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.