mentioning as antimalarial is

Artabotrys hexapetalus (Family Annonaceae). This is a Chinese plant from which an antimalarial compound Yingzhaosu A (29) has been isolated (Bepp-Oliver, 1983). Artabotrys species occuring in Kenya are A. nitidus Engl. and A. stenopetalus Engl. These are found in Kakamega and Nandi forests and also the Shimba Hills in the Coast Province of Kenya. No folkloric information on their local use as antimalarials is available.

1.4 SCREENING FOR ANTIMALARIAL ACTIVITY IN PLANTS

1.4.1 Selecting Plants for Screening

There are a number of approaches used to select plants for screening for biological and pharmacological activity. Information obtained from documented literature on plant families that have been found to possess the required activity can be used. From this, the genus and species of the family found in the respective country can then be screened for the activity. Literature might even narrow the gap by providing the genus with the required activity and the problem is narrowed to look for the species available in the country. For example the antimalarial activity of Artemisia annua a Chinese herbal remedy for malaria is well documented. The active principle from the plant has been isolated, and characterised as artemisinin. The compound has been tested in vitro and

in vivo and found to possess powerful antimalarial activity against chloroquine sensitive and resistant strains and also multidrug resistant strains.

The available *Artemisia* species in Kenya is *Artemisia afra* which has not been previously studied for antimalarial activity. The information available on the Chinese species can therefore be used as a model or basis for similar investigations of the Kenyan species.

Another approach is to investigate the plants that are commonly used by the local traditional herbalists for the treatment of the disease condition under question. This will involve interviewing the local herbalists, at their herbal clinics or at a recognised research institution. Here a large number of herbalists need to be interviewed so as to obtain adequate data on the specific plants used, number of herbalists using them, combination therapy if any and the method of preparation of the herbs.

Another very expensive and tedious method is blind screening. This method is economically limiting, is time consuming and the success rate is very low. It is therefore rarely used except for non-specified pharmacological investigations.

Methods one and two above were applied in this research. Literature survey method yielded four plants namely *Artemisia afra*, *Schkuhria pinnata*, *Caesalpinia volkensii* and *Warburgia ugandensis* as plants which,

among others are used in Kenya for treatment of malaria and other fever conditions. In addition to Caesalpinia volkensii and Warburgia ugandensis, whose use as antimalarial drugs was also supported by folklore, information obtained from local herbalists yielded another plant Ajuga remota (Benth) commonly known as "Wanjiru Waweru" (Kikuyu), "Kirurite" (Kimeru). Therefore a total of five plants were selected for screening for antimalarial activity.

Artemisia afra was finally chosen for more detailed phytochemical and pharmacological work because W. ugandensis and A. remota were identified for further investigations by other researchers.

1.4.2 Scientific methods used to evaluate antimalarial activity

There are a number of scientific methods that are used to screen for new drugs with antimalarial activity and also to check for sensitivity of malaria parasite to the already existing antimalarial drugs and hence detect development of resistance. These methods can be used in clinical malaria where human beings are used as the subjects or in experimental malaria where animals or in vitro parasites are used. In both clinical and experimental malaria the methods can be broadly classified into in vivo and in vitro methods.

1.4.2.1 IN VIVO

Techniques used in experimental malaria may not have direct relevance to humans because they make use of non human malaria parasites eg Plasmodium lophurae, P. yoelli, P. knowlesi, P. chabaudi, P. berghei, P. gallinaceum, P. relictum etc and animals such as mice, and other rodents, birds etc. Some of the methods that have been employed include:-

(i) The Roehl Test

This makes use of canaries infected with P. relictum inoculum by intramuscular injection. A period of 4-5 days is allowed for parasitaemia to build up before introducing the test substance. This method was used in the assessment of antimalarial activity of pamaquine. The disadvantage of this method is that the inoculum (malaria parasite count) introduced into the animal is not standardised and this makes reproducibility of results very difficult or impossible.

(ii) Davey Test

This method tests for activity of a drug against blood forms of malaria parasites. A standard inoculum (50 million parasitised cells) are injected intravenously into chicks. After 4 hours of injection a dose of the test drug is given. Subsequent drug doses are given on each of the next three days.

Percentage parasitaemia is assessed from blood slides made every day for 3 days. The disadvantage of this method is that the test drug is given early in the infection (4 hours after inoculation) when the parasitaemia is low. The results obtained give an indication of the effect of the drug on the course of infection, but do not tell the action of the drug in overcoming heavy parasitaemia as found in acute clinical malarial attack.

The disadvantage can be overcome by giving the test drug when the parasitaemia is high (10%), but some strains of P falciparum especially the chloroquine resistant type multiply slowly and immunity may influence the progress of parasite growth, thereby making interpretation of results difficult

(iii) Rane in vivo screening system

This is a more popular and recent method that is used at the Walter Reed Army Institute for Research in U.S.A. It utilises mice injected intraperitoneally with a known concentration of inoculum. After 3 days the test drug is given subcutaneously for another 3 days.

Blood films are made on days 6, 13 and 20 and parasitaemia determined. Also the survival time of the treated mice as compared to the control mice is an indicator of antimalarial activity. Control mice die after 6 to 7 days and treated mice survive up to over

12 days if the test drug is effective.

The disadvantage here is that some species of Plasmodium eg P. berghei that infect mice are not easily affected by some potent antimalarial drugs eg proguanil and the antimalarial activity of such substances may be missed if only the mouse model is used.

1.4.2.2 IN VITRO METHODS

The main advantage of these methods is that they can be used in the laboratory or in the field. Examples of such methods include:-

1. Macro-test (Rieckmann, 1978)

10-12 ml of patients' venous blood is withdrawn and defibrinated: 1 ml aliquots of the blood are added to vials containing known concentrations of the test drug. Control vials are also set without the drug. The vials are incubated at 37-38.5 degrees C for 24-48 hours after which thick blood films are prepared, stained with Giemsa stain and parasitaemia determined and the growth rate in relation to the control assessed.

Micro-test

This is a modification of the macrotest (Rieckmann, 1978). This method offers the following advantages over the macro-test:-

1. Microvolumes of blood are required, therefore it

can be applied in children by collecting blood by finger prick instead of vene_puncture. The blood is collected in heparinised or ethylenediamine tetraacetic acid sodium salt treated capillary tube.

2. A number of different drugs can be tested from a single specimen of blood.

3. Microvolumes of drugs are required.

4.Test is performed on flat bottomed culture plates with wells, so a wider range of drug concentrations can be tested.

5. The period of incubation can be extended to 48 hours to allow young ring forms to develop to mature schizonts and also assess effect of drug on parasite re-invasion of erythrocytes (Yisunsrih and Rieckman, 1980).

The disadvantage of this method is that it cannot be used to investigate the antimalarial activity of compounds that interfere with folic acid and folate synthesis and metabolism due to the high levels of para-amino benzoic acid and folic acid in the RPM1 1640 (Rowell Park Memorial Institute; hence the prefix RPM1) medium.

Visual Micro test (Rieckmann, 1982)

This method employs visual detection of a pigment that is formed when ring forms mature to schizonts. Presence and intensity of the pigment indicates the severity of infection. The absence of the pigment

implies absence of infection, or inhibition of ring from maturation to schizonts by the test drug. Advantage of this method is that it can be used in the field without the need for a microscope and is handy in those areas with limited diagnostic facilities.

Semi-automated microdilution technique (Desjardin's, 1979 Method).

This involves testing the activity of a drug by measuring the incorporation of a radioactive precursor into parasite molecules eg inhibition of uptake by the parasitised cells of a radio labelled nucleic acid precursor ^{3}H-hypoxanthine. The uptake of ^{3}H-hypoxanthine is measured using a scintillation spectrophotometer after a period of incubation of the parasitised cells with the drug and ^{3}H-hypoxanthine. Dose response data gives a quantitative measurement of the antimalarial activity.

For dihydrofolate reductase inhibitors and the para-amino benzoic acid analogues, the malaria parasites are cultured in medium containing neither folic acid nor para-amino benzoic acid. However the antimalarial test procedures are just as for the other antimalarials.

1.5 PHYTOCHEMISTRY AND PHARMACOLOGY OF THE SELECTED PLANT GENUSES

1.5.1 Phytochemistry of the genus Ajuga

This genus belongs to the Labiatae family. A number of chemical compounds have been isolated from it including steroidal lactones such as ajuga lactone (30) from Ajuga decumbens (Evans, 1989) diterpenoids, ajugapitin (31) and its 14, 15 dihydroderivatives from Ajuga chamaepitys (Amparo et al, 1982). The aerial parts of Ajuga ciliata has yielded a number of neo-clerodane diterpenes namely Ajugamarin B4, B5, ajumarin E1, E2, E3, F1F2 and F3 (Shimomura et al , 1989a).

Ajumarins A2,G1,H1,F4, and B2 have also been isolated from Ajuga decumbens (Shimomura et al, 1989b) while the same researchers have obtained 4-new bitter neo-clerodanes ajumarin C1, B2,B3,D, and an ajugarin I from Ajuga nipponensis (Shimomura et al, 1989c).Other substances isolated from the genus Ajuga, are triterpene alcohols eg alpha and beta amyrin (32) from Ajuga decumbens (Evans, 1989) and iridoid glycosides (harpagide, 6-deoxyharpagide and 8-O-acetyl harpagide) from Ajuga iva (L) (Assaad and Lahloub, 1988).

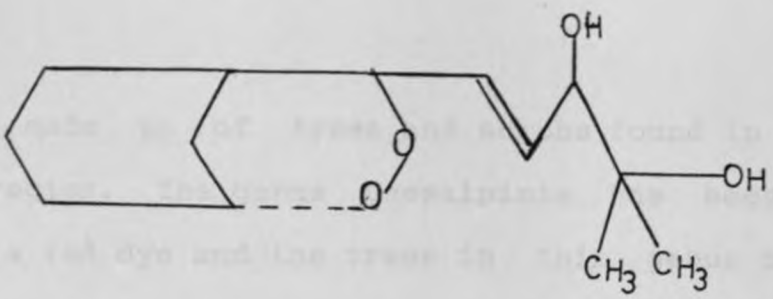
The genus Ajuga is known to have very bitter taste probably attributed to the ajumarins and ajugamarins present in the genus (Shimomura et al, 1989c). No wonder herbalists equate it to the bitter alkaloid

quinine obtained from Cinchona tree. Based on the use of quinine in the treatment of malaria the Kenyan herbalists have embarked on the use of the Kenyan species of Ajuga (Ajuga remota Benth) in the treatment of malaria and claims of its efficacy are common. However scientific investigations need to be carried out to ascertain these claims.

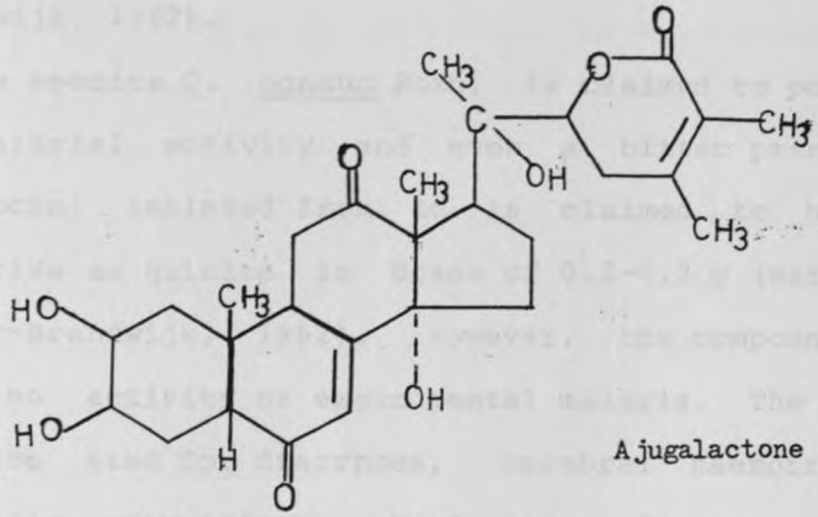
Aqueous extract of Ajuga ophrydis Burch has been used by the Sotho of Southern Africa as remedy for sterility, skin rashes and also to relieve painful mensturation (Watt and Breyer-Brandwijk, 1962). Ajuga remota Benth has also been shown to possess cardiostimulant and diuretic properties and has been claimed to be useful in management of congestive cardiac failure (Kuria and Muriuki, 1984). An aglycone of cardenolide type was isolated from this plant (Kuria and Muriuki, 1984). The plant has also been used as remedy for fever, toothache, dysentery and high blood pressure (Kokwaro, 1976). Ajugalactone (30) isolated from Ajuga decumbens has been shown to inhibit insect metamorphosis (Evans, 1989) and could possibly be used as a vector control agent.

1.5.2 Phytochemistry and Pharmacology of the genus Caesalpinia

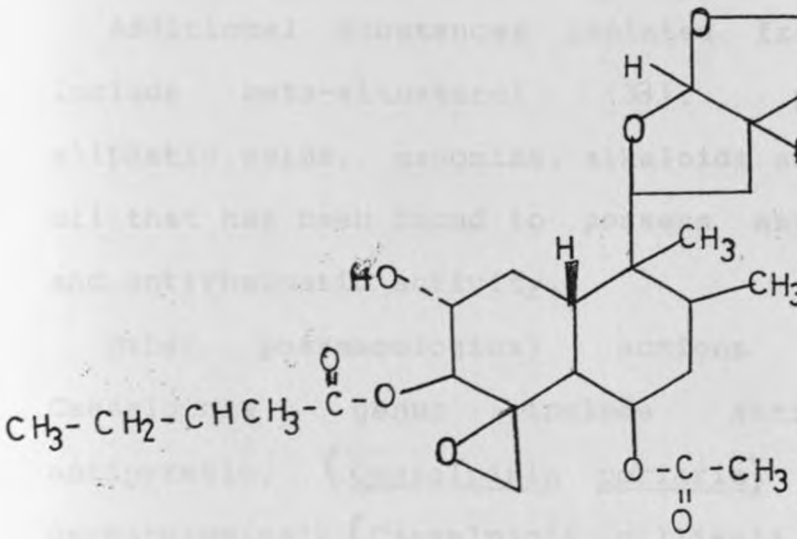
This genus belongs to the angiosperms, subclass archichlamydeae, order Rosales, suborder Leguminosae, Family Caesapinoideae. The family consists of at least 6 species made up of trees and shrubs found in the



Yingzhaosu (29)



Ajugalactone (30)



Ajugapitin (31)

6 species made up of trees and shrubs found in the tropical region. The genus Caesalpinia has been a source of a red dye and the trees in this genus have been referred to as redwood (Watt and Breyer-Brandwijk, 1962).

One species C. bonduc Roxb, is claimed to possess antimalarial activity and even a bitter principle (bonducin) isolated from it is claimed to be as effective as quinine in doses of 0.1-0.2 g (Watt and Breyer-Brandwijk, 1962). However, the compound has shown no activity on experimental malaria. The plant is also used for diarrhoea, cerebral haemorrhage, infantile convulsions, headaches, fever, stomach discomfort, and as an emetic and antihelmintic.

Additional substances isolated from C. bonduc include beta-sitosterol (33), phytosterolins, aliphatic acids, saponins, alkaloids and an essential oil that has been found to possess anti-inflammatory and antirheumatic activity.

Other pharmacological actions atributed to Caesalpinia genus include anti-haemorrhoids, antipyretic, (Ceasalpinia coriaria) arbotifacient, dermatological, (Caesalpinia gilliesii, Caesalpinia swart, Caesalpinia sappan). Caesalpinia volkensii also reputed to be a source of red dye (Watt and Brandwijk, 1962) is commonly used in Kenya as an antimalarial drug by the herbalists.

1.5.3 Phytochemistry and Pharmacology of the genus Schkuhria

Schkuhria is a member of the compositae family. Some members of this genus eg Schkuhria pinnata have been used as aqueous decoctions in management of malaria, influenza and for colds (Watt and Breyer-Brandwijk, 1962).

Schkuhria species has yielded germacranolides (11,13-dehydroeriolin), melampolides (Schkuhrioidin, Schkuhriolide). Similar compounds have been isolated from S. pinnata (Romo de vivar et al, 1982).

The full pharmacological potential of Schkuhria species has not been assessed especially Schkuhria pinnata, the only species found in Kenya. Though the plant is used to relieve gastrointestinal disturbances such as colic and constipation (Kokwaro, 1976) and as antimalarial no scientific literature exists citing either the constituents responsible for these activities or the mechanism of action of even crude plant extract.

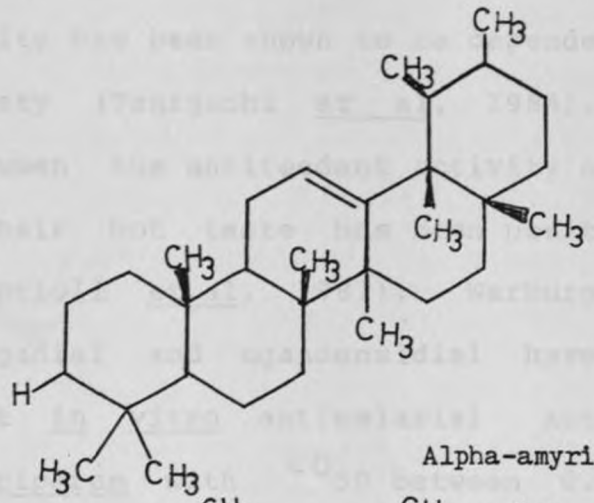
1.5.4 Phytochemistry and Pharmacology of the genus Warburgia

The genus Warburgia belongs to Canellaceae family. A few of its members are used as herbal remedies. W. stuhlmannii is used as remedy for toothache by the people of Tanzania while W. ugandensis Sprague commonly known as the "Fever tree" is used in East

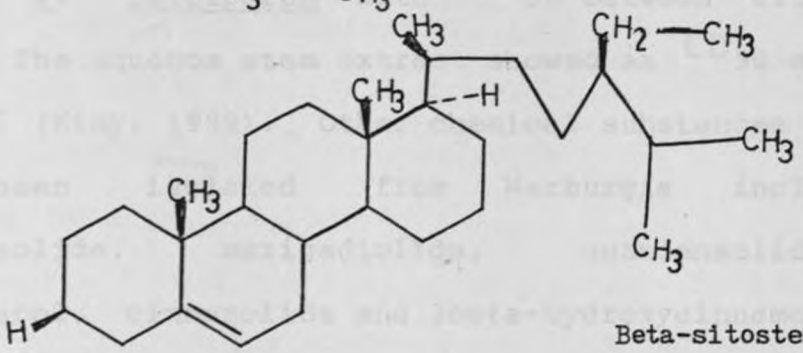
Africa as a purgative, expectorant, for chest complaints (Watt and Brayer-Brandwijk, 1962) and as malaria remedy (Watt and Brayer-Brandwijk, 1962; Kokwaro, 1976). Most of these activities are mainly attributed to folklore and most of them have not been scientifically established.

Various chemical constituents have been isolated from W. ugandensis including tannins, mannitol, essential oil (Watt and Breyer-Brandwijk, 1962; Kioy, 1989), mukaadial (34), warburganal (35) and ugandensidial (36) (Kubo et al, 1983; Kioy, 1989). Similar substances have been isolated from W. stuhlmannii (Kubo et al, 1983; Kioy, 1989). Mukaadial has shown in vitro molluscicidal activity (Kubo et al, 1983; Kioy, 1989) and also trypanosomicidal activity (Kioy, 1989). However no in vivo molluscicidal or trypanosomicidal activity was demonstrated at the doses tested (Kioy, 1989).

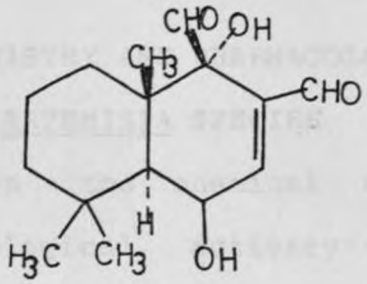
An antifungal potentiating agent polygodial (37) was isolated from Warburgia ugandensis and Warburgia stuhlmannii (Kubo and Tanigushi, 1988; Kioy, 1989). This substance was effective against Saccharomyces cererisiae Candida utilis and Sclerotinia libertiana. It was also found to enhance the fungicidal activity of actinomycin by enhancing its transmembrane permeability (Kubo and Tanigushi, 1988). Warburganol, mukaadial and muzigadial have also been shown to



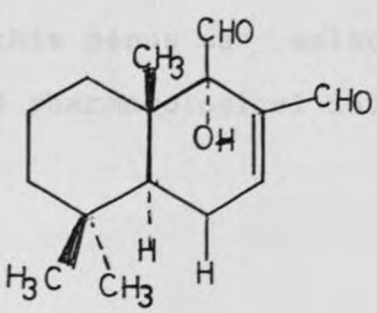
Alpha-amyrin (32)



Beta-sitosterol (33)



Mukaadial (34)



Warburganol (35)

possess potent insect antifeedant properties especially against the African army worms (Kubo et al, 1977). These dialdehyde sesquiterpenes were therefore patented protecting the general structure. The antifeedant activity has been shown to be dependent on the -CH=C-CHO moiety (Taniguchi et al, 1984). The relationship between the antifeedant activity of the aldehydes and their hot taste has been positively demonstrated (Caprioli et al, 1987). Warburganal, mukaadial, muzigadial and ugandensidial have been shown to exhibit in vitro antimalarial activity against P. falciparum with LD_{50} between 0.5-0.8 µg/ml. The aqueous stem extract showed an LD_{50} of 8-31 µg/ml (Kioy, 1989). Other chemical substances that have been isolated from Warburgia include, ugandensolide, muzigadiolide, ugandensolidiol, daucosterol, cinnamolide and 3beta-hydroxycinnamolide (Kioy, 1989).

1.5.5 PHYTOCHEMISTRY AND PHARMACOLOGY OF ARTEMISIA SPECIES

In this section the chemical composition and biological/pharmacological activity of various Artemisia species is reviewed in detail since A. afra, is a member of this genus was selected for further phytochemical and pharmacological investigations.

1.5.5.1 Phytochemistry

Of the various Artemisia species reported in literature, A. annua has aroused the greatest interest due to the antimalarial activity of its constituents. Artemisinin (24) a sesquiterpene lactone with an endoperoxide group was the first natural antimalarial isolated from A. annua of Chinese origin (Fourth Meeting of Scient. Working Group on Chemotherapy of malaria, 1981; Klayman et al, 1984). Cultivation of A. annua has been found to increase the yield of artemisinin with the highest content occuring at the beginning of budding (Shreter et al, 1988).

Not only has synthesis of artemisinin been achieved but its production in tissue cultures has also been effected (Nair et al, 1986).

In addition to artemisinin other compounds isolated from A. annua include Artemisitene (38) (Acton et al, 1985), Artemisic acid (39), beta-sitosterol , stigmasterol, 3,5-dihydroxy-6,7,3,4-tetramethoxyflavone (Tu et al, 1985), 5,4-dihydroxy-3,6,7,3-tetramethoxyflavone (Bhardwaj et al, 1985), Arteanuin B (40) (Misra, 1986; Elmerakby et al, 1987; Jeremic et al, 1973). Arteanuin B is an epoxy-methylene butyrolactone of the cadinane type of sesquiterpenes (Jeremic et al, 1973). Its microbial transformation studies have yielded products such as dihydroarteannuin B, 3B-hydroxy arteannuin B, and 13-

hydroxy 11-epi-dihydro-arteannuin B whose structures have been described (Elmarakby et al, 1987).

Arteannuin C (41) which is an isomer of Arteannuin B has been isolated from A. annua introduced in India from China (Misra, 1986).

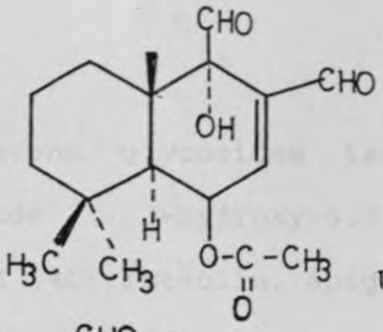
Other artemisinin derivatives include arte-ether (42) synthesised from dihydroartemisinin, artesunate (26) artemether (25) (Brossi et al, 1988).

Epoxy arteannunic acid has been isolated from A. annua and has also been synthesised by epoxidation of arteannuinic acid (Wu and Wang, 1984). Coumarins and methoxylated flavones have also been isolated from A. annua (Shilin et al, 1989). These include quercetagetin-3-Me-ether,2,4,5-trihydroxy-5,6,7-trimethoxy flavone, and 3,5,7,8-tetrahydroxy-3,4-dimethoxy flavone (Shilin et al, 1989).

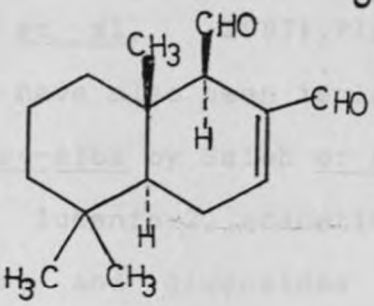
Apart from A. annua many other species of Artemisia have previously been worked on. The chemical composition of the isolated constituents will be discussed under broad chemical groups, namely 1. Flavones and flavonoids 2. coumarins 3. lactones 4. guaianolides.

(a) Flavones and flavonoids

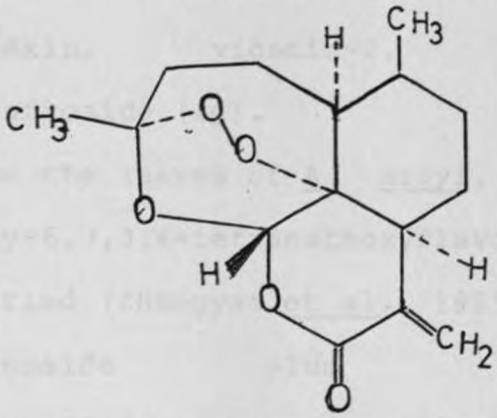
These groups of compounds share the basic chemical structure (43). While working on Artemisia assoana, Martinez et al (1987) isolated nine flavones and two flavone glycosides among other compounds. Examples of



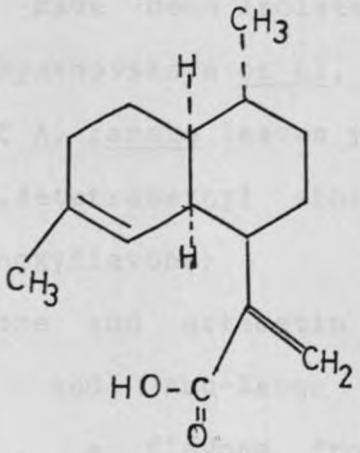
Ugandensidial (36)



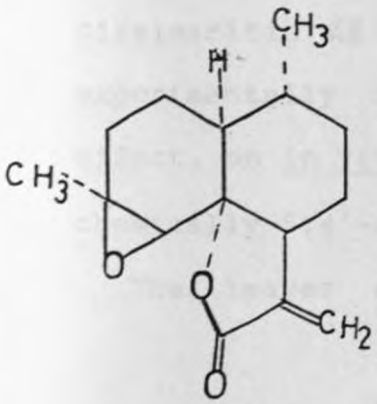
Polygodial (37)



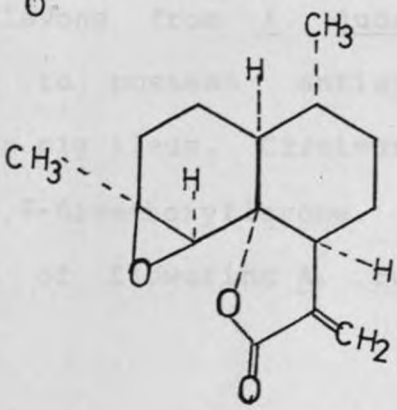
Artemisitine (38)



Artemisic acid (39)



Arteanuin B (40)



Arteanuin C (41)

flavone and flavone glycosides isolated from A. assoana include 3-hydroxy-6,7,3,4-tetramethoxy flavone, apigenin (44) luteolin, apigenin 7-rutinoside (Martinez et al, 1987).Flavonoid and flavonoid glycosides have also been isolated from A. monosperma and A. herba-alba by Saleh et al (1985). These include vicenin-2, lucenin-2, acacetin-7-glucoside, acacetin-7-rutinoside and glucosides and rutinosides of quercetin, patuletin, glucosides of isorhamnetin, isovitexin, vicenin-2, schaftoside (45), isoschaftoside (46).

From the leaves of A. argyi, eupatilin (47) and 5-hydroxy-6,7,3,4-tetramethoxyflavone have been identified (Zhongyao et al, 1985) and isorhamnetin 3-O-rutinoside plus 5,7,4-trihydroxy-6,3-dimethoxyflavone have been isolated from Artemisia sublessingiana (Ryakhovskaya et al, 1985).

Examination of A. lanata leaves yielded, gossypetin which is 7,8,3,4-tetramethyl ether, 3,5-dihydroxy-7,8,3,4-tetramethoxyflavone; 5-hydroxy-6,7,3,4-tetramethoxyflavone and artemetin (Esteban et al, 1986). Abdalla and Abu-Zarga (1987) isolated cirsimaritin (48), a flavone from A. judaica and experimentally found it to possess antispasmodic effect on in vitro guinea pig ileum. Cirsimaritin is chemically 5,4'-dihydro-6,7-dimethoxyflavone.

The leaves and stem of flowering A. rutifolia

contain chrystosplenetin , cirsilineol , tricin 49) and chrysoeriol as identified by infra red (IR), ultra violet (UV) and 1-H-NMR spectra (Chemesova et al, 1984).

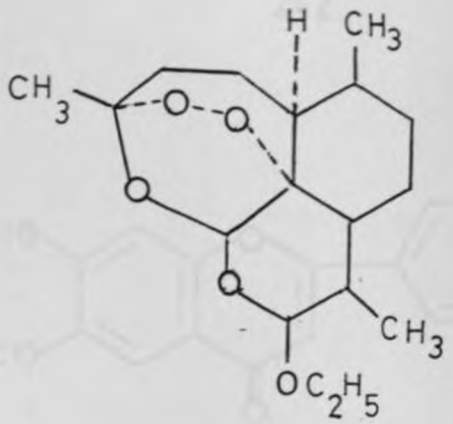
Known flavones cirsilineol, cirsimaritin rhamnocitrin plus a new flavone 8,2,4-trihydroxy-6,7,5-trimethoxyflavone have been isolated from A. capillaris (Namba et al, 1983).

Many more species of Artemisia eg A. campestris (Rauter et al, 1989), A. cina, A. scoparia, A. sublessingiana (Ryahovskaya et al, 1989), A. incanescans (Barbera et al, 1986) have been shown to contain flavones and flavonoids.

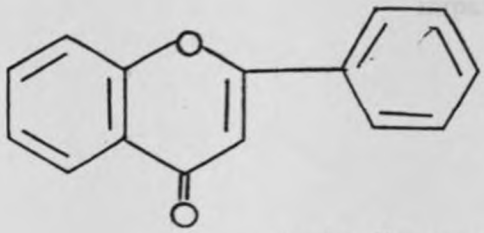
The medicinal value of the plants discussed above and many more that have been shown to contain flavones and flavonoids could possibly be attributed to these chemical constituents (Evans, 1989).

(b) Sesquiterpene Lactones

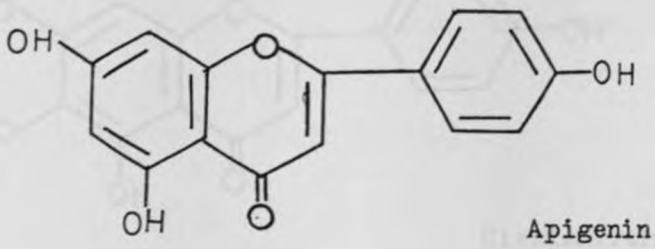
Over 500 compounds of this group are known. They are particularly characteristic of the Compositae but also occur sporadically in other families. The substances do not only have chemical and chemotaxonomic importance, but possess significnt medical properties eg antitumor, antimalarial, vasoactive, cytotoxic, antimicrobial activities plus insect feeding deterrent effects. Chemically the compounds can be classified according to their



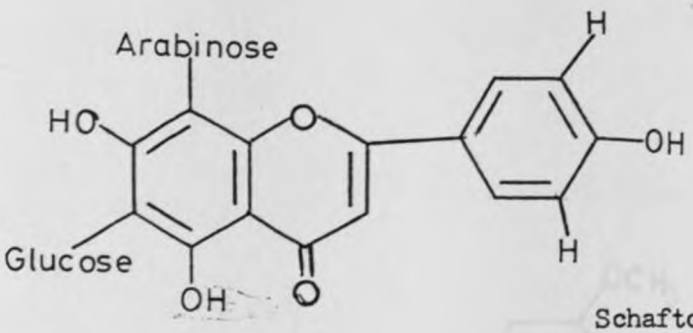
Artether (42)



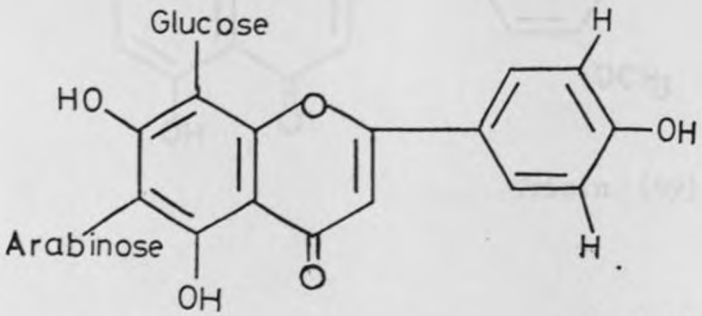
Basic flavone structure (43)



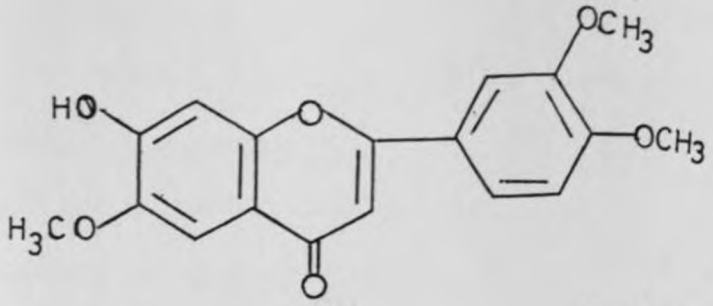
Apigenin (44)



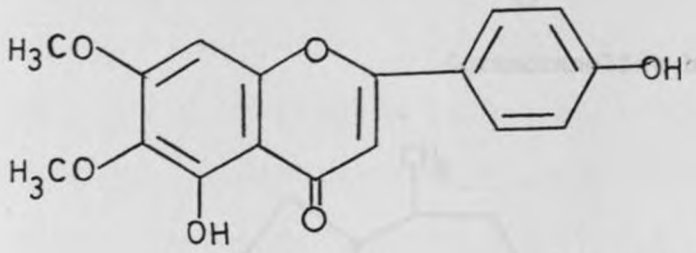
Schaftoside (45)



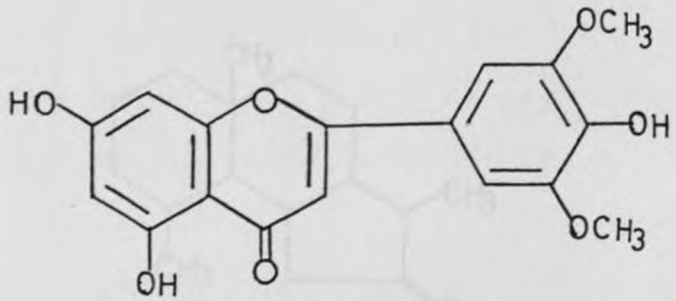
Isoschaftoside (46)



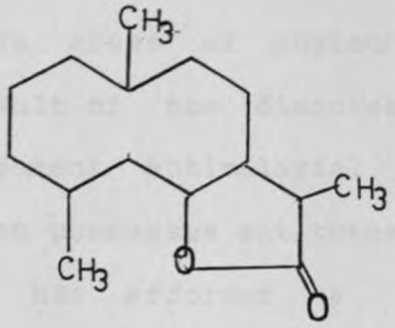
Eupatilin (47)



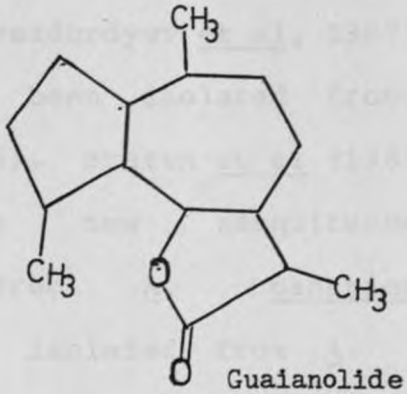
Cirsimaritin (48)



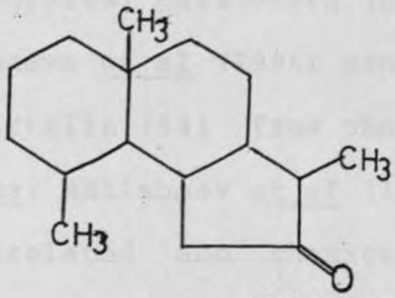
Tricin (49)



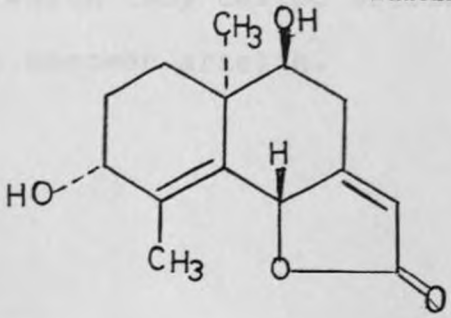
Germacranolide basic structure (50)



Guaianolide basic structure (51)



Eudesmanolide basic structure (52)

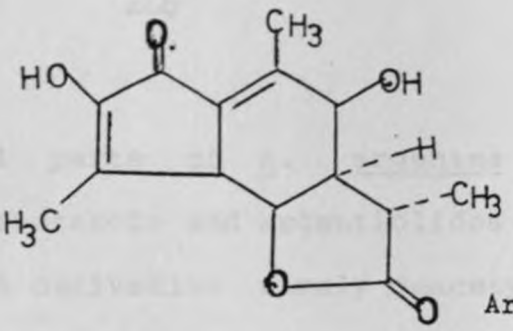


Herbolide E (53)

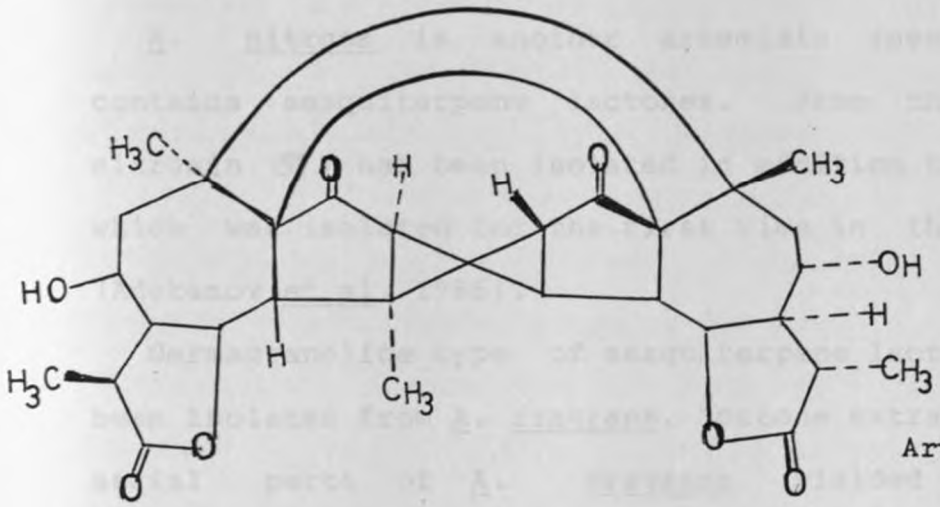
caboxylic skeletons. The germacranolides (55) give rise to guaianolides (56), pseudoguaianolides and eudesmanolides (52). Of course there are unusual sesquiterpene lactones like artemisinin which possesses an endoperoxide moiety.

Currently sesquiterpene lactones constitute one of the most active areas of phytochemical research probably as a result of the discovery of artemisinin which is a potent antimalarial agent and also arteannuin B which possesses antitumor activity. The genus Artemisia has afforded a wide range of sesquiterpene lactones. A. absinthium has yielded parishin B and C whereas A. argyi afforded matricarin and hanphyllin (Ovezdurdyev et al, 1987). Herbolides E (53) and F have been isolated from A. herba-alba (Segal et al, 1984). Breton et al (1985) isolated and characterised a new sesquiterpene lactone, heliangolidin from A. canariensis leaves. Arteanomalactone isolated from A. anomala was characterised as a sesquiterpene lactone using both chemical and physical parameters (Lin et al, 1985).

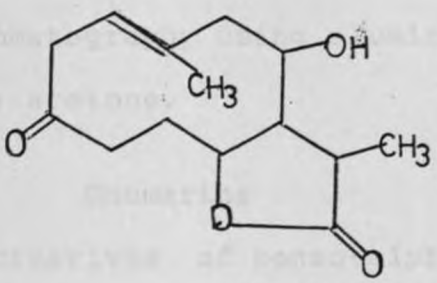
While Saitbaeva et al (1986) managed to isolate and characterise artelin (54) from the leaves and flowers of A. leucodes, Mallabaev et al (1986) working on the same plant isolated and characterised a dimeric lactone which they called artelein (55) a name derived from the monomer artelin.



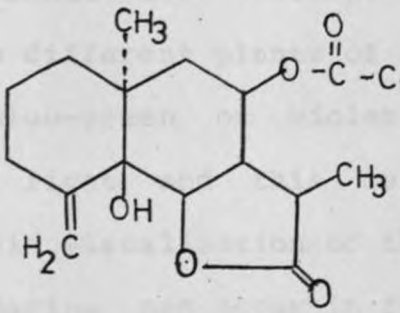
Artelin (54)



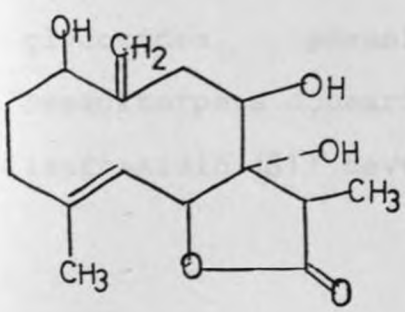
Artelein (55)



Deacetyl argentiolide B (56)



Nitrosin (57)



Shonachalin A (58)

The aerial parts of A. argentea afforded in addition to arborescin and argentiolides A and B a new ketopelenolide derivative namely deacetylargentiolide B (56) (El-Emary et al, 1986).

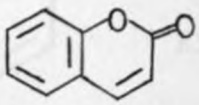
A. nitrosa is another artemisia species that contains sesquiterpene lactones. From this plant nitrosin (57) has been isolated in addition to artemin which was isolated for the first time in this plant (Adekenov et al, 1986).

Germacranolide type of sesquiterpene lactones have been isolated from A. fragrans. Acetone extract of the aerial parts of A. fragrans yielded a new germacranolide Shonachalin A (58) (Serkerov and Aleskerova, 1985) whereas Shonachalin C was obtained after column chromatography using aluminium oxide and 10:1 chloroform : acetone.

(c) Coumarins

These are derivatives of benzo-alpha-pyrone (59). All together about 1000 natural coumarins have been isolated from different plants of varying genera. They give blue, blue-green or violet fluorescence under ultra-violet light and this can be used for the chromatographic visualisation of the compounds (Evans, 1989). Coumarins can occur in free state, or as glycosides, phenolics or methoxy derivatives. Sesquiterpene coumarin ethers of scopoletin (60) or isofraxidin (61) have been identified in nature.

Coumarins have the basic structure:

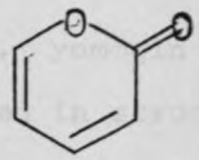


They include compounds such as scopoletin isofraxidin and umbelliferone (62) which are very common in the Compositae especially in the genus Artemisia (Heywood et al, 1977; Hegnauer, 1977; Esculin and aesculetin from A. vulgaris (Ikhsanova et al, 1986) are among other simple coumarins identified in the genus Artemisia.

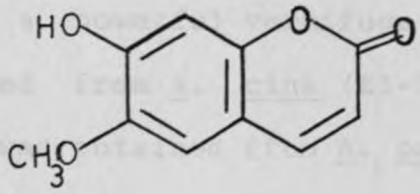
Others include scoparone (63) from A. capillaris (Yamahara et al, 1989), 7-methoxycoumarin , 7-isopentenyloxy-8-methoxycoumarin from A. apiacea (Wu and Tu, 1985), 5-dimethylallyloxy-7,8-dimethoxycoumarin derivatives (neoartanins), 5,6-dimethoxy-7,8-methylene dioxycoumarin, 7-dimethylallyoxy-8-methoxycoumarin (lacinartins) from A. lacinata (Hofer et al, 1986), 6-methoxy-7-methylene dioxycoumarin from A. dracunculoides and A. vulgaris (Murray and Stefanovic, 1986).

Naturally occuring sesquiterpene-coumarin ethers have also been shown to occur in Artemisia persica (Hoger and Greger, 1984). These include substances such as scopofarnol (6-methoxy-7-(3,7,11-trimethyl dodeca-2,6,10-trienoxy)-2H-1-benzopyran-2-one (64) and scopodrimol A (65) (Serkerov and Aleskerova, 1985b).

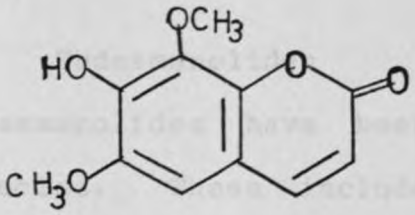
Artemisia montana has afforded an arteannuin type sesquiterpene lactone, yamayomoginin (66) in addition



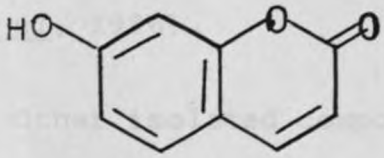
Alpha-pyrone (59)



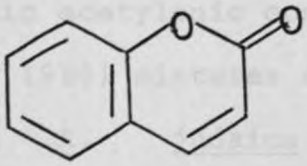
Scopoletin (60)



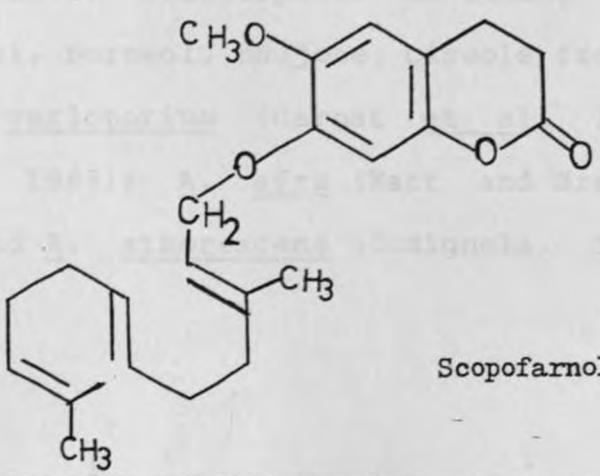
Isofraxidin (61)



Umbelliferone (62)



Scoparone (63)



Scopofarnol (64)

to other lactones, yomogin and yomogiartemin whereas feddeinin, related in structure to yamayomogimin has been isolated from A. fedder (Matsueda and Nagaki, 1984).

Santonin (67) a powerful vermifuge (Evans, 1989) has been isolated from A. cina (El-Sayed, 1988). Artepauline (68) was obtained from A. panciflora (Ade-Kenov et al, 1983).

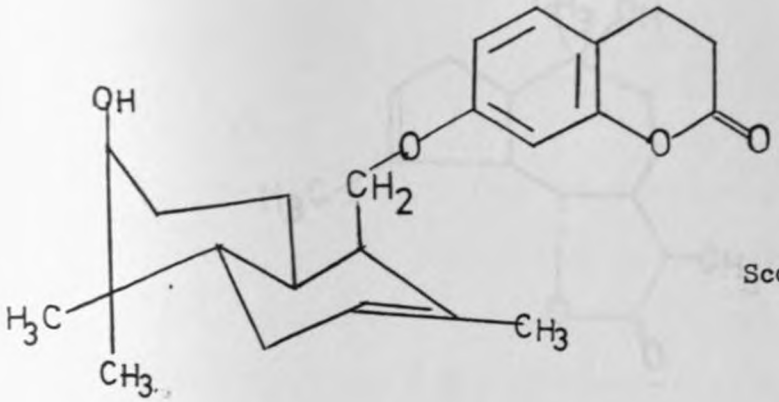
(f) Eudesmanolides

A few eudesmanolides have been identified in Artemisia species. These include hydroperoxides related to vulgarin isolated from A. judaica (Metwally et al, 1985) and also from A. umbelliformis (Cappelleti et al, 1986).

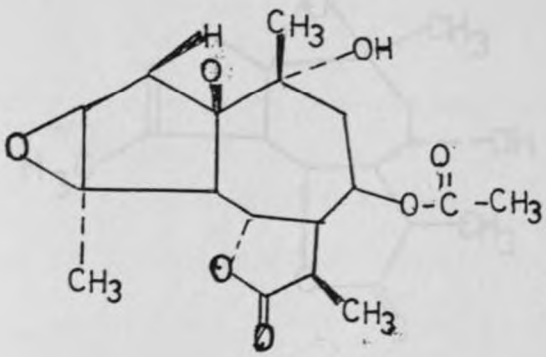
(g) Other isolated compounds

The genus Artemisia is rich in essential oil. Generally the oil consists of highly unsaturated hydrocarbons, aromatic acetylenic compounds eg from A. monosperma (Abba, 1985) mixtures of esters, ketones and aldehydes from A. judaica (Abba, 1985) monoterpenes from A. nilagrica (Uniyal et al, 1985).

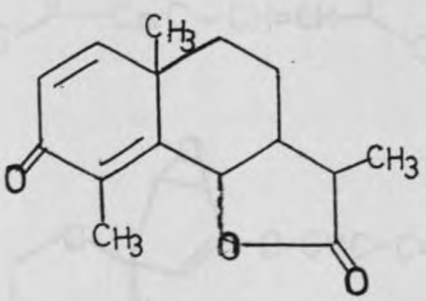
Oxygenated monoterpenes including camphor, 4-terpineol, borneol, thujone, cineole from A. vulgaris and A. verlotorium (Carnat et al, 1985); A. cina (Evans, 1989); A. afra (Watt and Breyer-Brandwjik, 1962) and A. arborescens (Codignola, 1984) have been



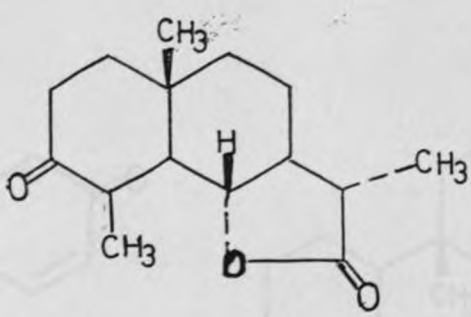
Scopodrimol A (65)



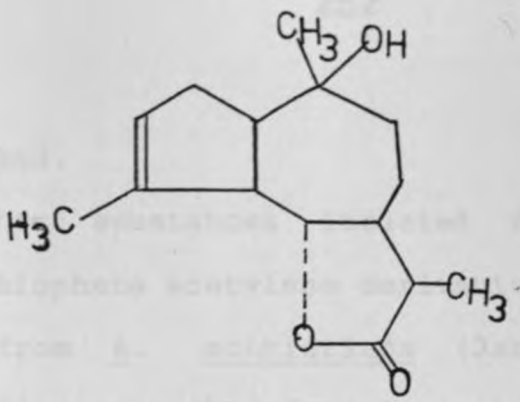
Yamayomoginin (66)



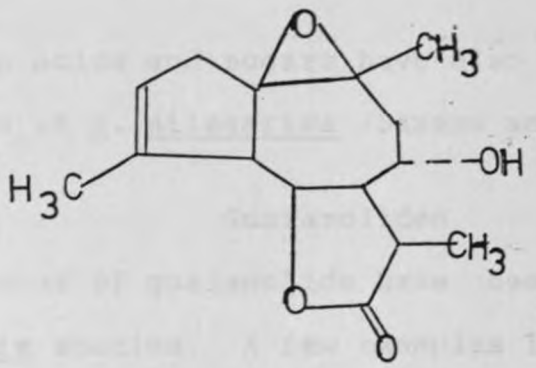
Santonin (67)



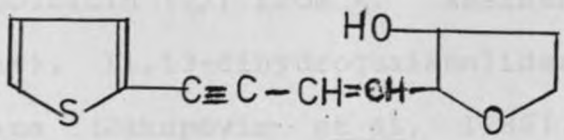
Artepauline (68)



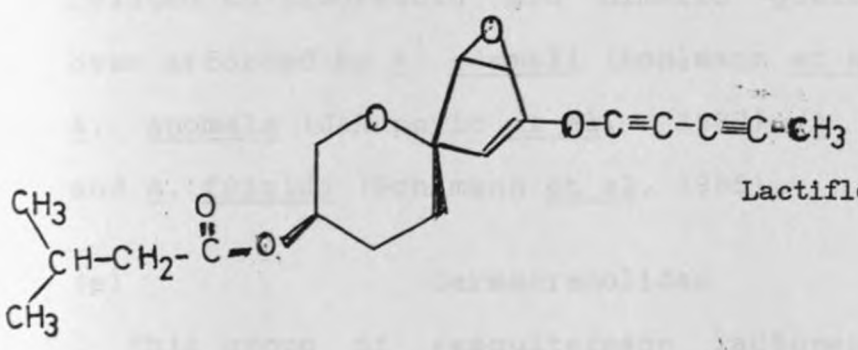
Artanolide (69)



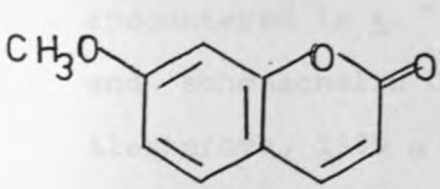
Deacetylglobicin (70)



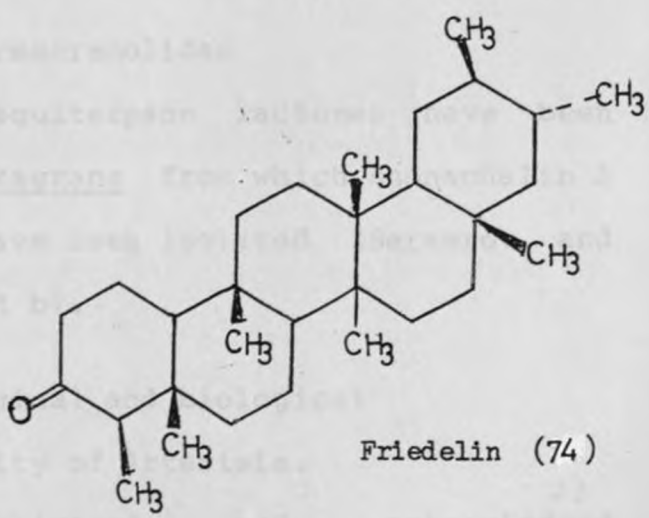
Schmidtiol (71)



Lactiflorasyne (72)



Herniarin (73)



Friedelin (74)

isolated.

Other substances isolated from Artemisia species are thiophene acetylene derivatives such as schmidtiol (71) from A. schmidtiana (Jakupovic et al, 1986). Lactiflorasyne (72) from A. lactiflora wall (Xui et al ,1986).

Amino acids and sugars have also been identified in the stem of A. nilegarica (Saxena and Samaiya, 1984).

(d) Guaianolides

A number of guaianolide have been isolated from Artemisia species. A few examples include artanolide, deacetylgolbicin (70) from A. Absinthium (Kasymov et al, 1984), 11,13-dihydroguaianolides from A. douglasiana (Jakupovic et al, 1986). Guaianolides related to arborescin and dimeric guaianolides have been afforded by A. adamsii (Bohlmann et al, 1985) and A. anomala (Jakupovic et al, 1987), A. sieversiana, and A. frigida (Bohlmann et al, 1985).

(e) Germacranolides

This group of sesquiterpene lactones have been encountered in A. fragrans from which shonachalin A and schonachalin C have been isolated (Serkerov and Aleskerova, 1985 a and b).

1.5.5.3 Pharmacological and biological activity of Artemisia.

The most publicised and acknowledged

pharmacological activity associated with Artemisia species is the antimalarial activity of a Chinese species Artemisia annua. After 2000 years of use as a Chinese herbal antimalarial remedy the active constituent artemisinin was isolated in 1972 (Fourth Meeting of Scient. Working Group on Chemotherapy of malaria, 1981). It is a sesquiterpene lactone with an endoperoxide group which is essential for antimalarial activity (Li et al, 1983).

Reduction of the keto group of the artemisinin molecule enhances in vitro antimalarial activity (Li et al, 1983) whereas breakage of the ring causes loss of activity (Lietinck and Dommisse, 1985).

Artemisinin is effective in all types of malaria and its potent inhibitory activity is comparable to that of the quinoline methanol, mefloquine (Klayman et al, 1984).

Ultrastructural studies have indicated that artemisinin is concentrated in only the infected red blood cells and affects the integrity of membranes and specifically parasite membranes (Warhurst et al, 1984).

Artemisinin and related compounds were found to cause rapid inhibition of 3-H-isoleucine uptake into human red cell infected with P. falciparum. Uptake of isoleucine is an index of protein synthesis and inhibition of its uptake is therefore an index of

inhibition of protein synthesis and hence inhibition of multiplication of malaria parasites (Gu et al, 1983).

Another mechanism of action of artemisinin may involve increasing oxidant stress in malaria parasite infected red cells. In vitro studies using artemisinin and its derivative artesunate showed that their antimalarial activity was enhanced by oxygen tension and was synergistic with miconazole or doxorubicin both of which increase oxygen stress (Krungkrai et al, 1987). However substances that decrease oxygen stress eg tocopherol, catalase, and dithiothreitol decreased the activity of artemisinin (Krungkrai et al, 1987).

Artemisinin has been demonstrated to possess gametocytocidal activity against P. cynomolgi B at a dose of 5 mg/kg intramuscularly (Dutta et al, 1989). However the drug has no sporontocidal action (Dutta et al, 1989).

Apart from its antimalarial activity artemisinin also possesses immunophamacologic activity (Lin et al, 1989). Intramuscular injection of artemisinin at a dose of 150 mg/kg twice daily for 7 days caused a decrease in serum immunoglobulin G (IgG) and decreased haemolysin forming capacity of mice sensitised with sheep red blood cells (SRBC) (Lin et al, 1989), however the same dose level of artemisinin caused an increase in the number of Thymus (T) cells of SRBC

sensitised mice and decreased the percentage phagocytosis of peritoneal macrophages (Lin et al, 1989). All this implies that artemisinin has a biphasic immunosuppressive and immunostimulant properties.

Many derivatives of artemisinin have been synthesised some of which surpass the parent compound in antimalarial potency. For example arteether a new antimalarial synthesised from dihydroartemisinin is 2-3 times more potent than artemisinin (Brossi et al, 1988). Its activity is comparable to other artemisinin derivatives, artesunate and artemether (Brossi et al, 1988).

Arteannuin B isolated from A. annua (Elmarakby et al, 1987) was found to possess potent antitumor activity (Jeremic et al, 1973).

Constituents of A. messerschmidiana and their derivatives were found to increase bile secretion after intravenous administration in dogs (Duk, 1966). The coumarins present in A. abrotanum were deemed to be responsible for the choleretic action of the extracts of this herb (Oscar et al, 1986).

Otto and Peter (1986) isolated isofraxidine, scopoletin and umbelliferone from A. abrotanum and found them to possess powerful choleretic effect. Similar pharmacological effect has been observed with constituents of A. capillaris Thumb in which scoparone

artepillin, capillartemisin B, and artepillin C, made overwhelming contribution (Okuno et al, 1988). Scopoletin, capillene and artepillin A, had a milder choleretic effect. Though all these compounds increased bile secretion, they had no effect on billiary acid, cholesterol or phospholipid excretion (Okuno et al, 1988).

Scopoletin a coumarin commonly found in the Compositae family especially Artemisia genus, possesses asthmolytic (Ojewole, 1983; Xaio Peigen, 1983), spasmolytic (Ojewole and Adesina, 1983) and antiarrythmic effects (Ojewole, 1983) 0.09 mg/ml scopoletin caused 50% contractile inhibition of estrone-primed and barium chloride stimulated rat uterus (Charles et al, 1967). This antispamodic effect was quantitatively similar when tested in vivo on oxytoxin and ergonovin promoted uterine contractions (Charles et al, 1967).

The compound also exhibits fungaltoxic properties when tested on Fusarium solani spores, by completely inhibiting the spore growth (Vasyukova et al, 1970). Scopoletin and scopolin possess enzyme inhibitory properties. The two compounds inhibited glucose 6-phosphate dehydrogenase (G6PD) enzyme from tobacco tissue culture (Shingo et al, 1971). Even other NADPH producing dehydrogenases such as NADP specific isocitrate dehydrogenase and 6-phosphogluconate and

NADP, specific malate dehydrogenase were inhibited (Shingo et al, 1971).

Crude extracts of A. princeps are used by the Chinese in the treatment of colic pain, vomiting, diarrhoea and irregular uterine bleeding (Yamada et al , 1983). Some of the compounds isolated from this plant consist of anti-complementary polysaccharides (acid heteroglycans) (Yamada et al, 1983).

Cirsimaritin a flavone isolated from A. judaica exhibited antispasmodic effect in vitro by inhibiting the smooth muscle contraction of the guinea pig ileum induced by histamine, acetylcholine or barium chloride (Abdalla and Abu-Zharga, 1987). The flavonoid group of compounds have the ability to influence arachdonic acid metabolism and this could contribute to the anti-inflammatory, anti-allergic, anti-thrombotic, antispasmodic, vasoprotective, antitumor properties commonly exhibited by this vast group of compounds (Evans, 1989).

The essential oil constituent of A. lactiflora (wall) is an effective asthmolytic, having expectorant properties, alleviating cough-induced inflammation (Xu Cheng-Jun et al, 1985) while oil obtained from A. asiatica and A. sieversiana showed marked anti-inflammatory activity when tested using the rat and mouse models (Saratikov et al, 1986). The activity was attributed to the azulenes present in the oil (Saratikov et al, 1986).

From the same plant a compound herniarin very effective in the treatment of chronic hepatitis was isolated from the whole plant with the leaves yielding the highest amount (29.4-94.1 mg/g) of leaf powder (Sun et al, 1987).

Extract of A. messerschmdtian (viridis) when injected intraperitoneally at a dose of 0.6 ml/kg/day for 3 days, 24 hours after intraperitoneal injection of carbon tetrachloride protected the rat from the hepatotoxic effects of carbon tetrachloride (Jeong and Khang, 1985). Similar antihepatotoxic properties were observed with flavonoids and coumarins extracted from A. capillaris buds, a plant reputed in oriental medicine for its liver protective effects (Kiso et al, 1984). The extract showed marked variance in its antihepatotoxic activity depending on the harvesting time and the 6,7-dimethyl esculetin content of crude preparations (Kiso et al, 1984).

Santonin a lactone glycoside is a common constituent of many Artemisia species. It is mainly obtained from the aerial parts of the plant. It is a potent anthelmintic mainly effective in the treatment of ascariasis (Tyler et al, 1976; Akhtar, 1984). At 15 mg/kg santonin is equipotent to piperazine at 88 mg in the treatment of ascariasis in buffalo calves (Akhtar, 1984). Though very effective, the use of santonin is restricted by the toxicity of the compound (Tyler et

al, 1976).

A. herba alba has yielded an anti-clotting principle, an acidic polysaccharide composed of galacturonic acid and rhamnose with a molecular weight of 10,000 (Han et al, 1984). When tested in rats made febrile by subcutaneous injection of beer yeast, alpha and beta santonin isolated from A. coerulescens subsp gallica was found to possess a dose dependent antipyretic activity decreasing the rectal temperatures (Martin et al, 1988).

A compound, 24-ethyl cholesta-7,22-dien-3beta-ol, isolated from A. absinthium, has also demonstrated antipyretic properties (Ikram et al, 1987).

Flavonoids artemisetin from A. absinthium, A. sieversiana and chrysosplenetin from A. sievers as well as eriodictoyl-7-methyl ester from A. xanthocrhoea exhibited marked antitumor activity against mice and rat experimental tumours (Chemesova et al, 1982).

The compounds were effective on melanoma B-16 but had no activity on sarcomas (Chemesova, 1987).

Antitumor activity was also exhibited by total flavonoids and acid phenols from A. cina, A. scoparia, and A. sublessingiana in a wide range of tumours in rodents (Ryahovskaya et al, 1989). The compounds also had antifungal activity against Fusarium bulbigenum blasticola in vitro (Ryahovskaya et al, 1989).

Capillarisin isolated from A. capillaris has also been found to possess cytocidal activity against L.29 and KB cells. In vitro and in vivo inhibition of the growth of experimental tumours in mice was also demostrated (Xu et al, 1989).

A synthetic derivative of capillarisin was even more potent in enhancing antitumor immune response and exhibiting direct tumoricidal activity in vivo (Xu et al, 1989).

Antimicrobial activity was exhibited by santonin isolated from A. cina. It was active against Bacillus subtilis ATCC6633 and Bacillus cereus with the alpha-form being the most active (El-sayed et al, 1988). Due to its toxicity on ingestion (Tyler et al, 1976), the compound may find use as a topical bacteriostatic agent (El-Sayed et al, 1988).

Members of Artemisia produce many terpenoid compounds in their glandular trichomes. Many of these especially the sesquiterpenoid lactones are biologically active as fungicides, herbicides, insecticides and insect antifeedants (Duke et al, 1988). They also offer potential as sources of new pesticides chemically related to or derived from the naturally occuring compounds (Duke et al, 1988). Examples of these compounds include substances such as toxamphene (insecticide cum herbicide), cinmethylin (a herbicide) and artemisinin antimalarial and phytotoxin

(Duke *et al*, 1988).

On the cardiovascular system and specifically the heart, a coumarin derivative, scoparone isolated from the Chinese *Artemisia capillaris* caused an increase in the coronary flow rate, with minimal effect on the cardiac output, left ventricular pressure or left ventricular work of an isolated perfused rat heart (Yamahara *et al*, 1989).

Not only is the *Artemisia* species important medicinally and biologically but it has also been shown to be a useful tool in prospecting for minerals (Busche, 1989) especially gold, in that the element is concentraed in the aerial part of the plant. A species of *Artemisia* that has been exploited for this purpose is *Artemisia californica* (Busche, 1989).

CHAPTER TWO

2 MATERIALS AND METHODS

2.1 Screening for antimalarial activity in Kenya plants

The screening was carried out using the micro-dilution method developed by Desjardin in 1979 adopted and modified by the Kenya Medical Research Institute, Nairobi. Details of the method are given below:

2.1.1 Preparation of Reagents

2.1.1.1 RPMI Medium (1640 HEPES)

Thoroughly cleaned glassware rinsed with distilled de-ionised water was used for preparing the above mentioned medium.

After cleaning the hands thoroughly with soap and water and finally rinsing them with distilled water, 100 ml distilled de-ionised water was carefully measured and poured into a 1000 ml graduated measuring cylinder.

Powder HEPES (5.49 g) was accuraely weighed into a clean weighing boat. This was poured into the measuring cylinder containing the water and the weighing boat washed thoroughly transferring all the washings into the cylinder.

One litre packet of powdered RPMI 1640 (not more than 6 months old) was removed from the refrigerator

and the contents carefully poured into the cylinder containing HEPES acid. The pocket was thoroughly rinsed with distilled de-ionised water and the rinsings poured into the cylinder.

The contents were made to 960 ml mark with the distilled de-ionised water. Using a piece of parafilm, the cylinder was covered and left to stand until all the powder dissolved.

The medium was then filtered while in a laminar flow hood using a 0.2 u filter unit under vacuum. Using the asceptic technique the filtered medium was poured into sterile 250 ml plastic flasks and stored at 4 degrees C until needed (up to 4 weeks).

2.1.1.2 5% Sodium bicarbonate solution

Only glassware meant for preparing media was used. After thoroughly washing hands, 5 g sodium bicarbonate powder was accurately weighed in a weighing boat. The powder was transferred through a funnel into a thoroughly cleaned 100 ml volumetric flask. The weighing boat and funnel were washed with the distilled and de-ionised water to remove all the powder into the flask. The solution was made to volume using additional water and the content thoroughly mixed and sterilised by filtering through 0.2 u filter. The solution was then poured into sterile 25 ml flask and stored at 4 degrees C for not more than 2 weeks.

2.1.1.3 Culture medium with 10% serum (CMS)

This medium was made by mixing RPMI 1640/HEPES with the 5% sodium bicarbonate and human serum (type/donor CDC1-AB+) using the formula below.

RPMI 1640 = 0.8640 x final CMS volume

5% bicarbonate = 0.036 x final CMS volume

serum = 0.1 x final CMS volume.

The hands were thoroughly washed and rinsed with distilled water. The required amount of serum was removed from the freezer and thawed in water bath at 37 degrees C. As soon as thawed, the serum was kept at room temperature. The RPMI - 1640/HEPES and 5% sodium bicarbonate were also removed from refrigerator. These together with the thawed serum were placed in a laminar flow hood. Also placed in the hood were 25 ml falcon flasks for packing the CMS in 25 ml portions.

Using the aseptic or sterile technique and the above formulae, the required amount of RPMI 1640/HEPES was added to each flask followed by the required amount of 5% sodium bicarbonate. The contents were shaken until the medium colour was uniform. The sodium bicarbonate pipette was used only once and discarded. The operation was repeated for all the other flasks.

The serum was mixed thoroughly by aspirating and expelling from the pipette five times. The required amount of serum was then aspirated and transferred to the flask containing RPMI 1640/HEPES and 5% sodium

bicarbonate and the contents thoroughly mixed by aspirating. The operation was repeated for all the other flasks. The flasks were then individually aerated by flushing with 3% carbon dioxide and 5% oxygen gas mixture and sealed tightly with caps and stored in the refrigerator at 4 degrees C until required.

2.1.1.4 Wash medium (WM)

The wash medium was made by mixing RPMI 1640/HEPES with 5% sodium bicarbonate solution using the formula below:

RPMI 1640/HEPES = 0.958 x final volume of medium

bicarbonate = 0.042 x final volume of medium.

Using thoroughly cleaned hands, 25 ml corning flask were placed in the laminar flow hood and the cap loosened. The RPMI 1640/HEPES and 5% sodium bicarbonate were removed from the refrigerator and also placed in the hood. Using the asceptic technique the required amount of RPMI 1640/HEPES was added to the flask followed by the required amount of the 5% sodium bicarbonate. The contents were thoroughly mixed until the medium was uniform (A separate pipette medium colour was used for each bicarbonate addition).

The flasks were flushed with 5% oxygen and 3% carbon dioxide gas mixture, sealed tightly and kept in

the refrigerator at 4 degrees C until required.

2.1.1.5 Washing fresh erythrocytes

The red blood cells (RBCs) used were of the type O+ from different donors designated as FK, SK and DK.

5 ml portions of whole blood were placed in 15 ml centrifuge tubes and centrifuged at 1000g x 10 minutes 4c. The required amount of wash medium (22.5 ml WM for each 5 ml of blood) was transferred into the laminar flow hood. The test tubes containing blood were also transferred to the hood with the cap(s) on the tube(s) loosened. Using vacuum suction, the supernatant fluid and the top 2 mm cell layer were gently aspirated.

10 ml of wash medium was added to the centrifuge tubes and the RBCs were gently suspended 5 times in a 10 ml pipette. The contents were centrifuged and the aspiration process repeated. The washing was repeated once more. Finally the packed cell volume (PCV) was estimated by holding the centrifuge tube alongside a calibrated tube. One PCV of wash medium was added and the cells suspended to form 50% suspension. If the cell were not being used immediately, the tubes were flushed with 3% carbon dioxide and 5% oxygen gas mixture and stored at 4 degrees C until required.

2.1.1.6 Preparation of Plasmodium falciparum cultures

These can be made either from old cultures which are being propagated or from cryopreserved infected

blood, or from fresh infected blood. The first two sources were used in this project.

(i) Cryopreserved infected blood

The blood was cryopreserved in Rowe's cryo solution (28% glycerol, 3% sorbitol and 0.65% sodium chloride). The cryopreserved blood was portioned in aliquots of 3 x 0.5 ml and recorded in a logbook. It was then packed in aluminium casing and frozen in a liquid nitrogen freezer until required.

When required, the specimen was identified in the logbook. The vial was removed from the aluminium case and thawed in a warm water bath at 37 degrees C. The contents were then transferred into a 15 ml centrifuge tube and centrifuged at 400g (1500 x pm for 5 minutes at 20 degrees C at room temperature) under sterile conditions. The supernatant was aspirated and 0.3 ml of sterilised 3.5% sodium chloride added. The contents were mixed and centrifuged. Aspiration of the supernatant was repeated and 1 ml culture medium was added followed by mixing of the contents, centrifuging and aspiration of the supernatant. This step was repeated once more. 50% RBCs and culture medium were added as shown below.

For 2 cultures of 5 ml at 6% haematocrit (hct)

0.25 ml PCV and 0.7 ml 50% RBC + 9.05 culture medium.

The cultures were placed in corning flasks 25 ml,

flushed with 3% carbon dioxide and 5% oxygen gas mixtures and capped tightly. The contents were mixed by agitating with circular motion and placed horizontally at 37 degrees C in an incubator.

(ii) Preparing cultures from old cultures

5 ml or 0.5 ml cultures were made depending on the number of the new cultures that would be required.

If the old culture was 5 ml volume and 6% haematrocrit (hct) the following formula was used:-

$$\text{Culture volume (CV)} = \frac{5}{D}$$

Where D is the reciprocal of desired dilution factor eg D = 10 for 1 : 10 dilution.

$$\text{50\% RBCs volume (RV)} = \frac{6}{50}(5-CV)$$

$$\text{Medium volume (CMS)} = 5-(CV + RV)$$

For 0.5 ml well cultures at 8% hct the following formul was applied:

$$\text{Culture volume (CV)} = \frac{0.5}{D}$$

$$\text{50\% RBC volume (RV)} = \frac{8}{50}(0.5-CV)$$

$$\text{Medium volume} = 0.5 - (CV + RV)$$

The value of D is dependent on the parasitaemia of the old culture. The new culture was always started with low parasitaemia to allow for adequate nutrition of the parasites so that they can multiply.

2.1.1.7 Preparation of slides and changing of medium of old culture

The CMS was removed from the refrigerator and warmed up for 20 minutes in a 37 degrees C incubator.

The valve of the gas cylinder containing the 5% oxygen and 3% carbon dioxide mixture was opened and the vacuum turned on. The pilot flame on the Bunsen burner was ignited.

The frosted edge of glass slide(s) was labelled with an identification number(s).

The flask(s) of warmed medium was placed in the laminar flow biological hood. Culture flask(s) was removed from the incubator and placed in the hood and the cap(s) loosened. A sterile unplugged pasteur pipette was attached to the vacuum and the distal half of it flamed by moving it back and forth in the flame for at least 2 seconds. After removing the cup from the culture flask, the flask was gently fitted so that the medium collected near the mouth. The pasteur pipette was inserted into the flask and the medium aspirated at a steady flow rate without creating bubles or turbulence. The aspiration was continued until the red cells were first drawn into the sloping

area of the flask, then promptly the pasteur pipette was removed to avoid aspirating the red blood cells. This left at least 1 ml of the medium and the RBCs in the flask. The pipette was discarded after use.

Another pasteur pipette was attached to a rubber bulb and flamed as above. The culture flask was was again tilted slightly and using a little pressure on the rubber bulb the medium was aspirated and expressed gently 4-6 times until a uniform red cell suspension was formed. With a little suspension at the tip of the pipette, a small drop of the suspension was applied to one end of the labelled glass slide and the pipette discarded. Using the end of another glass slide a thin smear of the red cell suspension was made and immediately the slide was placed in the stream of the air at the edge of the hood to facilitate drying. The slides were always made in duplicate.

The flask containing the culture medium (CMS) was uncapped and 5 ml of the medium aspirated into a pipette and the cap replaced on the flask. The tip of he pipette was placed on the wall of the flask containing the RBCs and the culture medium gently run into the flask.

The flask was capped and the RBC(s) suspended by gentle agitation of the flask. The whole process was repeated for any remaining culture flasks.

The flasks were then flushed with 3% carbon dioxide

and 5% oxygen mixture capped and placed horizontally in an incubator at 37 degrees C. The culture medium of the growing cultures was changed after every 24 hours.

2.1.1.8 Staining of the slides and determination of parasitaemia

The glass slide smear was fixed in methanol and stained with 4% Giemsa stain for 30 minutes (5 ml of the Giemsa stain was enough for 2 slides). The excess stain was washed off with a gentle stream of tap water and the slides allowed to dry.

The cell count was done using a Dialux 20 E8 (Leitz Wetzlar-Germany) microscope at 100 magnification. The immersion oil used was immersionoel (Zeiss West Germany). Using a cell counter at least 5,000 to 10,000 RBCs were counted and the parasitised cells in this population of cells determined as shown below.

If the total number of RBCs in two microscope fields was say 185, the average number of RBCs per field was equal to 93. To count 5,000 RBCs therefore one needed to count at least 54 fields.

If the number of parasites seen after counting the 5,000 RBCs was 98. The percentage parasitaemia was calculated to be

$$\frac{98}{5,000} \times 100 = 1.96\%$$

To prepare a new culture from the above old culture

a dilution was found necessary to provide nutrients to the erythrocytes and the growing falciparum parasites.

Usually a dilution to a parasitaemia level between 0.2% to 0.6% was found to be adequate for culture growth.

2.1.2 TESTING OF DRUGS (PLANT MATERIALS)

A total of five plants were screened for antimalarial activity. The plants are:

Ajuga remota Benth (leaves) (LAM 1)
Caesalpinia volkensii (seeds) (LAM 2)
Schkuhria pinnata (whole plant) (LAM 3)
Warburgia ugandensis (bark) (LAM 4)
Artemisia afra (leaves) (LAM 5).

(i) Preparation of the drug

The extracting solvent used for all the plant materials was 70% ethanol which also acted as a sterilising agent.

LAM 1

The leaves of Ajuga remota Benth were collected from upper Kabete area near the Kabete Goverment Veterinary laboratories' main water tank. The collection was done in the month of August 1986. The plant material was dried at room temperature (23-25 degrees C) for one week. After drying, the leaves were then ground into a fine powder using a coffee grinder.

5g of the dry leaf powder was extracted in 70% ethanol by cold percolation for 5 days. The material was then filtered through cotton wool first and then through Whatman filter paper No 1. The resulting filtrate was made to 50 ml with 70% ethanolic washings of the plant material. This formed the concentrate (C) designated as LAM1c.

LAM 2

The Caesalpinia volkensii seeds were obtained from Machakos District (Kenya). The hard seed kernel was broken to obtain the inner whitish cream cotyledons. These were ground to fine powder using a mortar and pestle. 5 g of powder was then weighed and treated as LAM 1 to finally yield LAM2c as the concentrate.

LAM 3

The Schkuhria pinnata whole plant were picked from the Nairobi Dam area in April 1986. The plants comprised of mature flowering plants and young unflowering plants. The plant material was dried at room temperature (23-25 dgrees C) for one week. The dry whole plant was then powdered and treated as LAM 1 above to finally yield the concentrate LAM3c.

LAM 4

The stem bark of Warburgia ugandensis was collected from a forest in Nkomo sublocation of Tigania division of Meru District (Kenya) in March 1986. The bark was

cut into small pieces and air-dried at room temperature for ten days. It was then ground into a fine powder which was treated as LAM 1 above to finally yield LAM4c as the concentrate.

LAM 5

Artemisia afra was collected from Kinungi area in Naivasha (Kenya). The aerial parts, mainly the leaves, were separated and airdried at room temperature for one week. They were then ground to fine powder and treated as LAM 1 above to finally yield the concentrate LAM5c.

(ii) Preparation of drug dilutions

Using the plant extract concentrates specified above, the following dilutions were asceptically made in culture medium (CMS). 1 : 100; 1 : 200; 1 : 400; 1 : 8,000; 1 : 80,000.

(iii) Setting the tests

The P. falciparum parasites were grown in vitro as indicated in section 2.1.1.6. After every 24 hours the culture medium was changed to provide a constant source of nutrients. After every 48 hours (latest 96 hours) slides were prepared as in section 2.1.1.7 and percentage parasitaemia determined (section 2.1.1.8). Based on the percentage parasitaemia, appropriate dilutions of the culture were made and new cultures started from the old ones. This process was continued

until a substantial crop of parasites was collected. From the initial and final parasitaemias, the growth rate was calculated using the formula:

$$GR = \left(\frac{Pf}{Pi} \times D\right)^{2/d}$$

Where GR = growth rate

Pf = final parasitaemia

Pi = initial parasitaemia

D = Dilution factor

d = number of days that the culture had grown up to the time of preparing the slides

The growth rate was constantly determined until it was equal or more than 3 fold indicating that the parasites had now been adapted to the in vitro growth conditions and testing of the drugs could now be carried out.

16 x 0.5 ml culture plates were used and the tests arranged as shown on the schematic diagram (Table 27).

0.4 ml of the drug dilutions in CMS were added to 0.5 ml wells to give the following final dilutions as shown (Table 28).

The range of drug concentration tested was 1:100 to 1:80,000 dilution.

Each plate was used to test varying concentrations of one plant extract at one given time. Each drug concentration was tested in duplicate (Table 27). The

TABLE 27 SCHEMATIC LAYOUT FOR ANTIMALARIAL DRUG TESTING

ROW NO	EXTRACT CONCENTRATIONS			
A	LAM 1	LAM 1	LAM 1	C-Et*
	1:00,000	1:10,000	1:250	
B	1:100,000	1:10,000	1:250	C-Et
C	1:1000	1:500	1:125	CN
D	1:1000	1:500	1:125	CN

*C-Et = Control with ethanol

CN = Normal control without ethanol.

16 x 0.5ml culture plates were used and the tests arranged as shown on the schematic diagram (Table 27).

TABLE 28 FINAL CULTURE WELL DRUG DILUTIONS

Extract concentration	Volume taken	Volume made up to(ml)	Final well concentration
1:100	0.4	0.5	1:125
1:200	0.4	0.5	1:250
1:400	0.4	0.5	1:500
1:8,000	0.4	0.5	1:10,000
1:80,000	0.4	0.5	1:100,000

The range of drug concentration tested was 1:125 to 1:100,000 dilution.

experiment was repeated at least three times giving a minimum of six determinations per drug concentration.

Control tests containing the highest concentration of ethanol (CEt) and another control (Normal) containing no ethanol or drug (CN) were set up.

Just as with normal cultures, the medium for the extract test cultures was changed every day (after 24 hours) to replenish the nutrient and add fresh supply. Slides were made after every 2 days (48 hours) latest 4 days (96 hours). Percentage parasitaemia was determined and compared to that of the control (CEt). Using the formula shown above the growth rate was calculated.

The parasitaemia and growth rates were finally expressed as a percentage of the normal. The percentage change in the growth rate was then determined by substracting the percentage of the normal from the test.

2.2 PHYTOCHEMISTRY OF ARTEMISIA AFRA

Artemisia afra Willd is an erect loose shrub that can grow to a height of 2 m with grey-green aromatic folliage of twice pinnate leaves. The leaves are ovate in outline with the ultimate segments linear. The flower heads, yellow in colur, appear in an elongated racemose panicle arrangement, each with very few outer female florets.

Artemisia afra is a common shrub in montane grassland areas in Kenya. These include the highlands of Mount Elgon, Cherangani, Tinderet, Aberdares, on the south eastern side of Mount Kenya (5000 ft) and on the northern and western sides of Mount Kenya (7000 ft) (Agnew, 1974).

The plant is also found in Kitale, Machakos, Kajiado and on the slopes of the Rift Valley (Agnew, 1974).

2.2.1 Collection of Plant Material

The plant material was collected from recorded (by East African Herbarium Nairobi) and unrecorded places. All the aerial plant parts were collected.

The recorded site was Limuru, at Kinungi East and West road junction on Nairobi Naivasha road. The plant was found mixed with Lippia ukambensis. This site is actually a settled area. Another collection site was in Nakuru on the slopes of Menengai Crater. Again the plant was found mixed with other plants like Lippia javanica.

A little amount was also collected on Ngoina hills in Sotik (Kericho) along the road.

The authenticity of all the plants was established by the East African Herbarium, Nairobi, Kenya, and voucher specimen were deposited at the herbarium and at the department of Pharmacy, University of Nairobi.

2.2.2 Cultivation of the plant

Artemisia afra from Kinungi was introduced into cultivation at Upper Kabete location, Nairobi in May 1984. Introduction of Artemisia afra into cultivation proved difficult either by layering, cutting or suckers. Out of 30 layerings, cuttings and suckers, only one sucker took root. All the others decayed. However the only one that took root did extremely well among onions, maize and cassava. It was planted close to a kitchen drain to avoid drying during the dry weather. No fertilizers or rooting hormones were used. The difficulties experienced in propagating the A. afra could probably be attributed to differences in climate of Kabete as compared to that of natural habitat. The differences in the soil composition may also have contributed to the difficulties. The soil found in the natural habitat is loose and sandy whereas that of the cultivation site is a mixture of red and black cotton soils making drainage less efficient.

2.2.3 EXTRACTION OF ARTEMISIA AFRA CONSTITUENTS

(a) Volatile oil

Preliminary work had indicated that Artemisia afra twigs, yielded only a trace of oil. Therefore only the leaves were used for extraction of the oil. The A. afra leaves previously air-dried for at least 7

days were exhaustively steam distilled for a minimum of three hours using the Clevenger-like apparatus. The essential oil content was expressed on dry plant material basis as an average of four determinations.

After hydro-distillation, the oil samples were dried with anhydrous sodium sulphate, and packed in glass bottles. The oil was stored at low temperatures (4 degrees C) until required.

Preliminary analysis of the A. afra oil

Preliminary analysis of the oil was carried out using gas liquid chromatography (GLC), Pye-Unicam model 104 Instrument fitted with a flame ionisation detector. A glass column 2 m x 4 mm), packed in our laboratory with 12% Carbowax 20 M on chromosorb W HP-DMCS 100-120 mesh was used. Nitrogen at a flow rate of 30 ml/min was used as the carrier gas with temperature programming at 2 degrees/min from 75 degrees C to 220 degrees C at 5 degrees C per minute. The peaks were recorded using Pye linear recorder type AR 55.

(b) Non-volatile constituents

(i) Petroleum ether extraction

Air-dried Artemisia afra leaves were finely ground. 2 kilogrammes was soxhlet-extracted with petroleum ether (60-80^{o}C) for a minimum of 48 hours.

The petroleum ether extract was vacuum dried to yield 120 g of dirty green solid.

(ii) Methanol Extraction

The petroleum ether exhausted A. afra material was further soxhlet extracted using 70% aqueous methanol for 48 hours.

The methanol extract was reduced by vacuum on a rotary evaporator until all the methanol had distilled off.

The remaining aqueous extract was treated as follows: Eight portions of 300 ml diethyl ether were used to exhaustively extract the aqueous portion. The diethyl ether portion was combined and dried with anhydrous sodium sulphate. The dry diethyl ether was then vacuum distilled to form extract DEM.

The remaining aqueous methanolic extract was further exhaustively extracted with ethylacetate (8 x 200 ml portions) and chlorform (8 x 200 ml portions) respectively. These portions were vacuum dried to yield METAc and CM extracts.

Treatment of the crude extracts

The petroleum ether semi-solid residue was triturated several times with ethanol to yield an ethanol insoluble dirty yellowish green solid which was filtered off.

The ethanol soluble petroleum ether extract was vacuum dried to yield a dark green residue which was column chromatographed as follows:

Column chromatography

40 g of above material was passed through a column packed with silica gel for column chromatography (Kieselgel 60, mesh 70-230, ASTM, Merck) and eluted with petroleum ether:ethylacetate in increasing polarity. The eluted fractions in 20 ml portions were monitored by thin layer chromatography (petroleum ether:ethylacetate in increasing polarity) and ultraviolet light followed by spraying with anisaldehyde reagent (composition: 0.5 ml anisaldehyde, 1 ml concentrated sulphuric acid in 50 ml glacial acetic acid) followed by heating in the oven at 110 degrees C for 10 minutes.

Identical column fractions were combined to form samples 1-10 which were vacuum dried and the resulting solid taken up in appropriate solvent and left to stand at low temperatures (4 degrees C) for some time.

Those samples that formed solids were further purified and the pure compounds characterised.

Methanol extract

The ethylacetate and chloroform fractions of the methanol extract did not yield much solid and were not followed further. However the diethyl ether fraction yielded 10.2 g of solid that was column-chromatographed (silicagel) and eluted with chloroform, methanol in increasing polarity. The eluted fractions were monitored by TLC (chloroform:methanol 10:1, to

chloroform: methanol 5 : 1) using UV light and anisaldehyde spray reagent for detection.

The identical fractions were pooled together and vacuum dried using a rotary evaporator. The resulting crude material was taken up in appropriate solvent (see below) and left to stand in the refrigerator at 4 degrees C until crystals deposited.

Details of purification and characterisation of the isolated compounds are given below:-

LONG CHAIN ESTERS

The ethanol insoluble dirty green solid obtained from the petroleum ether extract was washed several times with warm ethanol. After drying, a dull white powdery material was obtained (10 g, 0.5%) m.p 73-74 degrees C, IR (KBr)/cm 1730 (C=O) stretching vibrations, 2825 (C-H stretching), 1465 (C-O stretching).

To determine the structure, ^{1}H and ^{13}C-NMR spectra were performed, unless otherwise stated, measured on a JEOL FX 90Q instrument with TMS as internal reference. EIMS was measured on a Finnigan MAT 4500 instrument. Melting points were done on a Gallenkamp m.p apparatus, UV spectra were measured in chloroform on Pye Unicam Sp 8000 spectrophotometer, and IR measurements on a Perkin-Elmer Infrared spectrophotometer 727B. Chemical ionisation mass spectra (CIMS) measurements were also done.

D:A: FRIEDO-OLEANAN-3-ONE (FRIEDELIN)

Sample 2 made up of fractions 31-45 from the petroleum ether extract was reduced to dryness in rotary evaporator and the residue taken up in minimum amount of ethylacetate. The contents were left to stand in the refrigerator at 4 degrees C for 3-4 days and a solid was deposited. After filtration the solid was recrystallised twice in ethylacetate to yield white needle like crystals m.p 259-260 degrees C (lit 264-265; 260-261 degrees C; Carpenter et al, 1980;Addae-Mensah and Achenbach, 1985 respectively).

TLC was done in pure benzene; and/or benzene:methanol:chloroform 90: 0.5: 0.5 whereas IR was done using KBr disc.

The structure was elucidated by ^{1}HNMR in $CDCl_3$ at 400 mHz; ^{13}CNMR (in $CDCl_3$ at 22.5 MHz) and mass spectra (ei at 70 e.v), measurements using instruments previously mentioned.

7,4'-DIMETHOXY 5-HYDROXYFLAVONE (G-5)

The fifth sample of petroleum ether extract (86-99) column was reduced to dryness and the resulting residue was taken up in minimum amount of ethylacetate and left to stand at 4 degrees C for 3-4 days. Dirty green crystals were deposited. These were filtered and washed several times with ice cold ethylacetate. A further two recrystallisations in ethylacetate yielded yellow needles m.p 161-162 degrees C.

Further characterisation was done as before using UV, IR, ^{1}HNMR, ^{13}CNMR and mass spectrometry (see discussion).

ALPHA-AMYRIN

Sample 6 comprising of column fractions 100-110 from the petroleum ether extract was vacuum dried to form a lightly greenish residue which was taken up in minimum amount of ethylacetate and left to stand at 4 degrees C for at least a week. The fraction precipitated some dirty green solid which was recovered by filtration and taken up in hot methanol. After filtration, the methanol portion was stored at 4 degrees C for at least five days within which time dirty white solid was deposited. The solid recovered by filtration and further recrystallisation in ethylacetate yielded white crystals m.p 178-180 (lit 185-187) degrees C. IR (KBr)/cm 3625 (OH) (lit 3620-OH); $[\alpha]_D CHCl_3$ = + 79 (lit + 80.5, $CHCl_3$).

Further characterisation was achieved by comparative TLC with authentic alpha-amyrin, ^{1}HNMR ($CDCl_3$ at 90 MHz); ^{13}CNMR ($CDCl_3$) and electron impact (E.I) mass spectrometry (70 ev) (see discussion).

BETA-SITOSTEROL

The seventh fraction (111-125) from the column was pooled together and evaporated to dryness in a rotary eveporator (60-70 degrees C). The resulting residue

was taken up in a little ethylacetate and left to stand in the refrigerator at 4 degrees C. White needle like-crystals were deposited. After filtration a further two re-crystallisations in methanol/chloroform yielded very white neddle-like crystals m.p 139 degrees C; (literature values m.p 139-140, 141-142, 142-144 degrees C); $[\alpha]_D^{CHCl_3}$ -37.5 degrees (Addae-Mesah and Achenbach, 1985).

Other identification methods such as $^{1}HNMR$, $^{13}CNMR$ and E1-Mass spectrometry were done as before.

Scopoletin (6-methoxy-7-hydroxy coumarin).

The petroleum ether exhausted A. afra leaves were re-extracted by soxhlet for 48 hours using 70% aqueous methanol. The methanol extract was reduced to about 500 ml and extracted at least 8 times with diethyl ether. The diethyl ether portion was reduced to dryness and the resulting residue was triturated in methanol. The methanol soluble fraction was reduced to dryness. The resulting residue 57.22 g was columned through 70 g silica gel (70-230 mesh ASTM-Merck). The column was eluted with chloroform: methanol, 20: 1. The eluents were monitored by TLC. The first sample showed intense blue fluorescence under long UV. After pooling together the identical fractions, sample one (blue fluorescence) (fraction 1-96) was reduced to dryness and the resulting solid was taken up in minimum amount of ethylacetate and left to stand in the refrigerator at 4 degrees C. Dirty yellow crystals

were deposited. These were filtered and re-crystallised at least twice from a minimum amount of ethylacetate. The final crystallisation yielded amber coloured crystals. The melting point, UV and IR determinations were done. The capillary gas chromatography; was run under the following column conditions:- material: SE 54; size, 25 m; eluting gas He at 10 psig; injection temperature 280 degrees C; detector temperature 330 degrees C).

2.3 BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES OF A. AFRA

2.3.1 THE ANTIMICROBIAL ACTIVITY OF CONSTITUENTS OF ARTEMISIA AFRA

(i) The Agar Streak Method

The test organisms used in this experiment were Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas and Klebsiella species.

The modified method of Mitschler et al (1972) was applied. Trypticase soy agar was prepared and sterilised by autoclaving at 121 degrees C for 15 minutes in a portable autoclave.

The test organisms were maintained on Trypticase soy agar slants and were recovered for testing by growth in peptone water for 24 hours. Before testing, the organisms were diluted 1:100 using peptone water.

A stock solution of 50 mg/ml Artemisia afra essential oil was prepared from which the required dilutions were made as follows:-

To prepare 1000 µg/ml test drug concentration 10 ml of warm agar medium was poured asceptically into pre-sterilised petri dishes and before congealing 0.2 ml of the dissolved essential oil from the stock solution was added. The petri dishes were then gently and carefully swirled until the agar began to set, after which the test organisms were then streaked in a radial pattern on the agar containing the essential oil.

A negative control consisting of Trypticase soy agar without the drug, and positive control containing the standard antibiotic streptomycin sulfate at 10 µg/ml concentration were streaked before and after each test series to avoid cross contamination and hence ensure validity of the results.

In a similar way tests containing A. Afra essential oil ranging from 10-2000 µg/ml were set up. The plates were incubated at 37 degrees C and were examined after 24 hours for growth. Complete suppression of growth was necessary for the drug to be declared active at a given concentration. It should be noted that the solvent used (acetone) had been found not to cause any inhibition of microbial growth even at 2% concentration (Mitschler et al, 1972).

The above method was only used for testing for the antimicrobial activity of A. afra essential oil. Due to limited quantities of the other isolated A. Afra constituents, the filter paper disc method was applied for determining their antimicrobial activity.

(ii) The filter paper disc method

The modified Vincent and Vincent (1944) method later applied by others (Morris et al, 1979; Yousef and Towil, 1980; Ross et al, 1980) was used. Filter paper (Whatman No 1) disc 1cm diameter were sterilised by dry heat.

Preparation of test drug

7,4'-dimethoxy-5-hydroxyflavone

1.0 mg of 7,4'-dimethoxy-5-hydroxyflavone was accurately weighed and dissolved in 10 ml dimethylsulfoxide (DMS) to form 100 µg/ml solution. This formed the stock solution and lower dilutions were prepared by diluting the stock solution with distilled water as required. The range of drug concentrations tried were 10-100 µg/ml.

Mixture of long chain esters

10 mg of the long chain esters powder was weighed and triturated with 10 ml of 1% dimethylsufoxide (DMS) to form a 100 µg/ml solution. This being the stock solution, appropriate dilutions were made with 1% DMS to give a test drug range of 1-1000 µg/ml.

alpha-amyrin

The range of concentrations tested for alpha-amyrin was 1-1000 µg/ml. The drug had been prepared as detailed above for the long chain esters.

Ethanol soluble petroleum ether extract

The vacuum dried A. afra petroleum ether extract was triturated with ethanol until all precipitate was formed. The material was filtered and the filtrate consisting of the ethanol soluble substances was vacuum dried to form a greenish residue. 100 mg of the material was weighed and taken up in 100 ml sterilised distilled water to form a 10 mg/ml solution which was used as the stock solution from which lower dilutions were made using sterilised distilled water.

The range of concentrations tried was 10-1000 µg/ml. The sterilised filter paper discs were saturated with the drug concentrations specified above. They were removed from the solution asceptically with sterilised forceps and placed carefully on micro-organism seeded plates (1:100 dilution). The plates were incubated at 37 degrees C for 24 hours. The tests were set up in triplicate for each drug concentration.

Also a negative control consisting of filter paper discs saturated with the solvent used in the drug dilutions was also set up in every plate. Also included in every plate was a positive control

consisting of filter paper discs saturated with standard solution of streptomycin (10 µg/ml).

After the 24-hour incubation period, the average diameter of zone of inhibition was determined and the percentage inhibition was calculated with respect to that induced by the standard antibiotic.

2.3.2 MOSQUITO LARVICIDAL ACTIVITY OF CONSTITUENTS OF *ARTEMISIA AFRA*

Mosquito larvae (*Aedes aegypti*) were used. These were obtained from Kenya Medical Research Institute Laboratories, Nairobi, where they were bred under specified laboratory conditions. The third instar larvae were used instead of the fourth instar to avoid pupation during the experiment. The Larvae were counted in groups of twenty and each group placed in small size 50 ml beakers. Before and during the experiment the larvae were fed on powdered dessicated liver (Oxoid) suspended in water.

(a) Preparation of test samples

(i) *Artemisia afra* oil

To prepare the stock solution 50 mg of the neat oil was weighed and dissolved in 5 ml of acetone. The following dilutions were then made from the stock solution using water for diluting.

0.6 ml of stock solution made to 120 ml gave 50 ppm

1.2 ml of stock solution made to 120 ml gave 100 ppm

1.5 ml of stock solution made to 120 ml gave 125 ppm
1.8 ml of stock solution made to 120 ml gave 150 ppm.

Higher and lower concentrations were made as required. The control was prepared with acetone at the higher concentration used in the experiment (200 ppm) without the drug.

(ii) Long chain esters (AAG 1)

50 mg of AAG 1 powder was weighed and dissolved in 0.5 ml of warm chloroform. To this was added 4.5 ml of 2% Tween 80 solution forming a uniformly milky suspension. Using water for diluting, the following concentrations were prepared.

Stock solution	Diluted to	Final concentration (ppm)
0.4 ml	80	50
0.8 ml	80	100
1.0 ml	80	125
1.2 ml	80	150
1.4 ml	80	175
1.6 ml	80	200
2.0 ml	80	250

Cotrols were prepared to contain the highest concentration of chloroform (0.5 ppm) and 2% Tween 80 (4.5 ppm) used in the experiment.

(iii) Scopoletin

50 mg of scopoletin crystals were weighed and dissolved in 5 ml of acetone to form a stock solution

of 10 mg/ml concentration. From this, dilutions ranging from 50-200 ppm were made using water as the diluting solvent (see section (ii)). The control was set up as for the A. afra oil.

(iv) AA4 (not identified)

50 mg of AA4 crystals were weighed and dissolved in 1 ml chloroform. To this was added 4 ml of 2% Tween 80 solution and shaken thoroughly to form a milky suspension. From this, dilutions ranging from 50 to 250 ppm were made using water as the diluting solvent.

A control containing 2 ml of 2% Tweed 80 and 3 drops of chloroform was set up.

(v) Artemisia afra aqueous extract

5 g of dry Artemisia afra leaves were powdered and boiled in water for 40 minutes. The resulting extract was filtered with cotton wool and the residue washed with hot water to make 100 ml. From this stock solution, the following dilutions were made:

1:500; 1:250; 1:125; 1:100; 1:10

(b) Setting up the test

Before adding the test drugs to the beaker containing the mosquito larvae, all the water in the beakers was gently sucked out using a Pasteur pipette. Then at least 20 ml of the drug solutions was added to each beaker and any dead or sickly larvae and pupae in

the beaker replaced with normal larvae.

The beakers were left open in a humidified room for 24 hours after which the larvae mortality was assessed. The percentage larvicidal activity was calculated from the equation.

$$\% \text{ death} = \frac{D}{T} \times 100$$

where D = Dead larvae

T = Total larvae per sample.

For each drug concentration a minimum of six determinations were done.

2.3.3 DETERMINATION OF THE LETHAL DOSE-50 (LD-50) FOR AQUEOUS *ARTEMISIA AFRA* EXTRACT

To assess the safety of using aqueous extracts of *A. afra* as a herbal medicine the lethal dose-50 was determined in rats.

10 g of dry *Artemisia afra* leaf powder was boiled for 45 minutes in water. The extract was strained through cotton wool while still hot. The filtrate was made up to 40 ml with washings from the boiled plant material and formed a stock solution.

Seven groups of eight rats each were orally fed with varying amounts of the aqueous extract as stated below.

Group 1 5 ml/kg body weight

Group 2 10 ml/kg body weight

Group 3 15 ml/kg body weight

Group 4 20 ml/kg body weight

Group 5 30 ml/kg body weight

Group 6 40 ml/kg body weight

Group 7 50 ml/kg body weight.

After feeding, the animals were allowed free access to food and water. The number of deaths after 24 and 48 hours was determined.

2.3.4 EFFECT OF AN AQUEOUS EXTRACT OF ARTEMISIA AFRA AND SOME OF ITS CONSTITUENTS ON THE BLOOD SUGAR LEVELS OF NORMOTENSIVE RABBITS

As indicated earlier (section 1.5.5.3 Part II) A. afra is a plant with diverse medicinal attributes including alleviation of hyperglycaemia and glycosuria of diabetes mellitus (Watt and Breyer-Brandwijk, 1962). However apart from the above reference on its use as a hypoglycaemic agent, which can actually be considered as a record of folklore, scientific references to the aclaimed use do not seem to be available. In fact apart from the folklore claims made for A. afra, literature has not revealed use of any other Artemisia species in the management of diabetes mellitus.

The present study therefore is aimed at discovering that property of A. afra or its constituents that justify the use of infusions of this plant as an hypoglycaemic agent.

Materials and Methods

Only leaves of A. afra were used in this study. The leaves were air-dried and finely powdered.

(a) Preparation of test drugs

(i) Aqueous extract:

10 g finely powdered A. afra dry leaves were boiled in water for 30-40 minutes. The hot extract was then filtered through Whatman filter No 1 paper and the volume made up to 100 ml with the washings of the material. This formed AW 1 which was kept in the refrigerator at 4 degrees C as stock solution to be used within one week.

(ii) Long chain fatty esters (AAG_1)

1 g of AAG 1 powder, which is a mixture of the long chain esters was weighed and triturated in 2 ml of 2% aqueous acacia gum suspension until a fine suspension was made. The suspension was then carefully poured into a measuring cylinder and made up to 100 ml with washings from the used mortar and pestle. This formed a 10 mg/ml suspension.

(b) Animals

New Zealand white rabbits of both sexes weighing 2.0-3.5 kg were used. At least six animals were used for each set of determinations. The animals had been bred at the National Public Health Laboratories under standard laboratory conditions. Prior to testing, the

rabbits were starved for not less than 18 hours but water was allowed ad libitum.

(c) Collection of blood Samples

At the end of the starvation period and before drug administration zero time blood samples were collected from each rabbit. The blood was collected from the marginal vein using a heparinised needle. The concentration of blood sugar in that sample represented the initial glycaemia for that specific rabbit.

A control group of six animals starved for up to 48 hours were used to monitor changes (if any) in blood glucose levels that may arise as a result of starvation.

To evaluate the effect of the test drugs the rabbits were divided into groups of six and treated as follows:

Group 1

The animals in this group were starved for 48 hours after which they were fed orally on 2% aqueous acacia suspension and water ad libitum. This acted as the control.

Group 2

This group of animals was fed orally with 20 ml of AW 1 solution per kilogramme body weight of the animal plus water ad libitum.

Group 3

In addition to having free access to water, animals were fed on 10 mg/kg AGG 1 in 2% acacia suspension.

Group 4

4 mg/kg chloropropamide was administered orally to this group of animals. The animals had free access to water.

Preliminary experiments had indicated tha A. afra extract and AAG 1 had a slow onset and long duration of action. On this basis chlorpropamide, an oral hypoglycaemic agent with slow onset and long duration of action was chosen as the standard drug.

Blood samples were collected after every hour for 7 hours and after 24 hours for a further 3 hours.

Intramuscular route

New Zealand white rabbits previously starved for not less than 18 hours were used. Doses of 1 mg/kg and 2 mg/kg AAG 1 suspension in 2% acacia were tested. The drugs were administered using the thigh muscle. A total of six rabbits were used for each dose level.

Due to favourable rate of absorption of the drug through the parenteral intramuscular route as compared to the oral route, the onset of action of the drugs was shorter than for the oral route. For this reason

blood samples were collected after every 30 minutes for 180 minutes.

Analysis of blood samples

The blood samples were analysed for fasting blood sugar using the modified Landgrebe and Munday method (1954) outlined in part I, section 2.3.5.

2.3.5 Effect of *Artemisia Afra* Aqueous Extract on Neuromuscular transmission

Materials and Methods

The isolated rat phrenic-nerve diaphragm preparation was set up as in part I section 2.9 and tested using the same experimental procedure and conditions.

Preparation of Drugs

An aqueous extract of *Artemisia afra* was prepared by boiling 20 g of dried powdered leaves of the plant in 50 ml of water for 40 minutes. The extract was filtered while hot and the filtrate made up to 100 ml with hot washings of the extracted plant material. Other drugs prepared were chloroquine 10 µg/ml, gallamine 20 mg/ml and succinylcholine 100 mg/ml.

Testing

0.5 ml of the above resultant *A. afra* extract was tested on the phrenic-nerve diaphragm preparation and the response recorded on a Harvard recorder

(Bioscience USA 400).

The tissue was also challenged with standard neuromuscular junction blockers, gallamine 8 mg, and succinylcholine 5 mg in the presence of the extract. The effect of chloroquine/A. afra extract combination was also investigated.

2.3.6 Effect of A. afra and its constituents on the blood pressure of anaesthetised rabbit.

Material and Methods

New Zealand white rabbits of both sexes weighing between 1.5-3.0 kg were used. The animals were anaesthetised by intravenous administration (through the marginal ear vein) of urethane 0.6 ml/100g body weight of a 25% w/v solution in water.

The trachea was surgically exposed and cannulated with a tracheal tube to ensure adequate ventilation. The femoral vein and the carotid artery were carefully exposed, isolated and cannulated.

The femoral vein was used for drug administration and to ensure that no blood clotting or bleeding occurred, the cannula was connected to a syringe containing heparinised saline.

The carotid artery cannula was used for monitoring blood pressure changes. For this reason, it was connected to a pressure transducer and the whole closed system was filled with heparinised normal saline and all air bubbles were eliminated from the

saline and all air bubbles were eliminated from the system to avoid air embolism.

Drug Preparation and administration.

Long chain esters

The powder containing mixture of long chain esters is insoluble in water or normal saline. It was therefore formulated in the form of a suspension in 2% acacia. Being a suspension the preparation was not fit for intravenous administration and for this reason it was administered intramuscularly via the thigh muscle. Doses were limited to 1 mg/kg, 1.5 mg/kg and 3 mg/kg were as preliminary results had shown that higher doses were toxic.

Aqueous A. afra extract

The aqueous A. afra extract was prepared by boiling 20 g of the dried leaves, filtering and the filtrate made to 100 ml. Volumes ranging from 0.25 ml to 1.0 ml were administered through the femoral vein.

Volumes were restricted to the above range because higher volumes had been found to cause a momentary fall in blood pressure by inducing a volume effect eg 1.5 ml of warm water induced 45% and 50% fall in systolic and diastolic pressure respectively within 2 minutes.

The intravenous drugs were administered slowly over a period of 1 minute. After drug administration both

systolic and diastolic pressures were recorded after every 15 minutes and any change in the heart rate was also noted.

Controls were also set up using the highest volume (1.0 ml water) to assess the volume effect. All readings were expressed as percentages of the baseline values.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Screening for antimalarial activity in Kenya plants

Literature review and folklore interviews with herbalists provided at least fifty species of plants that are used by various ethnic groups in Kenya for diverse ailments. These plants species come from not less than twenty five different families (Appendix 1).

The efficacy of the different plant species as antimalarials could not be ascertained as most of the written information was also a collection of folklore that had been organised in a readable form and was not based on scientific findings.

It was also very difficult to assess which of the reported plant species had the highest activity in view of the shortcomings in the diagnostic skills of the herbalists who relied mainly on treating symptoms rather than the disease. It was also evident that as far as herbalists were concerned, stomach disturbances such as constipation, bloating and nausea were some of the major symptoms of malaria.

Any agent, that induced vomiting and hence relieved the stomach discomfort was therefore considered a good antimalarial agent. To qualify as an antimalarial agent, the herb must also have a bitter taste and "the

more bitter the better". Of course this is based on the very bitter taste of the original herbal antimalarial agent quinine, whose very bitter taste was associated with its potent antimalarial activity, as well as the fact that virtually all known synthetic antimalarials have very bitter taste.

For purposes of selecting plants for antimalarial screening, a plant that was widely used, preferably by different ethnic groups was considered a good candidate.

Appendix 1 shows the list of plants that are used in Kenya for management of malaria. The herbal decoctions are made from one or more substances depending on the degree of activity. Occassionally other herbs are added to the decoction to minimise the side effects associated with the active antimalarial herb(s).

Testing for antimalarial activity in five selected Kenyan plants.

From the literature and folkloric information five plants namely, Ajuga remota Benth (LAM 1); Caesalpinia volkensii (LAM 2), Schkuhria pinnata (LAM 3), Warburgia ugandensis (LAM 4) and Artemisia afra (LAM 5) were selected using the selection criteria detailed earlier (Part Two, chapter One).

Ethanolic extracts from dried ground material was tested for in vitro activity on a chloroquine

sensitive P. falciparum strain (M-24) provided by the Kenya Medical Research Institute.

The results from the in vitro experiments showed that, of the five selected plants, Warburgia ugandensis exhibited the highest activity inducing a mean % reduction in parasitaemia and growth rate of 64.60 (3.15) and 65.10 (2.89) respectively at a dilution of 1: 80.000 (Tables 29 and 30). The next most potent herb was Ajuga remota Benth achieving a mean % reduction in parasitaemia and growth rate of 52.88 (3.12) and 53.10 (3.17) respectively at a dilution of 1: 8,000.

This concentration is ten times higher than that of Warburgia ugandensis that induced a much higher antimalarial activity. This confirms the superior antimalarial activity of Warburgia ugandensis over Ajuga remota Benth. Lower dilution of the ethanolic extracts of the above two plants were almost equipotent (Table 29 and 30). At a dilution of 1: 200 Warburgia ugandensis achieved a 100% whereas the same dilution of Ajuga remota Benth achieved 98.53 (0.38) and 96.20 (2.17) mean % reduction in parasitaemia and growth rates respectively.

Artemisia afra exhibited low antimalarial activity at low concentration (1: 8,000 dilution) but the activity picked up with increase in dose comparing favourably with that of Warburgia ugandensis and Ajuga

TABLE 29 EFFECT OF FIVE KENYAN PLANTS ON IN VITRO PLASMODIUM FALCIPARUM (PARASITAEMIA)

PLANT	DILUTION	MEAN % REDUCTION IN PARASITAEMIA	MEAN DIFFERENCE IN PARASITAEMIA	LEVEL OF SIGNIFICANCE
Ajuga	1:80,000	21.73(2.52)	0.48(0.13)	p<0.05
remota	1:8,000	52.88(3.12)	1.04(0.16)	p<0.05
Benth	1:800	77.27(3.11)	1.67(0.36)	p<0.01
	1:640	89.95(0.71)	1.90(0.39)	p<0.001
	1:320	94.53(0.31)	2.04(0.43)	p<0.001
	1:200	98.53(0.38)	2.08(0.43)	p<0.001
	1:100	99.32(0.32)	2.11(0.44)	p<0.001
Caesalpinia	1:800	13.78(2.69)	0.50(0.10)	p<0.01
volkensii	1:400	18.13(2.14)	0.68(0.13)	p<0.01
	1:200	49.27(7.18)	1.80(0.34)	p<0.001
	1:100	54.87(2.96)	2.03(0.26)	p<0.001
Schkuhria	1:8000	13.90(2.31)	0.55(0.08)	p<0.05
pinnata	1:800	24.98(2.69)	1.03(0.15)	p<0.05
	1:640	48.97(3.26)	2.18(0.42)	p<0.01
	1:320	54.57(2.90)	2.47(0.46)	p<0.001
	1:200	76.55(4.77)	3.44(0.61)	p<0.001
	1:100	94.19(2.93)	4.21(0.71)	p<0.001

TABLE 29 CONT.

Warburgia	1:8000	76.37(2.76)	2.82(0.53)	p<0.05
ugandensis	1:800	90.17(4.22)	3.65(0.63)	p<0.001
	1:640	93.50(2.68)	4.20(0.68)	p<0.001
	1:400	98.23(0.38)	4.37(0.64)	p<0.001
	1:320	93.10(2.82)	4.59(0.63)	p<0.001
	1:200	100.00(0.00)	4.31(0.58)	p<0.001
Artemisia	1:8000	15.67(2.19)	0.63(0.08)	p<0.05
afra	1:3200	43.72(4.02)	2.95(0.54)	p<0.01
	1:1600	65.42(3.30)	3.42(0.58)	p<0.01
	1:800	75.65(2.66)	3.91(0.56)	p<0.001
	1:400	88.58(1.00)	4.40(0.64)	p<0.001
	1:320	95.35(0.16)	4.41(0.65)	p<0.001
	1:200	99.43(0.33)	4.43(0.65)	p<0.001

TABLE 30 EFFECT OF FIVE KENYAN PLANTS ON IN VITRO PLASMODIUM FALCIPARUM (GROWTH RATE)

PLANT	DILUTION	MEAN % REDUCTION IN GROWTH RATE	MEAN DIFFERENCE IN GROWTH RATE	LEVEL OF SIGNIFICANCE
Ajuga	1:80,000	22.12(2.48)	1.47(0.44)	p<0.05
remota	1:8000	53.10(3.17)	3.08(0.51)	p<0.01
Benth	1.800	77.28(3.07)	4.79(1.06)	p<0.001
	1:640	90.05(0.48)	5.48(1.12)	p<0.001
	1:320	94.50(0.28)	5.77(1.20)	p<0.001
	1:200	96.20(2.17)	5.89(1.26)	p<0.001
Caesalpinia	1:800	13.37(2.76)	1.58(0.35)	p<0.05
volkensii	1:400	19.53(1.24)	2.12(0.30)	p<0.05
	1:200	48.47(7.01)	5.78(0.94)	p<0.01
	1:100	54.63(2.94)	6.43(0.44)	p<0.01
Schkuhria	1:8000	17.25(2.43)	1.22(0.16)	p<0.05
pinnata	1:800	25.18(2.79)	2.32(0.31)	p<0.05
	1:640	49.07(3.28)	4.82(0.83)	p<0.01
	1:320	54.64(2.91)	5.47(0.97)	p<0.01
	1:200	77.15(5.06)	7.64(1.27)	p<0.001
	1:100	94.19(2.95)	9.34(1.51)	p<0.001

TABLE 30 CONT.

Warburgia	1:80,000	65.10(2.89)	7.60(0.96)	p<0.01
ugandensis	1:8000	76.35(2.76)	8.95(1.11)	p<0.01
	1:800	90.10(4.29)	10.32(1.19)	p<0.001
	1:640	93.58(2.65)	10.75(1.00)	p<0.001
	1:400	98.17(0.38)	11.37(1.13)	p<0.001
Artemisia	1:8000	15.62(2.24)	1.67(0.27)	p<0.05
afra	1:1600	43.75(4.06)	4.72(0.57)	p<0.01
	1:800	64.57(3.18)	6.85(0.32)	p<0.001
	1:400	75.63(2.60)	8.03(0.25)	p<0.001
	1:320	88.20(1.50)	9.42(0.18)	p<0.001
	1:200	99.37(0.16)	10.55(0.12)	p<0.001
	1:160	99.45(0.30)	10.58(0.11)	p<0.001

FIGURE 18 MEAN % REDUCTION IN PARASITAEMIA INDUCED BY ETHANOLIC EXTRACTS OF AJUGA REMOTA (LAM 1) CAESALPINIA VOLKENSII (LAM 2) AND SCKUHRIA PINNATA (LAM 3) ON IN-VITRO P. FALCIPARUM (Isolate M_{24})

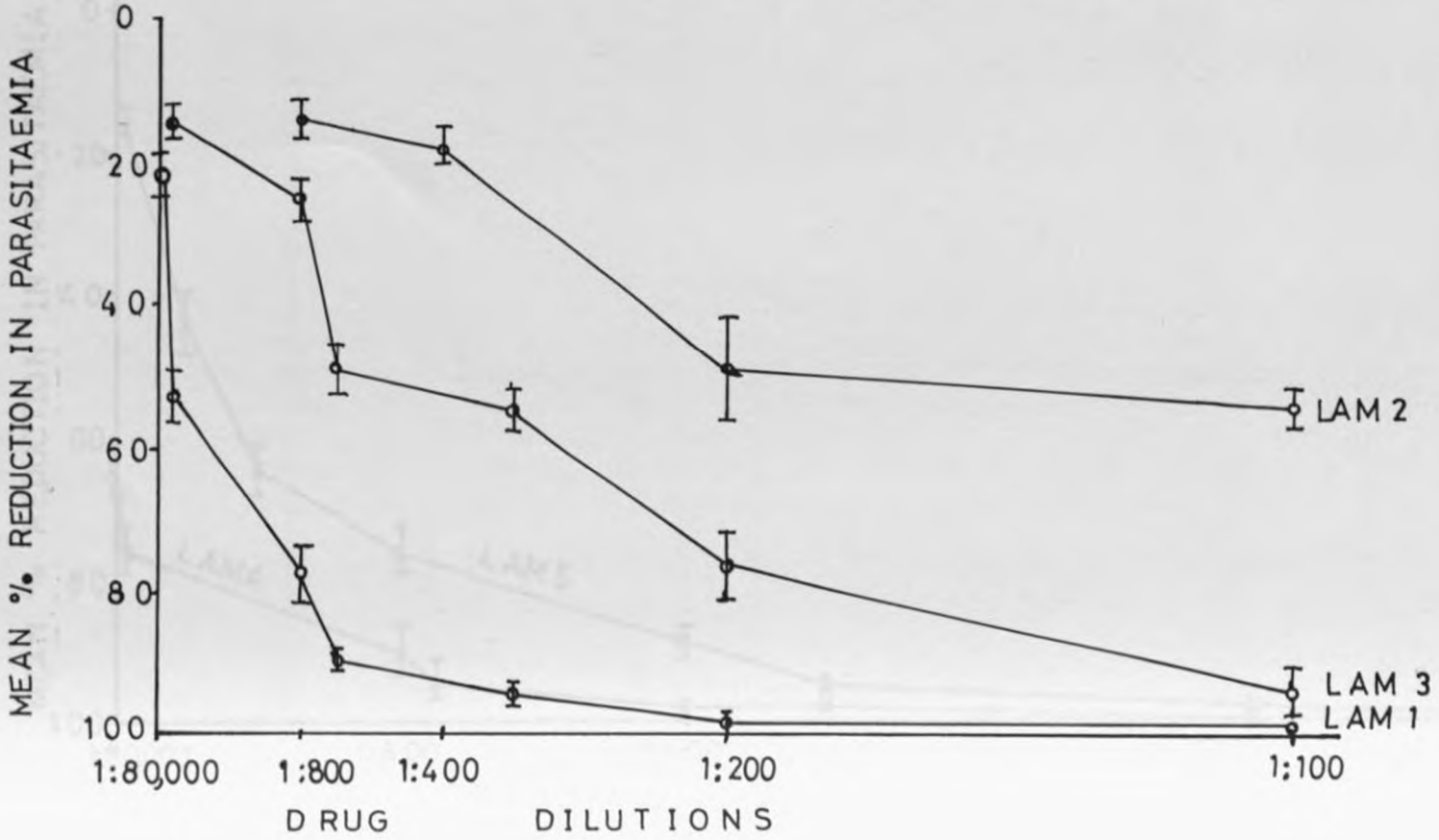


FIGURE 19 MEAN % REDUCTION IN PARASITAEMIA INDUCED BY ETHANOLIC EXTRACTS OF WARBURGIA UGANDENSIS (LAM4) AND ARTEMISIA AFRA (LAM5) ON IN VITRO P FALCIPARUM (Isolate M_{24})

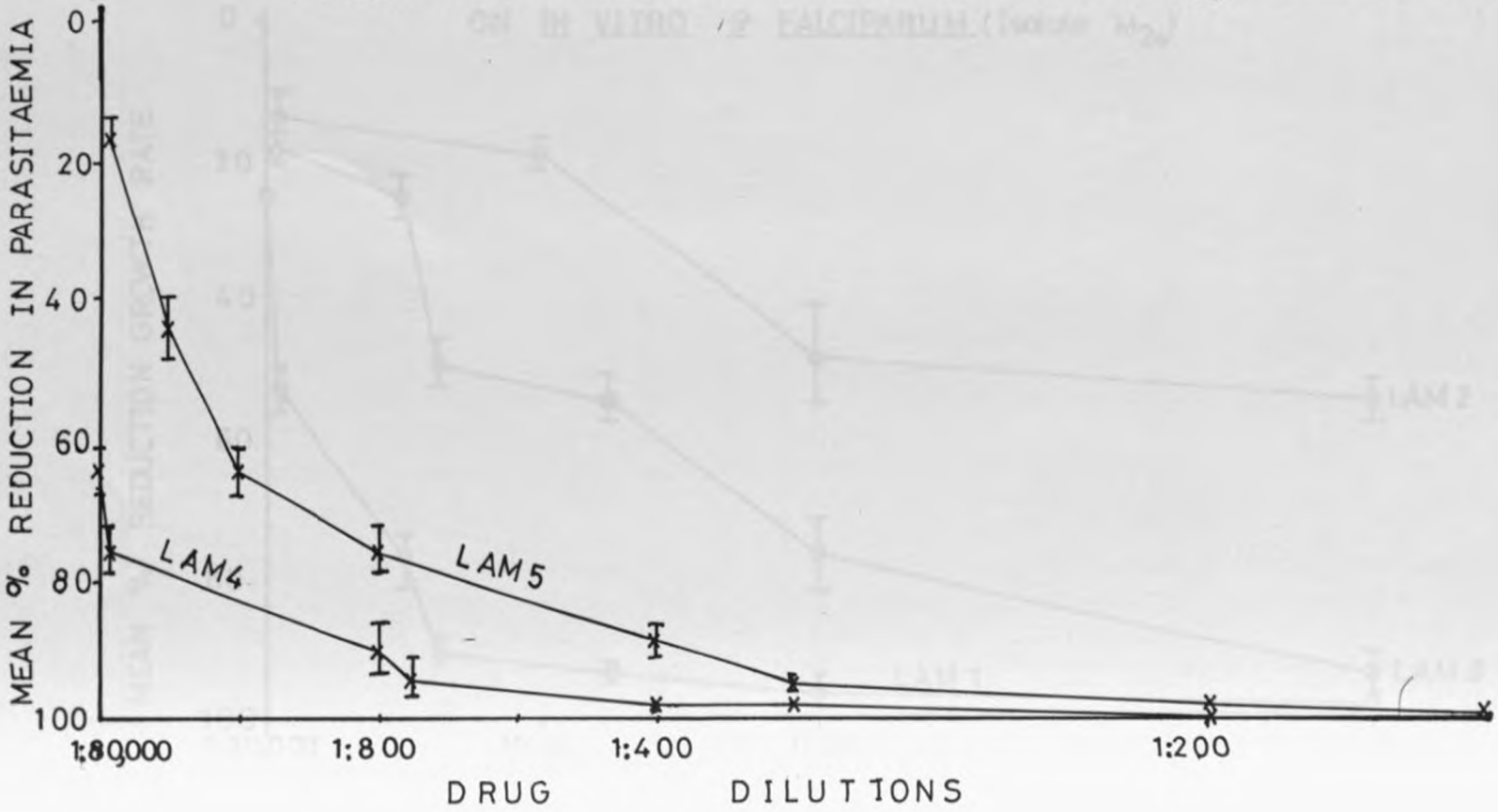


FIGURE 20 MEAN % REDUCTION IN GROWTH RATE INDUCED BY ETHANOLIC EXTRACTS OF AJUGA REMOTA (LAM 1) CAESALPINIA VOLKENSII (LAM 2) AND SCKUHRIA PINNATA (LAM 3) ON IN VITRO P. FALCIPARUM (Isolate M_{24})

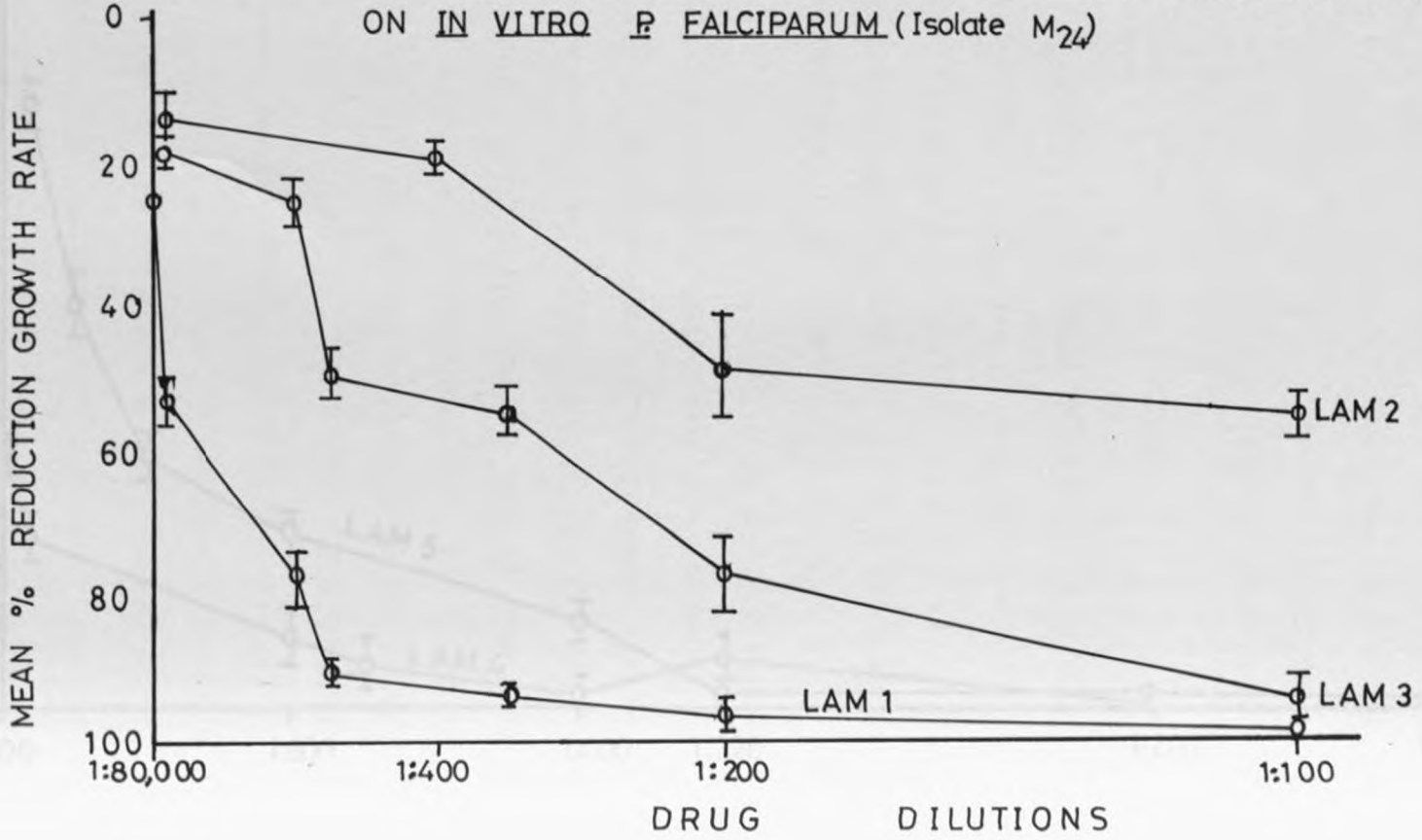
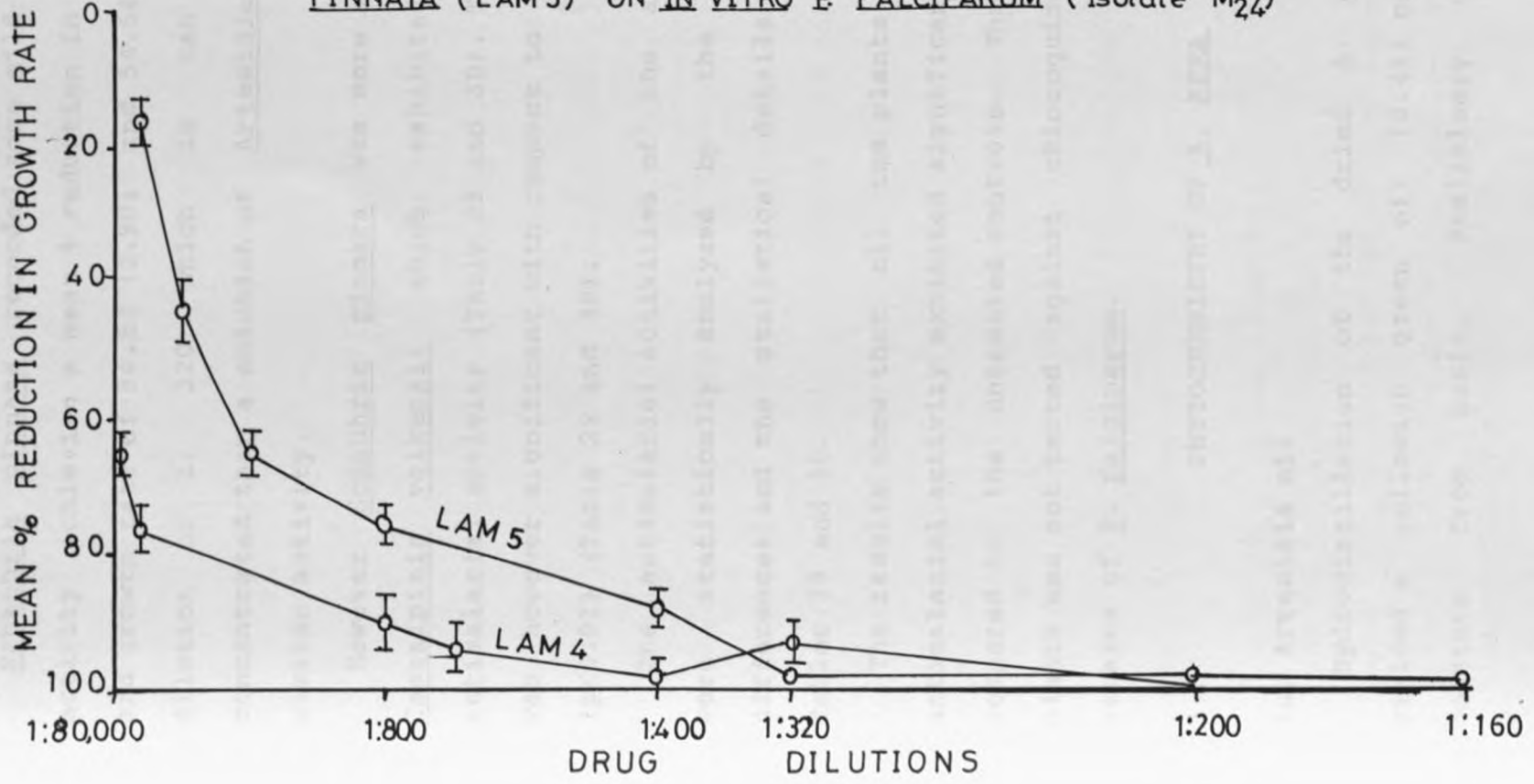


FIGURE 21 MEAN % REDUCTION IN GROWTH RATE INDUCED BY ETHANOLIC EXTRACTS OF WARBURGIA UGANDENSIS (LAM 4) AND SCKUHRIA PINNATA (LAM 5) ON IN VITRO P. FALCIPARUM (Isolate M_{24})



remota Benth.

Schkuhria pinnata recorded some mild antimalarial activity achieving a mean % reduction in parasitaemia and growth rate of 54.57 (2.90) and 54.64 (2.91) at a dilution of 1: 320 which is ten times more concentrated than a solution of Artemisia afra with similar activity.

However Schkuhria pinnata was more active than Caesalpinia volkensii which exhibited the least antimalarial activity (Table 29 and 30). This activity was however significant with respect to the control ($p<0.01$) (Table 29 and 30).

The antimalarial activities of the above plants were statistically analysed by the method of differences and the statistical details are shown on Tables 29 and 30.

The results show that all the plants screened for antimalarial activity exhibited significant effects as compared to the untreated controls. The activity of plants was not tested against chloroquine resistant strains of P. falciparum.

3.3 PHYTOCHEMISTRY OF A. AFRA

(a) Artemisia oil

Hydrodistillation of the dried A. afra leaves yielded a yellowish green oil (0.4%) calculated on moisture free basis. Preliminary gas liquid

chromatographic investigation revealed that the major constituents of the oil were camphor and 1-8 cineole as confirmed by peak enhancement method using authentic samples of camphor and 1-8 cineole.

The oil was used for biological and pharmacological investigations.

(b) Non-volatile constituents

Isolation and purification of the petroleum ether extract of the aerial parts of A. afra yielded (upon trituration with ethanol), long chain esters. The ethanol soluble fraction of the petroleum ether fraction upon column chromatography yielded 7,4'-dimethoxy-5-hydroxy flavone, D;A-Friedo-oleanan-3-one (Friedelin), alpha-amyrin, and beta-sitosterol. Except for the long chain esters, and 7,4'-dimethoxy-5-hydroxy flavone whose literature was not available, and could therefore be new compounds, the other isolated compounds had previously been isolated from other plants including other species of Artemisia.

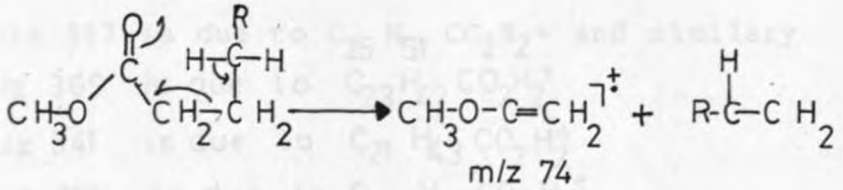
Solvent-solvent extraction of the methanol extract yielded a diethyl ether fraction (DEM) which upon column chromatography yielded a coumarin, scopoletin.

(i) Long Chain Esters (AAG_1)

The topical compound had a low melting point 73-74 degrees C and did not fluoresce under UV light suggesting lack of a conjugated system. The infra red (IR) spectral data showed peaks at 1730 cm-1 (C=O),

1465 cm-1 (C-0) stretching vibrations, and 2825 cm-1 (C-H stretch). The above data is suggestive of a saturated straight chain ester.

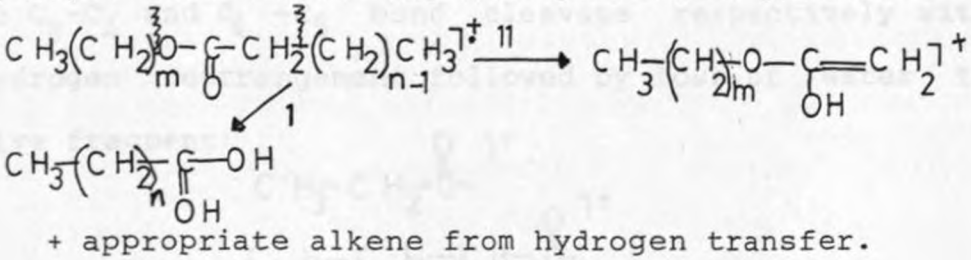
^{1}HNMR spectral data showed chemical shifts at delta 0.88 (CH_3 protons in a saturated environment; delta 2.39 ($-CH_2-C-$) and delta 4.05 ($-CH_2-O$ groups. These assignments fit well with the chemical structure of long chain esters. Electron impact (EI) mass spectrometry also suggests a long chain saturated ester. This is because mass spectrometry gave no base peak at m/z 60 or m/z 74 normally attributable to McLafferty rearrangement of free acids or methyl esters of long chain acids from C_6 to C_{26} (McLafferty, 1959). There was also no peak at m/z 88 characteristic of an ethyl ester.



m/z 74

(McLafferty arrangement of methyl esters from C_6 to C_{26})

) For esters with alkoxy groups longer than ethyl, alkyl-oxygen fission with rearrangement of the hydrogen atoms is the major fragmentation pattern ie



+ appropriate alkene from hydrogen transfer.

M = ion mass

61 = mass of (CH_3- + -C=O) group (Route 1)

14 = mass of (-CH_2-) group

74 = mass of (CH_3- + -O-$\overset{H}{\underset{|}{C}}$=$CH_2$) groups (Route II).

Therefore n and m can be found by the equation:

$$n = \frac{M-61}{14}$$

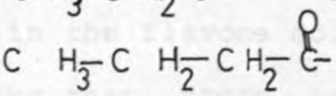
$$m = \frac{(M-74)}{14}$$

On this basis the peak m/z 397 on the mass spectrum of AAG_1 has

$$n = \frac{397-61}{14} = 24$$

m/z 397 is due to $C_{25}H_{51}CO_2H_2^+$ and similary
m/z 369 is due to $C_{23}H_{47}CO_2H_2^+$
m/z 341 is due to $C_{21}H_{43}CO_2H_2^+$
m/z 313 is due to $C_{19}H_{39}CO_2H_2^+$
No peaks associated with $\frac{M-74}{14}$
were evident on spectrum.

Peaks m/z 57 (100%) and m/z 71 could be attributed to C_3-C_4 and C_4 -C_5 bond cleavage respectively with hydrogen rearrangement followed by loss of water to give fragment:



respectively.

The appearance of two peaks at m/z 308 and m/z 336 corresponding to alkenes $C_{22}H_{44}$ and $C_{24}H_{48}$ suggest that these fragments bore the alkoxy group. This implied that the subject compound (AAG_1) was a mixture of esters of $C_{22}H_{45}OH$ and $C_{24}H_{49}OH$ and carboxylic acids $C_{19}H_{39}COOH$; $C_{21}H_{43}COOH$; $C_{23}H_{47}COOH$ and $C_{25}H_{51}COOH$ giving a possible mixture of eight esters.

However chemical ionisation mass spectrometry (CI-MS) and NMR measurements indicated that AAG_1 was a mixture of three long chain faty esters with molecular formulae;

$C_{44}H_{88}O_2$ (648) $C_{46}H_{92}O_2$ (676) $C_{48}H_{96}O_2$ (704)

The possible structural formulae were therefore deduced from the fragmentations as:

a) $C_{25}H_{51}COOR^1$ or $C_{23}H_{47}COOR^2$ (704)

b) $C_{23}H_{47}COOR^1$ or $C_{21}H_{43}COOR^2$ (676)

c) $C_{21}H_{43}COOR^1$ or $C_{19}H_{39}COOR^2$ (648)

where R^1 and R^2 are $C_{22}H_{45}$ and $C_{24}H_{49}$ respectively.

(ii) 7,4'-DIMETHOXY-5-HYDROXYFLAVONE

7,4'-dimethoxy-5-hydroxyflavone is a derivative of apigenin (21) and is structurally related to apigenin trimethyl ether (57). Due to chemical structure similarities and common molecular substituents, apigenin and apigenin trimethyl ether were selected as reference compounds in assigning the chemical shifts of the carbons in the flavone moiety in the ^{13}NMR.

Table 31 shows that there is general agreement in

the chemical shifts for the carbons in the flavone skeleton structures for the three compounds. The chemical shifts for the methoxy carbons in the test compound compare very well with those of the equivalent methoxy groups in apigenin trimethyl ether.

The mass spectrum of 7,4'-dimethoxy-5-hydroxyflavone shows a molecular ion as the base peak (M+) at m/z 298 (100%). Fisson of the two bonds beta to ring A yields two ions, a phenylacetylene ion m/z 167 (100%). Fisson of the heterocyclic ring is common with flavones in particular and flavonoids in general (Barnes and Ocolowitz, 1964).

The ^{1}HNMR spectrum for 7,4' -dimethoxy-5-hydroxyflavone shows six aromatic protons in the low field region with H-6 and H-8 appearing at delta 6.36 and delta 6.47 as interchangeable assigments. These appear as doublets due to coupling between them (J=2.7). The aromatic protons of the phenyl ring at 2' 3' 5 and 6 appear downfield between delta 6.95 and delta 7.88. The methyl protons at 7 and 4' positions are observed highfield as two singlet at delta 3.88 and delta 3.89 whereas the hydroxy proton shows a signal as a singlet at delta 12.80.

The signals for these protons were compared to those for salvigenin. Table 32 shows there is general agreement in the assigments of chemical shifts of 7,4' -dimethoxy-5-hydroxyflavone and those for salvigenin.

TABLE 31 ^{13}C-NMR Chemical shifts for 7,4'-Dimethoxy-5-Hydroxyflavone, Apigenin and Apigenin Tri-methyl ether

Carbon signments	7,4'-Dimethoxy 5-Hydroxyflavone	Apigenin	Apigenin Tri methyl ether
2	163.8	163.8	160.4
3	104.4	102.8	106.8
4	182.4	181.8	176.0
5	162.3	161.1	160.0
6	98.1	98.0	93.3
7	165.5	164.1	163.7
8	92.6	94.0	96.2
9	157.7	157.3	158.3
10	105.7	103.7	108.5
1'	123.7	121.3	123.2
2'	128.0	128.4	127.6
3'	114.5	116.0	114.4
4'	162.6	161.5	161.9
5'	114.5	116.0	114.4
6'	128.0	128.4	127.6
	*55.5 4'-OCH3		55.9 4'-OCH3
	*55.7 7-OCH3		55.5 7-OCH3

* - Interchangeable

TABLE 32 ^{1}HNMR SPECTRAL DATA FOR 7,4'-DIMETHOXY-5-HYDROXYFLAVONE AND THAT OF SALVIGEN IN $CDCl_3$ 90MHz 200MHz RESPECTIVELY

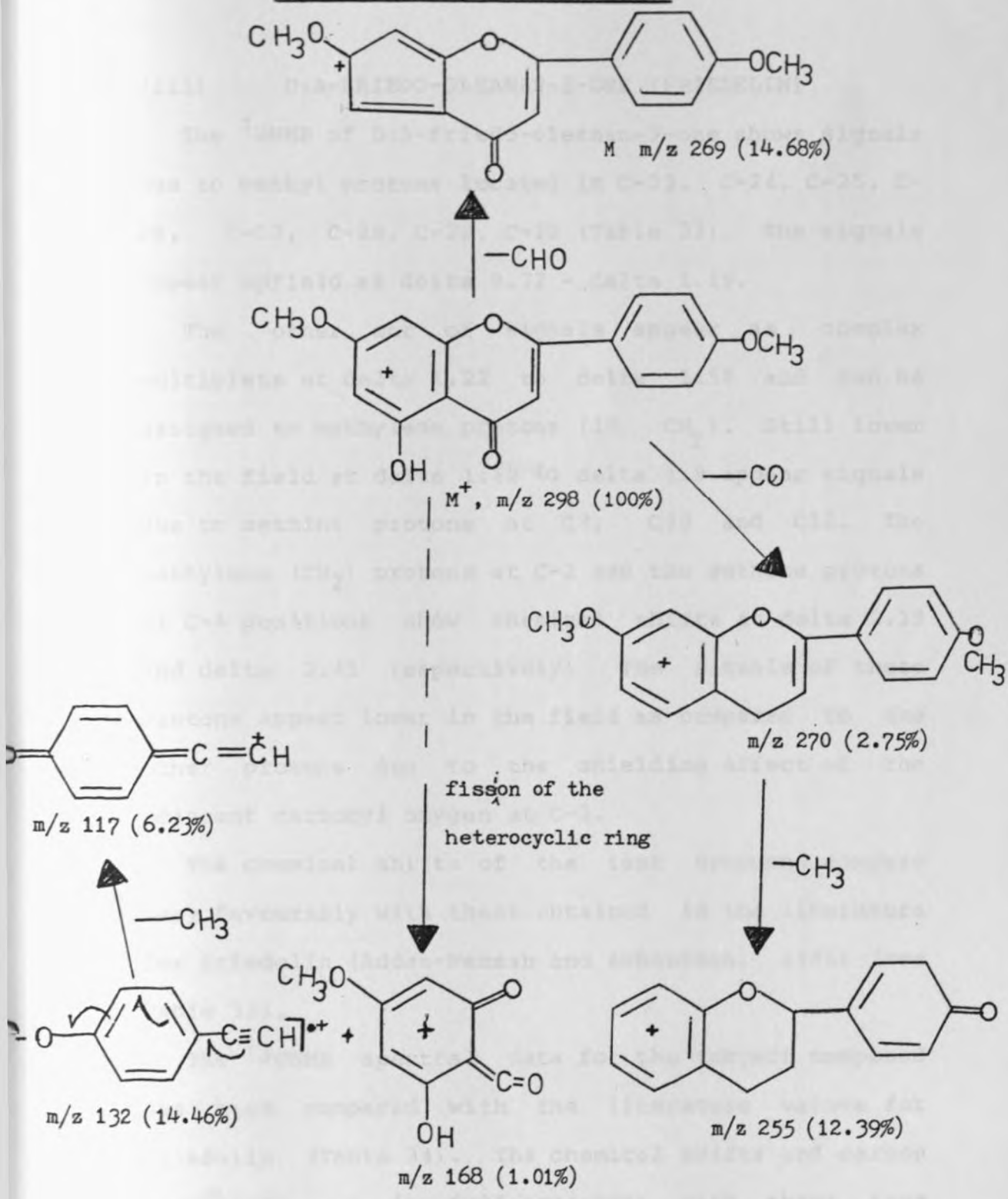
PROTON	7,4'-DIMETHOXY-5-HYDROXYFLAVONE	SALVIGENIN
H-3	6.56(s)	6.5(s)*
H-6	6.36(J=2.7)	
H-8	6.47(J=2.7)	6.52*(s)
H-2'	7.78(d)	7.83d(J=8.8)
H-3'	6.95(d)	7.00d(J=8.8)
H-5'	7.05(d)	7.00d(J=8.8)
H-6'	7.88(d)	7.83d(J=8.8)
5-OH	12.8(s)	12.8(s)
O-Me	3.89(s)	3.92
	3.88(s)	3.85
		3.73(s)

* - Interchangeable assignments within the corresponding spectra

J - Coupling constant in Hz

FIGURE 22 PROPOSED FRAGMENTATION PATTERN FOR

7,4'-DIMETHOXY-5-HYDROXY FLAVONE



(iii) D:A-FRIEDO-OLEANAN-3-ONE (FRIEDELIN)

The ^{1}HNMR of D:A-friedo-oleanan-3-one shows signals due to methyl protons located in C-23, C-24, C-25, C-26, C-27, C-28, C-29, C-30 (Table 33). The signals appear upfield at delta 0.72 - delta 1.19.

The other set of signals appear as complex multiplets at delta 1.22 to delta 1.58 and can be assigned to methylene protons (10, CH_2). Still lower in the field at delta 1.62 to delta 1.9 appear signals due to methine protons at C8, C10 and C18. The methylene (CH_2) protons at C-2 and the methine protons at C-4 positions show chemical shifts at delta 2.19 and delta 2.43 respectively. The signals of these protons appear lower in the field as compared to the other protons due to the shielding effect of the adjacent carbonyl oxygen at C-3.

The chemical shifts of the test compound compare very favourably with those obtained in the literature for friedelin (Addae-Mensah and Achenbach, 1985) (see Table 33).

The ^{13}CNMR spectral data for the subject compound has been compared with the literature values for friedelin (Table 34). The chemical shifts and carbon assignments are in full agreement with those from literature.

The presence of the six membered ring ketone was suggested by IR absorption at 1715 cm-1 in KBr. (Lit.

TABLE 33 ^{1}HNMR CHEMICAL SHIFTS FOR D:A: FRIEDO-OLEANAN 3-ONE (FRIEDELIN)

PROTON ASSIGNMENTS	FRIEDELIN EXPERIMENTAL	FRIEDELIN LITERATURE
CH3-24	0.71	0.72
CH3-23	0.87	0.86
CH3-25	0.89	0.89
CH3-29	0.97	0.96
CH3-26, 30	1.01	1.00
CH3-27	1.06	1.06
CH3-28	1.20	1.19
CH2(10)	1.21-1.60	1.22-1.58
CH(Methine) C8,C10 C18	1.63-1.79	1.62-1.76
CH2C=O	2.24-2.35	2.19-2.43
C-H-4	2.43	2.43

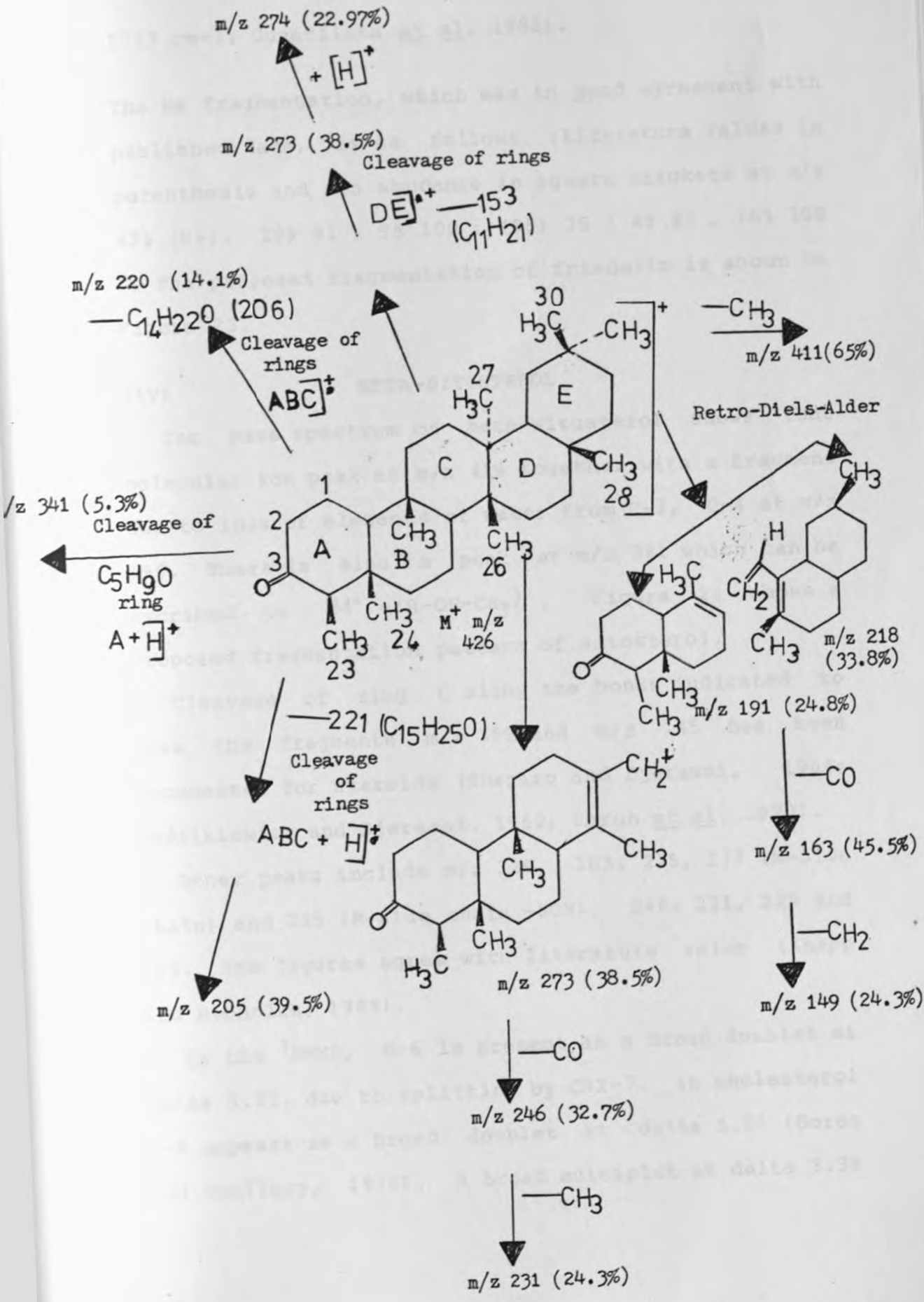
TABLE 34 ^{13}C-NMR SPECTRAL DATA FOR D:A: FRIEDO-OLEANAN-3-ONE EXPERIMENTAL AND LITERATURE

CARBON ASSIGNMENTS	FRIEDELIN EXPERIMENTAL	FRIEDELIN LITERATURE
1	22.3	22.3
2	41.5	41.5
3	213.0	212.6
4	58.2	58.3
5	42.1	42.1
6	41.3	41.4
7	18.2	18.3
8	53.1	53.2
9	37.4	37.5
10	29.5	29.6
11	35.6	35.7
12	30.5	30.6
13	38.3	38.4
14	39.7	39.8
15	37.4	37.5
16	36.0	36.1
17	30.0	29.7
18	42.8	43.0
19	35.4	35.4
20	28.1	28.2
21	32.8	32.9
22	39.2	39.3
23	6.8	6.8

TABLE 34 CONT.

24	14.6	14.7
25	17.9	18.0
26	18.6	18.6
27	20.3	20.3
28	32.1	32.1
29	31.8	31.8
30	35.0	35.0

FIGURE 23 PROPOSED FRAGMENTATION PATTERN FOR D:A: FRIEDO-OLEANAN-3-ONE (FRIEDELIN)



1713 cm-1; Gunatilaka et al, 1982).

The Ms fragmentation, which was in good agreement with published data, is as follows (literature values in parenthesis and ion abudance in square brackets at m/z 426 (M+), 109 91 , 95 100 ; (95) 75) 69 83 , (69 100). The proposed fragmentation of friedelin is shown on Figure 23.

(iv) BETA-SITOSTEROL

The mass spectrum of beta-sitosterol shows that molecular ion peak at m/z 414 together with a fragment due to loss of elements of water from C-3, C-4 at m/z 396. There is also a peak at m/z 381 which can be ascribed to $M^+ - (H-OH-CH_3)$. Figure 24 shows a proposed fragmentation pattern of sitosterol.

Cleavage of ring C along the bonds indicated to give the fragments m/z 159 and m/z 145 has been documented for steroids (Shapiro and Djerassi, 1964; Budzikiewicz and Djerassi, 1962; Unruh et al, 1970).

Other peaks include m/z 329, 303, 275, 173 (M-side chain) and 255 (M-side chain -HOH), 246, 231, 229 and 213. The figures agree with literature value (Knapp and Nicholas, 1969).

In the ^{1}HNMR, H-6 is present as a broad doublet at delta 5.35, due to splitting by CH2-7. In cholesterol H-6 appears as a broad doublet at delta 5.30 (Gorog and Szollosy, 1978). A broad multiplet at delta 3.36

TABLE 35 1-HNMR CHEMICAL SHIFT FOR BETA-SITOSTEROL

Proton	Beta-Sitosterol Experimental	Beta-Sitosterol Literature d-value
CH3-10	0.68	0.68
3CH3 (9H)	0.78-0.87	0.81 d
		0.83 d 2CH3
		0.84 t
CH3-25	0.94(d)	0.93 d
	J =	J =
CH3-19	1.01 (s)	1.01 (s)
(CH2)n	1.00 - 2.31	1.00 - 2.30
H - 3	3.36	3.54
H - 6	5.35 d	5.35

TABLE 36 13-CNMR SHIFTS FOR SITOSTEROL

Carbon	β-Sitosterol Experimental	β-Sitosterol Literature
1	37.3	37.3
2	31.8	31.7
3	71.9	71.7
4	42.4*	42.5*
5	140.8	140.6
6	121.7	121.7
7	31.8	31.8*
8	32.0	31.9*
9	50.3	50.1
10	36.6	36.4
11	21.1	21.4
12	39.9	39.7
13	42.4*	42.2*
14	56.9	56.8
15	24.3	24.3
16	28.2	28.1
17	56.2	56.0
18	12.0	12.3*
19	19.8	19.7
20	36.2	36.3
21	18.8	18.9
22	34.1	33.8
23	26.4	26.5
24	46.0	45.7

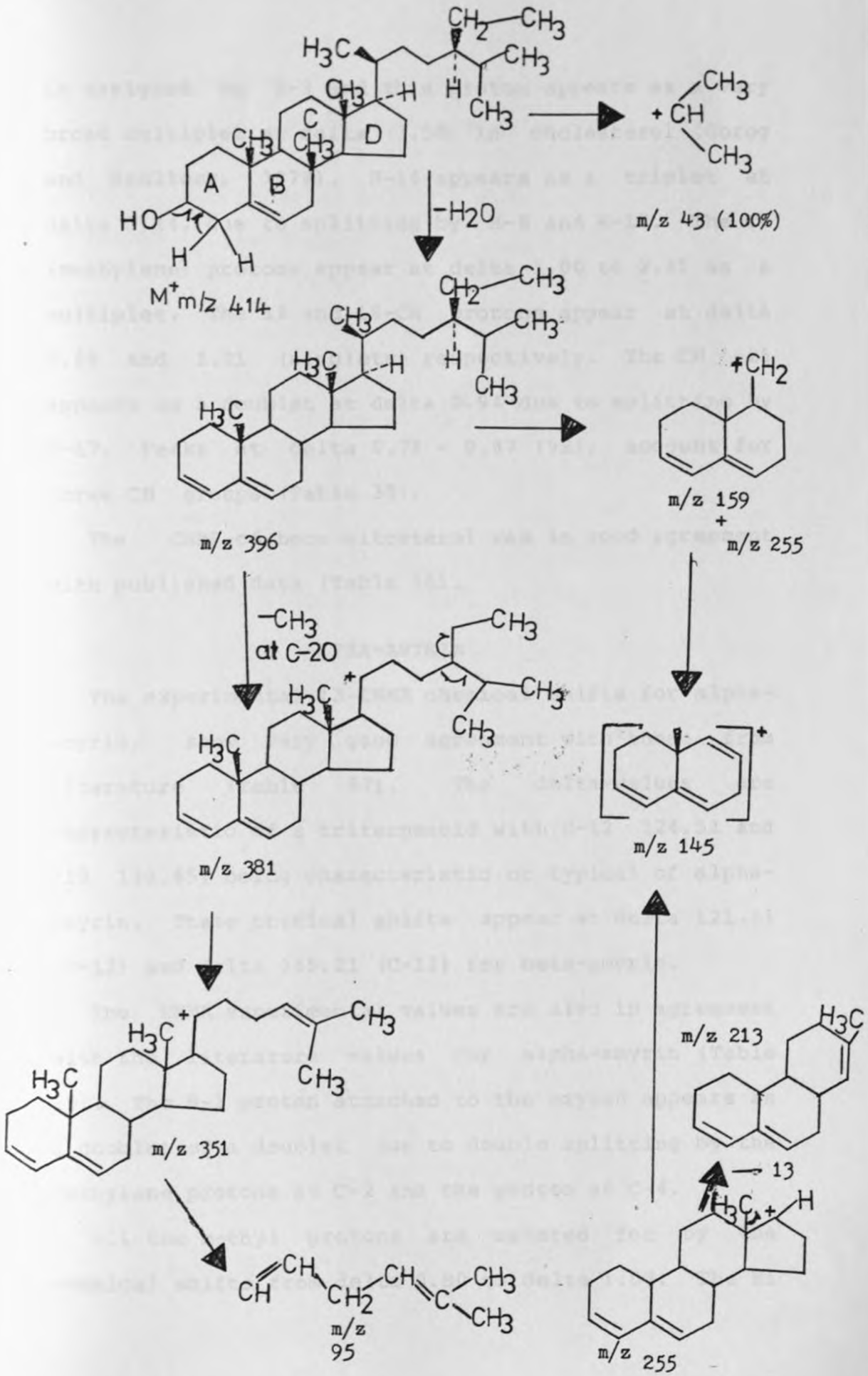
TABLE 36 CONT.

25	29.4	29.3
26	19.4	19.5**
27	19.1	19.0**
28	23.2	23.3
29	11.9	12.1

Characteristic peaks of Beta-sitosterol are d 71.9 (C-3), d 121.7 (C-6) and d 140.8 (C-5). Peaks 29.7 unaccounted for.

** or * - Interchangeable

FIGURE 24 PROPOSED FRAGMENTATION PATTERN FOR BETA-SITOSTEROL



is assigned to H-3 and this proton appears as a very broad multiplet at delta 3.50 in cholesterol (Gorog and Szollosy, 1978). H-14 appears as a triplet at delta 3.14, due to splitting by 'H-8 and H-15. The CH (methylene) protons appear at delta 1.00 to 2.31 as a multiplet. The 18 and 19-CH protons appear at delta 0.68 and 1.01 (singlets) respectively. The CH -25 appears as a doublet at delta 0.94 due to splitting by H-17. Peaks at delta 0.78 - 0.87 (9H), account for three CH groups (Table 35).

The CNMR of beta-sitosterol was in good agreement with published data (Table 36).

ALPHA-AMYRIN

The experimental 13-CNMR chemical shifts for alpha-amyrin, show very good agreement with those from literature (Table 37). The delta-values are characteristic of a triterpenoid with C-12 124.51 and C13 139.65; being characteristic or typical of alpha-amyrin. These chemical shifts appear at delta 121.83 (C-12) and delta 145.21 (C-13) for beta-amyrin.

The HNMR experimental values are also in agreement with the literature values for alpha-amyrin (Table 38). The H-3 proton attached to the oxygen appears as a doublet of a doublet due to double splitting by the methylene protons at C-2 and the proton at C-4.

All the methyl protons are catered for by the chemical shifts from delta 0.80 to delta 1.07. The EI

TABLE 37 ^{13}CNMR CHEMICAL SHIFTS FOR ALPHA-AMYRIN
EXPERIMENTAL AND LITERATURE VALUES

CARBON ASSIGNMENTS	CHEMICAL SHIFTS(d) EXPERIMENTAL	LITERATURE
1	38.92	38.87
2	27.35	27.34
3	79.09+	79.08
4	38.84	38.82
5	55.33	55.28
6	18.44	18.41
7	33.07	33.01
8	40.14	40.08
9	47.83	47.79
10	36.99	36.97
11	23.42	23.42
12	124.51	124.50
13	139.65+	139.64
14	42.19	42.15
15	26.73	26.68
16	28.16	28.16
17	33.79	33.80
18	59.20	59.15
19	39.73	39.66
21	31.30	31.30
22	41.60	41.58
23	28.19	28.16
24	15.62*	15.69

TABLE 37 CONT.

25	15.68*	15.69
26	16.95	16.91
27	23.31	23.31
28	28.76	28.78
29	17.46	17.49
30	21.36	21.40

+ Refers to peaks typical of alpha-amyrin

* Interchangeable peaks

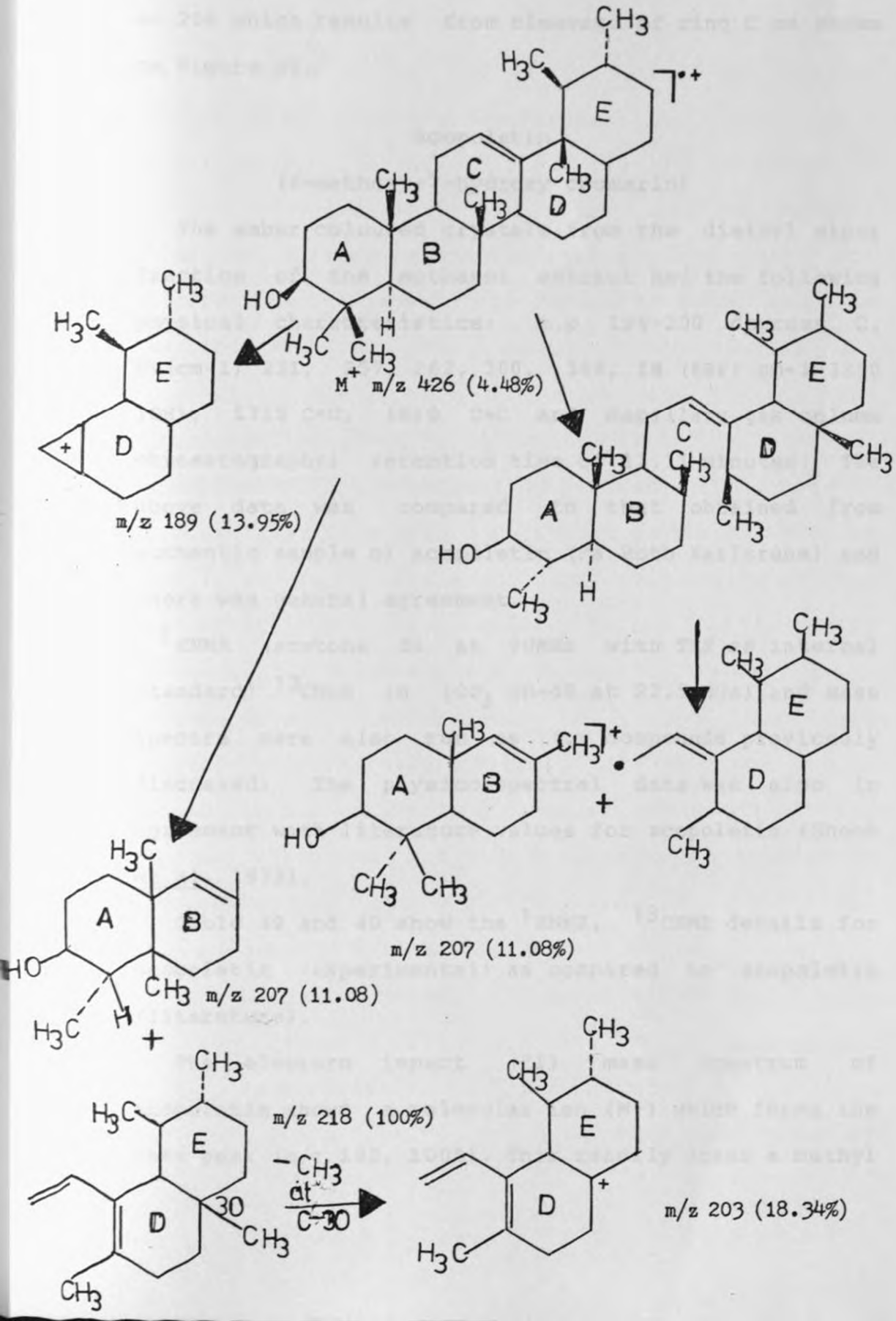
Two very minor peaks at d 121.83 and d 145.21 Characteristic of alpha-amyrin were discernible in the 13-CNMR and are characteristic of C-12 and C-13 of alpha-amyrin respectively.

A strong peak at d 29.68 could not be accounted for.

TABLE 38 ^{1}HNMR CHEMICAL SHIFTS (d VALUES) FOR ALPHA-AMYRIN

PROTON ASSIGNMENT	EXPERIMENTAL VALUE	LITERATURE VALUE
CH3-24		
CH3-29	0.80(s) & 0.87(d,J=6.5)	0.80 two singlets
CH3-28		superimposed on a
		doublet J=7
CH3-30	0.92(d J=4.1	0.91(d,J=7)
CH3-25	0.96 (s)	0.96 (s)
CH3-23	1.00 two singlets	1.00
CH3-26	1.01 fused together	1.01
CH3-27	1.07	1.08
(CH2)n & Methine	1.12 (2H)	
(CH2)	1.26-1.61 (18H)	1.1-2.2
	1.78-1.97 (3H)	
H-3	3.14-3.48	3.22
	(dd J1=8)	(dd J1=10)
	J2=7	J2=6
H-12	5.12(dd J1=3.8)	5.13(dd)
	J2=3.5	(J1-J2=3.5)

FIGURE 25 SUGGESTED FRAGMENTATION PATTERN FOR ALPHA-AMYRIN



mass spectral data shows a molecular peak (M+) at m/z 426 (4.48%). The base peak m/z 218 (100%) comes from m+ 208 which results from cleavage of ring C as shown on Figure 25.

Scopoletin

(6-methoxy-7-hydroxy coumarin)

The amber coloured crystals from the diethyl ether fraction of the methanol extract had the following physical characteristics: m.p 198-200 degrees C, UV(cm-1) 231, 257, 262, 300, 348, IR (KBr) cm-1 3350 (OH), 1715 C=O, 1610 C=C and capillary gas column chromatography; retention time of 11.11 minutes. The above data was compared to that obtained from authentic sample of scopoletin (Fa Roth Karlsruhe) and there was general agreement.

^{1}HNMR (acetone d6 at 90MHz with TMS as internal standard) ^{13}CNMR in (CD_3 OD-d6 at 22.5 MHz) and mass spectra were also run as for compounds previously discussed. The physico-spectral data was also in agreement with literature values for scopoletin (Shoeb et al, 1972).

Table 39 and 40 show the ^{1}HNMR, ^{13}CNMR details for scopoletin (experimental) as compared to scopoletin (literature).

The electorn impact (EI) mass spectrum of scopoletin shows a molecular ion (M+) which forms the base peak (m/z 192, 100%). This readily loses a methyl

fragment to give m/z 177 (64.6%). The molecular ion also loses CO to give m/z 164 (28.8%). The loss of CO from the molecular ion of scopoletin is either from the carbonyl group of the pyrone ring or from the hydroxy group. The m/z 164 fragment readily loses a methyl fragment to yield m/z 149 (52.5%). As predicted (Barnes and Occolowitz, 1963) the loss of methoxy group by fission of the phenyl-oxygen bond does not readily occur. However the remaining oxygen atoms in the ion m/z 149 are removed as CO (Barnes and Occolowitz, 1964) to give m/z 121 and finally the phenyl radical ion $C_6H_6^+$ at m/z 77 (5.6%).

Basically the fragmentation pattern of scopoletin follows that of simple substituted coumarins such as umbelliferone, herniarin and dimethylaesculetin (Barnes and Occolowitz, 1964). Figure 26 illustrates the suggested fragmentation pattern for scopoletin.

^{1}HNMR data for scopoletin in acetone shows two singlets at delta 6.80 and delta 7.19 which are due to single signals of the lone protons at positions 5 and 8. The proton at position 4 appears as a doublet of the doublet at delta 7.90 (J' = 9.8, J'' = 0.25) due to long range coupling with H-5 and H-3.

The signal at delta 3.90 (s) is due to the 3H from CH_3O at position 6 (lit delta 3.93 $CDCl_3$, Shoeb et al, 1973). H-3 appears as a doublet at delta 6.17 (J = 9.4) due to splitting by H-4. The ^{13}CNMR spectral

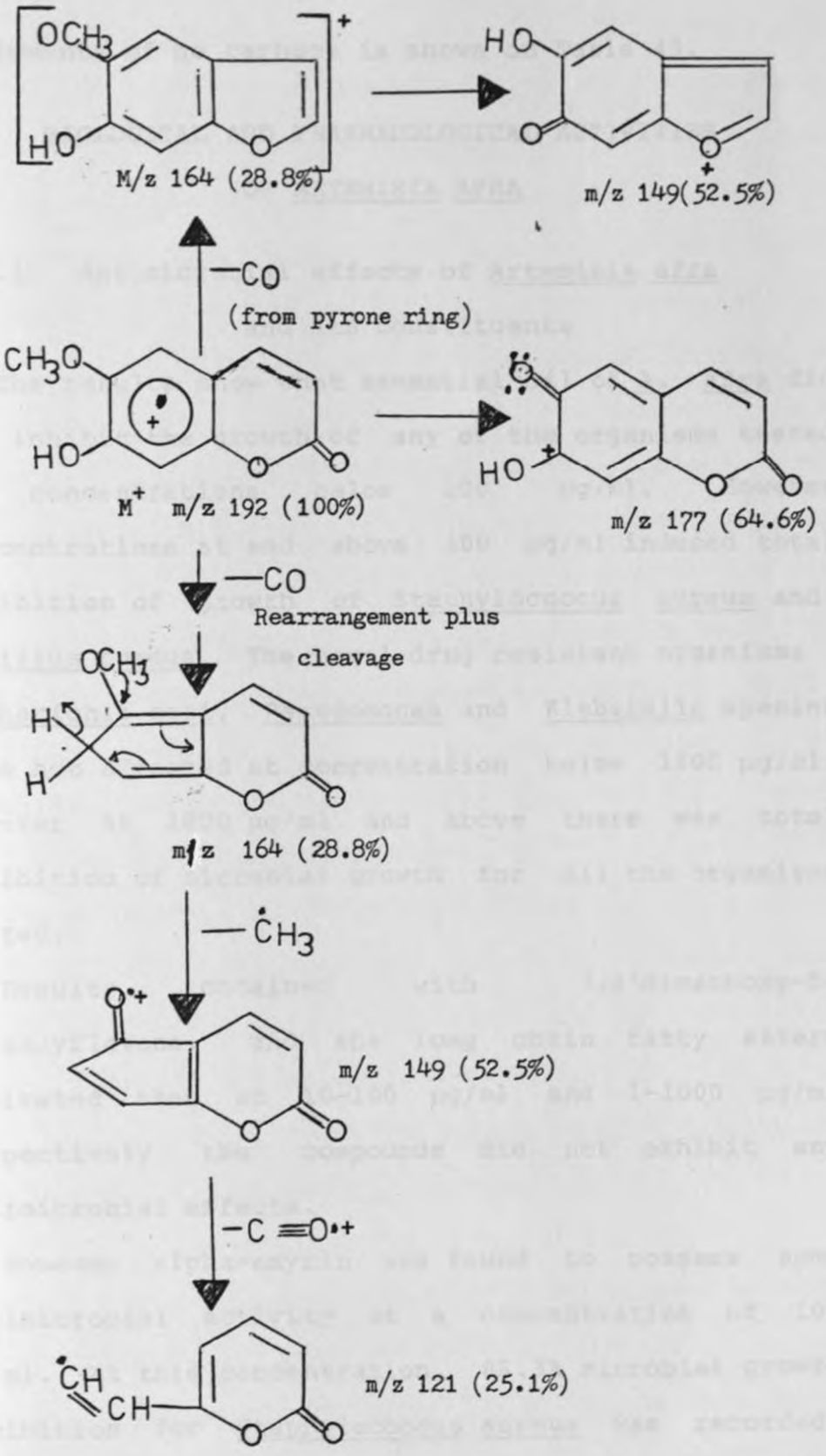
TABLE 39 ^{1}HNMR SPECTRAL DATA (d-VALUES) FOR SCOPOLETIN (EXPERIMENTAL AND LITERATURE VALUES)

PROTON ASSIGNMENTS	SCOPOLETIN EXPERIMENTAL (Acetone d6) (d value)	SCOPOLETIN LITERATURE (CDCl3) (d value) (Shoeb et al, 1973)
H-3	6.17(d) J=9.4	6.28(d) (J=9.5)
H-5	6.80(s)	6.85(s)
H-8	7.19(s)	6.92(s)
H-4	7.78-7.90(dd)(J=9.8,J2=0.25)	7.61(d)(J=9.5) H-4 long range couple to H-5 in addition to coupling to H-3
O-CH3	3.9	3.93

TABLE 40 ^{13}CNMR SPECTRAL DATA FOR SCOPOLETIN

CARBON ASSIGNMENT	CHEMICAL SHIFTS (d-VALUES)
O-CH3	56.94
C-8	104.0
C-5	110.2
C-6	153.0
C-7	112.7
C-3	146.0
C-2	164.0
C-4	147.1

FIGURE 26 SUGGESTED FRAGMENTATION PATTERN FOR SCOPOLETIN



assigments of he carbons is shown on Table 40.

3.4 BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES OF ARTEMISIA AFRA

3.4.1 Antimicrobial effects of Artemisia afra and its constituents

The results show that essential oil of A. afra did not inhibit the growth of any of the organisms tested at concentrations below 100 µg/ml. However concentrations at and above 400 µg/ml induced total inhibition of growth of Staphylococcus aureus and Bacillus cereus. The usual drug resistant organisms Escherichia coli, Pseudomonas and Klebsiella species were not affected at concentration below 1800 µg/ml. However at 1800 µg/ml and above there was total inhibition of microbial growth for all the organisms tested.

Results obtained with 7,4'dimethoxy-5-hydroxyflavone, and the long chain fatty esters indicated that at 10-100 µg/ml and 1-1000 µg/ml respectively the compounds did not exhibit any antimicrobial effects.

However alpha-amyrin was found to possess some antimicrobial activity at a concentration of 100 µg/ml. At this concentration, 83.3% microbial growth inhibition for Staphylococcus aureus was recorded. These percentage growth inhibitions were calculated

with respect to the standard antibiotic (streptomycin sulphate 10 µg/ml) and since the parameter was diameter of the zone of inhibition, a drug inducing a bigger zone of inhibition than the standard was considered to have more than 100% inhibition. On this basis, alpha-amyrin showed 116.7% growth inhibition at 500 µg/ml. When tested on Bacillus cereus alpha-amyrin was found to induce 60% and 78% microbial inhibition at 100 µg/ml and 500 µg/ml concentrations respectively. However at 1000 µg/ml concentration the antimicrobial activity of alpha-amyrin was found to decrease to 33.3% for Staphylococcus aureus and 10% for Bacillus cereus. No simple explanation could be given for this unusual phenomenon. However it is possible that at this high concentration, the solubility and hence diffusion of drug to the growth regions is considerably reduced.

The ethanol soluble fraction of the petroleum ether extract of A. afra was also found to possess some antimicrobial activity. At 50, 100 and 1000 µg/ml concentrations, antimicrobial activities of 16.7, 33.4 and 83.3% were recorded for Staphylococcus aureus and 10, 20 and 40% for Bacillus cereus.

Most clinically useful antibiotics are active at 10 µg/ml. A pure compound which is not active at 100 µg/ml is usually not a serious candidate as an antimicrobial agent (Mitschler et al, 1972), unless it

TABLE 41 THE ANTIMICROBIAL ACTIVITY OF SOME NON-VOLATILE PRINCIPLES OF ARTEMISIA AFRA

DRUG	CONCENTRATION in µg/ml	ORGANISMS TESTED AND ZONES OF INHIBITION WITH RESPECT TO STANDARD		
		Staph aureus	Bacillus cereus	Positive control
7,4'Dimethoxy-	10	0	0	0
5-hydroxyflavone	50	0	0	0
	100	0	0	0
*alpha-amyrin	1	0	0	0
	10	0	0	0
	100	0.5(83.3)	0.6(60)	0
	500	0.7(116.7)	0.78(78)	0
	1000	0.2(33.3)	0.1(10)	0
Long chain	1	0	0	0
fatty esters	10	0	0	0
	100	0	0	0
	500	0	0	0
	1000	0	0	0
Petroleum	10	0	0	0
ether extract	50	0.1(16.7)	0.1(10)	0
(Ethanol	100	0.2(33.4)	0.2(20)	0
soluble fraction)	1000	0.5(83.3)	0.4(40)	0
Streptomycin	10	0.6	1.0	0
sulphate 10ug/ml	10	(100)	(100%)	

has special pharmacological or biopharmaceutical properties.

Activity in plant extract is tested up to 1000 µg/ml concentration and very weak antimicrobial agents are unlikely to be tested within this concentration range. Plant extracts active at 100 µg/ml are considered of scientific interest as they suggest good potency (Mitschler et al, 1972).

Based on the above information then, the volatile and non-volatile constituenta of A. afra tested, that is A. afra essential oil, compound 7,4'-dimethoxy-5-hydroxyflavone and long chain esters had no antimicrobial activity. Alpha-amyrin was considered antimicrobial against Staphylococcus aureus and Bacillus cereus. Also the ethanol soluble fraction of the petroleum ether was considered fairly active.

3.4.2 THE MOSQUITO LARVICIDAL ACTIVITY OF THE CONSTITUENTS OF ARTEMISIA AFRA

Table 42 shows the larvicidal activity of constituents of A. afra. Figure 27 shows the plot of % mortality versus concentration while figure a & b show the respective probit versus log concentration graphs.

Comparison of the probit mortality versus log concentration was used to determine the LD-25, LD-50, LD-75 and LD-95. This information appears on Table 43. AA_4 (unidentified) was the most potent with an LD-

50 of 51.89 ppm as compared to 75.00 ppm 83.14 ppm for long chain fatty esters and artemisia oil respectively. Scopoletin was the least potent with an LD-50 of 221.5 ppm and never attained a 100% kill even at a concentration of 250 ppm. At high concentrations and Artemisia oil were equipotent with an LD-95 of 162.2 ppm for both, while that of the long chain esters was 186.5 ppm.

Comparison of the probits of long chain fatty esters and AA_4 by the method of differences showed that overall, there was a significant difference between the activities of the two substances at concentrations below 150 ppm with a $p<0.02$.

Substances can effect larvicidal activity by either being absorbed through the exoskeleton of the mosquito larvae or by being ingested by larvae as they feed on the water and food poisoned by the test substances. The route of entry is to a greater extent governed by polarity of the test compound. The non-polar substances eg volatile oil are more likely to pass through the exoskeleton and exert their larvicidal effects. The cuticle of the larvae and most insect exoskeleton is known to contain lipids and long chain hydrocarbons (Howard et al, 1988).

This may help to explain why the essential oil, the long chain fatty esters and AA_4 (unknown but with

a low melting point and insoluble in polar solvents) had higher larvicidal activity than the relatively polar coumarin, scopoletin.

There was no mosquito larvicidal activity detected with aqueous A. afra extract equivalent to concentration of up to 500 ppm. It therefore seems that the lipophilicity of the test substances is an important factor in determining the larvicidal activity. It has been demonstrated (Hassanali and Lwande, 1989) that introduction of polar groups eg hydroxy groups in a quinone nucleus decreases the larvicidal activity. The minimal activity detected with scopoletin is probably due to the presence of the hydroxy and methoxy groups in the coumarin nucleus.

The commercial mosquito larvicides eg malathion, chlorthion, diazinon (organophosphates), DDT, dieldrin, lindane (organochlorides) are in the concentration range of 0.01-0.23 ppm while pyrethrins at a concentration of 0.14 ppm attain a 50% kill (Pampane, 1969). Other miscellaneous mosquito larvicides are used in higher concentrations eg mineral oils (Kerosine, crude oil, diesel etc 1-2%). Paris green (a complex of copper metarsenite, arsenious oxide and copper acetate) is also used in the same concentration range of 1-2%.

Essential oils are known to possess potent mosquito larvicidal activity (Mwangi, 1989; Chavan et al,

TABLE 42 LARVICIDAL ACTIVITY OF CONSTITUENTS OF ARTEMISIA AFRA

MEAN % MORTALITY n = 6 mean (SEM)

DRUG conc ppm	25	50	75	100	125	150	175	200	250
Long chain esters	0.00	22.9 (2.61)	62.9 (4.20)	81.3 (2.40)	78.8 (1.60)	85.4 (2.10)	93.3 (1.50)	96.3 (1.00)	97.9 (0.70)
AA_4 (unidentified)	15.0 (2.10)	52.3 (2.70)	70.9 (2.90)	79.5 (2.00)	89.5 (1.40)	93.2 (3.00)	94.5 (1.60)	97.7 (1.00)	100 (0.00)
SCOPOLETIN	0.00 (0.00)	0.00 (0.00)	1.6 (0.90)	6.7 (1.40)	14.6 (1.90)	18.8 (3.10)	32.9 (3.10)	35.8 (3.60)	
ARTEMISIA OIL	0.00 (0.00)	11.5 (1.30)	33.3 (3.00)	50.6 (2.70)	85.6 (3.80)	92.5 (2.30)	97.5 (1.10)	100 (0.00)	

AQUOUS EXTRACT - No activity was detected with concentrations ranging from 100 - 500ppm

TABLE 43 LARVICIDAL ACTIVITY OF CONSTITUENTS OF ARTEMISIA AFRA

(LETHAL DOSES)

	LETHAL DOSES IN PPM			
DRUG	LD-25	LD-50	LD-75	LD-95
Long chain esters (AAG1)	50.22	75.00	118.4	186.5
Unidentified (AA4)	32.36	51.89	81.28	162.2
Scopoletin	151.6	221.5	250	250
Artemisia oil	61.65	83.14	105.9	162.2

FIGURE 27 MOSQUITO LARVICIDAL ACTIVITY OF SOME *A. AFRA* CONSTITUENTS

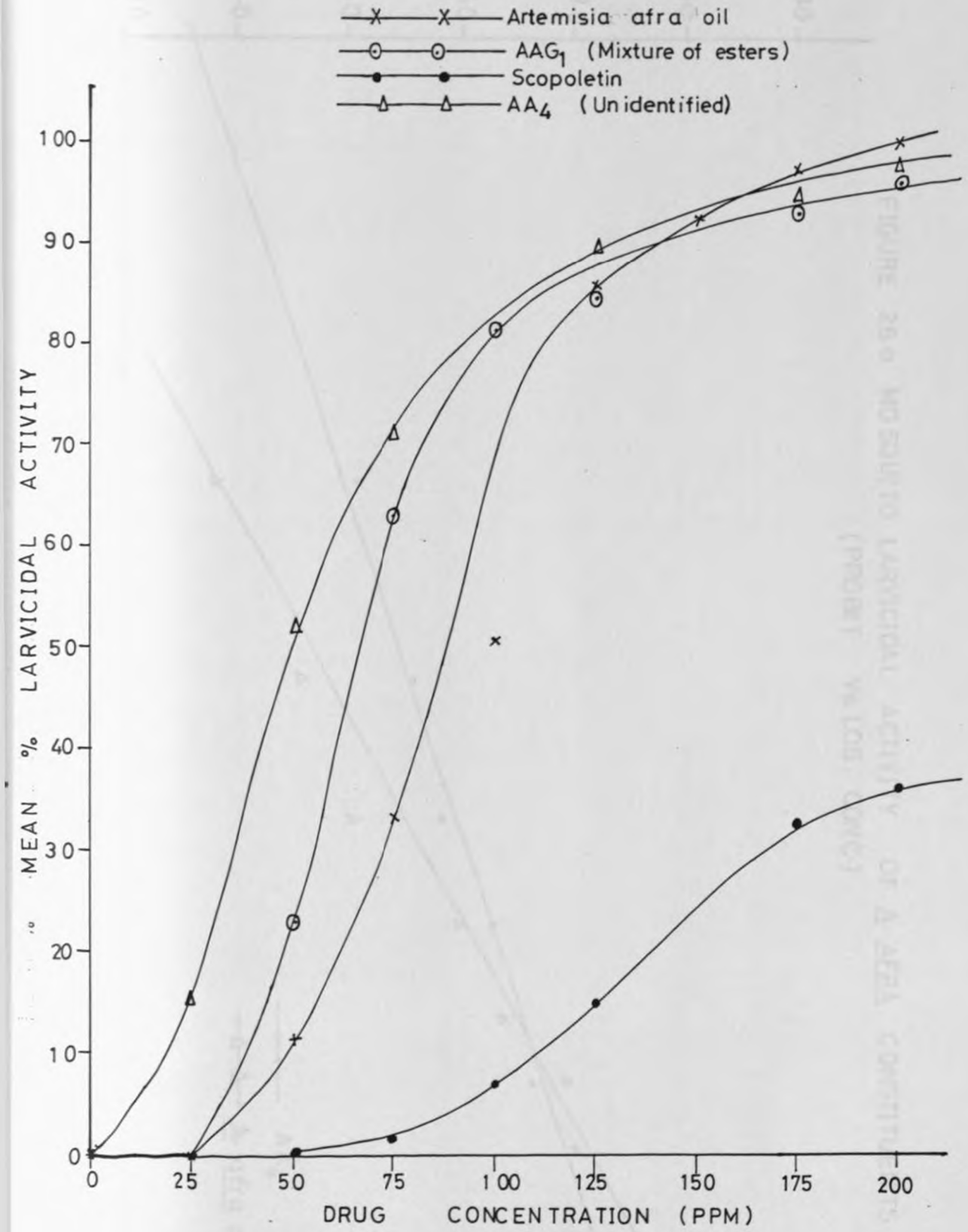


FIGURE 28 a MOSQUITO LARVICIDAL ACTIVITY OF A. AFRA CONSTITUENTS
(PROBIT Vs. LOG. CONC.)

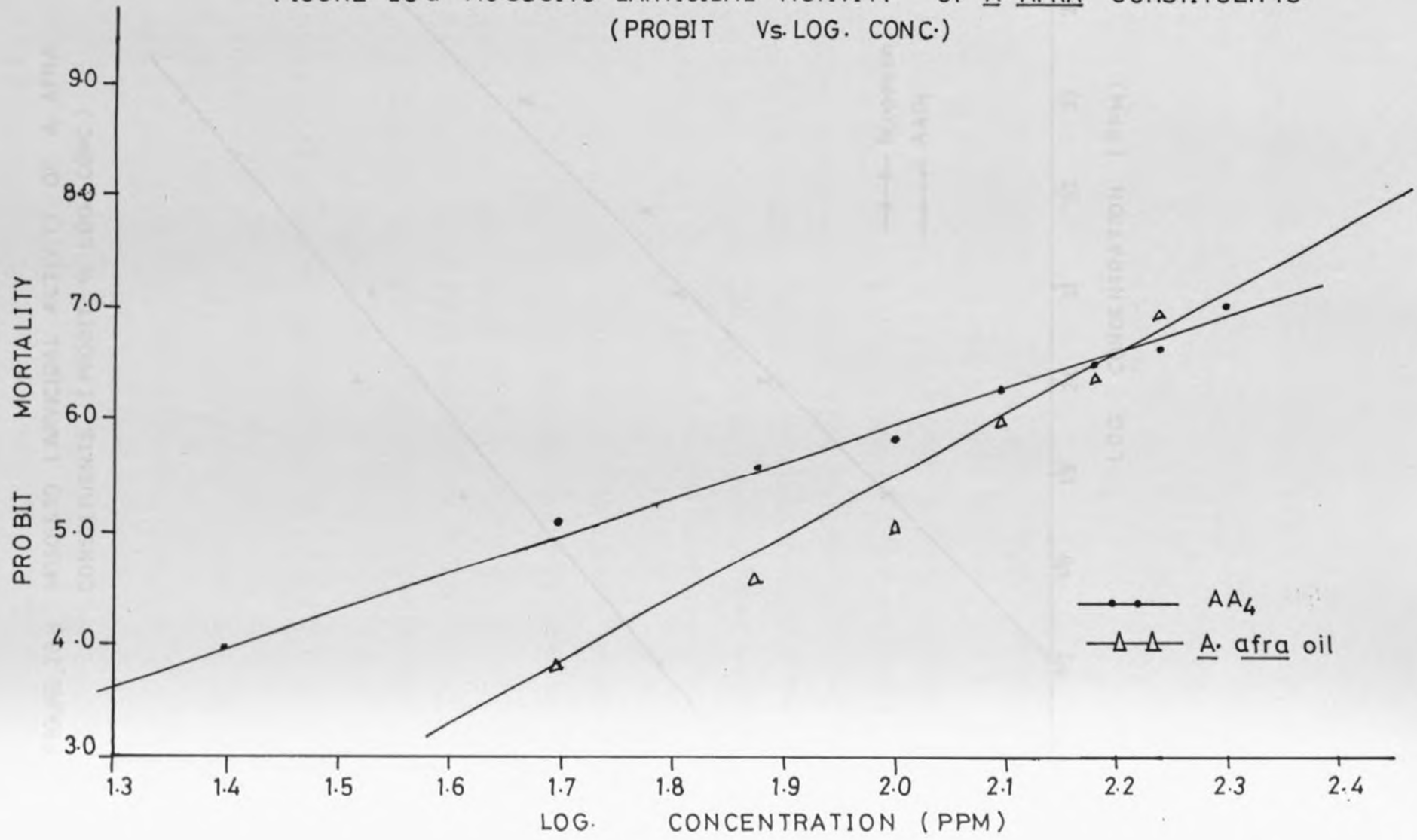
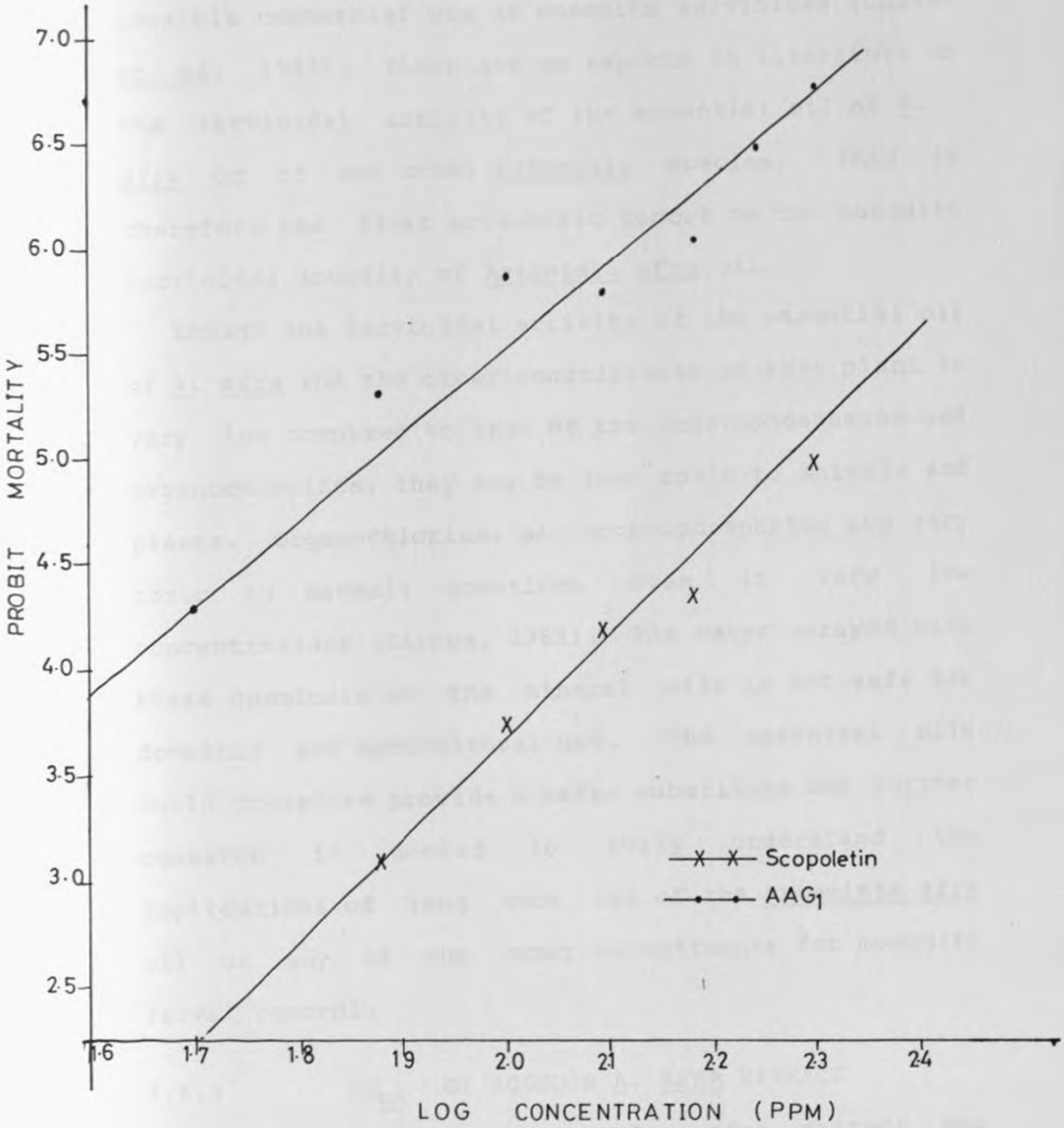


FIGURE 28 b MOSQUITO LARVICIDAL ACTIVITY OF A. AFRA CONSTITUENTS (PROBIT Vs LOG. CONC)



1983). In view of the pressing need for vector control from indigenous plants, essential oil from individual plants or their combinations are being studied for possible commercial use as mosquito larvicides (Chavan et al, 1983). There are no reports in literature on the larvicidal activity of the essential oil of A. afra or of any other Artemisia species. This is therefore the first scientific report on the mosquito larvicidal activity of Artemisia afra oil.

Though the larvicidal activity of the essential oil of A. afra and the other constituents of this plant is very low compared to that of the organophosphates and organochlorides, they may be less toxic to animals and plants. Organochlorides and organophosphates are very toxic to mammals sometimes even in very low concentrations (Gaines, 1969). The water sprayed with these chemicals or the mineral oils is not safe for domestic and agricultural use. The essential oils could therefore provide a safer substitute but further research is needed to fully understand the implications of long term use of the Artemisia afra oil or any of the other constituents for mosquito larval control.

3.4.3 LD_{50} OF AQUEOUS A. AFRA EXTRACT

The LD_{50} of the aqueous A. afra extract was carried out using rats. No deaths were recorded at any of the tested dose levels within 24 hours and even after 48 hours. This indicated that ingestion of an

aqueous extract of A. afra prepared by boiling powdered leaves of up to 12.5 g/kg is not acutely lethal to rats. The total leaf powder for an adult human being of average weight 70 kg would come to 875 g. This figure illustrates the relative safety of aqueous extracts of A. afra. This is an important property taking into consideration the use of the above plant as a herbal medicine by herbalists.

3.4.4 CARDIOVASCULAR EFFECTS OF ARTEMISIA AFRA CONSTITUENTS

(a) Effect of an aqueous Artemisia afra extract and the long chain esters on blood pressure of anaesthetised rabbits

The results are tabulated on Tables 44 and 45 and graphically presented on Figure 29 and 30. Long chain fatty esters at the dose range 1-3 mg/kg induced a progressive fall in the systolic and diastolic pressures. The drug had a slow onset but a long duration of action. Within the first three hours, 1mg/kg dose induced a mean % decrease in systolic pressure ranging from 7.8% for the first hour to 32.4% by the end of the third hour. The corresponding figures for 1.5 mg/kg are 6.1% and 34% whereas for 3 mg/kg the figures are 8.9% and 50.4% respectively.

As seen from the results there is no significant difference between the potency of the long chain esters at 1.0 mg and 1.5 mg/kg dose levels. The

difference between the potency of 1.5 mg and 3.0 mg/kg within the first 2 hours is also not significant. However after 3 hours, 3.0 mg/kg dose induced a mean % decrease in systolic pressure more than 1.5 times greater than 1.0 mg/kg and 1.5 mg/kg dose levels.

The effects on the diastolic pressures were more pronounced than those on the systolic pressures. The results obtained indicate that long chain esters induced a mean % decrease in diastolic pressure ranging from 6.4% to 44.6% for 1 mg/kg dose in the first and third hour respectively. The corresponding figures for 1.5 mg/kg and 3.0 mg/kg dose levels were 7.3% and 24.4%; 15.7% and 61.9% for the first and third hour respectively.

These results do not show a dose dependent response because the response obtained with 1.5 mg/kg is less than that obtained with 1 mg/kg. What is certainly evident is that the long chain esters have hypotensive properties reducing both the systolic and the diastolic pressures. The effect on the diastolic pressure is more than on systolic pressure.

Administration of adrenaline 4 µg/kg intravenously did not effectively antagonise the hypotensive effect of the esters. The antagonism was very brief (10.4% and 13.7% increase in systolic and diastolic pressures respectively) after which the hypotensive effect continued to be manifested.

The results obtained with the aqueous extract showed that at a dose of 0.25 ml there was no significant difference between the control readings and the test reading. However at a dose of 0.5 ml and 1.0 ml there was a significant mean % decrease in both the systolic and the diastolic pressures (Table 46). Figure 31 represents the change in systolic/diastolic pressures versus time.

Unlike the long chain esters which manifested hypotensive effect after about 15 minutes, the onset of action for the aqueous extract was fast and within 2 minutes there was an observable change of 8.9 (2.8)% and 8.9 (4.8)% reduction in systolic and diastolic pressures respectively for 1.0 ml dose level. No significant change was observed within this time for 0.5 ml dose. However for the two dose levels there was a progressive gradual fall in both systolic and diastolic pressures. Maximum values of 14.3% and 31.2% systolic for 0.5 ml and 1.0 ml respectively were attained within the first 20 minutes. Maximum diastolic mean % reduction values stood at 15.3% and 30.5% for 0.5 ml and 1.0 ml respectively within the first half hour.

Recovery was also gradual and even after 90 minutes there was significant depressor effect observed with 1.0 ml doses with figures standing at 14.5% systolic and 18.6% diastolic mean % reductions. From the

results obtained with the long chain esters and those of the aqueous A. afra extract one can conclude that the aqueous extract has a lower potency and a relatively shorter duration of action.

The decreased potency of the aqueous extract can be attributed to the inability of the solvent (water) to fully extract non-polar active substances from the plant material.

The significant hypotensive activity observed with the aqueous extract could be attributed to traces of non-polar active substances such as the long chain esters that went into solution in hot water and also to relatively polar substances such as scopoletin that have previously been shown to possess hypotensive properties (Adjewole and Adesina, 1983) and that have now been isolated from the plant under investigation and detected in the aqueous extract used for the blood pressure studies. But it is also possible that the hypotensive effects of the aqueous extract is due to totally different substances.

The present results have shown that A. afra is a plant with hypotensive properties. Its activity after oral administration, coupled with the low toxicity of the aqueous extract (see Part Two section 3.4.3) makes this plant a good candidate in management of hypertension. The long duration of action means fewer daily doses and hence increased patient compliance.

TABLE 44 MEAN % REDUCTION IN DIASTOLIC PRESSURE INDUCED BY INTRAMUSCULAR INJECTION OF AAG1

n = 6 mean (SEM)

TIME(MINS)	MEAN % REDUCTION IN DIASTOLIC RESSURE INDUCED BY VARYING DOSES OF AAG1		
	1.0mg/kg	1.5mg/kg	3.0mg/kg
0	0.0	0.0	0.0
15	2.1(1.3)	0.0	11.9(3.0)
30	3.0(1.9)	4.5(1.7)	13.7(2.6)
45	2.9(2.9)	6.1(2.5)	14.8(3.3)
60	6.4(2.3)	7.3(2.7)	15.7(2.7)
75	8.8(2.8)	9.5(2.3)	16.3(4.2)
90	10.9(3.5)	9.4(2.4)	20.7(3.7)
105	15.6(4.5)	10.0(2.7)	25.2(3.9)
120	21.0(3.9)	11.3(2.6)	26.7(4.0)
135	25.7(4.1)	13.4(4.0)	35.5(1.7)
150	27.9(4.7)	15.5(4.4)	42.5(4.2)
165	35.2(4.8)	17.5(5.3)	49.2(4.2)
180	44.6(2.7)	24.4(4.3)	61.9(6.5)
195	45.8(3.1)	43.1(3.5)	60.6(8.2)
210	44.7(3.1)	52.9(4.9)	65.3(7.1)
225	46.0(5.3)	57.9(4.4)	70.0(5.3)

TABLE 45 MEAN % REDUCTION IN SYSTOLIC PRESSURE INDUCED BY INTRAMUSCULAR INJECTION OF THE LONG CHAIN ESTERS (AAG1) IN ANAESTHETISED RABBITS

n = 6 mean (SEM)

TIME(MINS)	MEAN % REDUCTION IN SYSTOLIC PRESSURE INDUCED BY VARYING DOSES OF AAG1		
	1.0mg/kg	1.5mg/kg	3.0mg/kg
0	0.0	0.0	0.0
15	0.8(0.8)	0.0	1.8(1.1)
30	3.4(2.1)	6.1(1.2)	6.2(1.1)
45	8.6(1.7)	6.3(2.2)	8.2(1.0)
60	9.8(2.3)	6.1(2.8)	8.9(0.9)
75	12.7(2.6)	8.5(3.5)	10.6(1.4)
90	15.4(3.2)	13.5(4.5)	13.8(3.8)
105	15.6(2.9)	14.2(3.6)	19.6(3.3)
120	17.6(3.3)	19.4(5.9)	18.2(2.8)
135	21.4(3.5)	23.0(5.3)	28.2(0.8)
150	21.8(3.3)	27.9(5.2)	30.0(4.2)
165	29.1(5.3)	32.4(8.4)	38.9(3.6)
180	32.4(5.9)	34.6(7.2)	50.5(7.4)
195	36.8(5.0)	42.0(4.9)	44.8(6.7)
210	41.7(7.3)	55.4(4.6)	55.4(8.7)
225	41.2(11.5)	64.2(3.7)	57.7(5.9)

TABLE 46 MEAN % REDUCTION IN DIASTOLIC PRESSURE INDUCED BY INTRAVENOUS INJECTION OF *A. AFRA* AQUEOUS EXTRACT IN ANAESTHETISED RABBITS

n = 6 mean (SEM)

TIME(MINS)	CONTROL	0.5ml	1.0ml
	Normal saline	Aqueous extract	aqueous extract
1	0.0	0.0	4.5(1.8)
2	3.1(2.2)	3.1(2.2)	8.9(4.8)
3	0.0	4.5(2.3)	22.9(7.0)
4	3.1(2.2)	6.3(3.3)	20.9(0.4)
5	3.1(2.2)	9.0(4.1)	18.7(2.2)
6	3.1(2.2)	9.5(4.3)	18.6(1.8)
7	0.0	11.1(2.8)	22.9(7.0)
8	0.0	13.3(1.8)	23.3(2.2)
9	0.0	13.3(1.8)	21.1(6.0)
10	0.0	13.6(2.6)	21.1(6.0)
20	0.0	13.9(2.4)	28.2(9.1)
30	3.1(2.2)	15.3(4.6)	30.5(8.0)
40	3.1(2.2)	14.1(2.5)	28.2(10.0)
50	0.0	10.4(1.8)	23.3(5.2)
60	0.0	11.1(2.8)	21.1(6.0)
70	3.1(2.2)	12.4(1.7)	23.3(5.2)
80	0.0	8.9(4.8)	18.7(4.0)
90	3.1(2.2)	4.8(1.6)	18.6(1.8)

TABLE 47 MEAN % REDUCTION IN SYSTOLIC PRESSURE INDUCED BY INTRAVENOUS INJECTION OF *A. AFRA* AQUEOUS EXTRACT IN ANAESTHETISED RABBITS

n = 6 mean (SEM)

TIME(MINS)	Control	0.5ml	1.0ml
	Normal saline	Aqueous extract	Aqueous extract
1	0.0	0.0	3.6(1.5)
2	2.5(1.8)	0.0	8.9(2.8)
3	3.1(2.2)	8.6(1.4)	17.9(5.6)
4	5.6(0.4)	11.1(1.5)	19.9(2.6)
5	0.0	11.1(1.5)	16.4(0.2)
6	3.1(2.2)	9.0(2.8)	18.2(1.6)
7	0.0	12.5(1.7)	18.2(1.6)
8	0.0	13.6(2.3)	18.2(1.6)
9	5.6(0.4)	13.6(3.1)	21.9(2.8)
10	2.5(1.8)	14.3(3.1)	21.9(2.8)
20	3.1(2.2)	14.3(3.1)	31.2(8.2)
30	5.0(3.5)	10.6(3.9)	29.3(8.6)
40	0.0	9.4(3.1)	29.3(8.6)
50	0.0	12.3(5.6)	25.6(4.2)
60	0.0	11.1(2.4)	21.9(4.6)
70	2.5(1.8)	9.5(2.6)	20.1(3.2)
80	3.1(2.2)	8.6(1.4)	20.1(3.2)
90	0.0	2.5(1.3)	14.5(1.4)

FIGURE 29 EFFECT OF INTRAMUSCULAR AAG_1 ON BLOOD PRESSURE OF ANAESTHETISED RABBIT (MEAN % REDUCTION IN SYSTOLIC PRESSURE). (n =6 , Error bars = SEM)

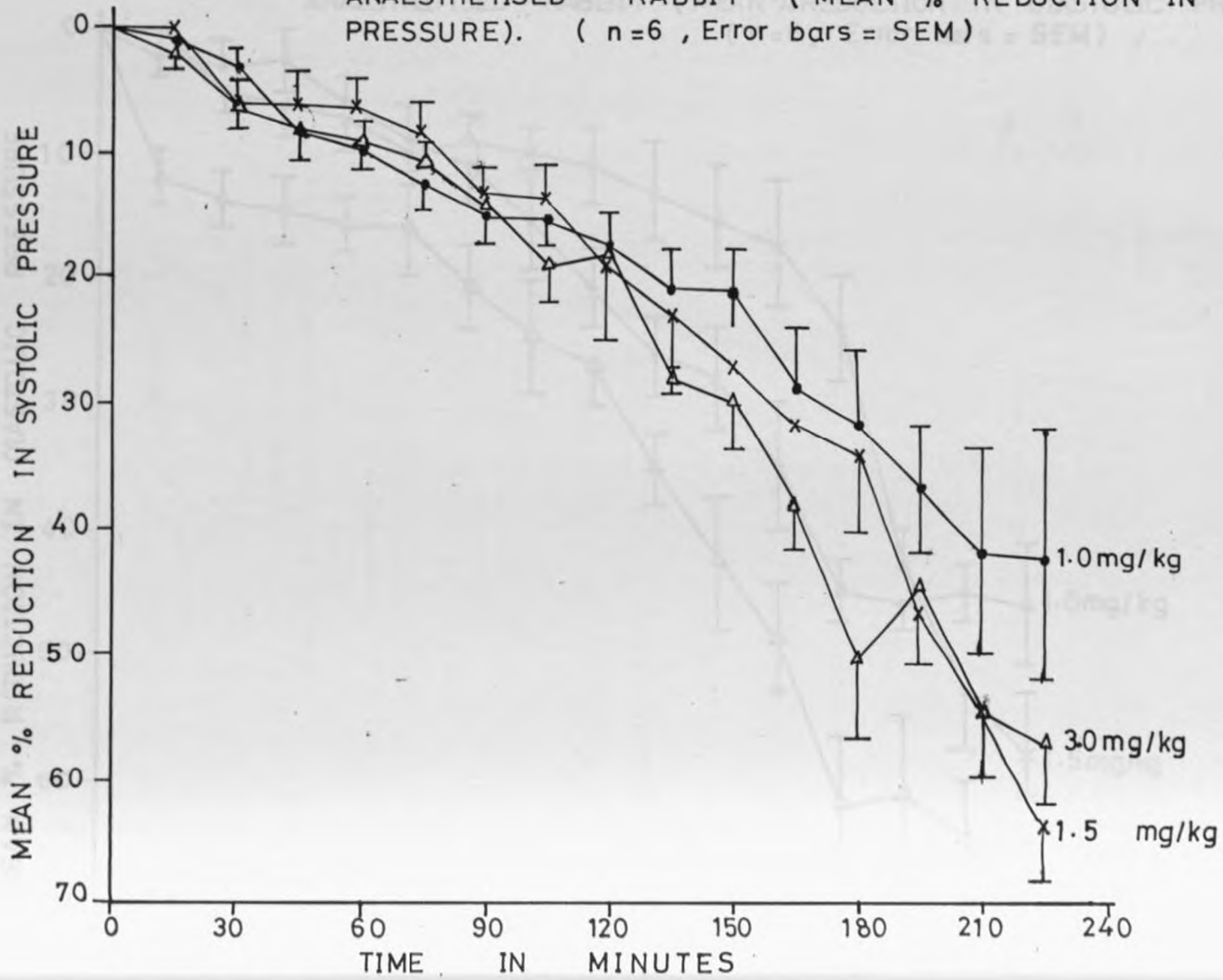


FIGURE 30 EFFECT OF INTRAMUSCULAR DOSES OF AAG_1 ON BLOOD PRESSURE OF ANAESTHETISED RABBIT (MEAN % REDUCTION IN DIASTOLIC PRESSURE)
(n=6, Error bars = SEM)

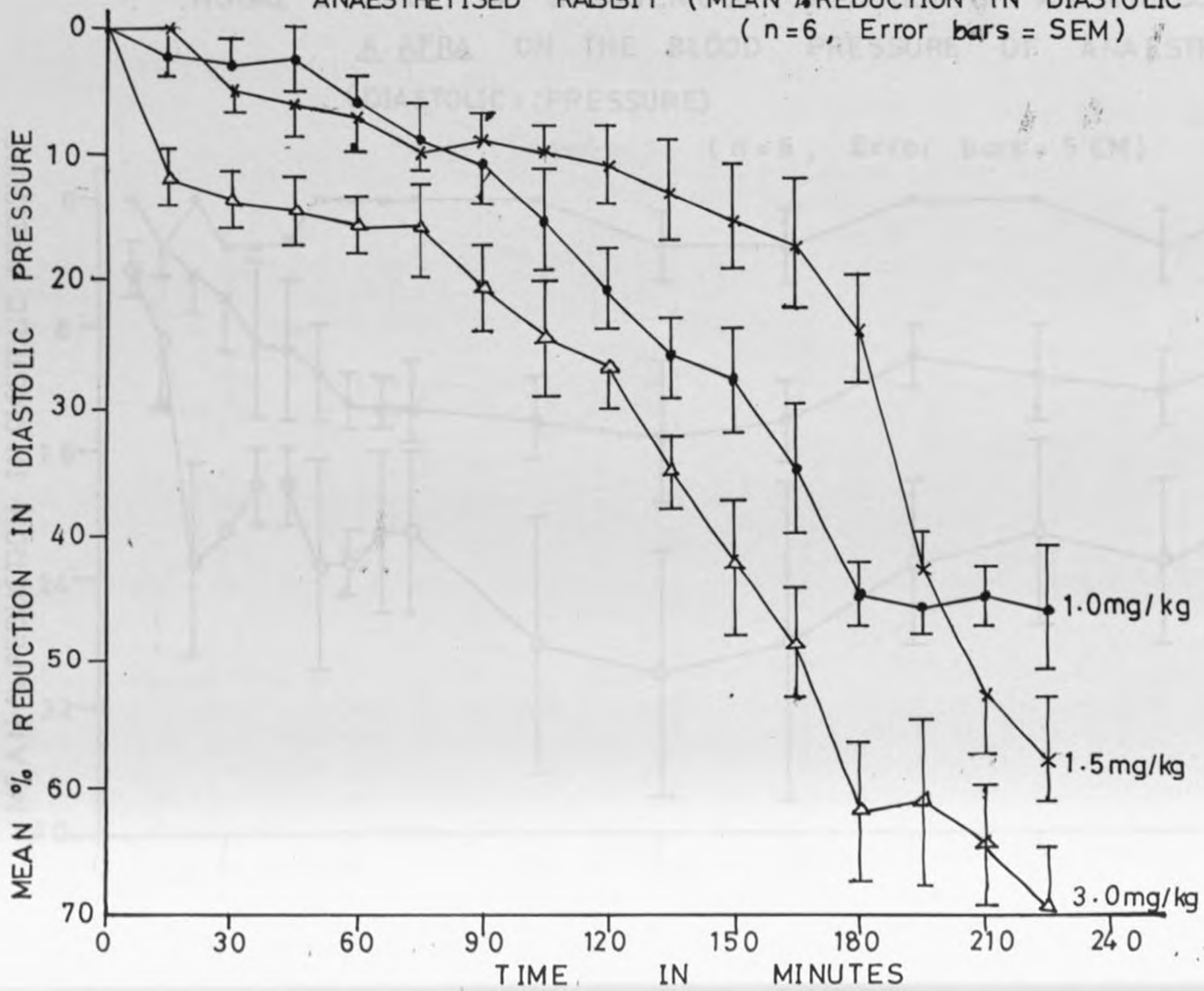


FIGURE 31 EFFECT OF INTRAVENOUS INJECTION OF AN AQUEOUS EXTRACT OF A. AFRA ON THE BLOOD PRESSURE OF ANAESTHETISED RABBIT (DIASTOLIC PRESSURE)

(n = 6, Error bars = S EM)

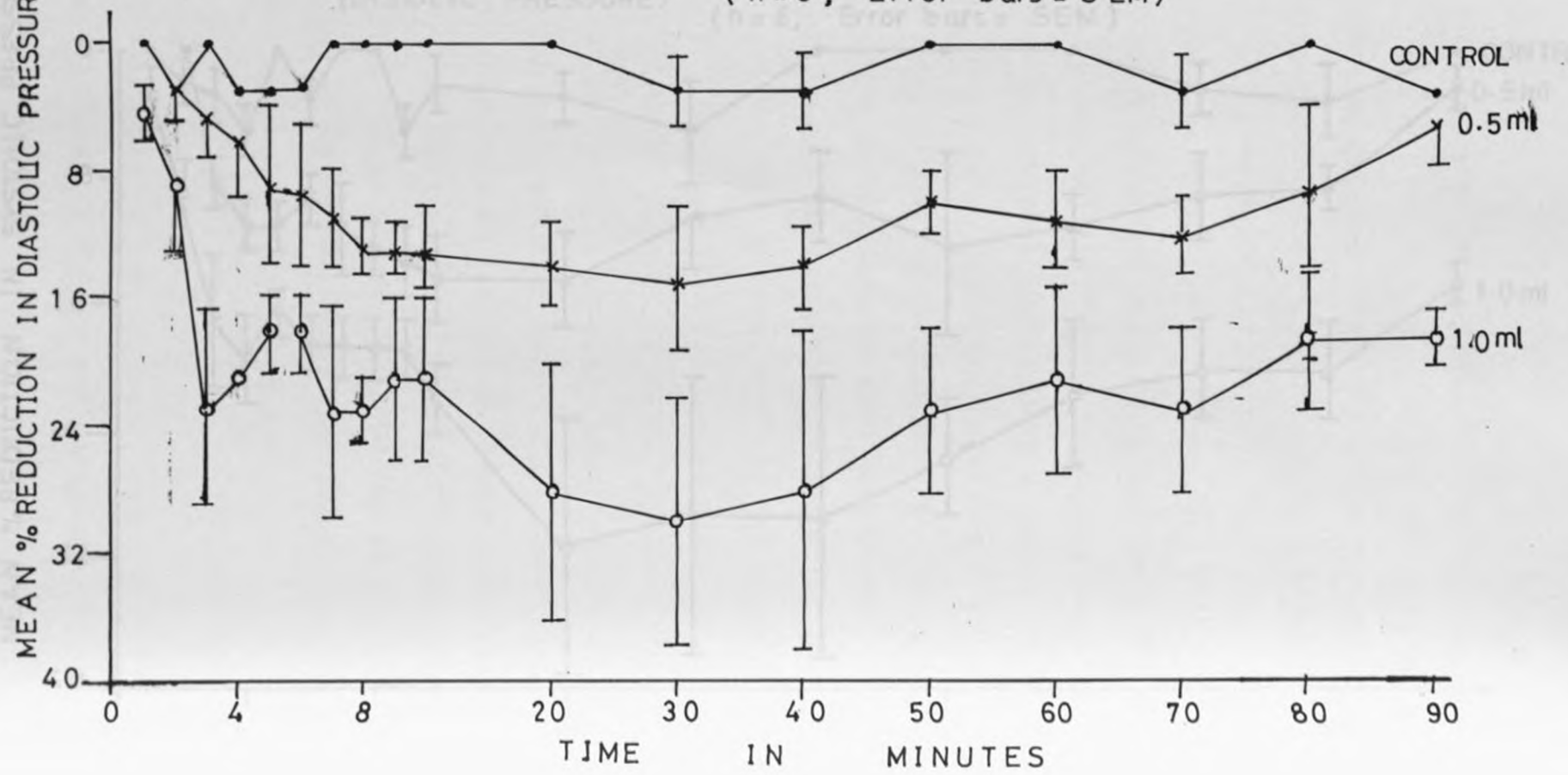
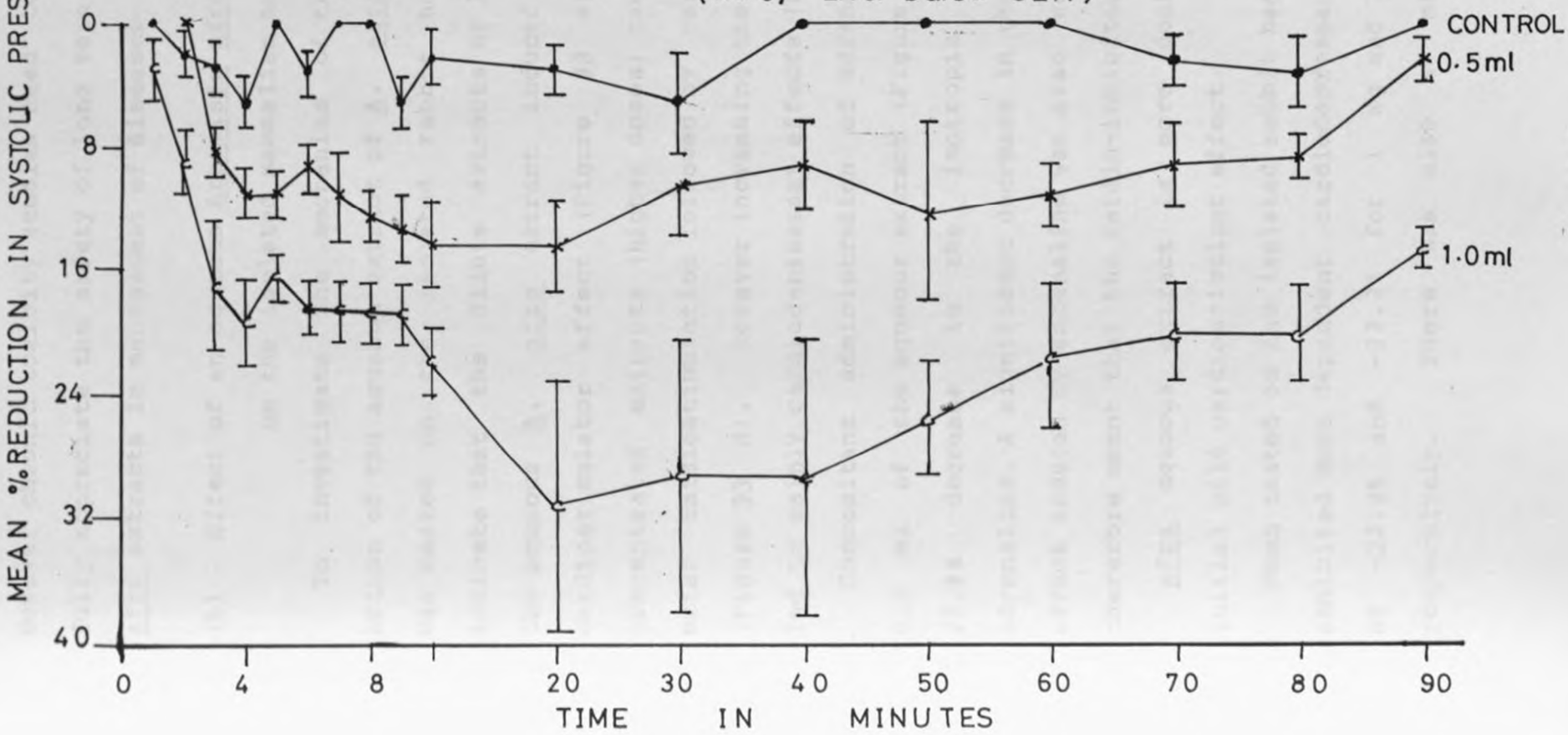


FIGURE 32 EFFECT OF INTRAVENOUS INJECTION OF AN AQUEOUS EXTRACT OF A AFRA ON THE BLOOD PRESSURE OF ANAESTHETISED RABBIT (SYSTOLIC PRESSURE)



However chronic toxicity studies need to be done to fully appreciate the safety of long term use of A. afra extracts in management of diseases.

(b) Effect of an aqueous Artemisia afra extract on the isolated mammalian heart

To investigate the mechanism of the hypotensive action of the aqueous extract of A. afra, the extract was tested on the isolated rabbit heart. Results indicate that the dilute extracts or lower doses of the aqueous A. afra extract induced mainly a mild cardiostimulator effect (Figure 33 e) whereas more concentrated extracts (higher doses) cause an initial brief cardiostimulation followed by cardiodepression (Figure 33 d). However increasing the doses further led to mainly cardiodepressant effects (Figure 33 c).

Concomitant administration of adrenaline 1 ug and 0.5 ml of the aqueous extract (Figure 33 f) caused 23.8% decrease in the inotropic response of adrenaline. A significant decrease in the chronotropic effect induced by adrenaline was also noticed. This therefore meant that the cardio-inhibitory effect of A. afra aqueous extract is more dominant than the initial mild cardiostimulant effect.

When tested on the isolated rabbit heart scopoletin exhibited dose dependent cardiodepressant properties of -22.2% and -33.3% for 1 mg and 2.5 mg doses respectively. There was also a mild negative

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FIGURE 33 EFFECT OF SCOPOLETIN AND AQUEOUS A. AFRA EXTRACT (AW_1) ON THE CONTRACTILE ACTIVITY OF THE HEART

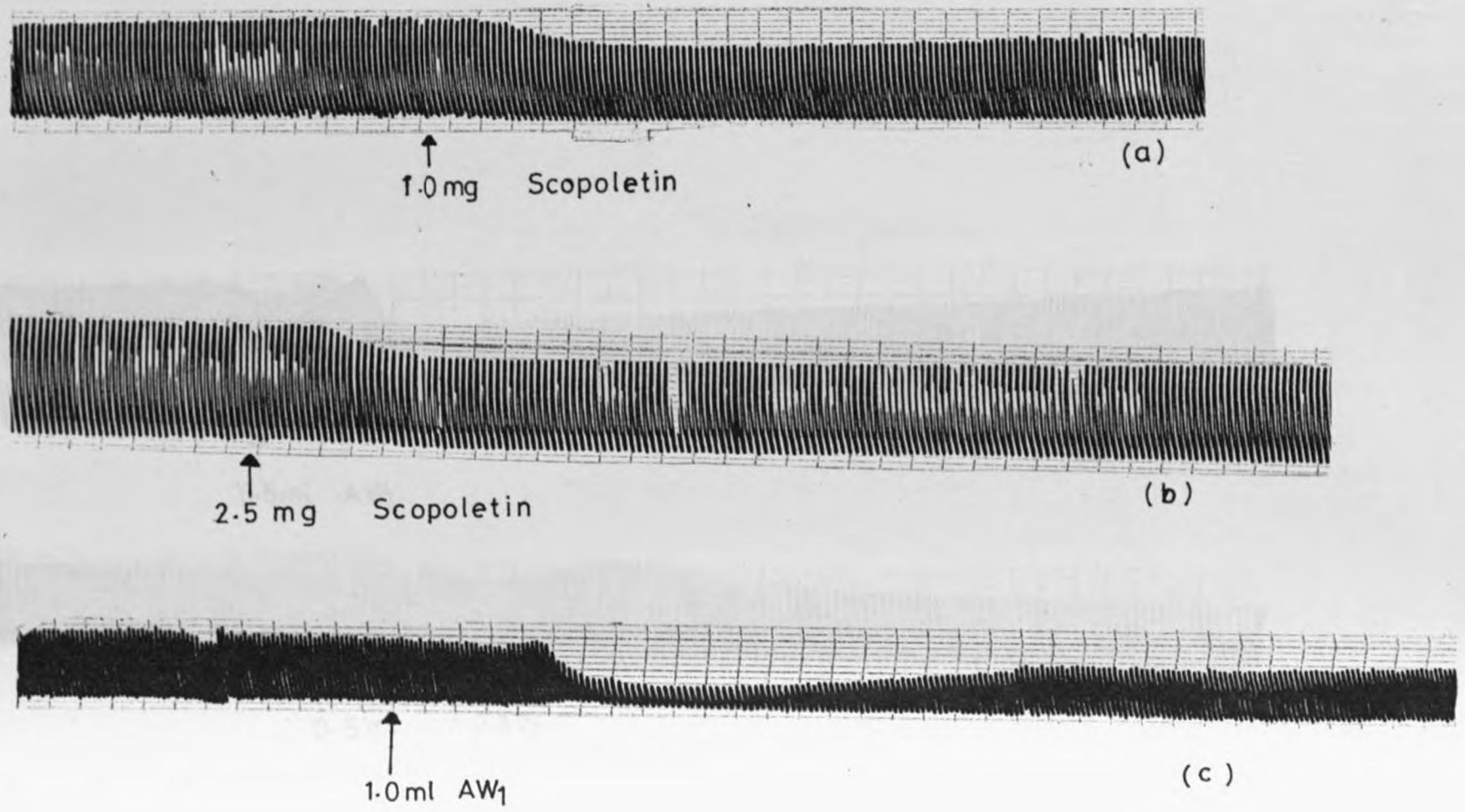
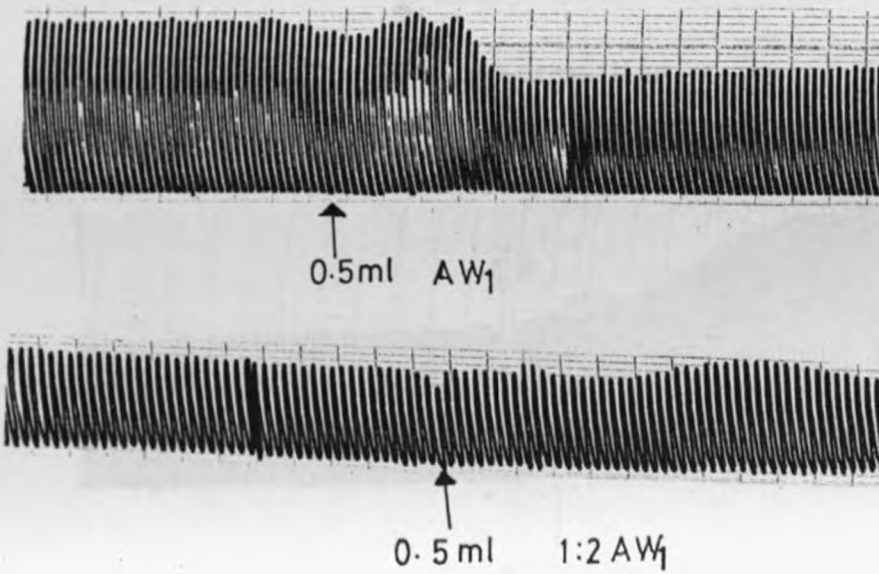
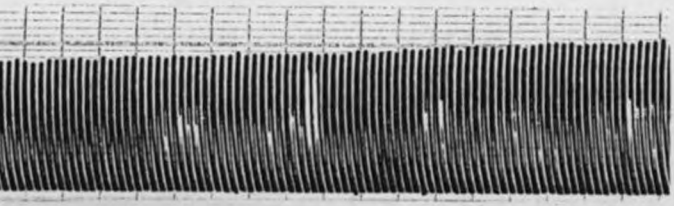


FIGURE 33 (Cont.)

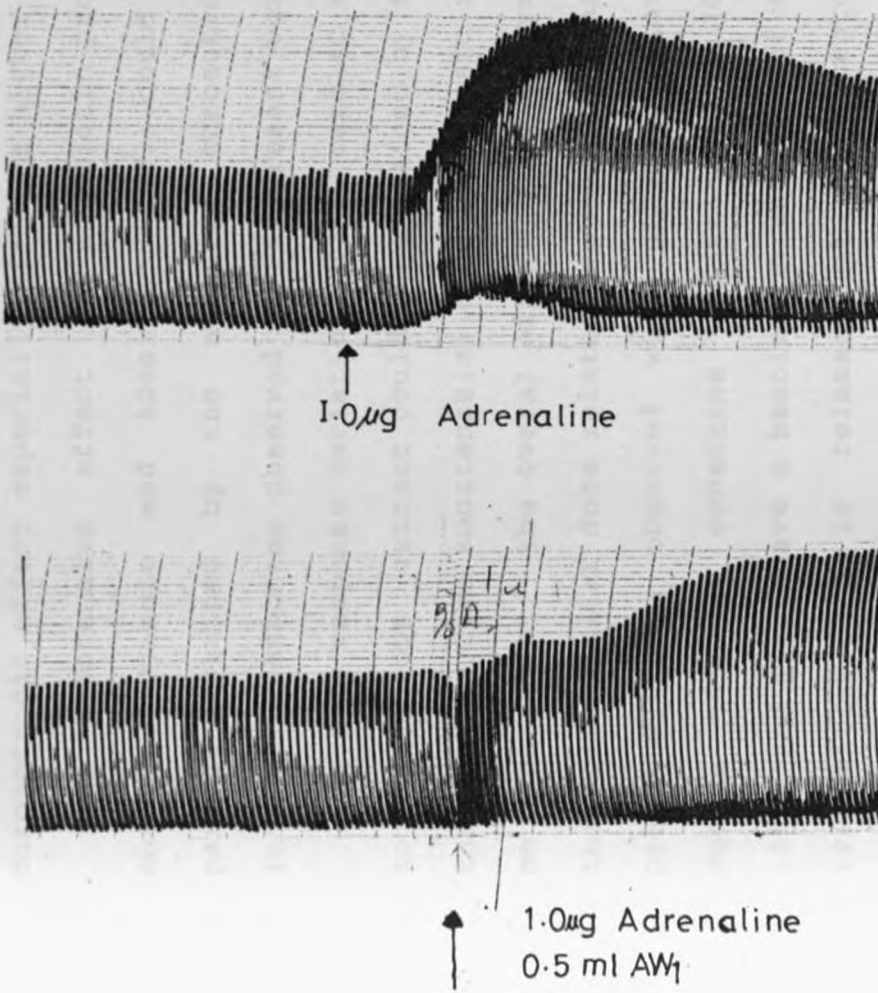


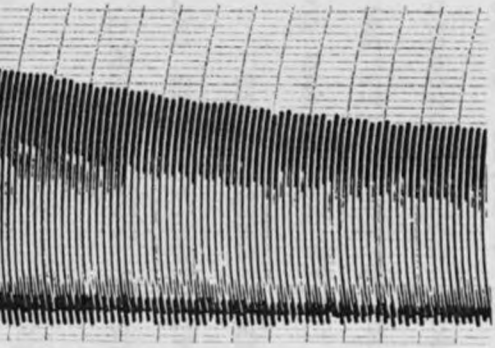


(d)

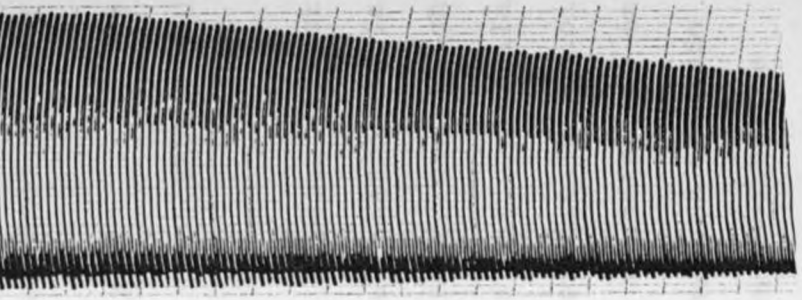
(e)

FIGURE 33 (Cont.)





(f)



(g)

chronotropic effect especially with the higher dose.

The hypotensive effect of scopoletin previously observed (Ojewole and Adesina, 1983) could in some part be explained by the negative chronotropic and inotropic responses observed in the present work.

The dose response variation in the cardiac response to the aqueous extract could be explained by the fact that, the active substance(s) have a biphasic response on the heart, the overal effect being determined by the dose. Similar dose related biphasic responses have previously been observed with other cardiovascular agents such as ephedrine and amphetamine (Guantai, 1982) and might have a bearing on the type of chemical transmitter that is released at the receptor sites upon drug receptor interaction.

3.4.5 EFFECT OF AQUEOUS A. AFRA EXTRACT ON NEUROMUSCULAR TRANSMISSION AND THE INFLUENCE OF COADMINISTERED DRUGS

Aqueous extract of A. afra was shown to possess weak neuromuscular blocking effect (23.3%). This effect was slightly enhanced by chloroquine 6 ug (26.7%). A similar dose of chloroquine caused 45.5% contractile inhibition. However gallamine 8 mg caused a substantial enhancement of the neuromuscular junction blockade (66.7%) induced by A. afra extract. Succinylcholine also induced a significant potentiation (56.7%) of the neuromuscular blockade

exerted by aqueous A. afra extract (Table 48).

Tested in presence of 5 mg succinylcholine, or 8 mg gallamine, 0.5 ml aqueous Artemisia afra extract induced a maximum of 56.67% contractile inhibition. A similar dose of succinylcholine induced 33.3% contractile inhibition, whereas when tested alone 8 mg gallamine induced 48.5% contractile inhibition of the isolated rat phrenic-nerve diaphragm preparation (Table 48).

The weak neuromuscular junction blocking effect of the aqueous extract implies that the extract is likely to induce slight muscle weakness and probably even mild respiratory distress when used therapeutically. However this fear is allayed by the fact that the neuromuscular blockade is fairly weak as compared to the blockade induced by the clinically useful neuromuscular blockers, gallamine and succinylcholine and might therefore not be of clinical significance.

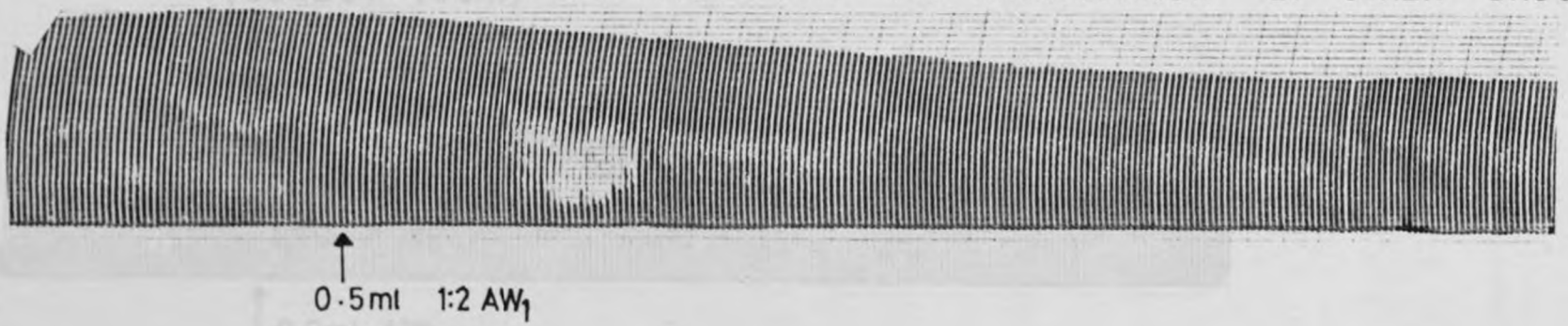
It is also known that not all drugs that depress neuromuscular transmission cause respiratory distress. For example chloroquine which is a potent neuromuscular junction blocker (Chinyanga et al, 1970) does not interfere with asthmatic patients but can actually cause alleviation of the asthmatic symptoms (Engeset, 1957; Sylvio de Carmargo, 1958).

TABLE 48 EFFECT OF AQUEOUS EXTRACT OF A. AFRA LEAVES ON NEURO-MUSCULAR TRANSMISSION AND THE INFLUENCE OF OTHER DRUGS

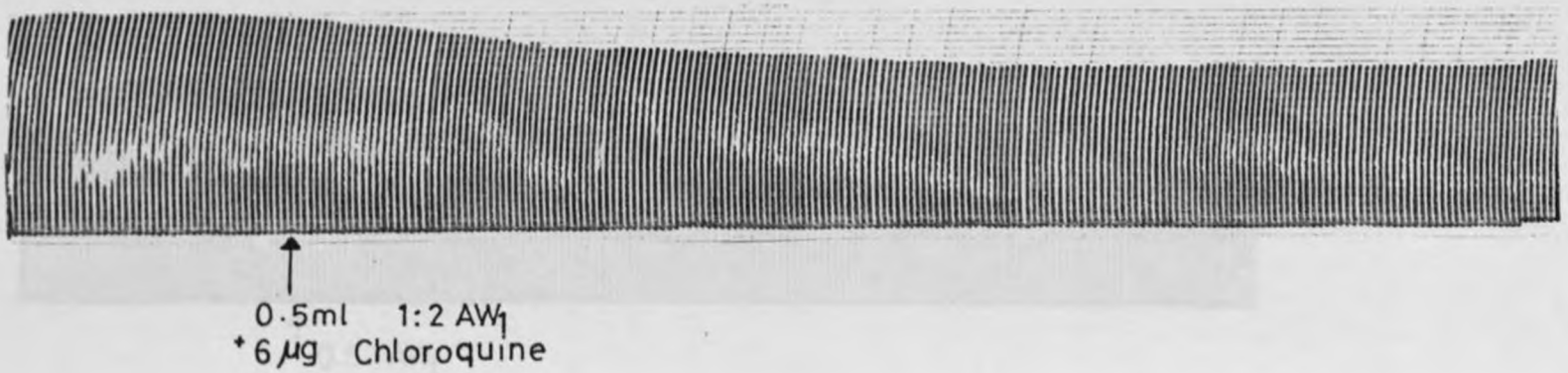
n = 6 mean (SEM)

DRUG OR DRUG COMBINATION	DOSE	MEAN % CONTRACTILE INHIBITION	% ENHANCEMENT OF INHIBITION
Aqueous A.afra extract	0.5 ml	-23.3(2.1)	-
Gallamine	8mg	-48.5(1.7)	-
Succinylcholine	5mg	-33.3(1.3)	-
Chloroquine	6ug	-45.5(2.1)	-
Gallamine+ extract	8mg+ 0.5ml	-66.7(2.1)	43.3to 43.6
Succinylcholine +extract	5mg+ 0.5ml	-56.7(4.0)	31.6 to 34.6
Chloroquine +extract	6ug+ 0.5ml	-26.7(3.3)	2.3 to 4.6

FIGURE 34 THE EFFECT OF AQUEOUS A. AFRA EXTRACT ON THE NEURO-MUSCULAR TRASMISSION AND ITS MODIFICATION BY OTHER DRUGS

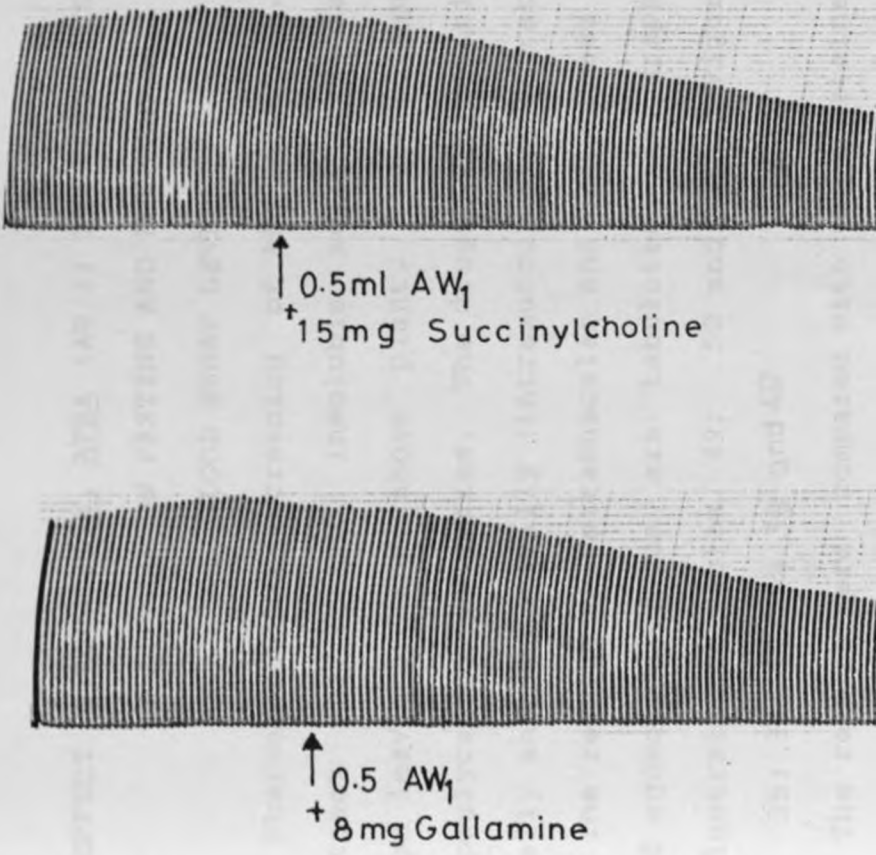


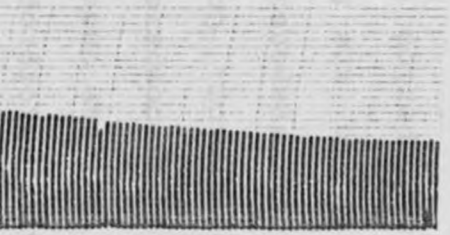
(a)



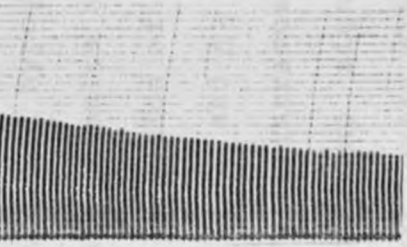
(b)

FIGURE 34 (Cont.)





(c)



(d)

3.4.6 EFFECT OF AQUEOUS *A. AFRA* (AW 1) AND CONSTITUENTS OF *A. AFRA* ON FASTING AND NON-FASTING BLOOD SUGAR LEVELS

Pharmacological screening of the aqueous *A. afra* extract and ethanol insoluble solid $(AAG)_1$ isolated from leaves of the above plant, were screened for hypoglycaemic properties. The drugs were administered orally and parenterally (intramuscularly) to rabbits.

The results of intramuscular and oral doses of AAG_1 and aqueous extracts are tabulated and graphically illustrated in Tables 49; 50 and 51 and Figures 35; 36; 37; 38 39 and 40

The results were compared with those obtained with a standard oral hypoglycaemic agent, chlorpropamide. Preliminary results had indicated that the onset and duration of action of chlorpropamide was comparable to that of AAG_1 and *A. afra* aqueous extract. Controls treated with intramuscular 2% acacia suspension in water showed no significant change in the fasting blood sugar levels within the treatment period (Figure 35).

However within 30 minutes of intramuscular administration of 1 mg/kg and 2 mg/kg AAG_1 a mean percentage drop in glycaemia of 8.78 (1.97) and 22.35 (3.8) respectively was recorded. The maximum hypoglycaemic activity was recorded after 2 hours and 2.5 hours for 1 mg/kg and 2 mg/kg of AAG_1 doses

respectively. The figures correspond to 20.1 (2.54) and 27.82 (5.36) for the two respective doses.

After 3 hours (180 minutes) there was a sign of recovery with the mean percentage drop in glycaemia changing from 19.68% to 15.18% for 1 mg/kg dose whereas for 2 mg/kg dose, the mean % drop in glycaemia changed from 27.82% to 24.62%.

Results obtained using oral route show that both the aqueous A. afra extract and AAG_1 induced a slow but sustained hypoglycaemia that was significant even 29 hours after drug administration. However the sustained effect was more pronounced with AAG_1 than with the aqueous extract.

The onset of drug action ranged from 1-2 hours whereas the duration was longer than 29 hours. This phenomenon compares well with that observed with chlorpropamide (Figure 36) an oral hypoglycaemic agent with delayed onset and long duration of action (Bowman and Rand, 1985).

It was not possible to monitor blood sugar levels at night. For this reason the nocturnal mean % glycaemia variation could not be deduced, hence the gap between 7 hours and 23 hours the following day indicated by the --- sign on the graphs.

However noting that even after 23 hours there was profound mean % reduction in glycaemia, one can conclude without quoting figures that the nocturnal

picture was characterised by decreased blood sugar levels.

The mean % maxima values were 56.42 (1.24) at 24 hours for the aqueous extract, 41.65 (1.63) at 24 hours for 10 mg/kg AAG_1 and 59.31 (1.74) at 23 hours for 20 mg/kg AAG_1 . The mean % maximum recorded after 4 mg/kg chlorpropamide was 68.43 (2.50) at 23 hours.

After oral administration of the aqueous A. afra extract, signs of recovery from hypoglycaemia were recorded after 25 hours (Figure 37). To investigate the effect of repeat dose, a booster dose equivalent to the first dose was administered immediately after drawing blood for the 26 hour sample. Hourly blood samples were obtained for a further 27 hours excluding nocturnal samples and analysed for variation in blood glucose levels.

The results indicate that a booster dose of AW1 given at the onset of recovery from hypoglycaemia arrested further recovery (Figure 36). There was an initial irregular, but sustained decrease in glycaemia which ranged from 51.78 (2.64)% to 68.78 (4.53)% decrease for 24 hours before any significant signs of recovery were recorded (Table 51). Similar effect was observed with a repeat dose of 10 and 20 mg/kg AAG_1 orally.

There were moderate signs of potentiation or enhanced activity. This observation therefore rules

out the possibility of cumulation if the drug is given once a day. More frequent doses should not be given to avoid cumulation and any accompanying adverse effects.

Due to the delayed onset of action of aqueous A. afra extract and AAG_1 after oral administration, these preparations may not be useful in management of a hyperglycaemic crisis in insulin dependent diabetes or in Juvenile onset diabetes mellitus. However if treatment is initiated with a drug having fast-onset, and short duration of action, then AAG_1 or the aqueous A. afra extract can be used as a single daily doses for maintenance therapy.

The shorter duration of action of AAG_1 after intramuscular administration as compared to the long duration after oral administration may be explained by the immediate exposure of the drug to metabolic and excretory mechanisms and other sites of loss after parenteral administration.

In conclusion, A. afra possesses hypoglycaemic properties. AAG_1 is one of the hypoglycaemic principles. Possible development of AAG and the crude A. afra extracts for management of chronic diabetic patients is proposed. The results also support the folkloric use (Watt and Breyer-Brandwijk, 1962) and also recent reports that some essential oils and long chain fatty esters show experimental hypoglycaemic activity (Anthony et al, 1988).

TABLE 49 EFFECT OF INTRAMUSCULAR DOSES OF AAG1 ON THE FASTING BLOOD SUGAR LEVELS OF NORMOTENSIVE RABBITS

n = 6 mean (SEM)

	MEAN % REDUCTION IN FASTING BLOOD SUGAR LEVELS INDUCED BY DRUGS BELOW		
TIME(MINS)	CONTROL	AAG1 1mg/kg	AAG1 2mg/kg
0	0.00(0.00)	0.00(0.00)	0.00(0.00)
30	0.83(0.50)	8.78(1.97)	22.35(3.80)
60	0.00(0.00)	16.30(3.01)	22.15(3.75)
90	0.00(0.00)	18.78(1.51)	19.85(3.48)
120	1.20(0.75)	20.10(2.54)	20.75(4.63)
150	0.84(0.25)	19.68(3.06)	27.82(5.36)
180	1.65(0.55)	15.18(1.60)	24.62(3.55)

TABLE 50 MEAN % REDUCTION IN FASTING BLOOD SUGAR LEVELS INDUCED BY ORAL DOSES OF AAG_1, AQUEOUS ARTEMISIA AFRA EXTRACT (AW_1) AND CHLORPROPAMIDE IN NORMOTENSIVE RABBITS

TIME	MEAN % REDUCTION IN FASTING BLOOD SUGAR LEVELS INDUCED BY DRUGS SHOWN BELOW				
	Control	AAG_1 10mg/kg	AAG_1 20mg/kg	Chlorpropamide 4mg/kg	Artemisia afra Aqueous extract 20 mls AW_1/kg
0	0.00	0.00	0.00	0.00	0.00
1	0.00	0.00	6.32(1.02)	0.00	4.18(0.55)
2	1.20(0.23)	4.20(0.25)	7.63(2.20)	5.25(0.65)	5.00(1.17)
3	1.65(0.36)	7.77(0.67)	10.80(2.29)	21.74(2.08)	12.78(1.38)
4	0.00	10.15(0.24)	16.98(2.26)	21.74(1.45)	19.32(2.54)
5	0.00	17.50(1.50)	29.42(2.32)	28.35(5.34)	24.68(1.33)
6	2.45(1.65)	23.48(2.15)	38.63(2.20)	43.48(3.28)	32.18(1.45)
7	3.40(1.74)	30.33(1.24)	46.98(1.45)	47.83(4.74)	40.46(2.58)
8	0.65(0.18)	-	-	56.90(3.15)	49.94(2.44)
23	2.30(1.26)	33.40(2.50)	59.31(1.74)	69.43(2.50)	55.42(1.24)
24	1.65(0.24)	41.65(1.63)	58.55(3.24)	59.38(3.45)	56.42(1.24)
25	3.81(0.25)	36.34(1.31)	50.61(3.93)	58.13(2.52)	45.74(1.68)
26	2.25(1.26)	29.56(2.70)	34.20(2.70)	39.42(1.43)	37.78(2.19)
27	1.65(0.28)	27.41(1.90)	32.60(4.04)	36.95(3.45)	26.90(2.19)
28	4.20(0.28)	25.85(1.52)	32.60(4.04)	38.72(2.17)	21.62(2.07)
29	5.00(1.29)	21.17(1.19)	-	36.42(1.52)	9.63(2.73)

TABLE 51 EFFECT OF REPEAT DOSE OF A. AFRA AQUEOUS EXTRACT (AW1) AND CHLORPROPAMIDE ON NON-FASTING BLOOD SUGAR LEVELS OF RABBITS n = 6 mean (SEM)

MEAN % REDUCTION IN NON-FASTING BLOOD SUGAR LEVELS IN RABBITS

TIME(HRS)	20ml AW1/kg	Chlorpropamide 4mg/kg	AAG1 10mg/kg	AAG1 20mg/kg
1	0.00	0.00	0.00	0.00
2	4.68(1.33)	0.00	4.00(0.34)	6.00(1.61)
3	12.18(1.45)	14.35(0.43)	7.50(0.25)	6.98(1.63)
4	25.74(1.68)	26.74(2.49)	10.30(0.41)	11.80(2.52)
5	21.35(1.50)	23.46(3.56)	18.00(1.51)	18.98(2.26)
6	35.42(1.21)	43.48(2.85)	23.45(2.15)	31.40(0.24)
7	41.56(1.74)	45.23(1.87)	31.43(1.51)	40.31(0.38)
23	54.16(2.17)	55.90(1.35)	33.40(1.30)	45.89(1.51)
24	52.99(3.35)	57.83(4.28)	43.40(3.00)	59.43(1.37)
25	47.18(3.15)	48.74(3.36)	37.23(0.55)	58.35(2.15)
26	38.76(2.44)	26.00(4.40)	20.50(1.48)	51.63(2.35)

TABLE 51 CONT.

REPEAT DOSE

27	41.77(3.85)	45.23(5.31)	21.92(2.50)	38.33(1.81)
28	46.31(2.66)	52.17(2.86)	30.51(2.61)	40.50(1.41)
29	51.78(2.64)	52.45(4.81)	36.16(3.86)	41.21(0.55)
30	56.07(3.54)	56.93(3.14)	40.50(0.25)	46.29(2.41)
31	61.77(2.04)	64.39(2.38)	42.31(1.42)	49.28(0.60)
32	62.07(2.64)	63.14(3.17)	45.83(3.35)	53.33(1.31)
33	63.50(0.95)	66.71(2.46)	48.44(1.93)	58.44(1.93)
43	63.45(4.37)	60.43(4.18)	53.15(0.85)	57.35(2.83)
44	63.86(0.77)	61.76(3.96)	40.65(1.63)	55.45(1.80)
45	67.64(5.06)	60.73(1.38)	40.65(1.63)	55.45(1.80)
46	68.78(4.53)	63.37(5.40)	31.56(2.73)	38.48(2.50)
47	62.02(4.66)	58.64(1.35)	28.85(1.81)	34.60(3.24)
48	51.42(4.16)	49.38(2.54)	26.80(1.50)	33.60(3.14)
49	40.18(3.54)	34.33(1.41)	22.80(1.20)	30.25(2.75)

FIGURE 35 MEAN % REDUCTION IN FASTING BLOOD SUGAR LEVELS INDUCED BY INTRAMUSCULAR DOSES OF AAG_1
(n=6, Error bars=SEM)

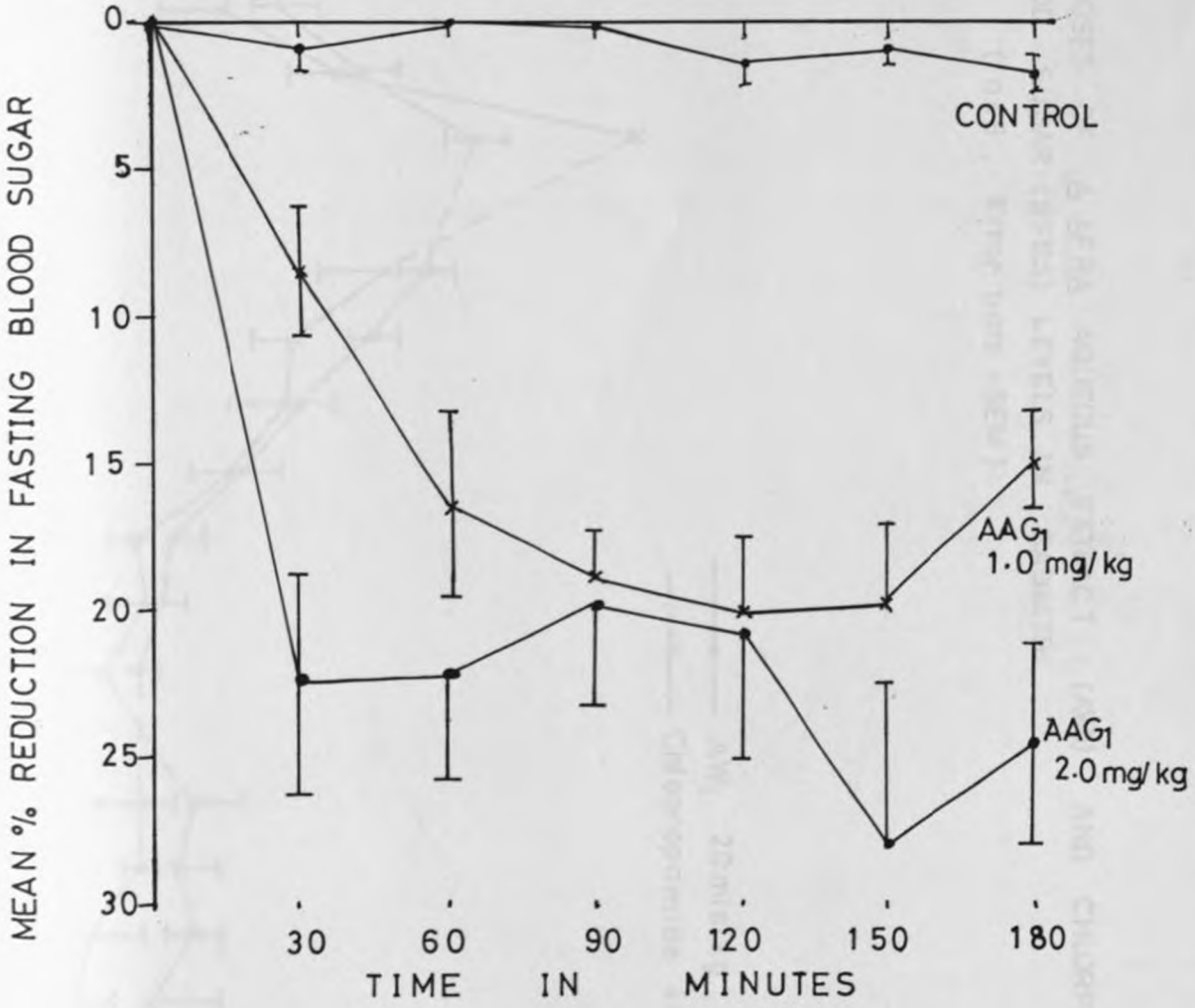


FIGURE 36 EFFECT OF REPEAT DOSES OF A. AFRA AQUEOUS EXTRACT (AW1) AND CHLORPROPAMIDE ON NON-FASTING BLOOD SUGAR (NFBS) LEVELS IN RABBITS

(n=6 , Error bars =SEM)

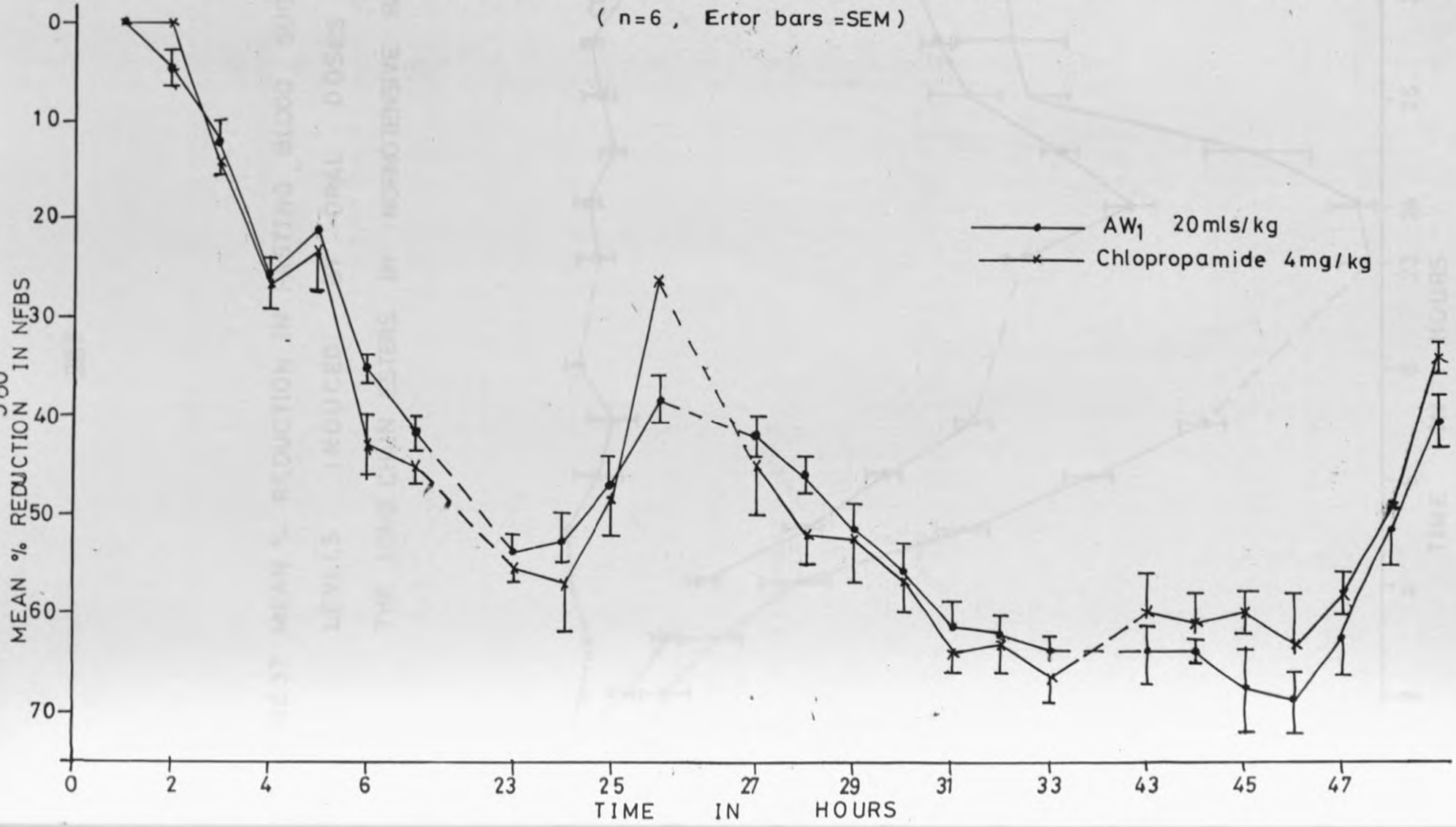


FIGURE 37 MEAN % REDUCTION IN FASTING BLOOD SUGAR(FBS) LEVELS INDUCED BY ORAL DOSES OF THE LONG CHAIN ESTERS IN NORMOTENSIVE RABBITS

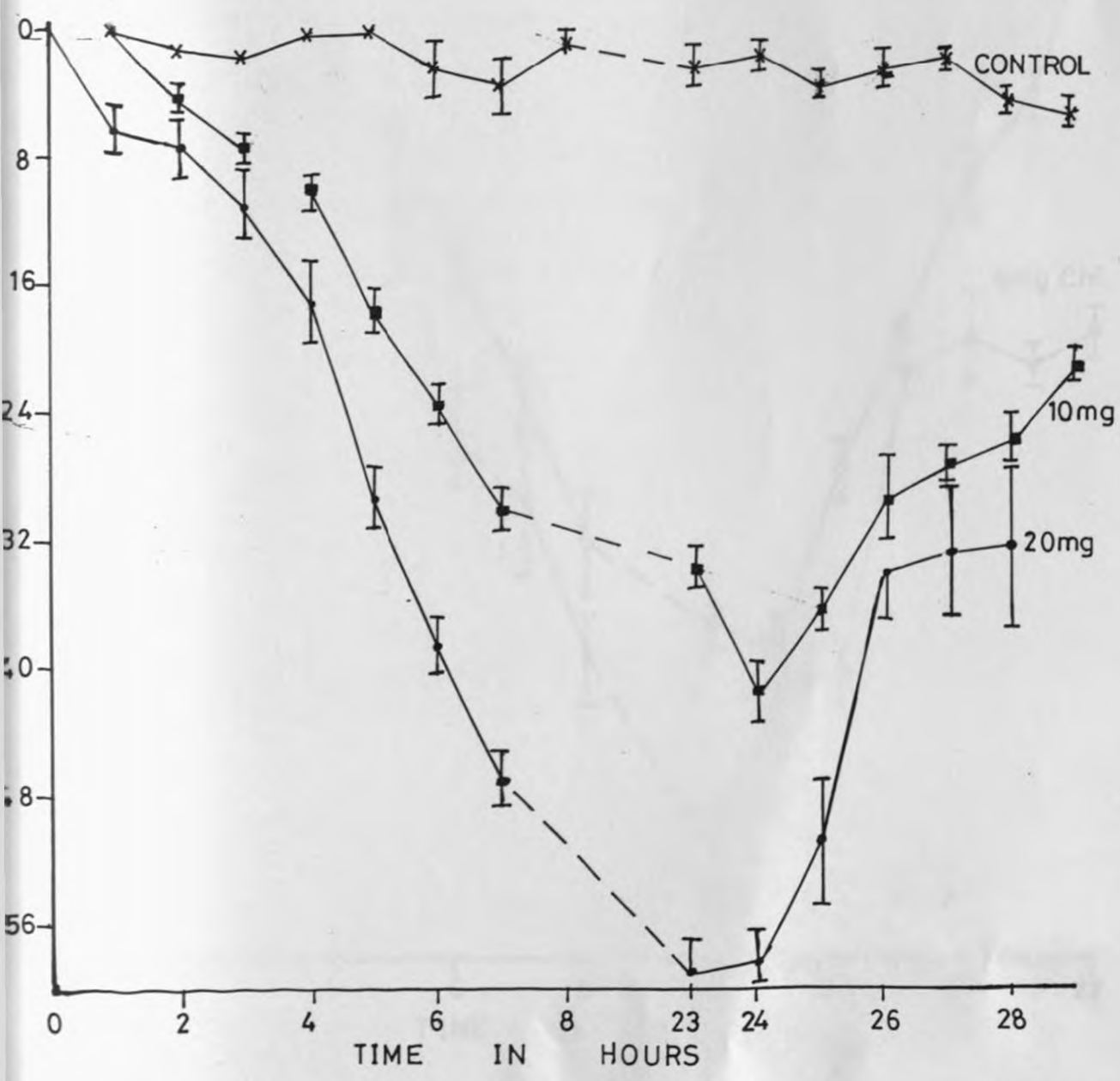


FIGURE 38 MEAN % REDUCTION IN FASTING BLOOD SUGAR (FBS) LEVELS INDUCED BY ORAL DOSES OF AW1 AND CHLORPROPAMIDE IN NORMOTENSIVE RABBITS

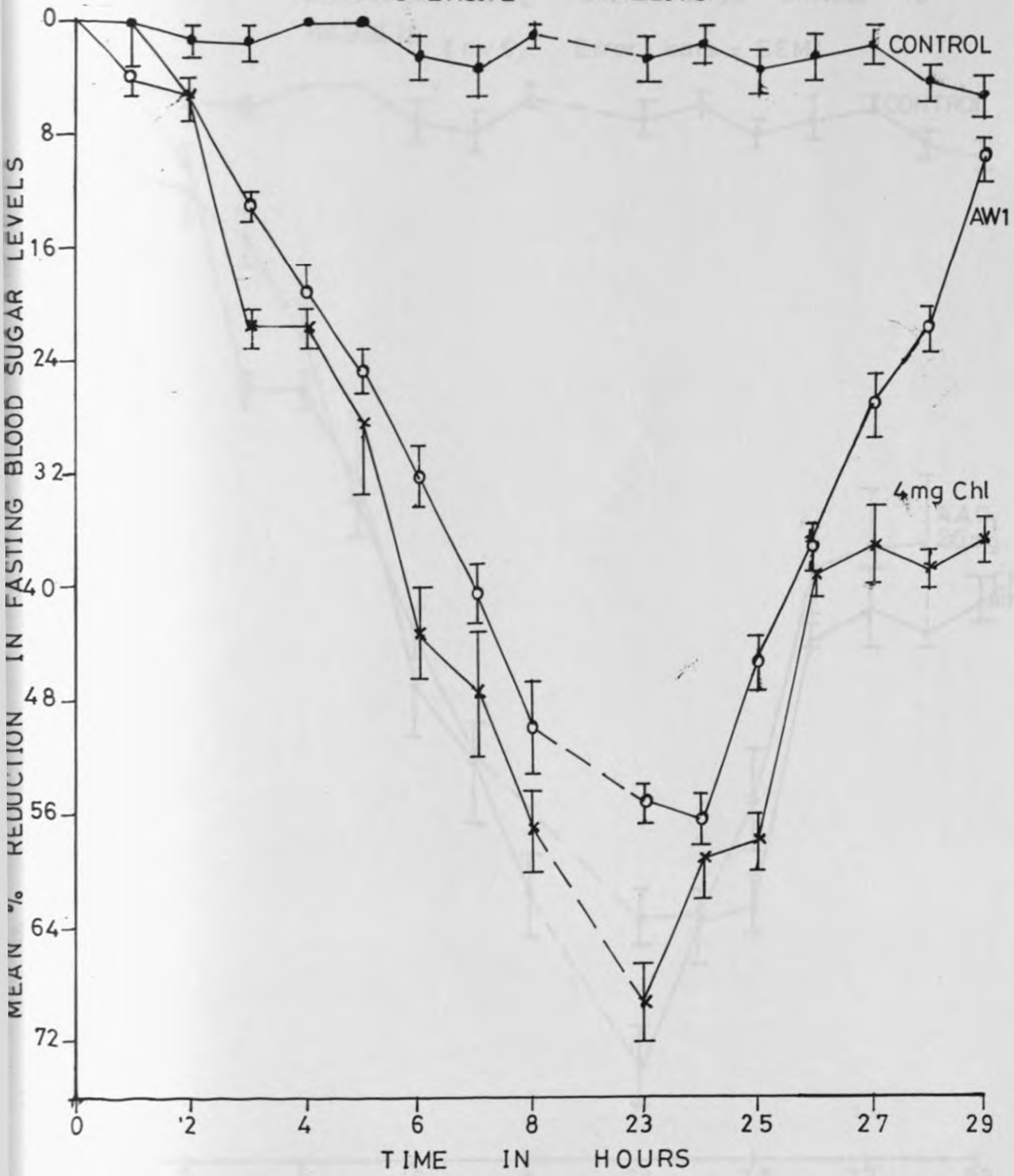


FIGURE 39 MEAN % REDUCTION IN FASTING BLOOD SUGAR (FBS) LEVELS INDUCED BY AAG_1 AND CHLORPROPAMIDE ADMINISTERED ORALLY TO RABBITS (n = 6, Error bars = SEM)

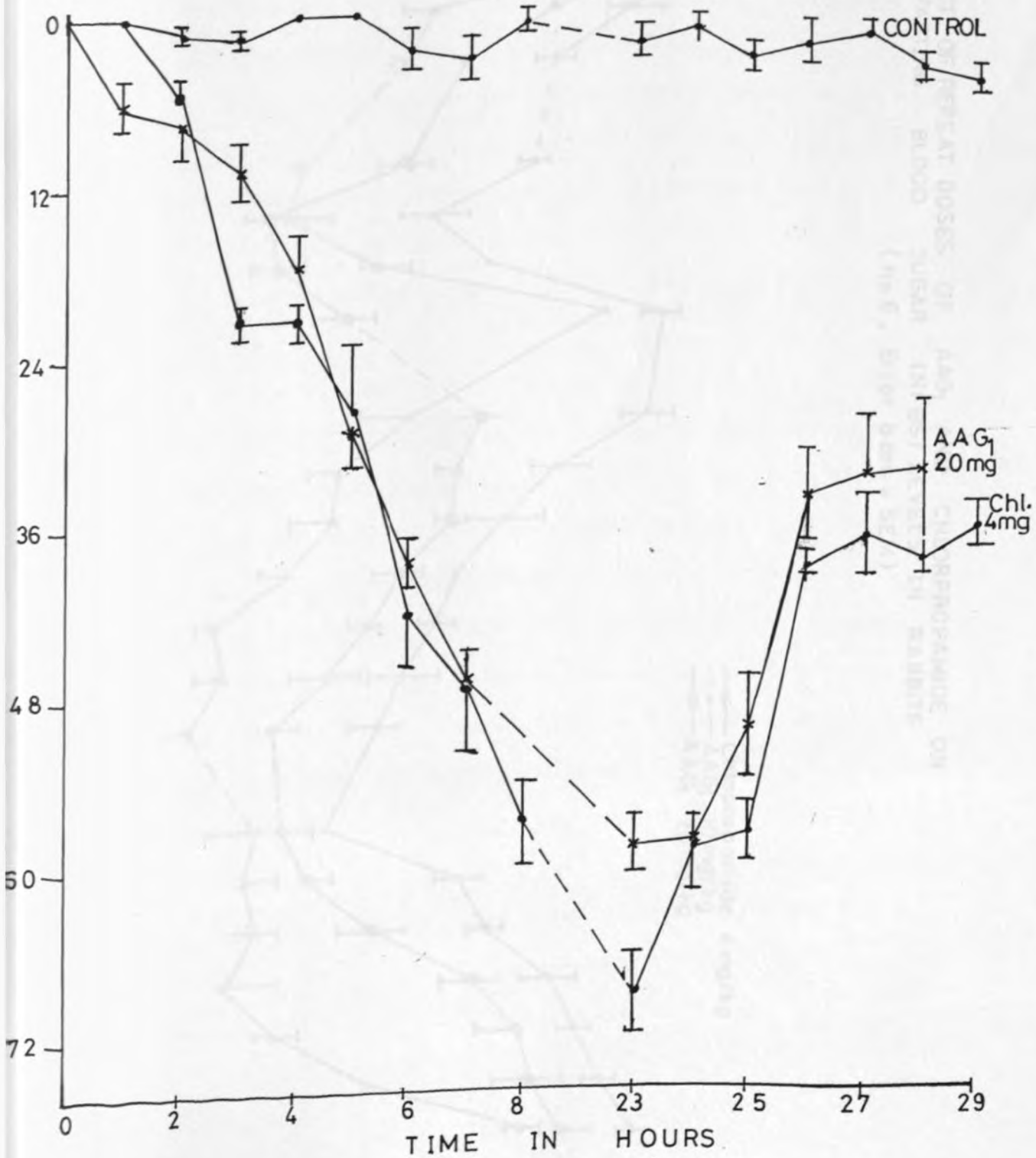
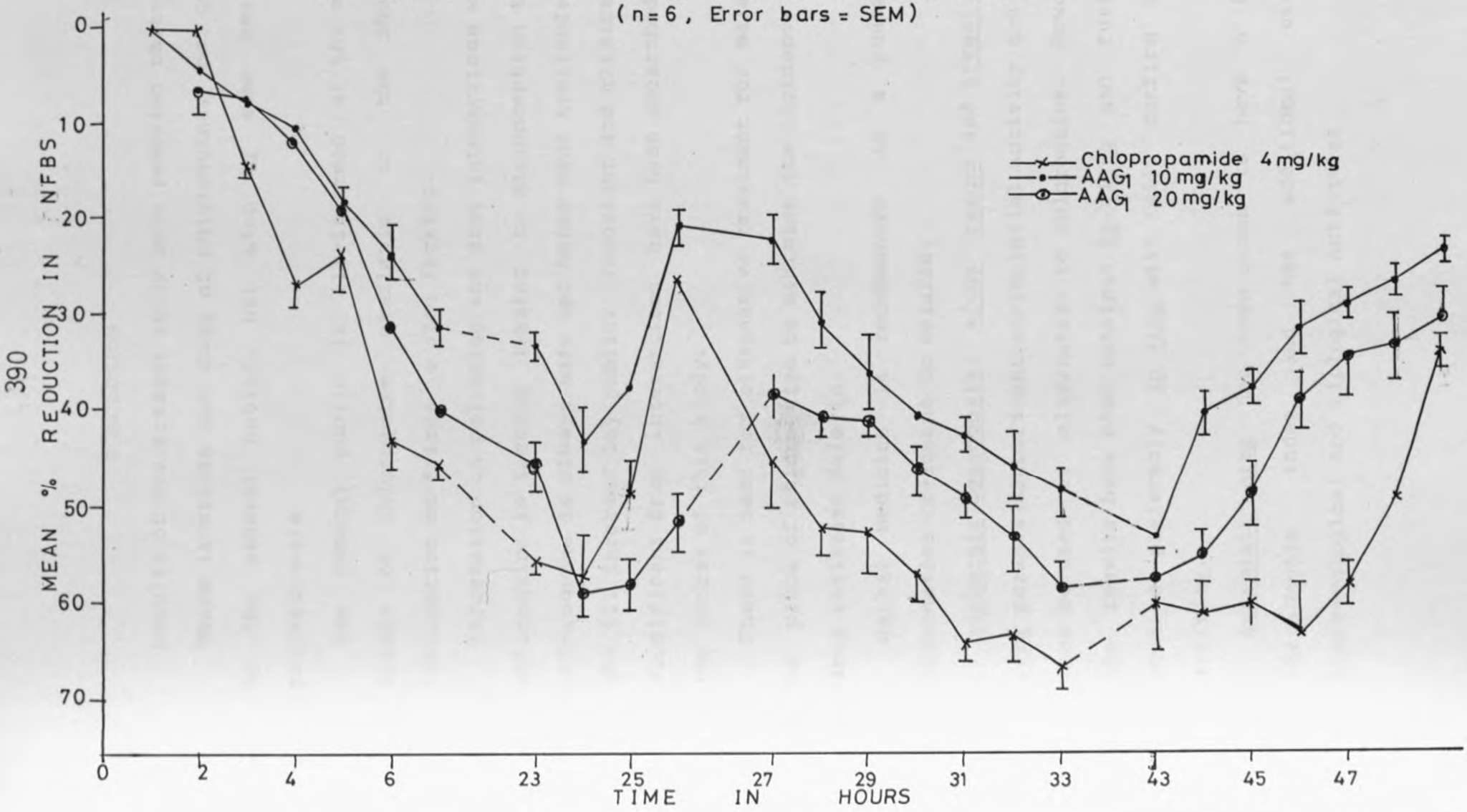


FIGURE 40 EFFECT OF REPEAT DOSES OF AAG_1 AND CHLORPROPAMIDE ON NON FASTING BLOOD SUGAR (NFBS) LEVELS IN RABBITS
(n = 6, Error bars = SEM)



CONCLUSION

Results of the present study have revealed that:-

There is misuse and abuse of chloroquine, not only by the general public, but also by some health professionals.

The general public is ill-informed of the many brands of chloroquine available in the shops. Information on dosage is also lacking.

Information on poisoning and drug interactions with chloroquine is lacking leading to misconception that chloroquine is always safe and hence made available to the ill informed lay public. Important and clinically significant drug interactions have been unearthed in the course of this study.

There is need for alternative treatment for malaria in place of chloroquine to minimise the incidence of drug resistant malaria.

Herbal medicine is recommended as a possible alternative treatment to malaria.

Warburgia ugandensis, Ajuga remota and Artemisia afra possess significant antimalarial activity and can thus be used as alternative to chloroquine. However the results have been obtained in vitro and further studies preferably in vivo will fully confirm this activity.

Artemisia afra has been shown to have a high therapeutic index and has additional useful pharmacological and biological activities.

RECOMMENDATIONS

From the results of the present investigations the following recommendations were made:

Greater surveillance should be excercised in the prescribing, distribution and sales of chloroquine so as to minimise misuse and abuse of the drug.

There should be continuous education provided to the health professionals and to the public at large on the proper use of not only chloroquine but other non-prescription drugs.

Patients should be encouraged to seek proper medical treatment as opposed to self medication that sometimes entails the use of drugs for wrong ailment.

Proper and quick diagnostic methods for malaria should be made available to the health professionals so as to assist in the accurate diagnosis of the malaria instead of relying on the symptomatology diagnosis commonly applied.

The health professionals especially the doctors and the pharmacists should be encouraged to report any suspected drug interactions or adverse effects attributed to the use of any drug to a central data collecting and disseminating centre such as the Drug Information and Poison Control Centre located in the Department of Pharmacy of the University of Nairobi.

Scientists and researchers especially the health

care professionals should continously review and update information on old and new drugs so as to keep abreast of any changes in the response of the population to drugs and also monitor chronic toxicities of commonly used drugs.

More research into herbal medicine should be encouraged. The information so obtained, especially as regards those herbs whose folklore use has been supported by scientific findings should be disseminated to the herbalists who actually need it to update information on the "tools of their trade" and hence provide proper service to their clients. This interaction between the scientists and the herbalists should help remove the stigma erroneously attached to the practice and use of herbal medicines.

REFERENCES

1. Abba, S.M. (1985) Volatile compounds of Artemisia monosperma and Artemisia judaica growing in Egyptian deserts. Biochem Syst-Ecol 13(3) 265-9.

2. Abdalla, S.S., Abu-Zarga, M.H. (1987) Effects of cirsimaritin, a flavone isolated from Artemisia judaica on isolated guinea pig ileum. Planta Med 53(4) 322-4.

3. Acton, N., Klayman, D.L. (1985) Artemisitene, a new sesquiterpene lactone endoperoxide, from Artemisia annua. Planta Med 5 44-2.

4. Addae-Mensah, I and Achenbach, H. (1985) Terpenoids and flavonoids of Bridelia ferruginea. Phytochemistry 24 8 1817-1819.

5. Adekenov, S.M.,Kagarlitskii. A.D., MuKhametzhanov, M.N., Kupriyanov, A.N. (1983) Sesquiterpenoid lactone of Artemisia panciflora. Khim Prir Soedin (2) 238-9, Cf Chem Abs. 99 35942C.

6. Adekenov, S.M., Kharasov, R.M., Kupriyanov, A.N., Turmukhambetov, A.Zh. (1986) Nitrosin, a new sesquiterpene lactone from Artemisia nitrosa. Khim Prir Soedin (5) 644-5 Cf. Chem Abs 106 116478y.

7. Adelusi, S.A.(1984) Adsorption, distribution and

elimination of chloroquine in presence of aspirin when administered into rabbits.

8. Ade-Serrano, O. (1982) Growth inhibitory and lymphocytotoxic effect of Azadirachta indica. Journal of African Medicinal Plants. 5.

9. Adjepon-Yamoah, K.K. (1986) The effects of chloroquine on paracetamol disposition and kinetics. Br. J. Clin. Pharmacol. 21(3) 322-4.

10. Adjepon-Yamoah, K.K. (1985) Chloroquine decreases oral-caecal transit time. Br. J. Clin. Pharmacol. 20 (4) 425-6.

11. Agnew, A.D. (1974). Upland Kenya Wild flowers a flora of the ferns and herbaceous flowering plants of Upland Kenya.
Oxford University Press pp. 471.

12. Agarwal, S.L., Arora, R.B. (1956) The anticholinergic actions of chloroquine and camoquine on smooth and cardiac muscles. Indian J. Med. Res. 44 631-36.

13. Akhtar, M.S. (1984) Chemotherapy of ascaris in livestock. Pak Vet J. 4(1) 75-81.

14. Ali, H.M. (1985) Reduced ampicillin bioavailability following oral co-administration with chloroquine. J. Antimicrob. Chemother. 15(6) 781-4.

15. Ali, H.M. (1981) The effect of Sudanese food and chloroquine on the bioavailability of ampicillin from bacampicillin tablets. Int. J. Pharm. AMST 9(3) 185-190.

16. Allison, J.L., Obrien, R.L., Hann, F.E. (1966) Nature of the deoxyribonucleic acid-chloroquine complex. Antimicrob. Agents. Chemother. 1965 (Sylvester, J.C ed) American Society for Microbiology. Ann Arbor, Mich, pp 310-314.

17. Allison, A.C. (1964) Polymorphism and natural selection in human population. Cold Spr Harb Symp. Quart Biol. 29 137-149.

18. Allison, A.C., Eugui, E.M. (1982) A radical interpretation of immunity to malaria. Lancet Dec. 25 1431-1433.

19. Allison, J.L., O'Brien,R.L., Hann, F.E. (1985) DNA reaction with chloroquine. Science. 149 1111-1113.

20. Alving, A.S., Eichelberger, L., Craige, B. Jr., Jones,R. Fr., Whorton, C.M., Pullman, T.N. (1948) Studies on the chronic toxicity of chloroquine (S.N-7618). J. Clin. Invest. 27 60-65.

21. Amparo, H., Pascual, C., Sanz, J., Rodriguez, B. (1982) Diterpenoids from Ajuga chamaepitys. Two neoclerodane derivatives. Phytochemistry. 21(12) 2909-

2911.

22. Andersson, A., Olsson, S., Tjalve, H. (1980) Chloroquine inhibits the insulin production of isolated pancreatic islets. Biochem. Pharmacol. 29 1779-1735.

23. Anthony, G.M., Morris, M. (1988) Plants of Dhofar, The Southern region of Omani Traditional, economic and medicinal uses. Publ. Office of the adviser for the conservation of the environment Diwani of the Royal Court Sultanate of Oman 1st Public. pp 327.

24. Arisz, L., Danker, A.J.M., BrentJens, J.R.H., Var Derhem, G.K. (1976) Effect of indomethacin on proteinuria and kidney function in nephrotic syndrome. Acta. Med. Scand. 199 121-125.

25. Aronow, W.S. (1973) The medical treatment of anginal pectoris VIII miscellaneous anti-anginal drug. Am. Hert. J. 85 275.

26. Aronsson, B., Bengtsson, E., Bjorkman, A., Wahlgren, M., Pehrson, P.O., Rombo, L. (1981) Chloroquine-resistant falciparum malaria in Madagascar and Kenya. Ann. Trop. Med. Parasitol. Aug. 75(4) 367-73.

27. Arora, R.B., Madan, B.R., Pathak, R.K. (1956) Chloroquine, amodiaquine, procainamide, quinidine in experimental auricular arrhythmias simulating clinical

disorders. Indian J. Med Res. 44 453-462.

28. Arora, R.B., Sharma, U.N., Madan, B.R. (1955) Antiarrhythmics Part 1:Chloroquine in auricular fibrillation. Indian J. Med Res. 43(4) 659-666.

29. Assaad, A.M., Lahloub, M.F. (1988) Iridoid glucosides of Ajuga iva (L). Alexandria J. Pharm. Sci. 2(2) 132-5.

30. Aviado, D.M., Sadavongvivad, C., Cambar, P. (1970) Cardiopulmonary effects of antimalarial drugs 4-aminoquinolines, chloroquine and quinetholate. Toxicol Applied Pharmacol. 17(1) 107-117.

31. Ayitey-Smith, E. (1976) Potentiation of pentobarbitone sleeping time by chloroquine in the rat. West. Afr. J. Pharmacol.and Drug Research 3(1) pp 83-84.

32. Ayitey-Smith, E. (1975) Dual action of chloroquine on frogs skeletal muscle contraction. Eur. J. Pharmacol. 30(1) 29-35 Jan.

33. Back, D.J. (1983) Inhibition of drug metabolism by the antimalarial drugs chloroquine and primaquine in the rat. Biochem. Pharmacol. Jan 15 32(2) 257-63.

34. Back, D.J. (1984) Pharmacokinetics of oral contraceptive steroids following the administration of the antimalarial drugs primaquine and chloroquine. Contraception 30(3) 289-95.

35. Bailey, D.M., Chakrin, L.W. (1981) Arachidonate lipoxygenase. Ann Repts Med. Chem. 16 213-227.

36. Bamber, M.G. (1986) Fatal Steven-Johnson's syndrome associated with fansidar and chloroquine. J. Infect. 13(1) 31-3 Jul.

37. Bamber, M.G., Redpath, A. (1987) Chloroquine and hypoglycaemia. (Letter) Lancet May 23 1211.

38. Bamgbose,S.D.A., Noamesi, B.K. (1981) Planta Medica 41 392-396.

39. Banyal, N.S., Fitch, C.D. (1982) Ferriprotoporphyrin IX binding substances and the mode of action of chloroquine against malaria. Life Science Sep 19 31 (11) 1141-4.

40. Barbera, O., Alberto, M.J., Sauz, J.F., Sanchez, P.J. (1986) 3-methoxyflavones and coumarins from Artemisia incanescan. Phytochemistry 25(10) 2357-60.

41. Barnes, C.S., Occolowitz, J.L. (1963) Aust. J. Chem. 16 219.

42. Barnes, C.S., Occolowitz (1964) The mass spectra of some naturally occuring oxygen heterocycles and related compounds. Aust. J. Chem. 17 975-86.

43. Beeler, G.W., Reuter, H. (1970) The relationship between membrane potential membrane currents and

activation of concentration in ventricular myocardial fibres. J. Physiol (Lond) 207 211-229.

44. Beep-Oliver, O. (1983) Meedicinal plants in tropical West Africa II. Plants acting on the nervous system (Elserier scientific publication Ltd. as in J. Ethnopharmacology 7 1-93 (Review paper).

45. Bennett, W.M., Irwin, S., Coggings, C.H. (1973) Guide to drug use in adult patients with impaired renal function (A supplement). J. Am. Med. Ass. 223 991.

46. Berthoud, F. (1976) Malarial prophylaxis for pregnant women. J. Pediatr. 88(2) 362 Feb.

47. Bertini, F., Daniel,R.B. (1970) The effect of drugs on the rate of proteolysis in vitro within secondary liver lysosomes. J. Cell Physiology 76(2) 225-230.

48. Bhardwaj, D.K., Jain, R.K., Jain, S.C., Manchanda, C.K. (1985) Constitution of Artemisia annua flavone. Proc India Natl Sci. Acad Part A 51(4) 741-5.

49. Black,R.H., Canfield, C.J., Clyde, D.F., Peters, W., Wernsdorfer, W.H. (1986) In chemotherapy of malaria. Bruce, L.J. Ed. pp 9 WHO Geneva.

50. Blazar,B.R., Whitley,C.B., Kitabchi, A.E. (1984) In vivo chloroquine induced inhibition of insulin degradation in a diabetic patient with severe insulin resistance. Diabetes Dec 33(12) 1133-37.

51. Boakye-Yiadom, K., Dwuma-Badu (1977) Antimicrobial action of cryptolepine hydrochloride, proceedings of 3rd symposium on medicinal plant. University of Ife,Ile-Ife, Nigeria.

52. Bohlmann,F.,Hartono, L., Jakupovic, J., Huneck, S. (1985) Guaianolides related to arborescin from Artemisia adamsii. Phytochemistry 24(5) 1003-7.

53. Bohlmann, F., Widayati, A., Trinks, C., Jakupovic, J., Huneck, S. (1988) Dimeric guaianosides from Artemisia sieversiana. Phytochemistry 24(5) 1009-15.

54. Bottiger, L.E. and Westerholm, B. (1973). Br. Med. J. iii 339.

55. Boudreau, E.F., Webster,H.K., Pavanand, K., Thosingha, L. (1982) Type II mefloquine resistance in Thailand. (Letters) Lancet Dec 11: 2 (8311) 1335.

56. Bowden, D.K., Basitien, P., Douglas, F.P., Muir., J.W., Tambisari, E. (1982) Chloroquine resistant Plasmodium falciparum malaria in Vanuata. Med. J. Austr. Dec 11-25 2(12) 561-2.

57. Bowman, W.C., Rand, M.J. (1980) Textbook of Pharmacology Blackwell Scientific Publications 2nd Edition. pp 36.1, 36.12, 10.5, 9.30, 22.54, 20.53.

58. Boye, G.L., and Ampofo, O. (1983) Clinical uses of Cryptolepis sanguinoleta (Asclepiadaceae). Proc. of

1st International Seminar on Cryptolepine 27th July (Kumasi, Ghana) 37-40.

59. Bresloff, D. (1977) Miscellaneous antirheumatic drugs and their possible modes of action. Adv. Drug Res. 11 1-21.

60. Breton, J.L., Camps, F., Coll, J., Eguren, L., Gavin, J.A., Gonzalez, A.G., Martorell, X., Miravitlles, C., Mullins, E., Torramilans, J. (1985) Isolation and structure elucidation of heliangolidin a new sesquiterpene lactone from Artemisia canariensis. Tetrahedron 41(15) 3141-6.

61. Brossi, A., Bvenugopalan, L., Dominguez., Gerpe, H. Jc., Yeh, J.L., Flippen-Anderson, P., Buchs, X.D., Milhous, W. and Peters, W. (1988) Arteether, a new antimalarial drug, synthesis and antimalarial properties. J. Med. Chem. 31(3) 645-650.

62. Brueton, M., Greenwood, B.M. (1987) (Letter) Lancet 1 281.

63. Bruno, R., Barriot, P., Rimailho, A., Band, F.J. (1988) Treatment of severe chloroquine poisoning. New. Eng. J. Med. 318(1) 1-6.

64. Budzikiewicz, H., Djerassi, C. (1962) Mass spectrometry in structural and stereochemical problems of steroid ketones. J. Am. Chem. Soc. 48 1430.

65. Busche, F.P. (1989) Using plants as an exploration tool for gold. J. Geochem. Explor. 32(1-3) 199-209.

66. Bygdeman, M. (1964) Acta Physiol. Scand. 63 243

Bygdeman, M. (1967) Prostaglandins 2 71.

67. Bygbjerg, I.C. (1983) Mefloquine resistance of falciparum malaria from Tanzania, enhanced by treatment. Lancet 1 774-775.

68. Brandling-Bennett, A.D., Oloo, A.J., Watkins, W.M., Boriga, D.A., Kariuki, D.M., Collins, W.E. (1988) Chloroquine treatmet of falciparum malaria in an area of Kenya of intermediate chloroquine resistance. Trans. R. Soc. Trop. Med. Hyg. 88(6) 833-7.

69. Brenziger, N.L., Becherer, P.R., Majerus, P.W. (1979) Characterisation of prostacyclin synthesis in cultured human arterial muscle cells, venous endothelial cells and skin fibroblasts. Cell 16 967.

70. Bruce-Chwatt, L.J. (1982) Transfusion malaria revisited. Trop Dis. Bulletin. Oct. 79(10) 827-40.

71. Cadigan, F.C. (1968) Trans. R. Soc. Trop. Med. Hyg. 62 562.

72. Camazine, B. (1985) Transfusion associated malaria. (Letters) Lancet July 6 2(8445) 37.

73. Campbell, G.D. (1968) Effect of indoleacetic acid on

glucosuria. Med Chem oral hypoglycaemics pp 211.

74. Canfield, C.J. (1980) Antimalarial amino-alcohol alternatives to mefloquine. Acta Trop (Basel) 37 232-237.

75. Cao, C., Manuel, M., Miguel, G.R., Miguel, J., Reyes, D., Fernando, S.G., Entique, M.C. (1970) The beta-adrenergic blocking action of chloroquine on the myocardium. Rev. Cubana Med. Trop 22(1-3) 63-76.

76. Caprioli, V., Cimino, G., Colle, R., Gavagni, M., Sodano, G., Spinella, A. (1987) J. Nat. Prod. 50(2) 146.

77. Cappelletti, E.M., Caniato, R., Appendino, G. (1986) Localisation of the cytotoxic hydroperoxy eudesmanolides in Artemisia umbelliformis. Biochem Syst. Eco 14(2) 182-90.

78. Carnat, A.P., Gueugnot, J., Lamaison, J.L., Guillot, J., Pourrath (1985) The mugwcrt, Artemisia vulgaris L and Artemisia verlotiorum Lamotte. Ann. Pharm Fr. 43(4) 397-405 Cf. Chem Abs 104 222004.

79. Carpenter, R.C., Southeeswaran, S., Sultan Bawawa M.U.S., Balasubramanian, S. (1980) Triterpenes of five Euphorbiaceae species of Sri Lanka. Phytochemistry 19 1171.

80. Carter, R., Miller, L.H., Rener, J., Kaushal, D.C.,

Kumar, N., Graves, P.M., Grotendorst, C.A., Gwadz, R.W., French, C., Wirth, D. (1984) Target antigens in malaria transmission blocking immunity. Philos Trans Roy Soc. Lond (Biol) Nov 13, 307(1131) 201-13.

81. Carter, R., Kaushall, D.C. (1984) Characterisation of antigens on mosquito midgut stages of Plasmodium gallinaceum III. Mol. Biochem Parasitol. Oct. 13(2) 235-41.

82. Cassaday, J.M., Suffness, M. (1989) Anticancer agents based on natural product models. Ed by Cassaday, J.M. and Duros, J.D., Academic Press Inc., New York pp 201.

83. Cattani, J.A. (1989) Malaria vaccines. Results of human trials and directions of current research. Exp. Parasitol. 68(2) 242-7.

84. Chapman, R.S., Ewen, S.W.B. (1969) Chloroquine induced myopathy. Br. J. Dermatology 81 217-219.

85. Charles, D., Bertrand, E. (1982) The heart and malaria. Med. Trop (Mars) July-August 42(4) 405-9.

86. Charles,H.J., Karimullah, A.Z., John, A.N., Charlotte,M.S. (1967) Scopoletin, an antispasmodic component of viburnum opulus and Prunifolium. J. Med. Chem. 10(3), 488-9.

87. Charmot, G., Couland, J.P., LeBcas, J. (1983) Chloroquine resistance of Plasmodium falciparum in

Africa: Current status and proposals for surveillance methods. Bull Soc. Pathol. Exot. Filiales. Mar. Apr. 76(2) 129-36.

88. Chatterjee, R. (1985) Chloroquine and premature evacuation of uterine conceptus in rats. Contraception 31(2) 173-7.

89. Chavan, S., Neena, R., Shah, P., Nikaw, S.T. (1983) Individual and synergistic activity of some essential oils as mosquito larvicidal agents. Bull. Haffkine Inst. 11(1) 18-21. Cf. Biol. Abstr. 78(3) 23106.

90. Chem, F., Zhang, G. (1987) Studies on several physiological factors on artemisinin synthesis in Artemisia annua. Zhiwu Shenglixue Tongxum 5 26-30

91. Chemesova, T.I., Belenovskaya, L.M., Markova, L.P. (1984) Flavonoids of Artemisia rutifolia. Khim. Priv. Soedin 2 249-50 Cf. Chem Abst. 101(7)

92. Chemesova, T.I., Belenovskya, L.M., Stukov, A.N. (1987) Antitumor activity of flavonoid, from some species of Artemisia L. Rastit Resur 23(1) 100-3 Cf. Chem Abst. 106(207296d).

93. "Chiang-su, I., Hsueh Ko Chi Tzuliao" (1962) (Chiang-Su Medical Science Information) 1 17.

94. Chianta, A., Chiono, L., Profumo, E., Testa, S. (1981) Renal changes in malaria. Arch. Sci. Med. (Torino) 138

(4) 459-66.

95. China Co-operartive Research group on Quinghaosu and its derivatives as antimalarials. (1982) J. Trad.China Med. 2, 3.

96. Chinyanga,H.M., Mapfumo, H., Vartanian, G.A.,Okai, E.A., Greenburger, D.V. (1972) Chloroquine induced depression of neuromuscular transmission. Eur .J. Pharmacol. 18(2) 256-260

97. Chinyanga, H.M. (1984) The inhibitory action of chloroquine to guinea pig uterine contractions. West Afr. J. Pharmacol Drug Res. 2(1) 50-57.

98. Chopra, R.N., Nayar, S.L., Chopra, J.C. (1956) in Glossary of Indian medicinal plants. C.S.I.R. Publications, New Dehli pp 31-32.

99. Chou, A.C., Chevli, R. and Fitch, C.D. (1980) Ferriprotoporphyrin IX fulfils the criteria for identification as the chloroquine receptor of malaria parasites. Biochemistry 19 1543-1549.

100. Choudry, V.P., Madan, N., Sood, S.K. (1980) Intravascular haemolysis and renal insufficiency in children with glucose-6-phosphate dehydrogenase deficiency following antimalarial therapy. Indian Med. J. 71 561.

101. Chulay, J.D. (1983) Plasmodium falciparum in Kisimu,

Kenya. Differences in sensitivity to amodiaquine and chloroquine in vitro. J. Infect. Dis. 148 732-36.

102. Clark, I.A., Cowden, W.B., Butcher, G.A. (1983a) Free oxygen radical generators as antimalarial drugs. (Letters) Lancet Jan 29 1(8318) 234.

103. Clark, I.A., Cowden, W.B., Butcher, G.A., Hunt, N.H. (1983b) Free oxygen radicals in malaria. (Letters) Lancet Feb 12 1(8320) 359-60.

104. Clark, I.A., Hunt, N.H. (1983) Evidence for reactive oxygen intermediates causing haemolysis and parasite death in malaria. Infect. Immun. Jan 39(1) 1-6.

105. Cliquart, E. (1929) Bull. Acad. Roy. Med. (Belg) 9, 627.

106. Cobden, I., Rothwell, J., Axon, A.T.R. (1980) Gut. 21 512-518.

107. Codignola, A. (1984) Essential oil of Artemisia arborescens L growing spontaneously in Italy and cultivated in Morocco. Allionia 26 89-95. Cf. Chem. abst. 103 42280z, 1985.

108. Cohen, S.N., Yielding, K.L. (1965) Inhibition of DNA and RNA polymerase reactions by chloroquine. Proc. Natl Acad Sci. USA. 54 521-27.

109. Conklin, K.A., Chou, S.C. (1970) Antimalarials:

Effects on *in vivo* and *in vitro* protein synthesis. *Science* (Washington) 170(3963) 1213-1214.

110. Conklin, K.A., Chou, S.C. (1972) The effects of antimalarial drugs on uptake and incorporation of macromolecular precursors by tetrahimena pyriformis. *J. Pharmacol Exp. Ther.* 180(1) 158-166.

111. Cook. L., Grant, P.T. and Kermack, W.O. (1961) *Exp. Parasitology II* 372-379.

112. Cook, I.F., McGregor, J.W. (1982) Inadequate prophylaxis of *Plasmodium faliparum* with once weekly malaprim therapy. *Med. J. Aust.* Dec 11-25 2(12) 563-4.

113. Cosgriff, T.M., Boudreau, E.F., Pamplin, C.L., Doberstyn, E.B., Desjardins, R.E. and Canfield, C.J. (1982) Evaluation of the antimalarial activity of the phenanthrene methanol halofantrine (WR 171,669. *Am. J. Trop. Med. Hyg*. Nov, 31(6) 1075-9.

114. Cotton, E.K., Fahlberg, V.I. (1964) Hypoglycaemia with salicylate poisoning. *Am. J. Dis Child* 108 171.

115. Cox, F.E.G. (1988) Vaccine development: which way for malaria? *Nature* 331 486-487.

116. Craven, S.A. (1974) (Letters) *Br. Med. J.* 2 556.

117. Cundall, R.D. (1964) Letter *Br. Med. J.* (i) 1638.

118. Danis, M., Felix, H., Brucker, G., Drulhe, P.,

Richard, L.E., Noble, D., Gentilin, M. (1982) Mefloquine in the treatment and prevention of malaria. Pathol Biol (Paris) Jun 30(6 part 2) 589-92.

119. Decker, W.J., Harold, F.C., Treuting, J.F., Treating, J.F., Banez (1971). Dialysis of drugs against activated charcoal. Toxicol. Appl Pharmacol 18(3) 573-578.

120. DeGeus, A., Meuwissen, J.H., Van Rijn, A. (1982) A case of fansidar resistant Plasmodium falciparum from Tanzania. Trop. Geogr. Med. 34(3) 261-3.

121. De Silver, D.H., Mendis, K.N., Premaratine, U.N., Jayatilleke, S.M., Soyza, P.E. (1982) Congenital malaria due to Plasmodium vivax. A case report from Sri Lanka. Trans R Soc. Trop. Med. Hyg. 76(1) 33-5.

122. Delmon, J., Ranque, P., Balique, H., Tounkara, A., Soula, G., Quilici, M., Pene, P. (1981) Influence d'une chimiopropita xie antipalidique sur letat de sante d'une communaute' rurale en Afrique de lovest Re' sultat Pre'liminaires. Bull Soc Path Exot Filiales Nov-Dec 74(6) 600-10.

123. Desjardin, R.E. (1979) Quantitative assessment of antimalarial activity in vivo by semi-automated microdilution technique. Antimicrobial agents and Chemotherapy 16 710-718.

124. Dintenfass, L. (1976) in Rheology of blood in diagnostic and preventive medicine. Butterworth & Co. Publishers Ltd. (London) pp 213.

125. Dixon, K.E., Williams, R.G., Pongsupat, T., Pitaktongu, P.P. (1982) Trans R. Soc. Trop Med Hyg. 76 (5) 664-7.

126. Dockrell, H.M., Playfair, J.H. (1983) Killing of blood stage murine malaria parasite by hydrogen peroxide. Infect. Imm. Jan 39(1) 456-9.

127. Duk, R.H. (1966) Biochemical studies on the constituents of Artemisia messerschimidiana and their derivatives. Yakhak Hoeji 10(2-3) 25-9. Cf. Chem Abst. 68 1968.

128. Duke, S.O., Paul, R.N.Jr., Lee, S.M. (1988) Terpenoids from genus Artemisia as potential pesticides. A.C.S. symp ser on Biol. Active Natural Products, potential use in agriculture 380 318-334.

129. Duriyananda, D., Noey-Patimanond, S. (1982) Quinine resistant falciparum malaria case report. J. Med. Ass. Thai Jul 65(7) 395-8.

130. Dutta, G.P., Renu, B., Viswakarma, R.A. (1989) Artemisinin (qinghaosu), A new gametocytocidal drug for malaria. Chemotherapy 35(3) 200-207.

131. Ebeigbe, A.B. (1986) Mechanism of chloroquine induced

inhibition of smooth muscle contraction. Arch. Int. Pharmacodyn and Ter. 280(2) 254-63.

132. Eckman, J.R., Modler, S., Eaton, J.W., Berger, E. and Engel R.R. (1977) J. Lab. Clin. Med. 90 767-770.

133. Eichenlaub,D., Rogler, G., Hoffmann, H.G., Weise, H.J. (1982) Falciparum malaria despite pyrimethamine/sulfadoxine in five tourists to East Africa. (Letter) Lancet Nov. 6 2(8306) 1041-2.

134. Eichenlaub, D., Hoffmann, H.G., Rogler, G., Weise, H.J. (1983) Falciparum malaria in East African Tourists inspite of fansidar prevention. A contribution on increased chloroquine and pyrimethamine/sulfadoxine resistance in areas of East Africa. Dtch Med. Wochenschr Mar.4 108(9) 338-43.

135. El-Emary, N.A., Makboui, M.A., Hamed, M. (1986) Sesquiterpene lactones from A. argentea. Phytochemistry 26(1) 314-15.

136. El-sayed, A.M., Aboutabi, E.A., Allazzuny, A.A. (1988) A contribution to the chemical and biological evaluation of santonin from Artemisia cina. Egypt J. Pharm. Sci. 29(1-4) 43-51.

137. Elmarakby, S.A., El-Feraly, F.S., Elsohly, H.N., Croom, E.M. and Hufford, C.D. (1987) Microbial Transformation studies of arteanuin B. J. Nat. Pro. 50

(5) 903-909.

138. Ekanem, O.J. (1978) Has Azadirachta indica (Dogonyaro) any antimalarial activity. Nig. Med. J. 8 8-10.

139. Elliot, S., Brimacombe, J. (1986) Pharmacy needs tropical forests. Manufacturing Chemist (Oct) 31-33.

140. Elmarakby, S.A., Raly El-Feraly, F.S., Elsohly, N.H., Groom, E.M., Hufford, C.D. (1987) Microbial Transformation studies of arteanuin. Br. J. Nat. Products 50(5) 903-909.

141. Embrey, M.P. (1969) The effect of prostaglandins on human pregnant uterus. J.Obs and Gyn (Brit. Commonwealth) 76 783-798.

142. Engeset, A. (1957) Quinacrin Og. Chloroquin Ved asthma bronchiale. Nord. Med. 281 1492-1494.

143. Esteban, M.D., Gonzalez, C.I., Macia, F.A., Massanet, G.M., Rodriguez, L.F. (1986) Structure and chemistry of secondary metabolites from compositae Part 7-Flavonoids from Artemisia lanata. Phytochemistry 25(6) 1502-4.

144. Etherington, D.J., Evans, P.J. (1977) The action of cathepsin B and collagenolytic cathepsin in the degradation of collagen. Acta. Biol. Med. Ger. 36 1555-1563.

145. Evans, W.C. (1989) Trease and Evans Pharmacognosy 13th Edn Bailliere Tindall London, Philadelphia, Toronto, Sydney, Tokyo. pp 419-421, 516.

146. Fabiato, A., Fabiato, F. (1977) Calcium release from the sarcoplasmic reticulum. Circ. Res. 40 119-129.

147. Famaey, J.P., Fontaine, J., Reuse, J. (1975) The inhibitory effect of morphine, chloroquine, non-steroidal and steroidal anti-inflammatory drugs on electrically induced contractions of guinea pig smooth muscle and the reversing effect of prostaglandins. Agents and action 5(4) 354-358.

148. Fandeur, T., Moretti, C., Polonsky, J. (1985) Planta Med. 51 20.

149. Fasan, P.O. (1971) Trimethroprim plus sulphamethoxazole compared to chloroquine in the treatment and suppression of malaria in African school children. Ann. Tro. Med. Parasit. 65(1) 1117.

150. Ferone, R., Burchall, J.J., Hitchings, G.H. (1969) Plasmodium berghei dihydrofolate reductase isolation, properties, and inhibition by antifolates. Mol. Pharmacol 5 49-59.

151. Finley, R., Weintraub, J., Louis, J.A., Engers, H.D., Zubler, R., Lambert, P.H. (1983) Prevention of cerebral malaria by adaptive transfer of malaria

specific cultured T. cell into mice infected with Plasmodium berghei. J. Immunol. Sep 131(3) 1522-6.

152. Fisher, C.W.S. (1983) Clinica curio: acidosis and hypoglycaemia in malaria. Br. Med. J. 286 1261.

153. Fitch, C.D., Chevli, R., Banyal, H.S., Phillips, G., Pfaller, M.A., Krogstad, D.J. (1982) Lysis of Plasmodium falciparum by ferriprotoporphyrin IX and a chloroquine ferriprotoporphyrin IX complex. Antimicrob. Agents Chemother. May 21(5) 819-22.

154. Fitzerald, D.E., Butterfield, J.H. (1969) A case of increased platelet anti-heparin factor in a patient with Raynulds phenomenon and gangrene treated with aspirin. Angiology 20 317-324.

155. Fourth meeting of the Scientific Working Group on the chemotherapy of malaria. Beijing People Republic of China. WHO Report TDR/CHEMOL SWG (4)/QHS 81.3P5.

156. Friedland, S.S., George, L.H., Longman, R.T., Train, K.E., O'Neal Jr. (1959) Mass spectra of steroids. Anal. Chem. 31 169.

157. Friedman, M.J. (1978) Erythrocytic mechanisms of sickle cell resistance to malaria. Proc. Nat. Acad. Sci. USA 75 1994-97.

158. Friedman, S.A., Bienen-Stock, H., Richter, I.H. (1969) Malignancy and arteriopathy. Angiology 20 136.

Friedman, M.J. (1979) Oxidant damage mediates variant red cell resistance to malaria. Nature 280 245-47.

159. Friedman, M.J. (1983) Control of malaria virulence by alpha-1-acid glycoprotein (orosomucoid) an acute phase (inflammatory) reactant. Proc Nat. Acad Sci. USA Sep 80(17) 542-4.

160. Fulton, J.D. and Remington, C. (1953). J. Gen. Microbiol. 8 157-159.

161. Fung, W.P. (1972) Malaria resistant to chloroquine and sulphamethoxine/pyrimethamine. Trop Disease Bull. 69 369.

162. Fung, W.P. (1971) Malaria resistant to chloroquine and sulphamethoxine pyrimethamine. Aust. N.Z.J. Med 1 262.

163. Gantt, C.I. (1972) Diuretic therapy. Rational Drug Ter. 6:1.

164. Garfield, R.M., Vermund, S.M. (1983) Changes in malaria incidence after mass drug administration in Nicaragua. Lancet Aug 27 2(83481) 500-3.

165. Garnier, R (1985) Haemoperfusion in chloroquine poisoning. (Letter) Br. Med. J. (Clin. Pos) 13 291(6488) 14.

166. Gavend, M., Voog, R., Thery, J.P., Bessard, G. (1971) Observation on the blood histamine levels in asthmatic

patients during antimalarial drug treatment. Therapie 26(5) 919-925.

167. Geary, T.G., Jensen, J.B. (1983) Lack of cross resistance to 4-aminoquinolines in chloroquine resistant plasmodium falciparum in vitro. J. Parasitol. Feb 69(1) 97-105.

168. Georghiou. G.P., Taylor,C.E. (1977) Operational influences in the evolution of insecticide resistance. J. Economic Entomology 70 655-658.

169. Gillin, F.D., Reimer, D.S., Suffness, M. (1982) Antimicrob. Agents Chother 22 342.

170. Giuffrida, A.M. Sjostrand, J., Cambria, A., Serra, I., Vanella, A., Avitable, M., Jarlstedt, J., Karlsson, J.O. (1976) Neuropharmacology 15 439.

171. Goldstein, R.E. (1972) Medical management of patients with angina pectoris. Prog. Cardiovasc. Dis 14 360.

172. Golenser, J., Miller, J., Auraham, H., Spira, D.T. (1983) The inhibitory effect of human sera upon the in vitro development of Plasmodium falciparum. Trop Georgr. Med mar 33(1) 15-20.

173. Gonasum, L.M., Potts, A.M. (1974) Invest. Ophthal. 13 107.

174. Gorog, S., Szollosy (1978) NMR spectroscopy,of steroid

hormone drugs in Analysis of steroid hormone drugs. Eds Gorog, S. and Szosz, C. Elsevier: Amsterdam Oxford and N.Y. pp 236-248.

175. Goodman, L.S., Gilman, A.G., Rall, T.W., Murad, F. (1985) In Goodman and Gilmans'. The Pharmacological basis of Therapeutics 7th Edn. Macmillan Publishing Company, New York. Chapter 65 pp 1519.

176. Gregory, M.H., Ruty, D.A., Wood, R.D. (1970) Differences in retinotoxic action of chloroquine and phenothiazine derivatives. J. Pathol. 102(3) 139-150.

177. Greilling, H., Kister, R., Vojtisek, O. (1969) The inhibition of deoxyribonucleic acid and ribonucleic acid in synovial cell by immunosuppressive substances. Korean J. Parasitol 7(3) 121-128.

178. Grundmann, M., Urublovsky, P., Demkova, V., Mikulikova, I., Pegrimova, E. (1972) Tissue distribution and urinary excretion of chloroquine in rats. Arzneim Forsch 22(1) 82-83.

179. Gu, H.M., Warhurst, D.C., Peters, W. (1983) Rapid action of qinghaousu and related drugs on incoporation of 3-H-Isolevicine by Plasmodium falciparum in vitro. Biochem. Pharmacol Sep 1 32(17) 2463-6.

180. Gu, H.M., Warhurst, D.C., Peters, W. (1984) Trans. R. Soc. Trop. Med Hyg 78 265-270.

181. Guantai, A.M. (1982) Catha edulis (Vahl) Forsk (Miraa) occurance, active constituents and pharmacological activity. MSc Thesis University of Nairobi, Kenya.

182. Gunatilaka, A.A.L., Nanayakkara, N.P.D., Sultanbawa, M.U.S. and Balasubramaniam, S. (1982). Friedelin, D:A-Friedo-oleanan-3,2,1-dione and 21alpha-hydroxy-D:A-Friedo-oleanan 3-one from Kokoona zeylanica. Phytochem 21(8) 2061-63.

183. Guru, P.Y., Warhurst, D.C., Harris, A., Phillipson, J.D. (1983) Ann. Trop. Med. Parasitol. 77 433.

184. Haataja, M., Fraki, J.E., Vaino, E. (1978) The effect of antirheumatic drugs on proteinases in synovia of patients with rheumatoid arthritis. Int. J. Clin. Pharmacol 16 417-419.

185. Habara, Y (1986) Antimuscarinic effects of chloroquine in rat pancreatic acini. Biochem. Biophysic Res. Commun. 13 137(2) 664-9.

186. Hall, A.P., Segal, H.E., Pearlman, E.J., Phintu-Yothin, P., Kosakal, S. (1975) Amodiaquine resistant falciparum malaria in Thailand. Am. J. Trop. Med. Hyg. 24 574-80.

187. Han, Y.N., Yang, H.O., Han, B.N. (1984) Studies on the anticoagulant component of A. herba. Yakhak Hoechi 28 (2) 69-77.

188. Harper, H.A. (1976) Review of physiological chemistry. Lange Medical Publications Maruzen Company Ltd 14th Edn. Cap. 19 409-416.

189. Hart, C.W., Naunton, R.F. (1964) The placental barrier and drugs. Arch Otolar 80 407, Per J. Am. Med Ass 190 392.

190. Hassanali, A.H., Lwande, W. (1989) Antipest secondary metabolites from African Plants. In Insecticides of Plant origin Eds Arnason J.T., Philogene, B.J.R., Morand, P. Am. Chem. Soc. Series No 187 pp 78-94.

191. Hearn, J. (1986) Myasthenia gravis caused by penicillamine and chloroquine therapy for rheumatoid arthritis. South Af. Med. J. 79(9) 1185-6 Sep. 1986.

192. Hegnauer, R. (1977) The Biology and Chemistry of Compositae Heywood, V.H., Harborne, J.B., Turner, B.L. eds Chap 10 Academic Press London.

193. Henquine, J.C., Horemans, B., Nenquin, M., Verniers, J. and Lambert, A.E. (1975) Quinine-indiced modification of insulin release and glucose metabolism by isolated pancreatic islets. FEBS Lett. 57 280-284.

194. Heywood, V.H., Harborne, J.B., Turner, B.L. (1977) The biology and Chemistry of compositae Heywood, V.H., Harborne, J.B., Turner, B.L. eds, Chap 1 academic Press London.

195. Hockmeyer, W.T., Dame, J.B. (1985) Recent efforts in the development of a sporozoite vaccine against human malaria. Adv. Exp. Med. Biol. 185 233-45.

196. Hofer, O., Greger, H. (1984) Naturally occuring sesquiterpene-coumarin ethers. Part 5: Scopoletin sesquiterpene ethers from Artemisia persica. Phytochemistry 23(1) 181-182.

197. Hofer, O., Szabo, G., Greger, H., Nikiforov, A. (1986) Leaf Coumarins from Artemisia laciniata.. Liebigs Ann Chem 12 2142-49. Cf. Chem Abs 106 2868V.

198. Hoffman, S.L., Rustama, D., Dimpudus, A.J., Punjabi, N.H., Campbell, J.R., Oetmo, H.S., Marwoto, H.A., Harun, S. (1985) RII and RIII type resistance of Plasmodium falciparum to combination of mefloquine and sulphadoxine/pyrimethamine in Indonesia. Lancet 9 2 (8463) 1039-40.

199. Hornstein, O.P. and Ruprecht, K.W. (1982) Fansidar induced Steven-Johnson syndrome. Letter New Engl. J. Med 307 (24) 1529.

200. Howells, R.E., Peters, W., Homewood, C.A., Warhurst, D.C. (1970) Theory for the mechanism of chloroquine resistance in rodent malaria. Nature (London) 228 (5272) 625-628.

201. Howard, R.T (1989) Malaria the search for vaccine antigens and new chemotherapeutic strategies. Blood Aug 1 74(2) 533-6.

202. Howard, R.W., Thorne, B.L., Levings, S.C., Mcdaniel, C.A. (1988) Culticular hydrocarbons as chemotaxonomic characters for Nasutitermes corniger (Motschulsky) and Nasutitermes ephrabe (Holmgren) Isoptera: Termitidae. Ann. Entomol Soc. Ang (3) 395-399.

203. Hughes, J.T., Esiri, M., Oxbury, J.M., Whity, C.W.M. (1977) Chloroquine myopathy. Quart J. Med. 40(157) 85-93.

204. Huo, I.W.S. (1953) A report from the malarial Therapeutic Hospital Shanghai First Medical College at Fo-Tzu-Ling.

205. Ikhsanova, M.A., Berocorskaya, T.R., Serykh, E.A. (1986) Coumarins of A. vulgaris. Khim. Prir. Soedin (1) 110. Cf. Chem Abs. 104 203928.

206. Ikram, M., Shafi, N., Mir, I., Do, M.N., Nguyen, P., Lee, Q.P.W. (1987) A possible antipyretic constituent of A. absinthiun. Planta Med 53(4) 389.

207. Inselburge, J., David, J.B., Toshihiro, H. (1987) Pyrimethamine resistant Plasmodium falciparum over production of dihydrofolate reductase by a gene mutation. Mol. Biochem. Parasitol 26(1/2) 121-134.

208. Irvin, H.M., Kyle, V., Diosi, D.T. (1972) An overdose of chloroquine. Forensic Sci. 1(2) 249-253.

209. Iwu, M.M., Obidoa, O., Anazodo, M. (1986) Biochemical

mechanism of the antimalarial activity of Azadirachta indica leaf extract. Proceedings of a workshop on medicinal plant research in Nigeria. Edited by Sofowora, A., University of Ife-Ile-Ife, Nigeria pp 369.

210. Jakupovic, J., Chen, Z.C., Bohlmann, F. (1987) Arteanomaloide, a dimeric guaianolide and phenylalanine derivatives from Artemisia anomala. Phytochemistry 26(10) 277-9.

211. Jakupovic, J., Chau-Thi, T.V., Warning, U., Bohlmann, F., Greger, H. (1986) 11-beta,13-dihydroguaianolides from Artemesia. douglasiana and a thiophen acetylene from A. schmidtiana. Phytochemistry 25(7) 1663-7.

212. James, M.J., Walsh, J.A. (1985) Effects of aspirin and alcohol on platelet thromboxane synthesis and vascular prostacyclin synthesis. Thromb. Res 39(5) 587-594.

213. Jayaweera, D.M.A. (1982) in medicinal plants used in Ceylon. The National Science Council of Sri-Lanka, Colombo. 4 53-54.

214. Jeong, H.S., Khang, D.V. (1985) Histopathological studies on the effects of phenobarbitone and Artemisia treatment upon acute hepatic lesions induced by chloroform. Chungnan Vidae Chapchi 12(1) 27-36. Cf. Chem abs 106 452035 1983.

215. Jeremic, D., Jokic, A., Behbud, A., Stefanovic, M. (1973) Isolation of a potent antitumor agent arteanuin-B from Artemisia annua. Tetrahedron lett. 3039.

216. Jeremy, R.T (1970) Interaction of aspirin and indomethacin in treatment of rheumatoid arthritis. Med J. Aust. 2 127.

217. Jindal, M.N., Patel, M.A., Joseph, A.D. (1960) Local anaesthetic action of antimalarials, chloroquine and amodiaquine. Arch. Int. Pharmacodyn 127 132-140.

218. Jindal, M.N. (1970) Adrenergic neurone blockade with chloroquine and amodiaquine. Indian J. Med Res 58(8) 1050-1056.

219. Johnson, D.E., Roendej, P., Williams, R.G. (1982) Falciparum malaria acquired in the area of Thai-Khymer-border resistant to treatment with fansidar. Am. J. Trop. Med. Hyg Sep. 31(5) 907-12.

220. Joseph A.J., Bush, L.H. Jr., Vaillancourt, R., Daykin, D. (1987) Effects of chronic low dose aspirin on platelet and vascular eicosanoid metabolism in non human primates (Macoca fascicularis). Arteriosclerosis 7(6) 599-604.

221. Jutamaad, S.O., Wongsawatkul, O., Bunnag, D., Tan-Ariya, P. and Brockelman, C.R. (1987) Flunarizine and

verapamil inhibit chloroquine resistant Plasmodium falciparum growth in vitro. South East. Asian J. Trp Med Publ Health 18(2) 253-258.

222. Kabir, S.M.A. (1969) Transac. Roy Soc Trop Med. Hyg 63 983.

223. Kahles, H., Riegger, A.J.G. (1987) Indomethacin and frusemide in patients with heart failure. Renal function, renin-angiotensin system and renal prostaglandin. Dtsch Med Wochenschr 112(45) 1737-1760. Cf. Biol abs. 85(8) April 1988.

224. Karim, S.M.M. (1968) Appearance of prostaglandins F2alpha in human venous blood during labour. Brit. Med. J. 4 618-621.

225. Karim, S.M.M., Truseel, R.R., Hillier, K., Patel. R.C. (1968) Response of pregnant human uterus to prostaglandins F2alpha induction of labour. Brit. Med. J. 4 621-623.

226. Kasymov, SH.Z., Abdullaev, N.D., Yusupov, M.I., Sidyakin, G.P., Yagudaev, M.R. (1984) New guaianolides from Artemisia absinthium Kim Prir Soedin (6) 794-5. Cf. Chem Abs. 102 14 6143V 1985.

227. Keen, A.T., Harris, A., Phillipson, J.D., Warhurst, D.C. (1986) Planta Med. 52 278.

228. Kent, A.K. (1987) Indomethacin anatagonises frusamides

intratubular effects during loop segment microperfusion. J. Pharmacol Exp. Ther 243(3) 881-884.

229. Khalid, R., Colema, R. (1987) Effect of chloroquine on biliary lipid and lysosomal enzyme output in isolated perfused rat liver at low bile salt output rates. Biochim Biophys. Acta 922(3) 395-397.

230. Kiel, F.W. (1964) Chloroquine suicide. J. Am. Med. Ass. 190 398-400.

231. Kioy, D.W. (1989) A study of the chemistry and pharmacology of phytochemicals from three species of Canellaceae. PhD Thesis University of Strathclyde pp 258, 294, 295.

232. Kiso, Y., Ogasawara, S., Hiroth, K., Watanabe, N., Oshima, Y., Konno, C., Hikino, H. (1984) Validity of oriental medicine: 56 liver protective drugs: 10 Antihepatotoxic principles of Artemisia capillaris buds. Planta Med 50(1) 81-5.

233. Klayman, D.L., Lin, J., Acton, N., Scovill, J.P., Hoch, J.M., Milhous, W.K., Theoharides, A.D. (1984) Isolation of artemisinin (Qinghaosu) from Artemisia annua growing in the United States. J. Nat Prod 47(4) 715-717.

234. Knapp, F.E., Nicholas, H.J. (1969) The sterols and triterpenes of Banana Peel. Phytochemistry 8 207 214.

235. Koda-Kimble, M.A., Katlher, B.S., Young, L.Y. (1980) Applied Therapeutics for Clinical Pharmacists 2nd edn. Applied Therapeutics Inc. San-Fransisco California pp 226.

236. Kokwaro, J.O. (1976) Medicinal Plants of East Africa. E. Afr. Lit. Bureau pp 45, 58, 69, 106, 116.

237. Krogstad, D.J., Ilya, Y.G., Dennis, E.K., Ayoade, M.J., Samuel, K.M., Wilbure, K.M., Paul, H.S. (1987) Efflux of chloroquine from Plasmodium falciparum mechanism of chloroquine resistance. Science (Wash. D.C) 238(4831) 1283-1286.

238. Krotoski, W.A., Bray, R.S., Garnham, P.C. and Gwadz, R.W. (1982) Observations on early and late post sporozoite tissue stages in primate malaria II. The hypnozoite of Plasmodium cynomolgi bastianelli. Am. J. Trop. Med. Hyg. 31 211-225.

239. Krungkrai Sudaratana Rochanakiji, Yuthavong, Yongyuth. (1987) In vitro activity of qinghaosu against Plasmodium falciparum was enhanced by oxygen tension. Trans. R. Soc. Trop. Med Hyg 81(5) 710-714.

240. Kupferschmidt, H.G., Schroder, K., Beltzner, B. (1988) Chloroquine and fansidar resistance of Plasmodium falciparum now also in Ghana. Agnew Parasitol Nov. 29 (4) 211-3.

241. Kubo, I., Miura, I., Pettei, M.J., Lee, Y.W., Pilkewicz, F., Nakanishi, K. (1977) Tetrahedron letts 4553.

242. Kubo, I., Taniguch, M. (1988) Polygodial, an antifungal potentiator. J. Nat. Prod. 51(1) 22-9.

243. Kubo, I., Matsumoto, I., Kakooko, A.B., Mubiru, N.K. (1983) Structure of mukaadial a molluscicide from the Warburgia plants. Chem Letts (7) 979-80.

244. Kuria,K.A.M., Muriuki, G. (1984) A new cardiotonic agent from Ajuga remota Benth (Labiatae). E. A. Med. Journal 533-538.

245. La, H. (1982) A comparative trial of oral chloroquine and oral co-trimoxazole in vivax malaria in children. Am. J. Trop. Med. Hyg May 31(1) 438-40.

246. Ladda, R. and Sprinz, H. (1969). Pro. Soc. Exp. Biol. Med. 130 524-527.

247. Langrave, M. (1985) The influence of various types of breakfast on chloroquine levels (Letter). Trans R. Soc. Trop. Med Hyg 79(4) 559.

248. Landgrebe, F.W., Munday, K.A. (1954) Quart. J. Exp. Physiol 38 17.

249. Lafler, C.F., Lilja,H.S., Holbrook, D.J. Jr. (1973) Biochem Pharmacol 22 715.

250. Lee, K., Tani, S., Imakura, Y. (1987) Antimalarial agents, 4: synthesis of brusatol analog and biological activity of brusatol related compounds. J. Nat. Prod 50(5) 847-851.

251. Lagros, J., Jean. (1971) Action of three 4-aminoquinolines on the electroretinograph of the pigmented rat. J. Pharmacol (Paris) 2(3) 327-336.

252. Lagros, J., Rosner, I. (1971) Electroretinographic modifications in albino rats after chronic administration of toxic doses of hydroxychloroquine and desethylhydroxychloroquine. Arch. Ophthalmol. Rev. Gen. Ophthalmol (analys) 31(2) 165-180 illus.

253. Levy, M.R. and Chou, S.C. (1973). J. Parasitol, 59 1064-1070.

254. Li, Z.L., Gu, H.M., Warhurst, D.C., Peters, W. (1983) Trans. Roy. Soc. Trop. Med Hyg 77 522-523.

255. Li, H.T. (1987) Developing new drugs related to Chinese Medicinal plants. Lecture at IIIrd Int. Congress Pharm. Sci. Barcelona 12th June pp 46-54.

256. Li, G., Guo, X., Jin, R., Wang, Z., Jian, H. and Li, Z.J. (1982). Traditional Chinese Medicine 2 125.

257. Lin, P.Y., Pan, J.Q., Feng, Z.M., Zhang, D., Yand, W.L. (1989) Immunopharmacologic activity of artemisinin (Qinghaosu). Asia. Pac J. Pharmacol 3(4)

197-200.

258. Lin, X., Sang, S. (1985) Crystal structure and absolute configuration of arteanomalactone. Huaxue Xuebao 43(8) 724-7. Cf. Chem Abs 104 197454W, 1985.

259. Liu, N.M., Fan, J., Tu, Y., Wu, Z., Qu, Y. and Chou, W. (1979). Acta Chim Sinica 37 129.

260. Loftus, L.R. (1963) Peripheral neuropathy following chloroquine therapy. Can. Med. Ass. J. 89(917) 603-605.

261. Rollo, I.M. (1975). In the pharmacological basis of Therapeutics 5th edn (Goodman, L.S. and Gilman, A., eds) pp 1049-53. Macmillan, N.Y.

262. Lottenbach, K. (1967) Periphere Durchblutung and ischaemische attacke beim morbus Raynauds. Angilogica 4 161.

263. Lucas, E. (1969) Pyrimethamine resistant strain. Trans. Roy. Soc. Trop Med Hyg 63 216.

264. Macdonald, R.D., Andrew, G.E. (1970) Experimental chloroquine myopathy. J. Neuropathol Exp. Neurol. 29 (3) 479-499.

265. Macomber, P.B., Sprinz, H. and Tousimis, A.J. (1967). Nature (London) 214 937-39.

266. Madow, B.P. (1960) Use of antimalarial drugs as

'desludging' agents in vascular disease process. J. Am. Med. Ass. 172 1630.

267. Maitai, C.K., Guantai, A.N., Mwangi, J.W. (1981) Self medication in management of minor health problems in Kenya. E. A. Med. J. 58(8) 593-599.

268. Mallabaev, A., Tashkhodzhaev, B., Saitbaeva, I.M., Yagudaev, M.R., Sidyakin, G.P. (1986) Stucture of artelein - a new dimeric lactone from Artemisia leucodes. Khim Prir Soedin (1) 46-52. Cf. abs. 105 75873g.

269. Martin, V.A. (1974). J. ir. Med. Ass. 67 46 as per Pharm. J. ii/1974 159.

270. Martin, M.L., Morgan, A., Carron, R., Monterio, M.J., Roman, L.S. (1988) Antipyretic activity of alpha and beta santonin. J. Ethnopharmacol 23(2-3) 285-90.

271. Martindale: The Exra-Pharmacopoeia (1989) The Pharmaceutical Press (London), 29th edn pp 505-521.

272. Martindale: The Extra-pharmacopoeia. (1977) 7th Edn. The Pharmaceutical Press (London) pp 346.

273. Martinez, V., Barbera, O., Sanehez-Parareda, J., Marco, J.A. (1987) Phenolic and acetylenic metabolites from Artemisa assoana. Phytochemistry 26(9) 2619-24.

274. Matsueda, S., Nagaki, M. (1984) Studies on

sesquiterpene lactones XII. Chemical constitution of Artemisia montana (Nakai) pamp. Yukugaku Zasshi 104(7) 753-6. Cf. Chem Abs. 101(7) 1984.

275. Matz, G.J., Naunton, R.F. (1968) Ototoxicity of chloroquine. Archs Otolar 88 370. Cf. J. Am. Med Ass. 206 910.

276. McCann, W.P. (1975) Fatal chloroquine poisoning in a child. Experience with peritoneal dialysis. Pediatrics 55(4) 536-8.

277. McGrath, M.A., Tracy, G.D., Lord, R.S., Penny, R. (1973) Peripheral ischaemia caused by blood hyperviscosity. Aust. N.Z.J. Surg 43 109-113.

278. McGrath, M.A., Penny, R. (1974) The mechanisms of Raynaud's phenomenon Parts 1 and 2. Med. J. Austr. 2 328 and 367.

279. McLafferty, F.W. (1959) Mass spectrometric Analysis; molecular rearrangements. Anal. Chem 31 82-87.

280. Metwally, M.A., Jakupovic, J., Youns, M.I., Bohlmann, I. (1985) Eudesmanolides from Artemisia judaica. Phytochemistry 24(5) 1103-4.

281. Michael, T.A., Don, A., AIwazzadeh, S. (1970) The effects of acute chloroquine poisoning with special reference to the heart. Am. Heart J. 79(6) 831-42.

282. Misra, L.N. (1986) Arteannuin C - sesquiterpene latone from Artemisia annua. Phytochemistry 25 2892-3.

283. Mitscher, L.A., Leu, P., Mohindar, S., Wu, B.W., Beal, J.L., Roger White. (1972) Antimicrobial agents from higher plants, introduction, rationale and methodology. Llyodia (J. Nat. Prod) 35(2) 157-166.

284. Muhlens, P. (1926) Die Behandlung der naturlichen menschlichen malaria. infektion mit plasmochin. Naturwissenschaften 14 1162-1166.

285. Moreno, A., Patarroyo, M.E. (1989) Development of an asexual blood stage malaria vaccine. Blood Aug 1 74(2) 537-46.

286. Murray, R.D.H., Stefanovic, M. (1986) 6-methoxy-7,8-methylenedioxy coumarin from Artemisia dracunuloides and Artemisia vulgaris. J. Nat. Prod. 49(3) 550-1.

287. Mwangi, J.W. (1989) Pharmacognostical and biological studies of Kenyan Lippia species with special reference to their essential oil content. PhD thesis, University of Nairobi.

288. Murray, R.D.H., Mendez, J., Brown, S.A. (1982) The Natural Coumarins: John Wiley and Sons, Chichester pp 481-484.

289. Nadkarni, K.M. (1954). Indian Materia Medica 1 776-784.

290. Nair, M.S.R., Acton, N., Klayman, D.L., Kendrick, K., Basile, D.V., Mante, S. (1986) Production of artemisinin in tissue cultures of Artemisia annua. J. Nat Prod. 49(3) 504-7.

291. Nakamura, J., Yoshizaki, Y., Yasuhara, M., Kimura, T., Muranishi, S. and Sezaki, H. (1976a) Chem Pharm Bull 24 683-690.

292. Nakamura, J., Yoshizaki, Y., Yasuhara, M., Kimura, t., Muranishi, S. and Sezaki, H. (1976b) Ibid 24 691-697.

293. Nakamura, J., Takada, S., Ohtsuka, N., Heya, T., Yamamoto, A., Kimura, T., Sezaki, H. (1983) Assessment of indomethacin induced gastrointestinal mucosal damage in vivo enhancement of urinary recovery after oral administration of phenolsulfonphthalein in rats. J. Pharm. Pharmacol 35 369-372.

294. Namba, T., Hattori, M., Takehana, Y., Tsunezuka, M., Tomimori, T., Kizu, H., Miyaichi, Y. (1983) A flavone from Artemisia capillaris. Phytochemistry 22(4) 1057-8.

295. Neblock, D.S., Richard, A.B. (1982) Lysosomotropic agents ammonium chloride and chloroquine inhibit both synthesis and secretion of freshly isolated embryonic chick tendon cells. Biochem Biophys Res. Commun. 105 (3) 902-908.

296. Nussenzweig, R.S., Nussenzweig, V., (1989) Antisporozoite vaccine for malaria: experimental basis and current status. Rev. Infect.Dis. May-Jun. 11pp 1 3 5579-85.

297. Odetola, A.A. (1975) "Biochemical studies on some plant extracts in use as antimalarials in Western Nigeria". PhD Thesis, University of Ibadan, Ibadan.

298. Odetola, A.A., Bassir, O. (1986) Evaluation of anti-malarial properties of some Nigeria medicinal plants. The proceedings of a workshop on medicinal plant research in Nigeria. Edited by Sofowora, A., University of Ife. Ile-Ife. Nigeria pp 275.

299. Ogbuokiri, J.E. (1987) (Letter) Lancet 1 281.

300. Ojewole, J.A.O., Adesina, S.K. (1983) Mechanism of the hypotensive effect of scopoletin isolated from the fruits of Tetrapleura etraptra. Planta Medica 49 46-50.

301. Ojewole, J.A.O. (1983c) Scopoletin as a bronchodilator and antiarrhythmic agent. Fitoterapia 54 153-161.

302. Okuno, I., Uchida,K., Nakumura, M. and Sakurawi, K. (1988) Studies on choleretic constituents of Artemisia capillaris Thunb. Chem. Pharm Bull. 36(2) 769-775.

303. Olatunde, I.A. (1972) The present status of chloroquine in drug treatment of malaria. Afr. J. Med.

Sci. 3(1) 77-91.

304. Ojewole, J.A.O. (1983a) Scopoletin as a spasmolytic. Fitoterapia 54 203-211.

305. Oloo, A.J., Bennett, A.D., Kamunvi, F., Koech, D.K. (1986) Falciparum malaria highly resistant to chloroquine in Western Kenya case report (letter). Trans. R. Soc. Trop. Med Hyg 80(1) 166-7.

306. Onori, E. (1981) Resistance of Plasmodium falciparum to anti-malarials. Practical implications and control prospects. Parasitologia Dec 23(1-e) 31-62.

307. O'Neill. M.J., Bray, D.H., Boardman, P, Chan, K.L., Phillipson, J.D. (1987) Plants as sources of antimalarial drugs. Part 4. Activity of Brucea javanica fruits against chloroquine. Chloroquine-resistant Plasmodium falciparum in vitro and against Plasmodium berghei in vitro. J. Natural Products 50(1) 41-48.

308. O'Neill, M.J., Bray, D.H., Boardman, P., Phillipson, J.D., Warhurst, D.C., Peters, W., Suffness. (1986) Antimicrobial agents and Chemother 30 101.

309. Onori, E., Grab, B., Ambroise, T.P., Thelu, J. (1982) Incipient resistance of Plasmodium falciparum to chloroquine among semi-immune population of the United Republic of Tanzania. The impact of chloroquine used

as a chemosuppressant on the immune status of the population. Bull WHO 60(6) 899-906.

310. Oscar, B. (1986) 3-methoxyflavones and coumarins from Artemisia ivearrescens. Phytochemistry 25(10) 2357-2360.

311. Otto, N., Peter, S. (1969) Choleretic agents from Artemisia abrotanum. Arzneim-Forsch 18(10) 1330-6. Cf. Chem Abs, 70 10194a.

312. Ovezdurdyev, A., Zakirov, S., Kh, Yussupov, M.I., Kasymov, Sh.Z., Abdusamatov, A.T., Malikov, V.M. (1987) Khim Prir Soedin 11(4) 607-8. Cf. Chem Abs. 108 34794a 1987.

313. Oyekan, A.O., Laniyona, A.A. (1986) Interaction by bendrofluazide with acetylsalicylic acid. Gen. Pharmacol 17(2) 251-254.

314. Pampana, E. (1969) A textbook of Malaria Eradication. 2nd Edn. Oxford University Press London pp 122, 139, 149.

315. Pannacciulli, I., Salvidio, E., Tizianello, A., Parravidino, G. (1969) Hemolytic effects of standard single dose of primaquine and chloroquine on G-6-PD deficient caucasians. J. Lab Clin Med 74 653.

316. Pasvol, G., Weatheral, D.J., Wilson, R.J.M. (1978) Cellular mechanisms for the protective effects of Hbs

against P. falciparum. Nature 274 701-703.

317. Peltola, H., Case, S.E., Siddiqui, W.A., Perri, S.F. (1984) Plasmodium falciparum merozoite vaccine in Acotus monkeys. An evaluation of tolerableness of three types of adjuvants. Scand. J. Infect Dis. 16(4) 393-402.

318. Perez-Stable, E., Caralis, P.V. (1983) Thiazide-induced disturbances in carbohydrate, lipid and potassium metabolism. Am. Heart J. 106 245-251.

319. Peters, W. (1982) Antimalarial drug resistance, An increasing problem. Br. Med. Bull. 38 1987-92.

320. Peters, W. (1980) Trop Dis. Bull 77 555-558.

321. Peters, W. (1964) Nature (London) 203 1290-1291.

322. Pfaller, M.A., Krogstad, D.J. (1983) Oxygen enhanceds the antimalarial activity of the imidazoles. Am. J. Trop. Med Hyg July 32(4) 660-5.

323. Phillips-Howard, P.A., Behrens, R.H., Dunlop, J. (1989) Stevens-Johnson syndrome due to pyrimethamine/sulphadoxine during presumptive self-therapy of malaria. (Letter) Lancet Sep 30; 2(8666) 803-4.

324. Phillipson, J.D. (1990) Kumasi Seminar, Sep. 25.

325. Pichon, P. (1984) Myasthenic syndrome induced by

chloroquine poisoning: an unusual clinical form confirmed by ocular involvement. Bull Soc Ophthalmol Fr Feb 84(2) 219-22.

326. Pietras, R.J., Szego, C.M., Mangan, C.E., Seeler, B.S., Burtnett, M.M. (1979) Elevated serum cathepsin B-like activity in women with neoplastic disease. Gynaecol. Oncol. 7 1-17.

327. Pichon, P. (1984) Myasthenic syndrome induced by chloroquine poisoning: an unusual chemical form confirmed by ocular involvement. Bull Soc Ophthalmol Fr 84(2) 219-222.

328. Poncin, E., Bonneau, D., Galenne, B., Jacquemin, J.L., Belq-Giraudon, B. (1983) Plasmodium falciparum malaria acquired in Kenya with type II or type III resistance to 4-aminoquinolines. Sem. Hop. Paris. April 28, 59 (17) 1331.

329. Poole, A.R., Tiltman, K.J., Recklies, A.D., Stoker, T.A.M. (1978) Differences in secretion of the proteinase cathepsin B at the edges of human breast carcinomas and fibroadenomas. Nature (London) 273 545-547.

330. Preston, F.E., Emmanuel, T.G., Winfield, D.A., Malia, R.G. (1974) Essential thrombocythaemia and peripheral gangrene. Brit. Med. Journal 3 543-551.

331. Pringle, R., Walder, D.N., Weaver, J.P. (1985) Blood viscosity and Raynauds disease. Lancet 1 1086.

Prochazka, J. (1972) Inhibitory effect of antimalarial drugs on platelet adhesiveness. Acta Univ. Carol Med Monogr. (52) 93-7.

332. Puri, S.K., Dutta, G.D (1982) Antibiotics in the chemotherapy of malaria. Prog. Drug Res. 26 167-205.

Qinghaosu antimalarial Co-ordinating Research Group. (1979) Chin Med. J. 92 811.

333. Ragab, A.H. (1973) (Letter) Lancet 1 1061.

Ramanathan, V.D. (1985) In vitro inhibition of the activation of the human complement and coagulation systems by chloroquine. Int. J. Immunopharmacol 7(5) 769-73.

334. Ramsey, M.S., Bloodworth, J.M.B.Jr., Engerman, R.L. (1970) Chloroquine retinopathy in the rabbit. Can. J. Ophthalmol 5(3) 264-273.

335. Ramsey, M.S., Ben, S. (1972) Chloroquine toxicity in the human eye. Histopathological observationa. Am. J. Ophthalmol 75(229-235.

336. Rang, H.P., Dale, M.M. (1987) Pharmacology. 1st Publication Churchill Livingstone pp 663.

337. Rauter, A.P., Branco, I., Tostao, Z., Pais, M.S.,

Gompmejo, J.B. (1989) Flavonoids from Artemisia campestris subsp. Maritima Phytochemistry 28(8) 2173-2175.

338. Rawlings, J.S., Beall, S.C. (1982) Transfusion malaria in premature infant. Clin. Pediatr. (Phila) Oct. 21 (10) 638-9.

339. Reacher, M., Campbell, C.C., Freeman, J., Doberstyr, E.B., Brandling-Bennett, A.D. (1981) Drug therapy for Plasmodium falciparum malaria resistant to pyrimethamine-sulfadoxine (Fansidar) A study of alternative regimens in Eastern Thailand, 1980. Lancet Nov 14: 2(8255) 1066-9.

340. Rees, R.G., Smith, M.J. (1987) Beneficial response to chloroquine in a patient with type I diabetes. Br. Med J. 249 900.

341. Reilly, Q. (1982) Danger: Haemolysis from primaquine use Papua New Guinea Med. J. 25(3) 452-3.

342. Reyes, S. (1981) Malarial infection caused by Plasmodium falciparum resistant to chloroquine treatment. The situation in Brazil 1960-1981. Rev. Braz. Malariol-Doencas Trop. 33 109-30.

343. Rieckmann, K.H. (1982) Visual in vitro test for determining the drug sensitivity of Plasmodium falciparum. Lancet 1 1333-1335.

344. Rieckmann, K.H. (1978) Drug sensitivity of Plasmodium falciparum. An in vitro microtechnique. Lancet 1 22-23.

345. Rigdon, R.H. (1950) A consideration of the phenomenon of sludged blood in disease. A.J. Clin. Path. 20 946.

346. Rombo, L. (1985) Does chloroquine contribute to the risk of serious adverse reactions to fansidar. (Letter) Lancet Dec 7: 2(8467) 1298-9.

347. Romo de Vivar, A., Ali, P.C., Leon, N.C., Delgedo, G. (1982) 11,13-Dehydroeriolin, schkuhriordin and schkuhriolid, germacranolides from Schkuhria species. Phytochemistry 21(12) 2905-8.

348. Roskoski, R., Jaskunas, S.R. (1972) Biochem. Pharmac. 21 391.

349. Rothermich (1967) Treatment of chloroquine poisoning. (Letter) Br. Med. J. i 700.

350. Ryakhovskaya, T.V., Ushbaeva, G.C., Zhemaletdinov, F.G. (1989) Antitumor activity of phenolic compounds from some Artemisia L. species. Rastit Resur 25(2) 249-53. Cf. Chem Abs. III(70445h) 1989.

351. Ryakhvskaya, T.V., Manadilova, A.M., Sapko, O.A. (1985) Flavonoids from Artemisia sublessingiana. Khim. Prir. Soedin (3) 85072t.

352. Ryhage, R., Stenhagen, F. (1963) Mass spectrometry of long chain esters in Mass spectrometry of organic ions Ed. by MacLafferty, F.W., Acad. Press. New York and London pp 399-452.

353. Saitbaeva, I.M., Abdullaev, N.D., Mallabaev, A., Sidyakin, G.P., Yagudaev, M.R. (1986) Artelin, a new sesquiterpene lactone from Artemisia leucodes. Khim. Prir. Soedin (1) 115-16. Cf. Chem Abs. 105 94464.

354. Salako, L.A., Sangodey, J.M. (1976) Effect of chloroquine on isolated guinea pig atria. West African J. Pharmacology Drug. Res. 3(1) 85-86.

355. Saratikov, A.S., Prishchep, T.P., Vengerovskii, A.I., Taran, D.D., Bersovskaya, T.P., Kalinkina, G.J., Serykh, E.A. (1986) Khim-Farm Zh 20(5) 58508. Cf. Chem Abs. 105 3530ft.

356. Sassan, J.W. (1983) Progressive chloroquine retinopathy. Ann Ophthalmol Jan 15(1) 19-22.

Savin, J.A. (1970) Br. J. Derm. 83 546.

357. Savion, N. (1985) Chloroquine and primary amines inhibit the internalisation of anti-thrombin III trypsin complex in cultured cells. Thromb. Res. Sep. 15: 39(6) 671-82.

358. Saxena, V.K., Samaiya, G.C. (1984) Chemical study of the stem of Artemisia nilegarica. Acta Cienc. Indica

10(4) 272-5. Cf. Chem Abs. 103 102042c.

359. Schanker, L.S., Shore, P.A., Brodie, B.B., Hogben, C.A.M. (1957) J. Pharmacol. Exp. Ther. 120 528-539.

360. Shaker, L.S., Shore, P.A., Brodie, B.B., Hogbe, C.A.M. (1958) Ibid 123 81-87.

361. Schellenberg, K.A., Coatney, G.R. (1960) The influence of antimalarial drugs on nucleic acid synthesis in Plasmodium gallinaceum and Plasmodium berghei. Biochem Pharmacol 6 146-153.

362. Schmidt, L.H., Crosby, R., Rasco, J., Vaughan, D. (1978) Antimalarial activities of various 4, quinoline-methanols with special attention to WR-142, 490 (melfloquine). Antimicrob. Agents Chemother. 13 1011-1030.

363. Schmidt, L.H., Vaughan, D., Mueller, D., Crosby, R., Hamilton, R. (1977) Activities of various 4-aminoquinolines against infection with chloroquine resistant strains of Plasmodium falciparum. Antimicrob. Agents. Chemoth. 11 826-43.

364. Schmidt, G. and Hofheinz, W. (1983) J. Am. Chem. Soc. 105 624.

365. Schneider, J. (1954) Plasmodium berghei and chemotherapy. Indian J Malar. 257-279.

366. Schwartz, I.K., Payne, D., Campbell, C.C., Khatib, O.J. (1983) In vivo and in vitro assessment of

chloroquine resistant Plasmodium falciparum malaria in Zanzibar. Lancet 1003-05.

367. Segal, R., Eden, L., Danin, A., Kaiser, M., Duddeck, H. (1984) Sesquiterpene lactones from a further population of Artemisia herba-alba. Phytochemistry 23 (12) 2954-6.

368. Sellioglu, B. (1986) Malaria vaccine research. Mikrobiyol Bul Jan: 20(1) 37-41.

369. Serkerov, S.V., Aleskerova, A.N. (1985) Structure of a new germacranolide shonachalin C from A. fragrans. Khim Prir. Soedin. (6) 787-9(b). Cf. Chem Abs. 105 3493k, 1986.

370. Serkerov, S.N., Aleskerova, A.N. (1985) Structure of shonachalin A, a new germacranoide from Artemisia fragrans. Khim Prir. Soedin. (2) 196-9a. Cf. Chem Abs. 103 85045m, 1984.

371. Shapiro, R.H., Djerassi, C. (1964) Fragmentation and hydrogen migration reactions of beta-unsaturated 3-ketosteroids. J. Am Chem Soc. 86 2825.

372. Shen, T.Y. (1980) Toward more selective anti arthritic therapy. J. Med. Chem. 24 1-5.

373. Shilin, Y., Roberts, M.F., Phillipson, J.D. (1989) Methoxylated flavones and coumarin from Artemisia annua. Phytochemistry 28(5) 1509-11.

374. Shimomura, H., Sashid, Y., Ogawa, K. (1989a) Neoclerodane diterpenes from Ajuga ciliata var villosior, Chem. Pharm. Bull 39(4), 988-92.

375. Shimomura, H., Sashida, Y., Ogawa, K. (1989b) Neoclerodane diterpenes from Ajuga decumbens. Chem Pharm Bull. 39(4) 93-96.

376. Shimomura, H., Sashida, Y., Ogawa, K. (1989c) Neoclerodane diterpenes from Ajuga nipponensis. Chem Pharm Bull. 37(2) 354-7.

377. Shingo, K., Wenzer, S.H., Smith, E.C. (1971) Inhibition of glucose-6-phosphate dehydrogenase from tobacco by scopoletin and scopolin. Phytochemistry 10 (7) 1501-3.

378. Shoeb, A., Kapil, R.S., Popli, S.P. (1972) Coumarins and alkaloids of Aegle marmelos. Phytochemistry 12 2071-2073.

379. Shreter, A.I., Rhbalko, K.S., Knovalova, O.A., Derevinskaya, T.I., Maisuradze, N.J. (1988) Ra stit Resur 24(1) 66-72.

380. Sitprija, V., Indraprasit, S., Pochanugool, C., Benyajati, C., Piyarata, P. (1967) Renal failure in malaria. Lancet 1 185-188.

381. Sixsmith, D.G., Spencer, H.C., Watkins, W.M., Koech, D.K., Chulay, J.D. (1983) Changing in vitro response

to chloroquine of Plasmodium falciparum in Kenya. Lancet II 1022.

382. Smith, G.D., Amos, T.A.S., Mahler, R., Peters, T.J. (1987) Effect of chloroquine on insulin and glucose homeostasis in normal subjects and patients with non-insulin dependent diabetes mellitus. Br. Med. J. 294 465-67.

383. Smith, R.S., Eliot, L.B. (1971) Acute toxic effects of chloroquine on the cat retina: ultrastructural changes. Invest. Ophthalmol 10(4) 237-246.

384. Snellman, A.M. (1985) Potentially severe and often insiduous adverse psychological effects of chloroquine. Lakartidningen 82(21) 1962.

385. Sorimachi, K. (1987) Increase in insulin binding affinity by chloroquine in cultured rat hepatoma cells. Endocr. Res 13(1) 49-60.

386. Sowunmi, A., Walker, O., Salako, L.A. (1989) Pruritis and antimalarial drugs in Africans. (Letter) Lancet July 22 213.

387. Spencer, H.C., Kariuki, D.M., Koech, D.K. (1983) Chloroquine resistance in Plasmodium falciparum from Kenya infants. Am. J. Trop. Med Hyg 32 922-25.

388. Spencer, C.F., Koniuszy, F.R., Rogers, E.F. (1947) "A survey of plants for antimalarial activity" Lloydia 10

145-176.

389. Spencer, N.C., Kipngor, T., Agure, R., Koech, D.K., Chulay, J.D. (1983) Plasmodium falciparum in Kisumu, Kenya. Differences in sensitivity to amodiaquine and chloroquine in vitro. J. Infect. Dis 148 732-36.

390. Stuart, M.J., Miller, M.C., Davey, F.R., Wolk, J.A. (1979) The post-aspirin bleeding time, a screening test for evaluating haemostatic disorders. Brit. J. Haem. 43 649-659.

391. Sun, J., Maxin, Wei, D., Huang, L. (1987) Studies on the quantitative change of 7-methoxy coumarin in A lactiflora. Zhongcaoyao 18(10) 449-450. cf Chem Abs. 108 11069d.

392. Surrey, H. (1946) J. Am. Chem. Soc. 68 113.

393. Swaiman, K.F. (1986) Chloroquine reduces neuron and glial iron uptake. J. Neurochem. 46(2) 652-4 Feb.

394. Sylvio de Camargo, J. (1958) A chloroquina na doenca asthmatica. Arg. Med Municipale 10 111-120.

395. Szczeklik, A. (1980) Analgesics, allergy and asthma. Brit. J. Clin. Pharmacol 10 4015.

396. Szilagyl, T., Kaval, M. (1970) The effect of chloroquine an antigen-antibody reaction. Acta Physiol. Acad. Sci. Hung 38(4) 411-417.

397. Taniguchi, M., Adachi, T., Oi, S., Kimura, A., Katsumura, S., Isoe, S., Kubo, I. (1984) Agric. Biol Chem. 48(1) 73.

398. Thaithong, S., Beale, G.H., Chutmong, K.M. (1983) Susceptibility of Plasmodium falciparum to five drugs an in vitro study of isolated mainly from Thailand. Trans R. Soc. Trop. Med Hyg. 77(2) 228-31.

399. Tin, F., Hlaing, N., Lasserre, R. (1982) Single dose treatment of falciparum malaria with mefloquine: field study with different doses in semi-immune adults and children in Burma. Bull WHO 60(6) 913-7.

400. Trnavsky, K., Trnavska. (1970) The effect of chloroquine on metabolism of collagen proteins. Fysiat Reumatol Vestin 48(4) 169-175.

401. Trojan, H.J. (1982) Eye damage as a result of long term malaria prophylaxis with chloroquine. Klin Monatsbl Angenheikd. 180(3) 232-6.

402. Tu, Y., Zhu, Q., Shen, X. (1985) Constituents of young Artemisia annua Zhongyao Tongbao 10(9) 419720. Cf. Chem Abs. 104 31768b.

403. Turner, G.D. (1984) Kenyan chloroquine, fansidar and quinine resistant P. falciparum. Centr. Afr. J. Med. 30(7) 136-7.

404. Tyler, V.E., Brady, L.R., Rubbers, J.E. (1976)

Pharmacognosy 7th edn Lea and Febiger. Philadelphia pp 100, 156.

405. Uniyal, G.C., Singh, A.K., Shah, N.C., Naqui, A.A. (1985) Volatile constituents of Artemisia nilagirica. Planta Medica (5) 457-8.

406. Unruh, V.G., Friedmann, S.M., Spiteller, G. (1970) Tetrahedron 26 3039.

407. Udalova, L.D. (1967) The effect of chloroquine on the embryonal development of rats. Pharmacol. Toxicol. 2 226-228.

408. Varga, F. (1966) Effect of chloroquine on gastric emtying in rats. Archives of Int. Pharmacodyn. 163 38-44.

409. Varga, F. (1975) Effects of gastric emptying rate on the intestinal absorption of chloroquine in rats. Pharmacology 13(5) 401-8.

410. Vartanian, G.A., Chinyanga, H.M. (1972) The mechanism of acute neuromuscular weakness induced by chloroquine. Can. J. Physiol. Pharmacol 50(II) 1099-1103.

411. Vasyukova, N.I., Ozeretskovskaya, O.L., Metlitskii, L.V. (1970) Phytoalexins of potatoes. Prik (Biokhim, Mikrobiol) 6(4) 431-6. Cf. Chem Abs. 73.

412. Verbov, J.L. (1968) Br. J. Clin. Pract. 22 229.

413. Virgilio, L.A., Reddy, S. and Edge, M. (1982) Congenital malaria in Delaware Case Report. Del Med. J. 54(3) 159-160.

414. Vleugels, M.P., Westeyn, J.C., Meuwissen, J.H. (1982) Fansidar resistant Plasmodium falciparum infection from Tanzania. Trop Geogr. Med 34(3) 263-5.

415. Vlietinck, A.J. and Dommisse, A.R. (1985) Advances in medicinal plants research plenary lectures of the 32nd International Congress of Medicinal Plant Research Wissenschaftliche Verlagsgese Uschaft Mb H, Stuttgart 1985.

416. Vreeken, J., Van Aken, W.G. (1971) Spontaneous aggregation of blood platelets as a cause of idiopathic thrombosis and recurrent painful toes and fingers. Lancet 2 1394-1397.

417. Walker, B. (1974) A preliminary investigation of theteratogenic effects of chloroquine in the rat. West Afr. J. Pharmacol Drug Res 2(1) 61-62 Dec.

418. Ward, W.F. (1975) Accelerated haemostasis in chloroquine treated rats. Thromb. Diath Haemorrh 34(2) 545-7, 15 Nov.

419. Watkins, W.M., Sixsmith, D.G., Spencer, N.C., Boriga, D.A., Kariuki, D.M., Kipingor, T. (1984) Effectiveness

of amodiaquine as treatment for chloroquine resistant Plasmodium falciparum infections in Kenya. Lancet 1 357-359.

420. Watkins, W.M., Oloo, J.A., Lury, J.D., Mosoba, M., Kariuki, D., Mjomba, M., Koech, D.K., Gilles, H.M. (1988) Efficacy of multiple-dose halofantrine in treatment of chloroquine resistant P. falciparum malaria in children in Kenya. Lancet Jul 30: 2(8605) 247-50.

421. Watt, J.M., Breyer Brandwijk. (1962) The medical and poisonous plants of Southern and Eastern Africa. E & S Livingston 2nd Edition pp 514.

422. Wells, R.S., Schmid Schobein, H. (1968) Effect of red cell deformability upon capillary flow. Circulation Res. 38 Suppl. 6, 205.

423. Weniger, B.G., Blumberg, R.S., Campbell, C.C., Jones, T.C., Mount, D.L., Friedman, S.M. (1982) High level chloroquine resistance of Plasmodium falciparum malaria acquired in Kenya. N. Eng. J. Med. Dec 16: 307 (25) 1560-2.

424. Whichard, L.P., Morris, C.R., Smith, J.M., Holbrook, D.Jr. (1968) The binding of primaquine, pentaquin, pamaquine and plasmocid to deoxyribonucleic acid. Mol. Pharmacol 4 630-639.

425. White, N.J., Miller, K.D., Marsh, K., Berry, C.D., Turner, R.C., Williamson, D.H., Brown, J. (1987) (Letter) Lancet Aug. (1) 281-282(b).

426. White, N.J., Miller, K.D., Marsh, K., Berry, C.D., Turner, R.C., Williamson, D.H., Brown, J. (1987a) Hypoglycaemia in African children with severe malaria. Lancet March 28: 1 708-11.

427. Wirima, J., Khoromana, C., Molyneux, M.E., Gilles, H.M. (1988) Clinical trials with halofantrine hydrochloride in malaria. Lancet Jul 30, 2(8505) 250-2.

428. WHO (1980) Technical Report Series No. 655. Resistance of vectors of disease to pesticides. Fifth report of the WHO Expert Committee on Vector Control.

429. WHO (1984) Technical Report Series No. 711. Advances in malaria Chemotherapy: Report of a WHO Scientific Group.

430. WHO (1973) Chemotherapy of malaria and resistance to antimalarials. Technical Report No 529 WHO, Geneva 1973.

431. WHO (1986) Expert Committee of malaria 18th Report. Tech. Report Series No 735, WHO Geneva.

432. WHO (1984) Report of the Steering Committees of the Scientific Groups working on malaria. WHO Geneva.

433. WHO (1983) Development of mefloquine as an antimalarial drug. Bull. WHO 61 169-178.

434. WHO (1987) Report on the 9th meeting of the Scientific working group on the immunology of malaria TDR/IMMAL/SWG(9)/86. 3.

435. Wibo, M., Poole, B. (1974) Protein degradation in cultured cells II. The uptake of chloroquine by rat fibroblasts and the inhibition of cellular protein degradation and cathepsin B. J. Cell. Biol 63 430-440.

436. Willerson, D. Jr., Rieckmann, K.H., Carson, P.G., Frischer, H. (1972) The effect of minocycline against chloroquine resistant falciparum malaria. Am. J. Trop. Med Hyg 21(6) 857-862.

437. Wu, C., Tu, Y. (1985) Studies on the chemical constituents of A. celery wormwood Artemisia apiacea. Zhongcaoyao 16(6) 242-3. Cf. Chem Abs. 104 39561z.

438. Wu, Z., Wang, Y. (1984) Structure and synthesis of arteannuin and related compounds XI. Xuaxue Xuebao 42 (6) 596-8.

439. Wyler, D.J., Oster, C.N., Quinn, J.C. (1979) The role of the spleen in malaria infection. In Torrin G. ed. The role of the spleen in immunology of parasitic disease. Basel: Schwabe 183-204.

440. Xaio Peigen. (1983) Recent developments on medicinal

plants in China. J. Ethnopharmacol 7 95-109.

441. Xu, C., Sun, X., Yang, J., Yu, D., Lii, Q., Zhang, Y., Dou, S. (1986) The structure of lactiflorasyne isolated from Artemisia lactiflora Wall. Yaoxue Xuebao 21(10) 772-5. Cf. Chem Abs. 106 135232e, 1985.

442. Xu, Q., Mori, H., Sakamoto, O., Koda, A., Nishioka, I., Ogawa, Y., Hosaka, K. (1989) Antitumor principles of Artemisia capillaris Herba and its related comound. Wakan lyaku Gakkaish 6(1) 1-7. Cf. Chem. Abs. III 208780x 1989.

443. Xu-Cheng-Jun, Sun Xiao Fang: Seng-Xiaan 4 Zhou Ying-xin. (1985) Studies on the chemical constituents of Artemisia lactiflora Wall. Intern. Symp Organic Chem of Medicinal Natural Products IUPAC B-164 Nov 10-14 Shanghai China. 1985.

444. Yamada, H., Otsuka, Y., Omura, S. (1983) Structural characteristics of anticomplimentary polysaccharides from the leaves of Artemisia princeps. Planta Med. 4 311-314.

445. Yamahara, J., Kobayashi, G., Matsuda, H., Katayama, T., Fujimura, H. (1989) The effect of scoparone, a coumarin derivative isolated from the Chinese crude drug Artemisia capillaris Flos on the heart. Chem Pharm Bull 37(5) 1297-9.

446. Yisunsrih, L., Rieckmann, K. (1980) In vitro microtechnique for determining the drug susceptibility of cultured parasites of Plasmodium falciparum. Trans. Roy. Soc. Trop. Med Hyg 74 809-810.

447. Zekert, F., Kohn, P., Vormittag, E., Piza, F., Thien, M. (1975) Zur acetylsalisaure-prophylaxie von sofortuerschvissen nach gefasschirurgi schern eingriffen in Colfarit symposium III. Bayer Cologne, pp 109-119.

448. Zubarev, V.T., Polyantseva, L.R. (1972) Ophthalmologic complications after Resochin (chloroquine) therapy in nephrotic patients. Acta Ophthalmol 50(1) 18-25.

APPENDIX

I. List of Kenyan plants claimed to have anti-malarial activity

II. SPECTRAL DATA

- IR
- MASS
- ^{1}H NMR
- ^{13}C NMR

APPENDIX 1 LIST OF KENYAN PLANTS CLAIMED TO HAVE ANTI-MALARIAL ACTIVITY.

BOTANICAL NAME	LOCAL NAME	FAMILY	SOURCE OF LITETATURE
Acacia clavigera	Mugunga (Swahili)	Leguminosae	Kokwaro (1976)
			Folklore Watt (1962)
Albizia*anthelmintica	Muowa (Kamba)	Leguminosae	Kokwaro (1976)
Ajuga remota	Kirurite (Meru)	Labiatae	Folklore
	Wanjiru waweru (Kikuyu)		
Albizia	Mshai (Shamba)		Kokwaro (1976)
gummifera	Sect (Kipsigis)	Leguminosae	
Albizia zygia	Sesut (Sebei)	Leguminosae	Kokwaro (1976)
Alchornea		Euphorbiaceae	
Aloe secundiflora	Kiluma (Kamba)	Liliaceae	Kokwaro (1976)
	Kiruma (Kikuyu)		Folklore
Bidens pilosa	Munyugunyugu (Meru)	Compositae	Kokwaro (1976)
	Munzee (Kamba)		
	Onyiego (Luo)		
Boscia-angustifolia	Misingesa (Gogo)	Capparaceae	Kokwaro (1976)
Caesalpinia volkensii	Muchuthi (Kikuyu/Meru)	Leguminosae	Folklore
	Mubuthi (Kikuyu/Meru)		Kokwaro (1976)
	Mkomwe (Swahili)		
Carissa edulis	Mukalama (Kamba)	Apocynaceae	Kokwaro (1976)

APPENDIX 1 CONT.

Cassia didy	Ithaa (Kamba)
mobotrya	Murao (Kimeru)
	Owinu (Luo)
Catha edulis	Mairungu (Kikuyu)
	Miraa (Kimeru)
Clausena anisata	Matathi (Kikuyu)
	Misimbari (Kakamega)
Clemantis hirsuta	Mugayangondu (Kikuyu)
	Bisida (Kipsigis)
Clutia abbysinica	Muthimambari (Kikuyu)
	Sambukwe (Kakamega)
Clerodendrum sp	Muumba (Kamba)
	Mhunhanharia (Nyamwezi)
Conyza pyrrhopappa	Kwamereryet (Kipsigis)
Cordia sinensis	Cesege (Masai)
	Mudawe (Gogo)

Leguminosae	Kokwaro (1976)
Celastraceae	Kokwaro (1976)
Rutaceae	Kokwaro (1976)
Ranuncu	Kokwaro (1976)
Caceae	Folklore
Euphorbiaceae	Kokwaro (1976)
	Folklore
	Watt (1962)
Verbenaceae	Kokwaro (1976)
Compositae	Kokwaro (1976)
	Watt (1962)
	Folklore
Boraginaceae	Kokwaro (1976)

Croton macrostachyus	Mutuntu (Meru)
	Mutundu (Meru)
	Mutundu (Kikuyu)
Cucumella engleri	Mandusyet (Kipsigis)
Cyathula cyclindrica	Matindii (Kimeru)
Cyathula schimperana	Ngatunyat (Kipsigis)
Draceana deremensis	Kimemue (Shambaa)
Dryopteris inaequalis	
Ethulia scheffleri	
Fagara chalybea	Mugucwa (Kikuyu)
	Mukenia (Kamba)
	Cloisuki (Masai)
Gardenia jovis tonantis	Kulutimi (Meru3Tigania)
	Geninyyet (Masai)
	Odwong (Acholi)
Hagenia abyssinica	Majogajoga (Kimeru)
	Muinyeri (Kikuyu)

Euphorbiaceae	Kokwaro (1976)
	Folklore
Cucurbitaceae	Kokwaro (1976)
Amaranthaceae	Kokwaro (1976)
Agavaceae	Kokwaro (1976)
(Liliaceae)	
Aspidiaceae	Kokwaro (1976)
(Filices)	
Compositae	Kokwaro (1976)
Rutaceae	Kokwaro (1976)
	Folklore
	Kokwaro (1976)
	Folklore
Rubiaceae	
Rosaceae	Kokwaro (1976)
	Folklore

APPENDIX 1 CONT.

Harungana madagascarensis	Aremo (Luo)
	Munyamwe (Kimeru/Tankiira area)
	Muitathwa (Kikuyu)
Kigelia africana	Murantina (Kimeru)
	Yago (Luo)
	Muratina (Kikuyu)
	Morabe (Kakamega)
Lippia javanica	Muthiriti (Kikuyu)
	Muthithi (Kyulu) (Kamba)
	Angwe Rao (Luo)
	Ol sinoi (Masai)
	Orwo (Acholi)
Ludwigia erecta	Munyaschaka (Somali)
Microglossa sp	Muhinga/Muteci (Kikuyu)
	Nyatung-Odide (Luo)
Momordica friesiorum	Simete (Kipsigis)
Ocotea usambarensis	Muura (Kimeru)
	Kivumba/Manyoda (Taita)
	Muthaiti (Kikuyu)

Hypericeae	Kokwaro (1976)
Bignoniaceae	Kokwaro (1976)
	Folklore
Verbenaceae	Kokwaro (1976)
	Watt (1962)
Onagraceae	Kokwaro (1976)
Compositae	Kokwaro (1976)
Cucurbitaceae	Watt (1962)
Lauraceae	Kokwaro (1976)

Pentas longiflora	
Pittosporum lanatum	Chemaroryet (Kipsigis)
Plectranthus sylvestris	Muoro (Kimeru)
	Nginga (Taita)
Rauvolfia mombasiana & Rauvolfia natalensis	Kibombo (Digo)
Sacciolepsis curvata	Ukoka Dumes (Swahili)
Sphaeranthus suaveolens	Cheptogonda (Kipsigis)
	Njogu-la-iria (Kikuyu)
Turraea mombassana	Ol-nyerima (Masai)
Tridax procumbens	
Vangueria acutiloba	Mubiru (Kikuyu)
Vernonia brachycalyx	Mwoka Ndigu (KImeru)
	Mucatha (Kikuyu)
Warburgia ugandensis	Muthiga (Kikuyu)
	Thurunui Kimeru/Tigania)
	Olsogonoi (Masai)

Rubiaceae	Kokwaro (1976)
	Watt (1962)
Pittosporaceae	Kokwaro (1976)
Labiatae	Kokwaro (1976)
Apocynaceae	Kokwaro (1976)
Gramineae	Kokwaro (1976)
Meliaceae	Kokwaro (1976)
Compositae	Kokwaro (1976)
Rubiaceae	Kokwaro (1976)
	Watt (1962)
Compositae	Kokwaro (1976)
	Watt (1962)
	Watt (1962)
Canellaceae	Kokwaro (1976)
	Folklore

Schkuhria pinnata	Onyalo biro (Luo)
Artemisia afra	Karema-iyu (Kikuyu)
Azadirachta indica	Marubaine (Swahili)/ (Kikuyu)

Compositae	Folklore	
	Watt (1962)	
Compositae	Folklore	
	Watt (1962)	
Melliaceae	Folklore	
	Watt (1962)	

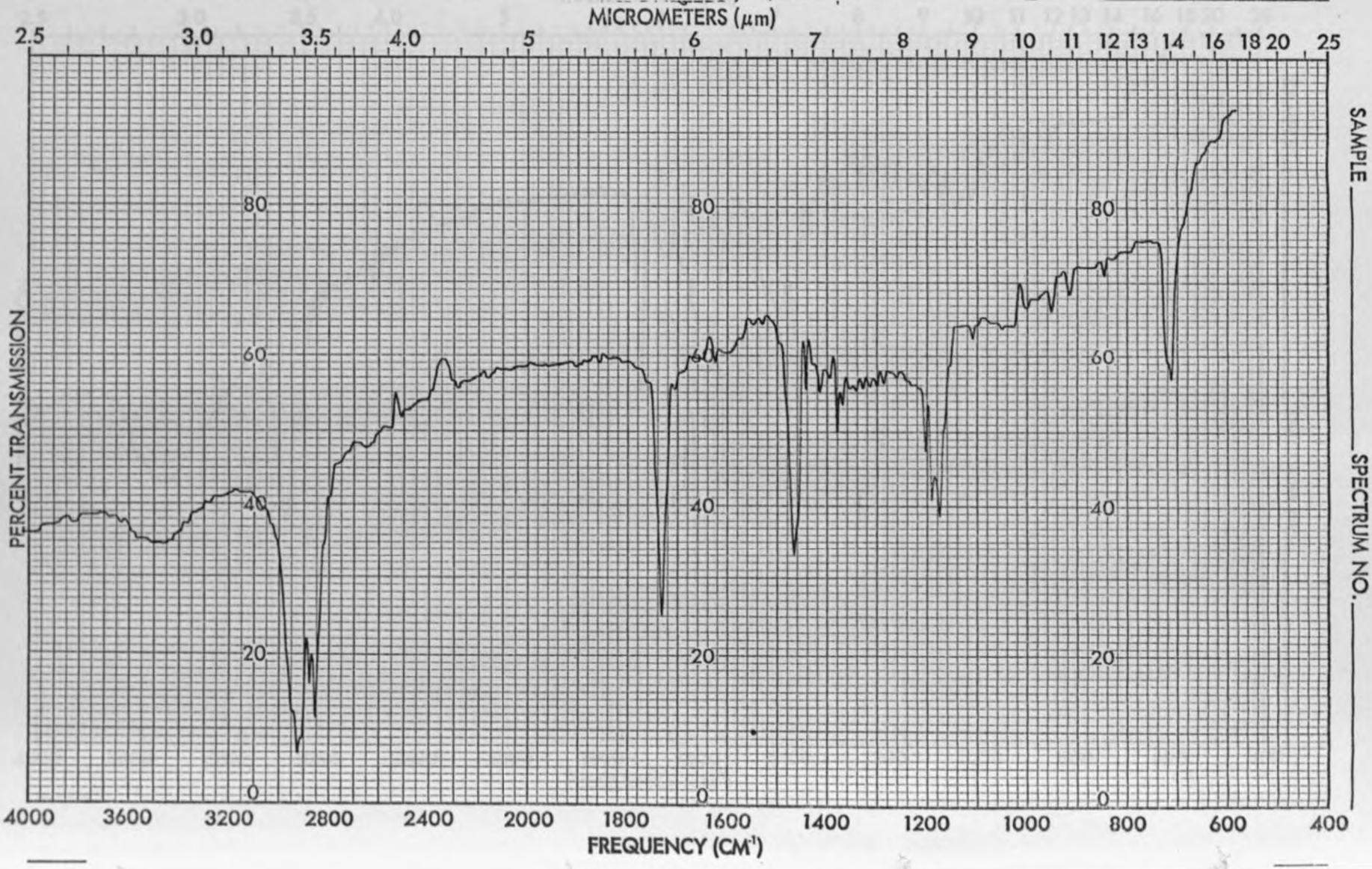
NO. 007-1493

PERKIN-ELMER

CONCENTRATION DILUTE
THICKNESS THIN FILM
PHASE KBr
REMARKS MELTING POINT 73—74° C

SCAN MODE ACCY. ☐ SURVEY ☐
HI ENERGY ☐ CAL. ☐
RESOLUTION ☑
OPERATOR THURANIRA DATE

SPECTRUM NO.
SAMPLE AAG1
LONG CHAIN ESTERS
ORIGIN A.AFRA



463

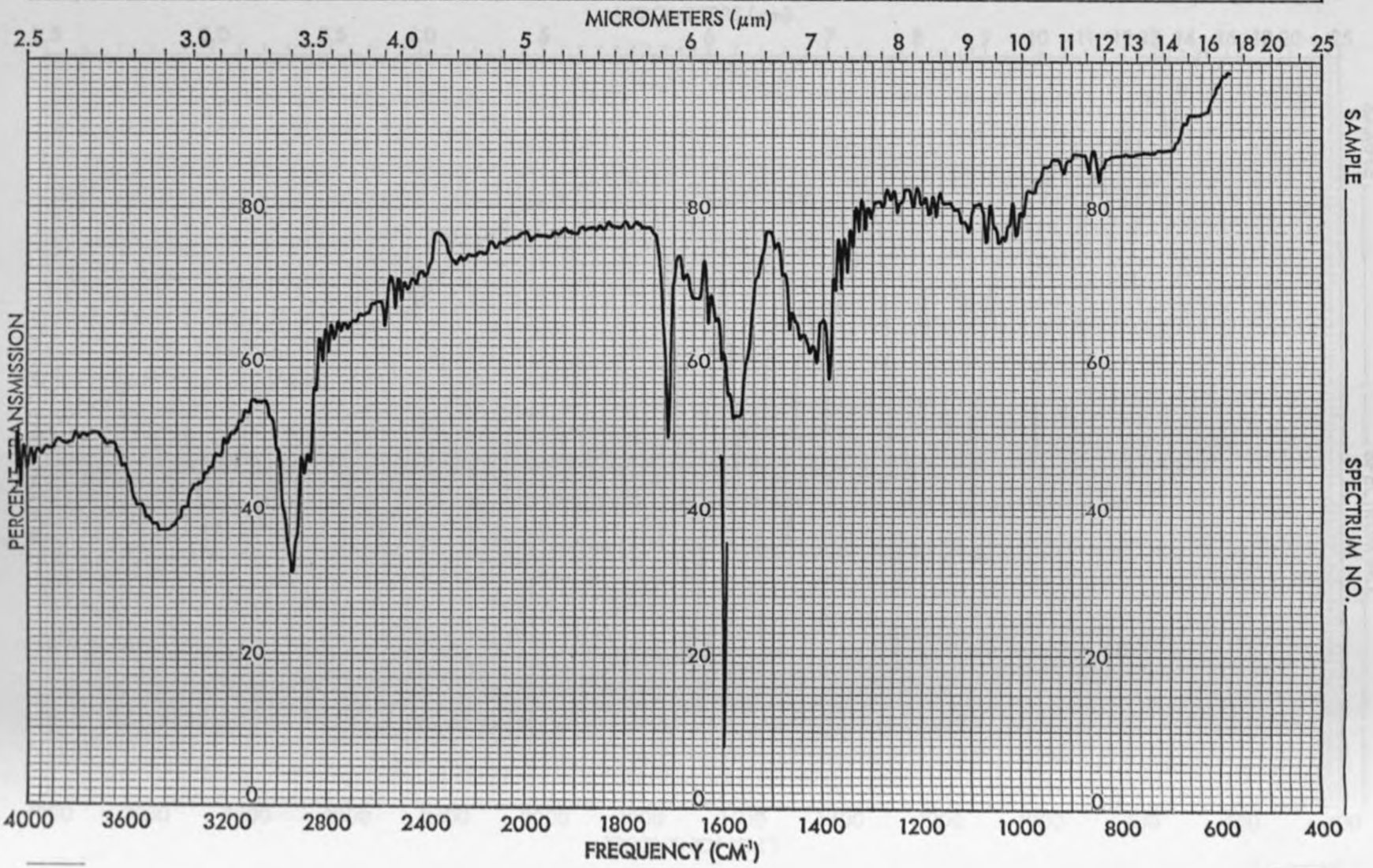
NO. 007-1493

PERKIN-ELMER

CONCENTRATION DILUTE
THICKNESS THIN FILM
PHASE K Br
REMARKS MELTING POINT 259-260°C

SCAN MODE ACCY. ☐ SURVEY ☑
HI ENERGY ☐ CAL. ☐
RESOLUTION ☑
OPERATOR Mwentai DATE 26/11/86

SPECTRUM NO.
SAMPLE G2 (32-37)
FRIEDELIN
ORIGIN A. AFRA



NO. 007-1493

PERKIN-ELMER

CONCENTRATION DILUTE

THICKNESS THIN FILM

PHASE KBr

REMARKS MELTING POINT 198-200°C

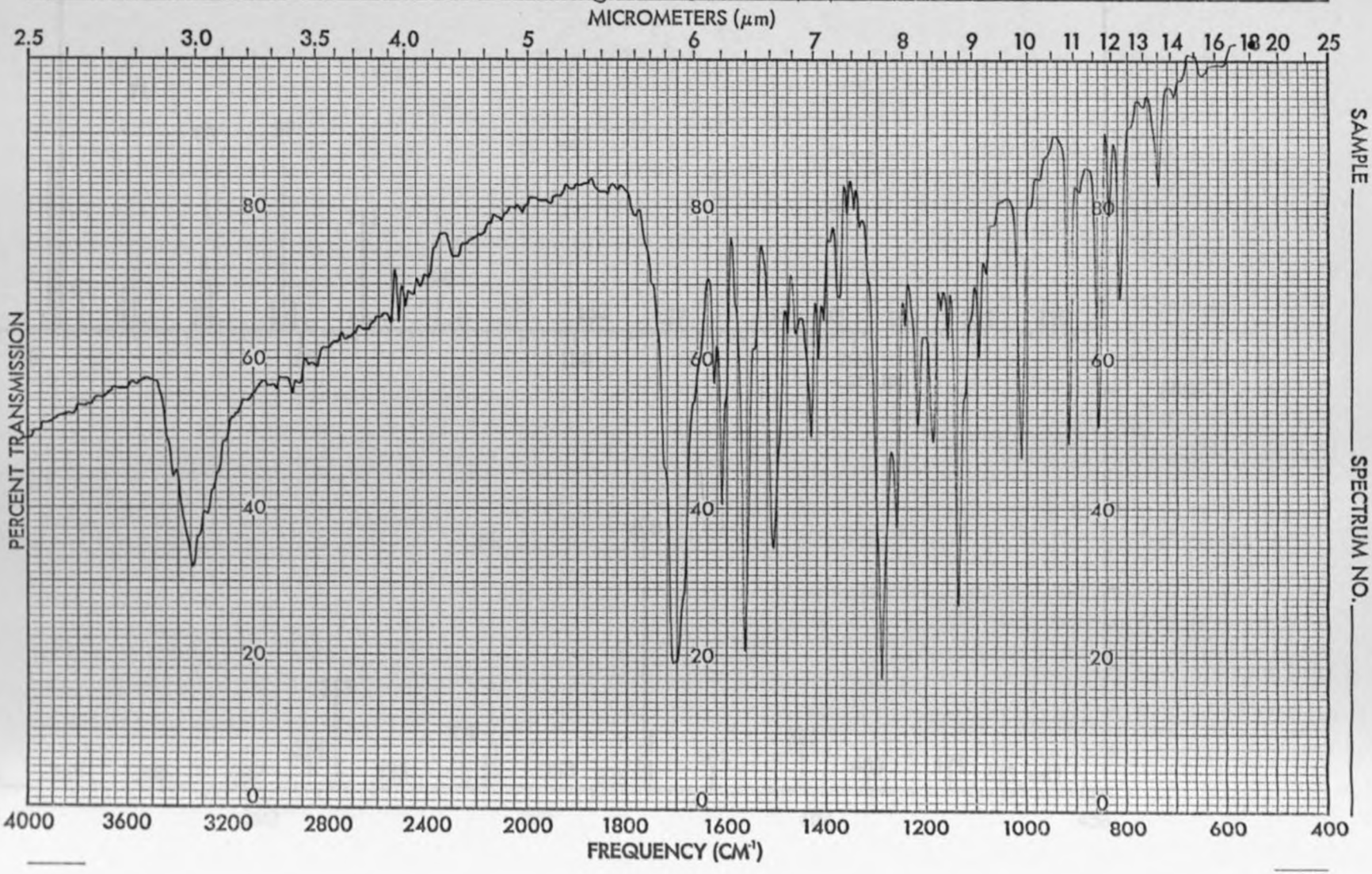
SCAN MODE ACCY. ☐ SURVEY ☑ HI ENERGY ☐ CAL. ☐ RESOLUTION ☑

OPERATOR RG Murahighy DATE 4/12/86

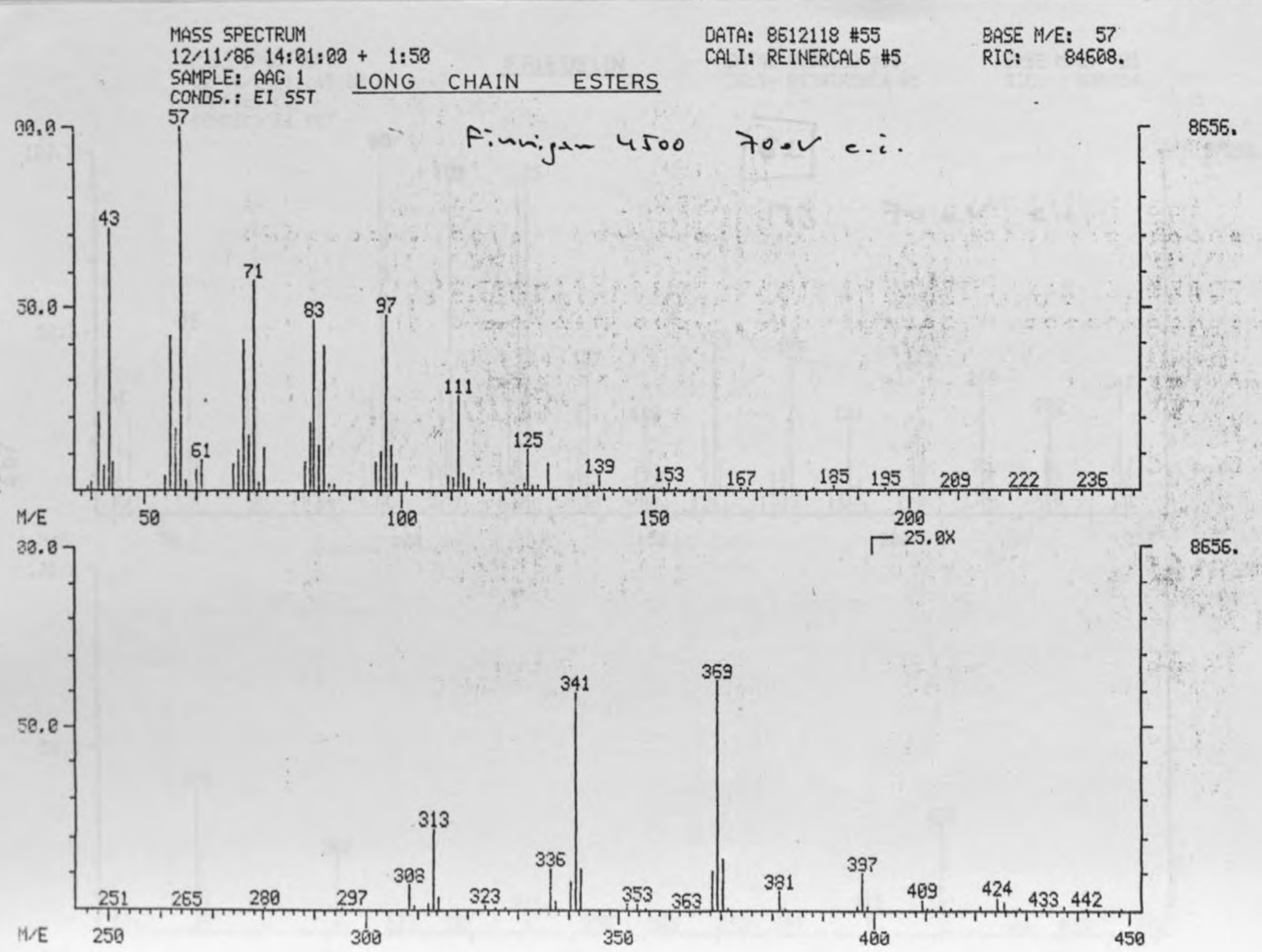
SPECTRUM NO.

SAMPLE SCOPOLETIN AAG2'

ORIGIN A. AFRA



465


MASS SPECTRUM
12/11/86 14:01:00 + 1:50
SAMPLE: AAG 1
CONDS.: EI SST
LONG CHAIN ESTERS
DATA: 8612118 #55
CALI: REINERCAL6 #5
BASE M/E: 57
RIC: 84608.
Finnigan 4500 70eV e.i.
100.0
50.0
M/E
8656.
25.0X
57
43
71
83
97
111
125
61
139
153
167
185
195
209
222
235
341
369
313
336
308
251
265
280
297
323
353
363
381
397
409
424
433
442
50
100
150
200
250
300
350
400
450

MASS SPECTRUM
02/12/86 11:45:02 + 8:46
SAMPLE: 02
CONDS.: EI SST

FRIEDELIN

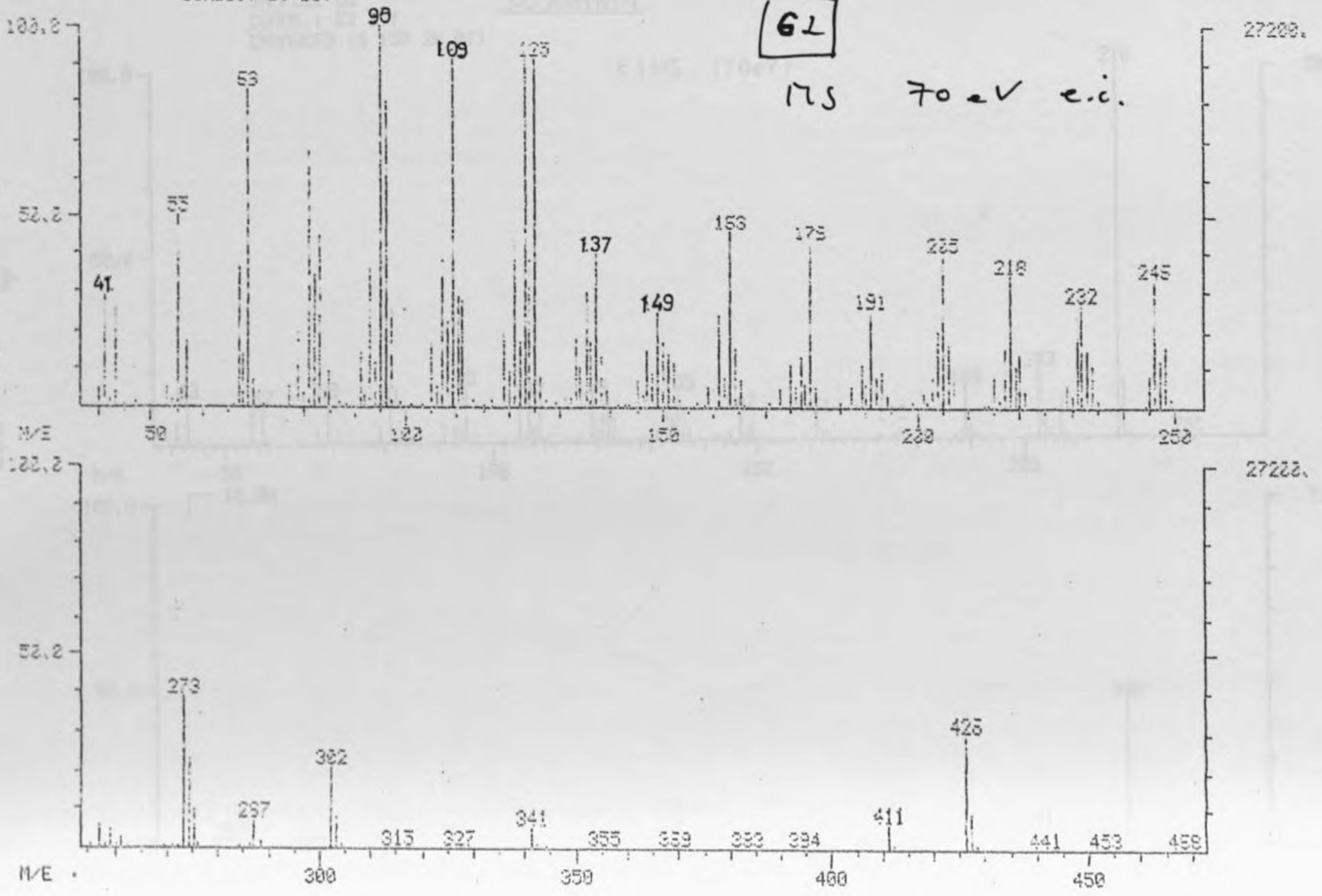
DATA: 8802122 #23
CALI: REINERCAL4 #1

BASE M/E: 95
RIC: 535504.

61

MS 70 eV e.i.

467



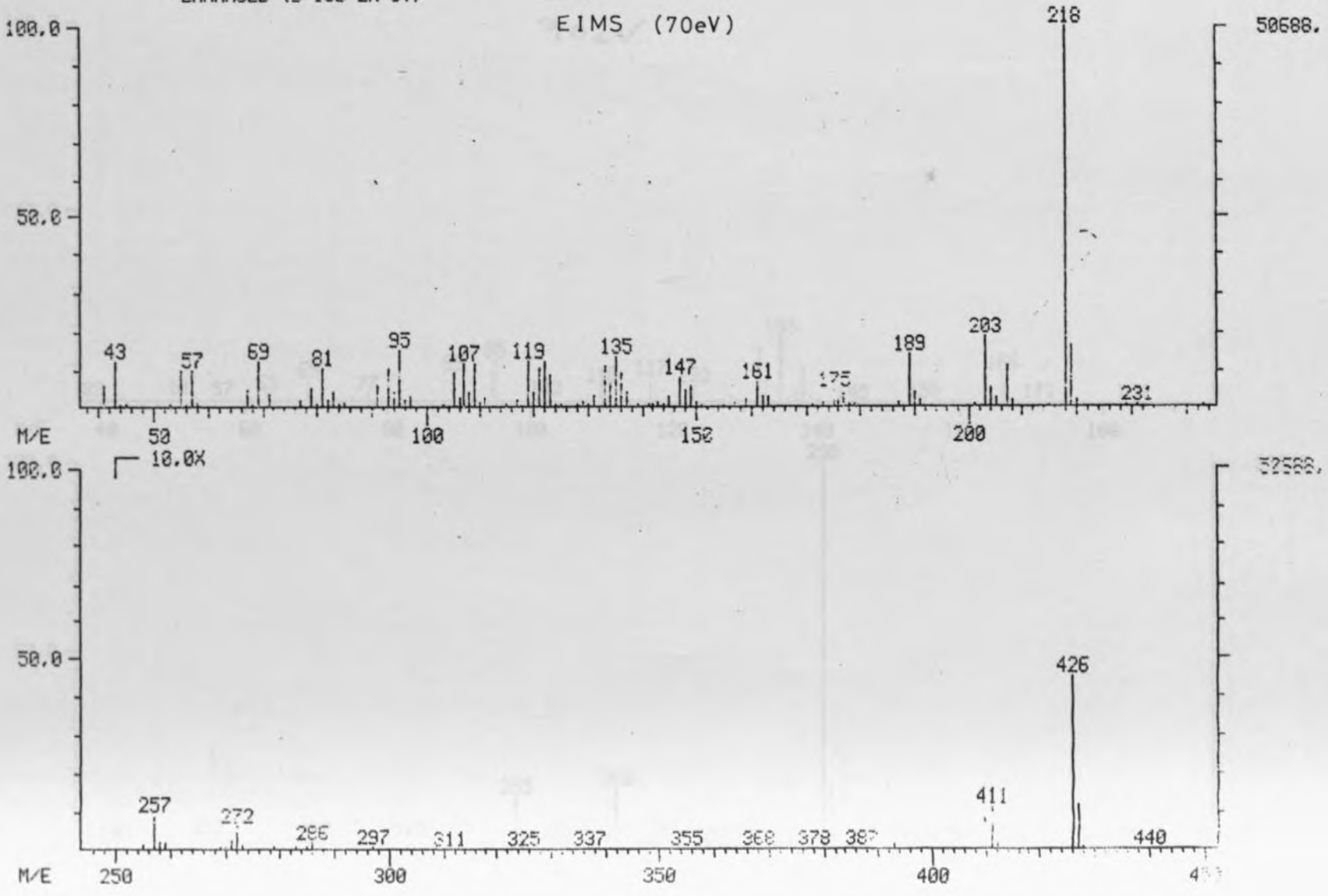
MASS SPECTRUM
06/26/87 13:31:30 + 0:34
SAMPLE: G6
CONDS.: EI 55T
ENHANCED (S 15B 2N 0T)

DATA: 8706262 #17
CALI: REINERCAL1 #1

BASE M/E: 218
RIC: 265216.

α-AMYRIN

EIMS (70eV)

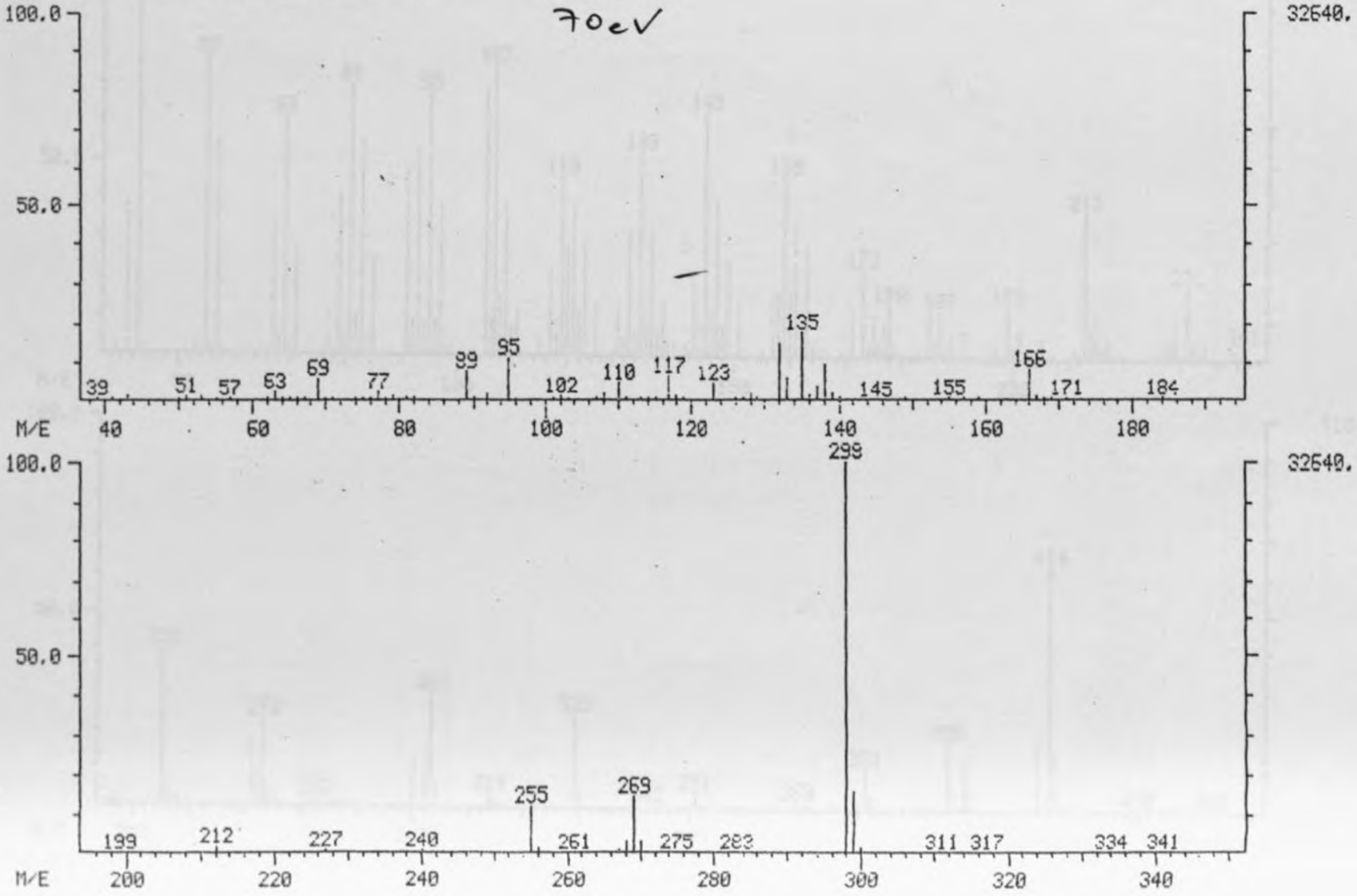


MASS SPECTRUM
06/24/87 14:50:00 + 0:40
SAMPLE: G5 7,4-DIMETHOXY-5-HDROXYFLAVONE
CONDS.: EI SST
ENHANCED (S 15B 2N 0T)

DATA: 8706245 #20
CALI: REINERCAL1 #1

BASE M/E: 298
RIC: 98944.

e.i.
70eV



694

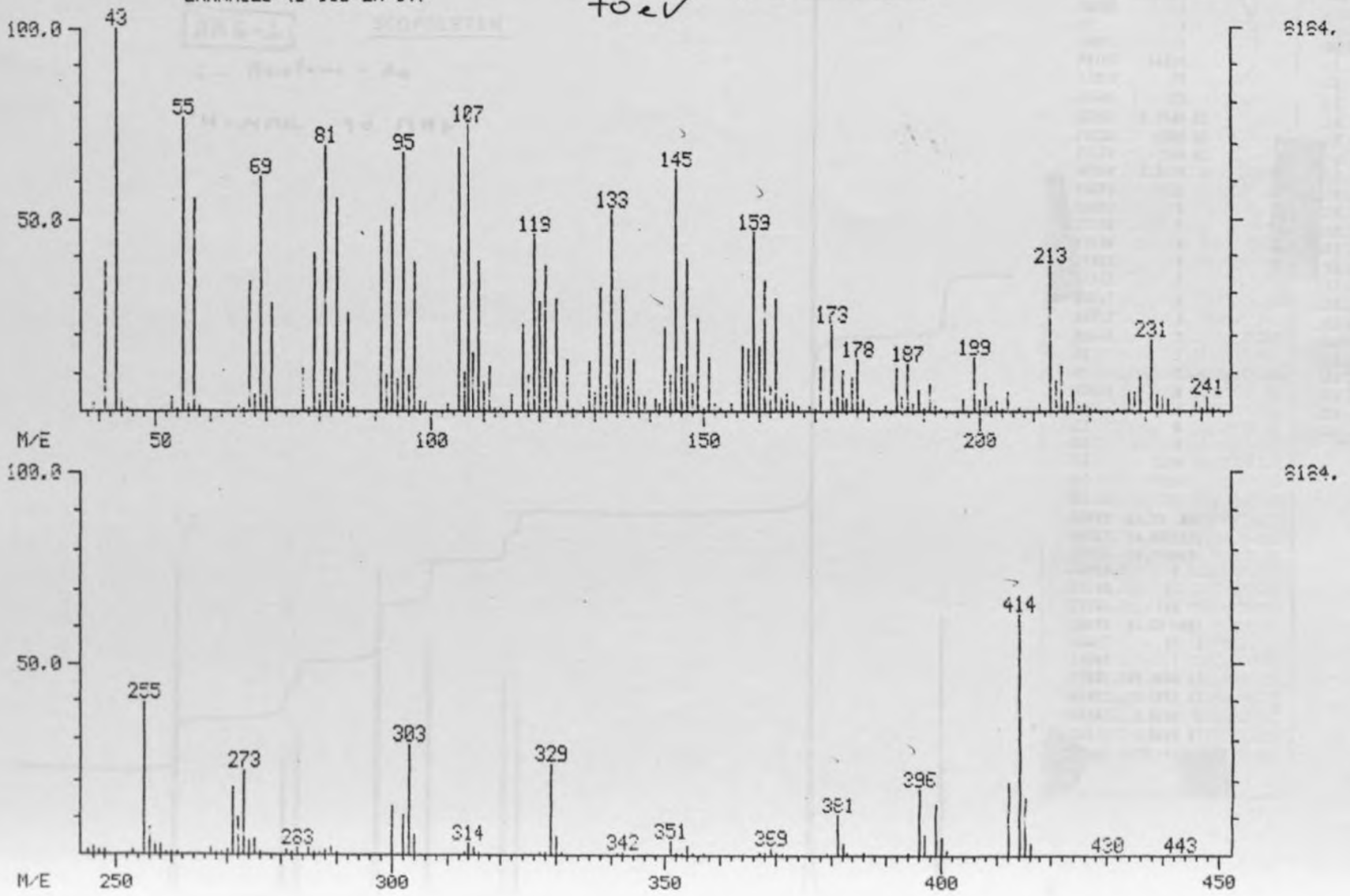
MASS SPECTRUM
06/26/87 11:53:00 + 0:38
SAMPLE: G7
CONDS.: EI 5ST
ENHANCED (S 15B 2N 0T)

DATA: 8706261 #19
CALI: REINERCAL1 #1

BASE M/E: 43
RIC: 205056.

e.i
70eV

β-SITOSTEROL



PW1##	15.0000 US
PW2##	20.0000 US
PI###	1.0000 S
PD###	100.0000 MS
PUMOD	1
HS	0
LOOP:	1
POINT	16384
TIMES	25
SCANS	25
DELAY	0.9500 MS
FREQU	1000 HZ
FILTR	500 HZ
ACQTM	3.9680 S
SAMPO	7936
DUMMY	0
FTIME	1
FSCAN	0
IRNG1	0
IRNG2	8
OVFL1	0
OVFL2	0
NGAIN	7
YG	3
XE	1
ADOWN	0
EX	0
T1	0
T2	0
T3	3520
T4	4096
RS	30
OBFRQ	89.55 MHZ
OBSET	54.0000KHZ
IRSET	54.0000KHZ
IRMOD	0
IRPOW	63
EXTNS	160
IRFRQ	89.55 MHZ
#WAIT	60 S
TODAT	1
PREDL	200.0000 US
DEADT	50.0000 US
ININT	1.0000 S
SPOIL	1.0000 MS
COMNT	????????

TOTAL 20
RESOL12207# -5 HZ
EXREF 0.0000PPM
OBS 466.0644 MZ
NGAIN 2

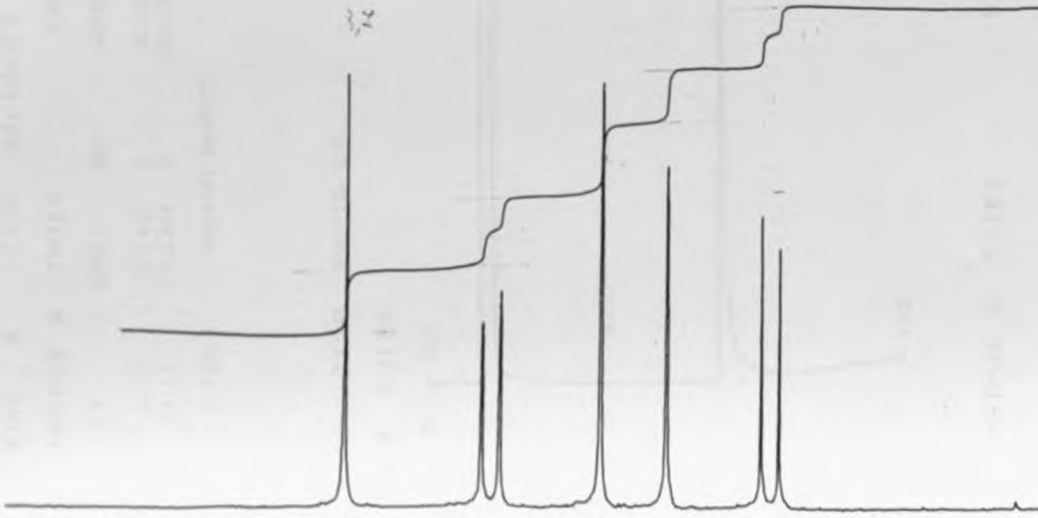
NO	FREQ(HZ)	PPM	INT#
1	824.21	9.198	31
2	781.12	8.717	2302
3	707.27	7.893	993
4	707.03	7.890	1084
5	697.75	7.787	1097
6	697.50	7.784	1160
7	656.61	7.328	54
8	644.84	7.197	2249
9	608.88	6.795	1807
10	557.86	6.225	1535
11	548.46	6.121	1369
12	433.71	4.840	29
13	362.54	4.046	130
14	349.97	3.905	10440
15	259.52	2.896	1957
16	226.68	2.529	32
17	186.27	2.078	160
18	184.20	2.055	227
19	182.80	2.031	159
20	0.00	0.000	39

ABC-2
heiße gesättigte Lösung

AAG-2 SCOPOLETIN

in Acetone - d_6

^1H-NMR 90 MHz



RUN 4 7:20 86/10/23

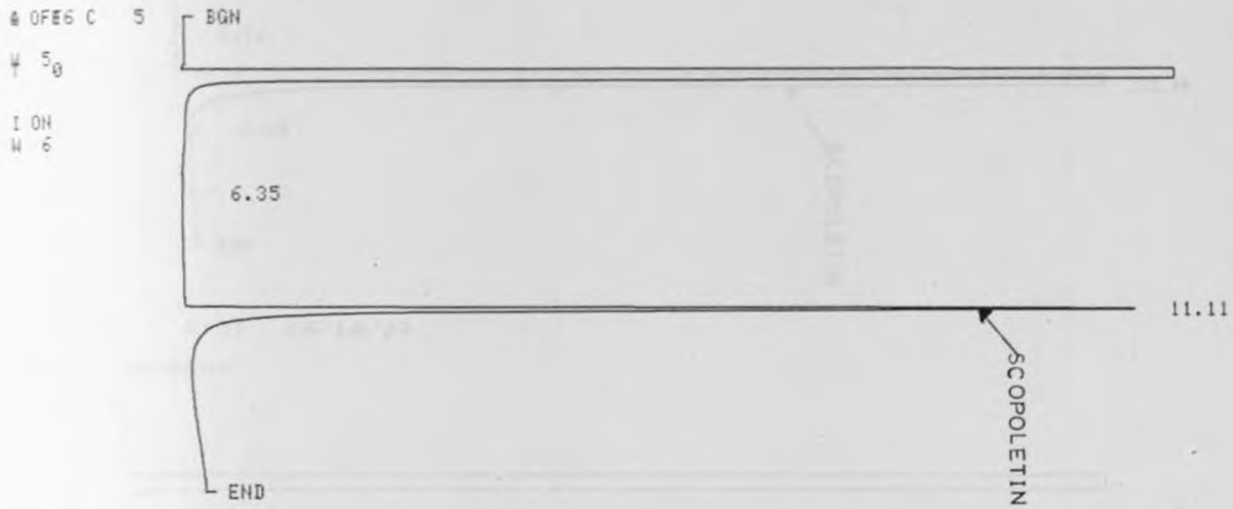
METHOD 4 THIES CALCULATION: %

RT	AREA	BC	AREA %
6.35	0.1422	V	0.6086
11.11	23.2346	V	99.3914

2 PEAKS > AREA/HT REJECT

RUN 4 7:20 86/10/23

METHOD 4 THIES



METHOD 4 THIES DATE LAST WRITTEN 86/10/23

SCOPOLETIN
(EXPERIMENTAL)

GC - capillary column

AAG-2

25m SE54

SECTION 1 GC CONTROL

	1	2
OVEN TEMP (DEG C)	200	280
ISO TIME (MIN)	6.0	2.0
RAMP RATE (DEG C/MIN)	8.0	

FID SENS HIGH FID ZERO ON

DET TEMP 330
AUX 1 TEMP 0

PRESSURE 10.1 PSIG

EQUILIB TIME 0.5 MIN
TOTAL RUN TIME 18.0 MIN

SECTION 2 TIMED EVENTS

TIME	EVENT	
- 0.40	HOLD	
- 0.30	PTV TEMP	280
- 0.20	RELAY 1	ON
- 0.10	INTEG	OFF
2.00	PTV TEMP	0
4.00	INTEG	ON

SECTION 3 DATA HANDLING

DATA ACQUISITION

START TIME 0.00 MIN
END TIME 18.00 MIN

WIDTH 4
AREA SENS 200
BASELINE SENS 4
SKIM SENS 0
BASELINE CORR B-B

REPORT

CALC TYPE %
AREA/HT CALC AREA
PRINT TOL 0.0000
OUTPUT DEVICE SCREEN

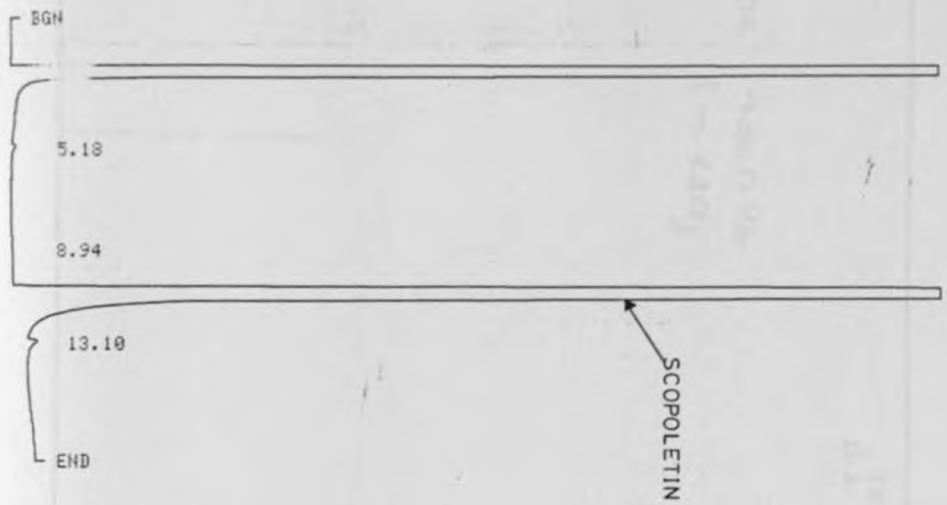
PEAK IDENTIFICATION

UNRETD PEAK TIME 0.00 MIN

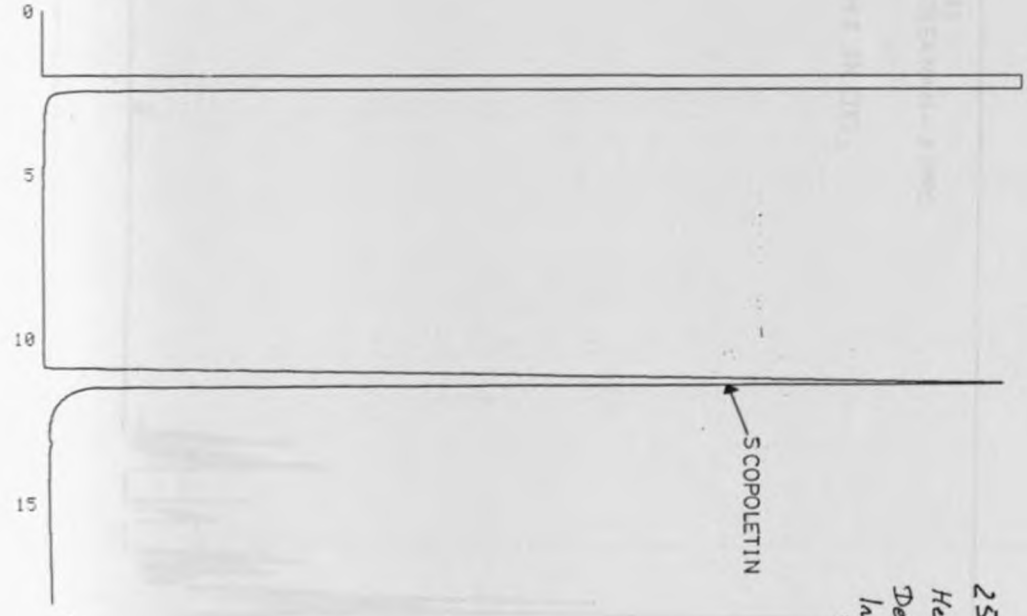
QUANTITATION

SCALING FACTOR 1.0000

RUN 3 6:21 86/10/23
METHOD CURRENT



RUN 3 6:21 86/10/23
METHOD CURRENT



GC - capillary column

Scopoletin Synthetic
Fa. ROTH, Karlsruhe

25 m SESY 200° 1 min/8°/min/ 280° 2 min halten
He 10 psig
Det. t° 330°
Inj 280°

RUN 3 6:21 86/10/23
METHOD CURRENT CALCULATION: %

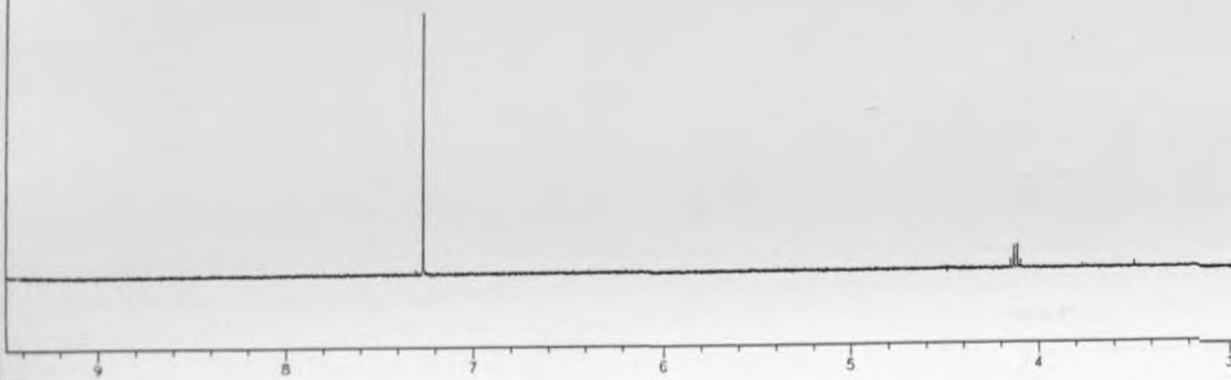
RT	AREA	BC	AREA %
5.18	0.1149	V	0.0583
8.94	0.1424	V	0.0722
11.44	195.5173	T	99.2239
13.10	1.2717	T	0.6453

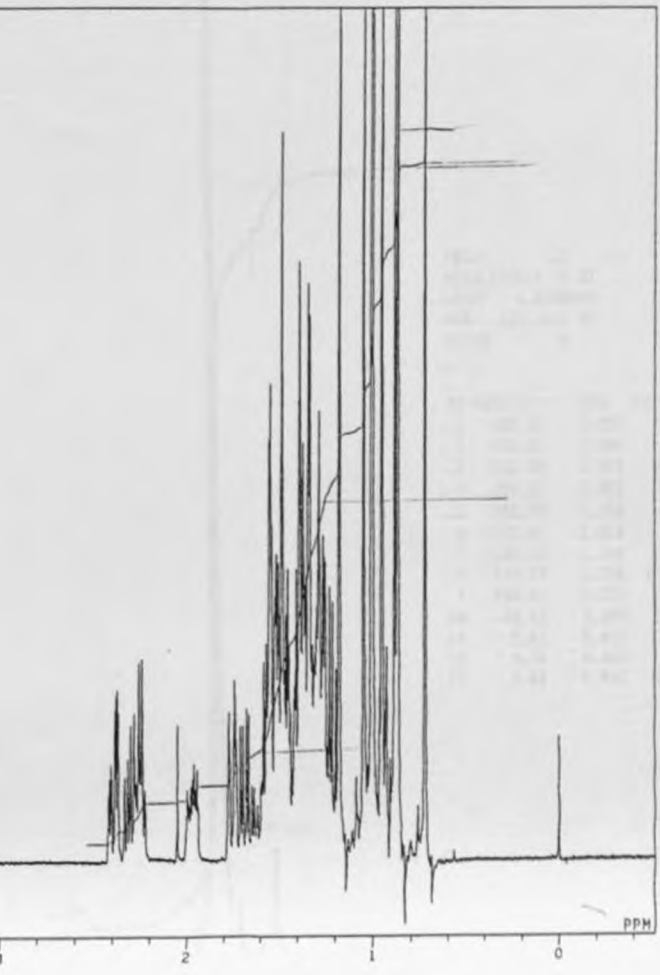
4 PEAKS > AREA/HT REJECT

(FRIEDELIN)
D: A- FRIEDO-OLEANAN - 3-ONE

400 MHZ IN $CDCl_3$

^{1}H-NMR 400 MHz
in $CDCl_3$



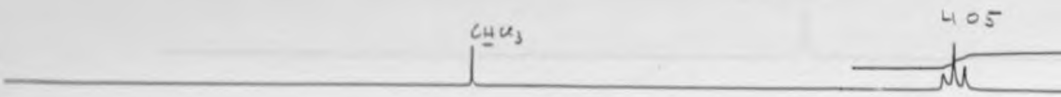


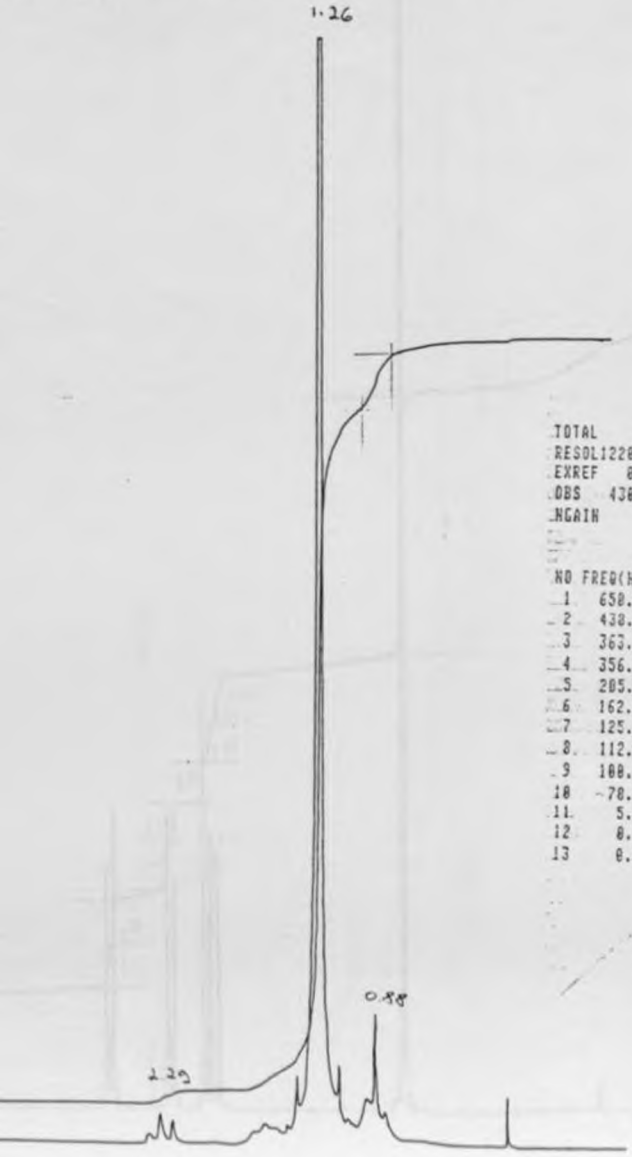

```
 1 FEB-88 15:51:54
OBNUC 1H
OBFRQ        399.65 MHz
OBSET        124.00 kHz
OBFIN      10300.0 Hz
PW1            4.0 us
PW2           33.0 us
PW3           33.0 us
P11        142.330 ms
P12         75.264 ms
P13        390.000 ms
POINT        32768
SAMPO        32768
TIMES            8
SCANS            8
DUMMY            0
FRQDU        4000.0 Hz
FILTR        2000 Hz
ACQTM        4.096 sec
PD           0.500 sec
RGAIN    12
NGAIN           10
BF           0.00 Hz
GF           0.00 Hz
WNDMD            0
EXMOD  SGNON
IRFRQ        399.65 MHz
IRSET        124.00 kHz
IRFIN      10300.0 Hz
IRRPW           42 us
IRATN           30
DFILE  DM1:VALH
SLVNT  CDCL3
LKSET          61.4 kHz
LKFIN          37.0 Hz
XRANG   50.0 cm
XSHIF    1.0 cm
XC       4000.0000 Hz
XS          0.0000 Hz
YG            12.00
YG2         12.0000
YRANG   25.0 cm
YSHIF    2.0 cm
YBIAS    2.0 cm
SLEVL   -2.0 cm
PO      -157.00 deg
 P1      -40.00 deg
 PP          14119
 TMSP        15541
 EXREF        0.00 ppm
```

LONG CHAIN ESTERS

^{1}H-NMR (90 MHZ, $CDCl_3$)

475





TOTAL	13
RESOL122071	-5 HZ
EXREF	0.0000PPM
OBS	430.5419 HZ
NGAIN	11

NO	FREQ(HZ)	PPM	INT%
1	650.51	7.259	99
2	438.66	4.806	-6
3	363.40	4.055	113
4	356.93	3.983	61
5	205.20	2.290	67
6	162.96	1.818	-6
7	125.12	1.396	154
8	112.54	1.256	9600
9	100.09	1.117	177
10	78.85	0.880	284
11	5.61	0.062	-8
12	0.36	0.004	56
13	0.00	0.000	111

AAC-1
Gesättigte
Lösung
in $CDCl_3$

PW1##	15.0000 US
PW2##	20.0000 US
PI###	1.0000 S
PD###	1.0000 S
PUMOD	1
HS	0
LOOP:	1
POINT	16384
TIMES	32
SCANS	32
DELAY	8.4500 MS
FREQU	2000 HZ
FILTR	1000 HZ
ACQTM	1.9840 S
SAMPO	7936
DUMMY	0
FTLME	1
FSCAN	0
TRNG1	0
TRNG2	0
OUFL1	0
OUFL2	0
NGAIN	0
YC	3
XE:	1
ADOWN	0
EX	0
T1	0
T2	0
T3	3520
T4	4096
RS	30
OBFRQ	89.55 MHZ
OBSET	54.0000KHZ
IRSET	54.3000KHZ
IRMOD	0
IRPOW	63
EXTNS	160
IRFRQ	99.55 MHZ
#WAIT	60 S
TODAY	1
PREDL	200.0000 US
DEADT	50.0000 US
INIWT	1.0000 S
SPOIL	1.0000 MS
COMNT	????????

TOTAL 25
RESOL24414# -5 HZ
EXREF 8.0000PPM
OBS 930.9002 HZ
NGAIN 0

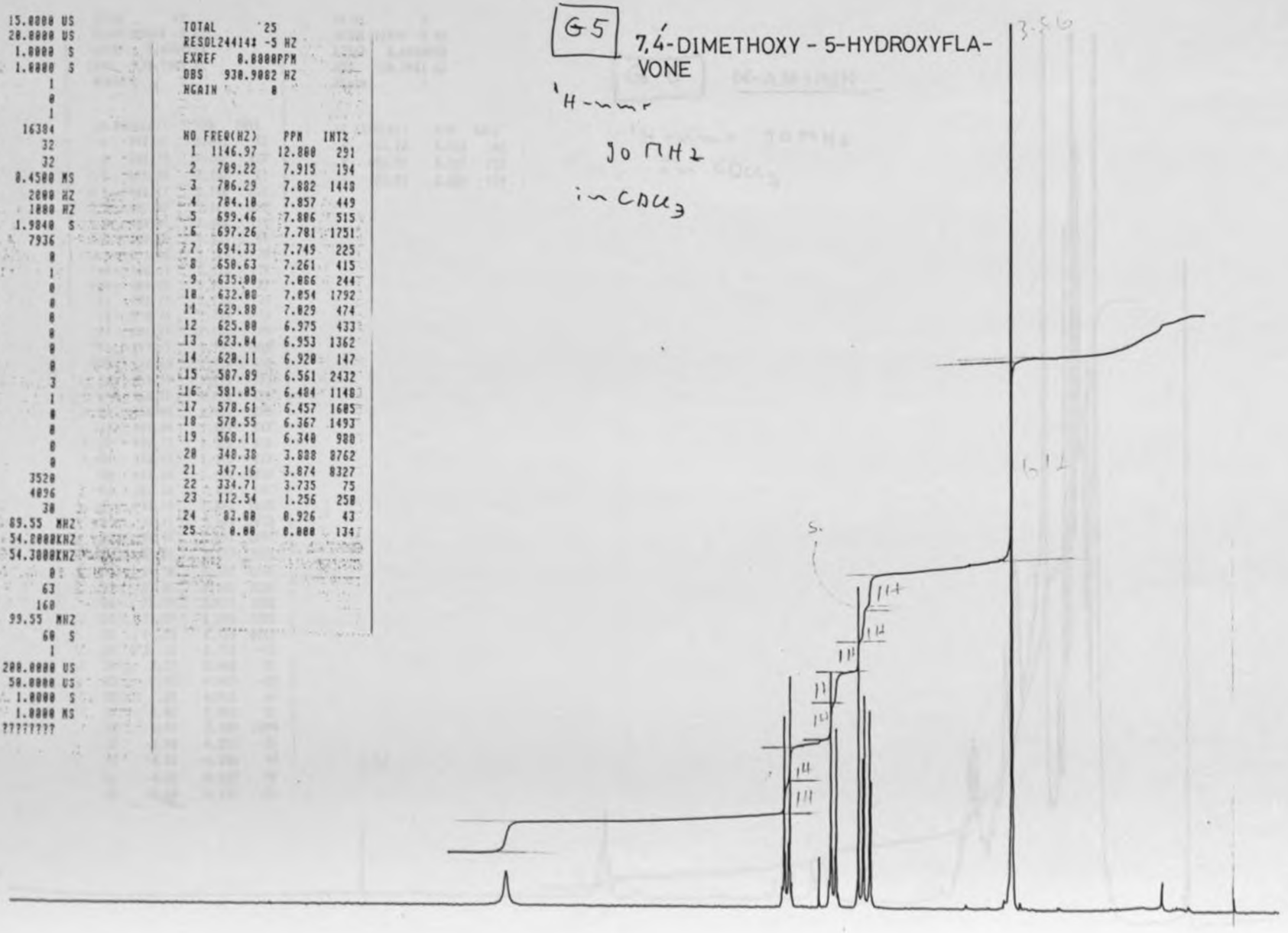
NO	FREQ(HZ)	PPM	INT#
1	1146.97	12.880	291
2	789.22	7.915	194
3	786.29	7.882	1440
4	784.10	7.857	449
5	699.46	7.806	515
6	697.26	7.781	1751
7	694.33	7.749	225
8	650.63	7.261	415
9	635.00	7.086	244
10	632.80	7.054	1792
11	629.88	7.029	474
12	625.00	6.975	433
13	623.04	6.953	1362
14	620.11	6.920	147
15	587.89	6.561	2432
16	581.05	6.484	1140
17	578.61	6.457	1605
18	570.55	6.367	1493
19	568.11	6.340	980
20	348.38	3.888	8762
21	347.16	3.874	8327
22	334.71	3.735	75
23	112.54	1.256	258
24	82.88	0.926	43
25	0.00	0.000	134

G5

7,4'-DIMETHOXY - 5-HYDROXYFLAVONE

^{1}H-nmr

10 mg in $CDCl_3$



G5
10 mg / $CDCl_3$
17.06.89 Bo

TOTAL 42
RESOL12287: -5 HZ
EXREF 0.0000PPM
OBS 430.7861 HZ
NGAIN 7

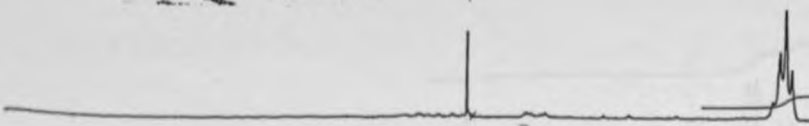
NO	FREQ(HZ)	PPM	INT%
1	885.81	9.037	-12
2	650.75	7.262	190
3	645.98	7.253	21
4	463.13	5.160	156
5	459.59	5.129	250
6	455.81	5.086	119
7	325.68	3.634	64
8	311.76	3.479	75
9	296.99	3.314	114
10	289.55	3.231	122
11	281.25	3.138	139
12	250.36	2.794	-15
13	194.09	2.166	40
14	179.19	1.999	158
15	172.60	1.926	412
16	169.55	1.892	453
17	166.01	1.852	426
18	156.00	1.741	280
19	144.84	1.607	717
20	136.23	1.520	950
21	123.65	1.380	777
22	112.54	1.256	5219
23	101.56	1.133	687
24	96.19	1.073	1895
25	90.45	1.009	2610
26	89.47	0.998	2897
27	85.81	0.957	2144
28	84.18	0.938	1760
29	82.03	0.915	1299
30	78.12	0.871	1520
31	73.85	0.824	1205
32	71.65	0.799	3660
33	35.15	0.392	-74
34	3.29	0.036	20
35	2.19	0.024	-30
36	1.09	0.012	44
37	0.85	0.009	51
38	0.00	0.000	1609
39	-0.40	-0.005	-36
40	-0.61	-0.006	27
41	-0.85	-0.009	50
42	-2.69	-0.030	-36

TOTAL 3
RESOL12287: -5 HZ
EXREF 0.0000PPM
OBS 430.7861 HZ
NGAIN 7

NO	FREQ(HZ)	PPM	INT%
1	463.13	5.160	156
2	459.59	5.129	250
3	455.81	5.086	119

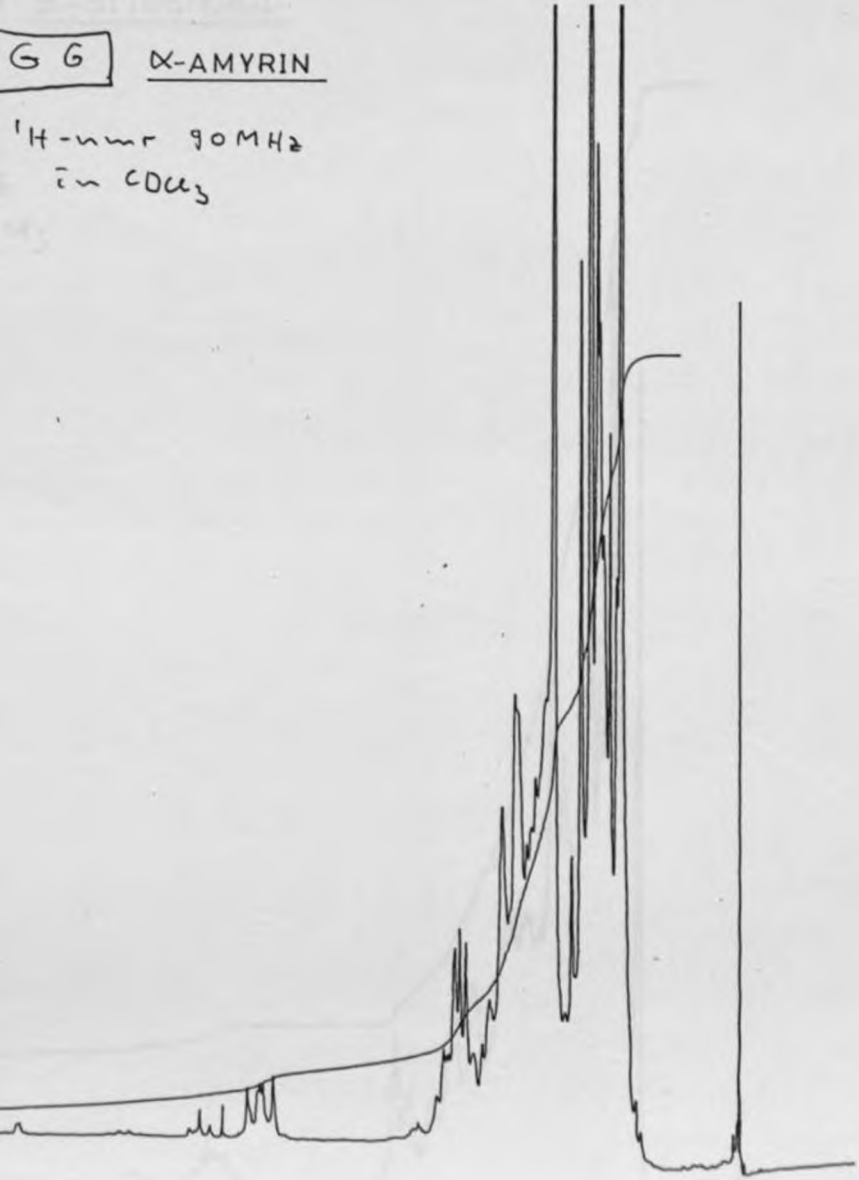
477

7.26



GG α-AMYRIN

^{1}H-nmr 90MHz
in $CDCl_3$

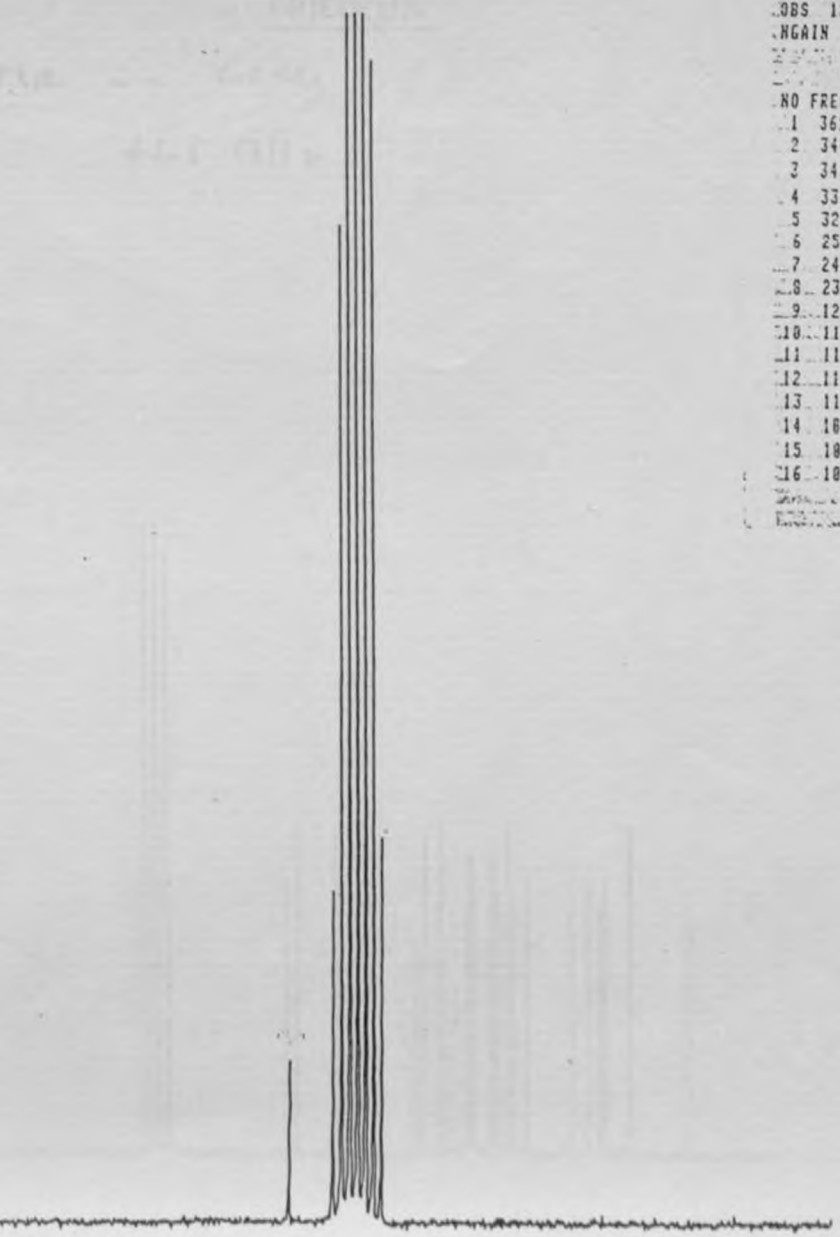


GG
27mg/$CDCl_3$
23.6.87
Th

AA6-2

^{13}C-NMR 22.5 MHz SCOPOLETIN

in CD_3OD*



TOTAL 16
RESOL 6103: -4 HZ
EXREF 49.000@PPM
OBS 1228.6376 HZ
NGAIN 13

NO	FREQ(HZ)	PPM	INT%
1	3695.68	164.012	170
2	3446.66	152.960	264
3	3413.09	151.471	214
4	3314.82	147.110	241
5	3289.80	145.999	589
6	2538.45	112.655	783
7	2482.91	110.190	587
8	2344.36	104.041	668
9	1282.96	56.936	679
10	1168.82	51.871	1322
11	1146.85	50.896	3928
12	1125.49	49.948	8286
13	1104.13	49.000	10019
14	1082.76	48.051	8892
15	1061.40	47.103	4578
16	1040.04	46.155	1533

AAC-2

TOTAL 34
RESOL 61831 -4 HZ
EXREF 77.0000PPM
OBS 655.5175 HZ
NGAIN 12

NO	FREQ(HZ)	PPM	INTZ
1	4791.09	212.625	649
2	1766.80	78.400	5485
3	1735.06	77.000	5477
4	1702.71	75.564	5255
5	1343.02	59.637	2411
6	1313.93	56.310	2905
7	1198.55	53.190	2911
8	960.45	42.980	1876
9	949.53	42.140	1874
10	935.49	41.517	2854
11	933.05	41.409	2497
12	896.43	39.783	2198
13	885.47	39.296	2909
14	865.33	38.402	1655
15	845.80	37.535	1585
16	813.45	36.100	2191
17	804.98	35.720	2592
18	798.19	35.422	2691
19	788.42	34.989	2333
20	741.43	32.903	2227
21	732.68	32.524	2587
22	723.73	32.118	2349
23	716.48	31.793	2384
24	688.29	30.545	2849
25	677.31	30.058	2369
26	668.76	29.679	349
27	635.19	28.189	2419
28	502.14	22.284	2715
29	456.36	20.252	2392
30	419.74	18.627	2164
31	412.42	18.302	2225
32	404.48	17.950	2431
33	338.63	14.675	2896
34	152.41	6.766	2332

COMNT 210

480

G2

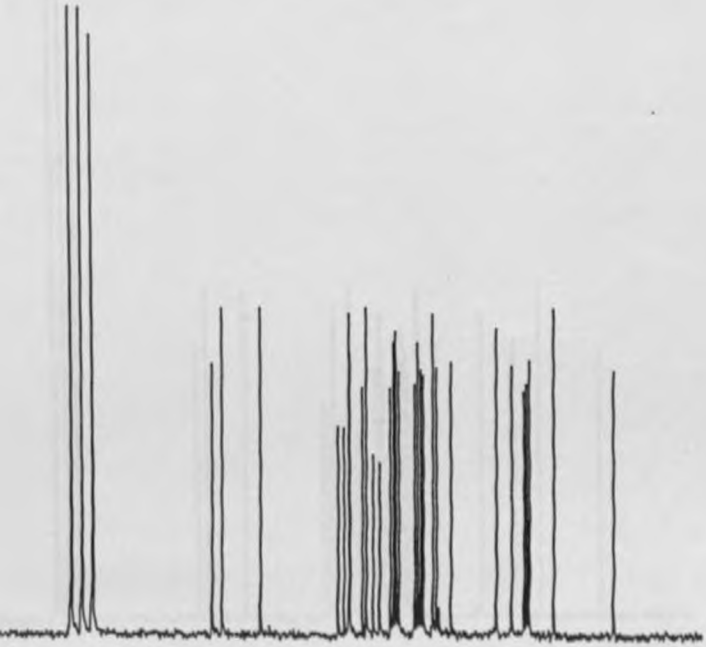
^{13}C - NMR

145.34

FRIEDELIN

in $CDCl_3$

44.5 MHz



G2
28 mg
$CDCl_3$
11.02.88
TR

TOTAL 34
FZ3DL 61831 -4 HZ
EXREF 77.0000PPM
OSO 655.5175 HZ
NGAIN 12

NO	FREQ(HZ)	PPM	INTS
1	4791.89	212.625	619
2	1766.80	78.400	5485
3	1735.86	77.000	5477
4	1702.71	75.564	5255
5	1343.82	59.637	2411
6	1313.93	58.310	2905
7	1198.55	53.198	2911
8	968.45	42.980	1876
9	949.53	42.140	1874
10	935.49	41.517	2854
11	933.85	41.489	2497
12	896.43	39.793	2198
13	885.47	39.295	2989
14	865.33	38.402	1655
15	845.80	37.535	1585
16	813.45	36.100	2191
17	804.98	35.728	2592
18	798.19	35.422	2631
19	788.42	34.989	2333
20	741.43	32.903	2227
21	732.68	32.524	2587
22	723.73	32.118	2345
23	716.48	31.793	2384
24	688.29	30.545	2843
25	677.31	30.058	2369
26	668.76	29.679	345
27	635.19	28.189	2419
28	502.14	22.284	2715
29	456.36	20.252	2392
30	419.74	18.627	2164
31	412.42	18.302	2225
32	404.48	17.950	2431
33	330.63	14.675	2826
34	152.41	6.766	2332

CONNT 218

481

G2

FRIEDELIN

^{13}C-NMR in $CDCl_3$

44.5 MHz

145.34

G2
28 mg
$CDCl_3$
11.02.88

G 6 α- AMYRIN

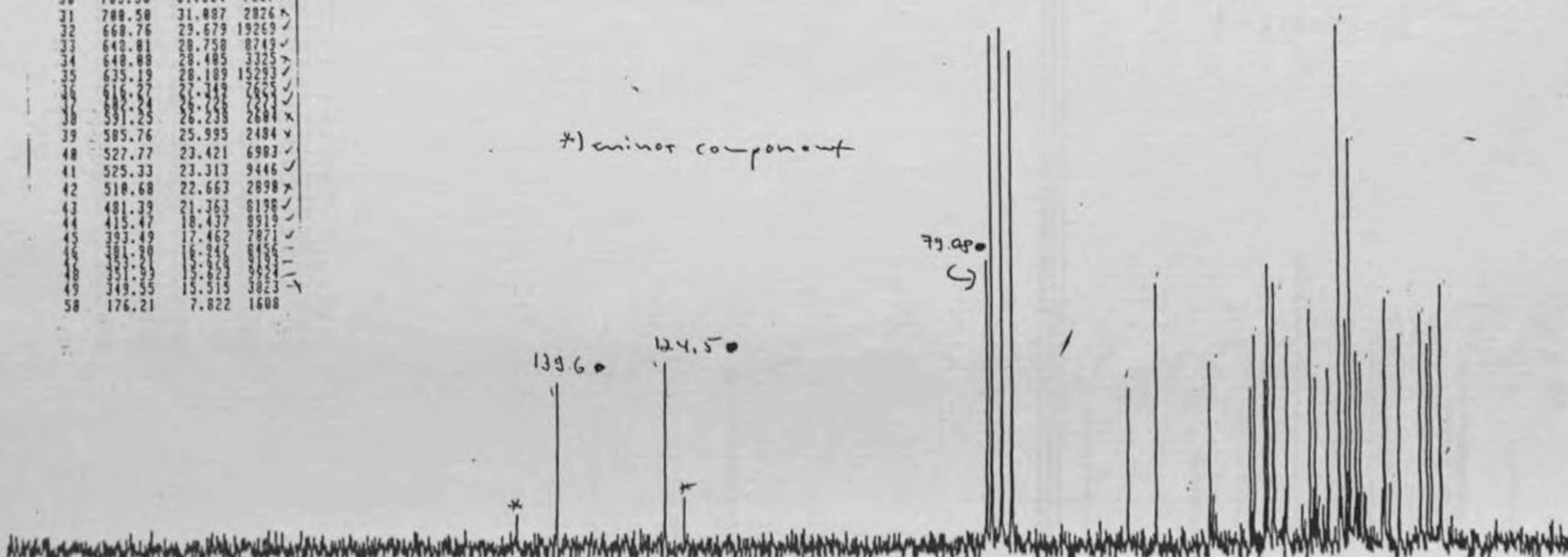
^{13}C-nmr in $CDCl_3$

TOTAL 50
RESOL 6103: [illegible] HZ
EXREF 77.0000PPM
OBS 655.5175 HZ
NGAIN 1

NO	FREQ(HZ)	PPM	INT2
1	4759.35	211.217	1286
2	4498.12	199.623	1419
3	4055.01	179.958	1533
4	3271.92	145.205	2046
5	3146.00	139.652	6803
6	2805.62	124.511	7512
7	2745.19	121.829	2634
8	2468.09	109.531	1311
9	1782.06	79.085	18989
10	1766.80	78.400	18993
11	1735.06	77.000	19269
12	1703.32	75.591	18444
13	1334.06	59.244	6390
14	1246.29	55.331	10850
15	[illegible]	47.822	2330
16	1067.33	47.366	2553
17	950.75	42.194	6353
18	942.82	41.842	1989
19	937.32	41.598	8219
20	904.36	40.136	6677
21	895.24	39.729	7944
22	894.01	39.675	10742
23	876.92	38.917	8422
24	875.09	38.835	10875
25	[illegible]	[illegible]	[illegible]
26	[illegible]	[illegible]	[illegible]
27	745.09	33.066	6703
28	732.88	32.524	2505
29	719.45	31.928	2216
30	705.38	31.304	7037
31	700.50	31.087	2826
32	668.76	29.679	19269
33	648.01	28.758	8749
34	640.00	28.405	3325
35	635.19	28.189	15293
36	616.27	27.349	7625
37	[illegible]	[illegible]	[illegible]
38	591.25	26.238	2684
39	585.76	25.995	2484
40	527.77	23.421	6983
41	525.33	23.313	9446
42	510.68	22.663	2898
43	481.39	21.363	8198
44	415.47	18.437	8919
45	393.49	17.462	7871
46	381.90	16.947	8456
47	[illegible]	[illegible]	[illegible]
48	351.93	15.623	9924
49	349.55	15.515	3823
50	176.21	7.822	1608

• signals characteristic for α-amyrin

*) minor component



G 6
27mg 1 $CDCl_3$

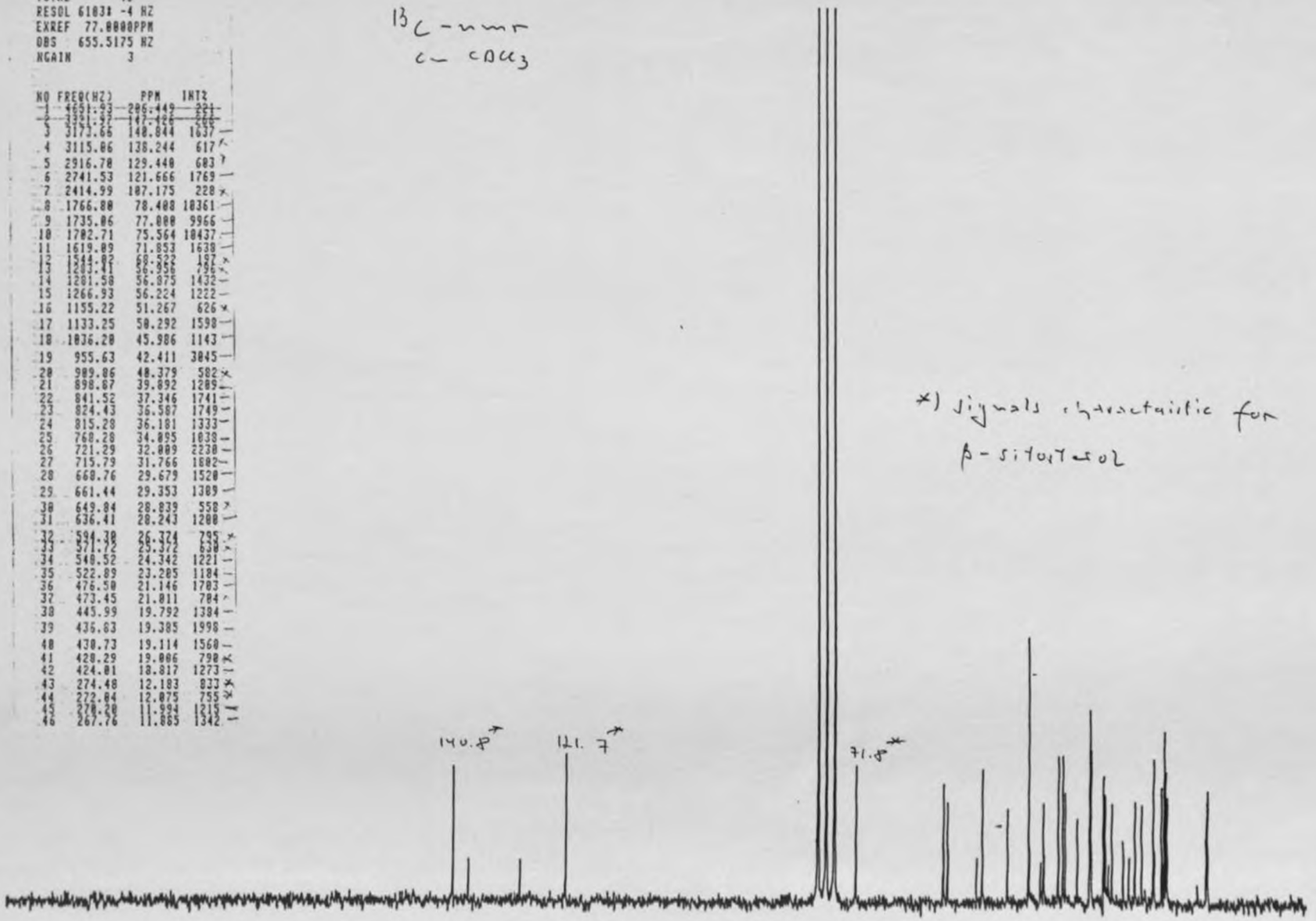
G7

β-SITOSTEROL

^{13}C-nmr
in $CDCl_3$

TOTAL 46
RESOL 6183 -4 HZ
EXREF 77.0000PPM
OBS 655.5175 HZ
NGAIN 3

NO	FREQ(HZ)	PPM	INTS	
1	4651.43	206.449	221	
2	3391.42	141.126	265	
3	3173.66	140.844	1637	
4	3115.86	138.244	617	*
5	2916.78	129.440	683	?
6	2741.53	121.666	1769	
7	2414.99	107.175	228	*
8	1766.80	78.408	10361	
9	1735.86	77.000	9966	
10	1702.71	75.564	10437	
11	1619.89	71.853	1638	
12	1544.82	68.522	187	*
13	1283.41	56.956	1956	*
14	1281.58	56.875	1432	*
15	1266.93	56.224	1222	
16	1155.22	51.267	626	*
17	1133.25	50.292	1598	
18	1036.28	45.986	1143	
19	955.63	42.411	3845	
20	909.86	40.379	582	*
21	898.87	39.892	1289	*
22	841.52	37.346	1741	
23	824.43	36.587	1749	
24	815.28	36.181	1333	
25	768.28	34.095	1838	
26	721.29	32.009	2230	
27	715.79	31.766	1882	
28	668.76	29.679	1520	
29	661.44	29.353	1389	
30	649.84	28.839	558	*
31	636.41	28.243	1200	
32	594.30	26.374	295	*
33	571.72	25.372	630	*
34	548.52	24.342	1221	
35	522.89	23.205	1184	
36	476.50	21.146	1703	
37	473.45	21.011	784	*
38	445.99	19.792	1384	*
39	436.83	19.385	1998	
40	430.73	19.114	1560	
41	428.29	19.006	798	*
42	424.81	18.817	1273	*
43	274.48	12.183	833	*
44	272.84	12.075	755	*
45	270.20	11.994	1215	*
46	267.76	11.885	1342	*



*) signals characteristic for β-sitosterol

483

G7
5mg / 1CDCl3