A TOXICOLOGICAL INVESTIGATION OF CATHA EDULIS FORSK (MIRAA)

By

CHARLES KARIMI NAITAI

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

Signed.....

(C.K. MAITAI)

This thesis has been submitted for examination with my approval as University Supervisor.

Signed.....

(PROF. G.M. MUGERA).

ABSTRACT

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By

Charles Karimi Maitai

Catha edulis Forsk, also known as Miraa, grows in the Eastern part of the African continent from Ethiopia to South Africa. The young twigs of this plant are masticated to give a central stimulant effect. The leaves, shoots and roots have also been used in herbal medicine preparations.

The central stimulant effect of Catha edulis has been known for a long time. However, details of its discovery have been lost in legends and obscurity. The first reference to the use of Catha edulis was in a prescription by Naguib Ad-Din in 1237 A.D. Later, there were numerous references to it especially in the fourteenth Century.

Catha edulis became known in Europe through the writings of Vaugham who was a Port Surgeon at Aden in the 1850s. The medicosocial repercussions arising from the indiscriminate use of Catha material in Arabia and Eastern Africa were generally well recognised by the beginning of the 20th Century. For example, control measures were enacted in 1921 and 1939 by the British Colonial Government, in what was then British Somaliland and Kenya Colony respectively.

The first concerted effort to control the indiscriminate use of Catha material was made in 1956 when the United Nations Drug Commission at the

request of the Arab League considered the problem posed by the excessive use of Catha material. Shortly afterwards, the United Nations Economic and Social Commission requested the World Health Organisation to study the medical aspects of the consumption of Catha edulis. Since then, publications on the effect of chewing Catha material have increased considerably, but not much definite and authoritative documentation exists about its toxicity. It was in recognition of this fact that the present study was undertaken to provide additional valuable and comprehensive information on all aspects of Catha edulis toxicosis.

Toxicology is not a distinct discipline in its own right. It is a hybrid of many disciplines, among them chemistry, pharmacology, psychiatry, pathology, physiology, and biochemistry. A toxicological study of any problem is, therefore, bound to be biased depending on the specific interest of the research worker. The present study is a compromise of many interests all aimed at providing as much information as possible on the toxicity of Catha material.

The impetus for research on Catha material toxicosis was a recognition of possible harmful effects in those people who indulge in excessive chewing of the material. For example, there has been speculation that Catha material might be a contributory factor in the aetiology of oesophageal cancer and in cirrhosis of the liver.

Prior to starting the experimental work, a survey was carried out with a view to obtaining a basis for experimental work and also to ascertain or refute certain empirical information that are quoted freely in literature.

During the survey of literature, it became evident that there still exists a controversy as to whether there are more than one alkaloid in Catha material. It was, therefore, decided to investigate this aspect of the problem using chromatographic and spectrophotometric techniques.

Catha material was incorporated into food in concentration of 1, 5, 10, 15, 25 and 50% and fed to weanling rats 3-4 weeks old. The rats were observed for behavioural changes and for general signs of toxicity. The toxicity of Catha material in rats was also evaluated by its effect on the growth rate, symptomatology, change in organ-body weight ratios, gross pathology and histopathology. Rats feeding on various concentrations of Catha material (extract or non-extracted) were sacrificed at different experimental stages and examined for gross and histopathological lesions.

The transmission of the toxic principle(s) of Catha material through the placental tissue and mammary glands was evaluated by gross and histopathology of young rats immediately after birth and of sucklings approximately nine days after birth respectively.

Rats fed 5, 10, 15 and 25% Catha extract for more than 3 months were mated using male rats fed a corresponding percentage of Catha material and the new born examined for morphological deformities immediately after birth.

The average weight and number in each litter was recorded and the data from ten litters analysed and compared with that of control rats. Approximately one half of the litter was left to grow and examined for any deformities that might not have been detected at birth.

Rabbits were also fed Catha material extract in the concentration of 15 and 25% and their blood pressure determined, using Grants ear capsule method. Similarly, daily excretions of Na^+ and K^+ were determined. After about four months, blood was obtained from rabbits and examined for haematological and biochemical changes.

The pharmacology of the active principle of Catha material was studied, the emphasis being on those aspects which relate to the toxicity of Catha material. In particular, the effect on the smooth muscles (GIT, trachea, uterus), the cardiovascular system (heart and peripheral blood vessels) and central nervous system was considered important in this respect.

Results of the survey indicated that the active principle present in Catha material is a psychotropic compound which also induces some hallucinations. The effect is biphasic, the first phase is one of stimulation, and this is followed by the second phase of depression. The effect on sexual behaviour closely follows that of the central nervous system, i.e. intial stimulation followed by depression.

The survey also showed that people who indulge in excessive chewing of Catha material develop a form of dependence which was considered "habituative" rather than "addictive". The precise contribution of Catha material to the overall health of the individuals was uncertain but it appeared to vary considerably, depending on the feeding habits. The possible contribution of Catha material to schizophrenia was considered minimal. It was noted that several non-Moslems who chew Catha material also smoke bhang and indulge in heavy drinking. In nearly all cases, those who chew Catha material (but do not drink or smoke bhang) were found to be generally calm, withdrawn and tended to avoid noisy places. This finding is in sharp contradiction to published reports, mostly by non-Africans, which tend to portray those who chew Catha material as being "quarrelsome and antagonistic" to all forms of authority and generally as being "predisposed to maniacal outburst".

Chemical analysis of Catha material showed that there is only one alkaloid, d-norpseudoephedrine. It was present in all parts of the plant examined.

Feeding rats on Catha material caused retardation in growth even when no significant gross or histopathological lesions were noted. There was an indication of development of tolerance with respect to inappetite and central nervous system effects in rats fed Catha material extract.

The most consistent finding in rats fed high concentrations of Catha material was extensive desquamation and ulceration, sometimes haemorrhagic, especially in the stomach, and duodenum. Catha material extract incorporated in food caused little or no gross or histopathological lesions as compared to the bulky, non-extracted Catha material incorporated in food. It appeared that the difference could partly be accounted for in terms of effective contact between the toxic material and the glandular mucosal epithelium. This is consistent with information gained during the survey which indicated that the stomach discomfort experienced by Miraa chewers was especially marked when the material was ingested before eating food.

Centrolobular necrosis in hepatic lobules and coagulative necrosis of renal cortex were observed in the liver and kidney respectively associated with ulceration of gastrointestinal tract (GIT). Some rats which apparently died suddenly and which showed haemorrhagic ulceration in the GIT had no lesions in the liver and kidney. These lesions were directly attributed to tannins and it appeared that the tannins first impaired the integrity of mucosal epithelium and were subsequently absorbed into the body where they caused lesions.

Coagulative necrosis was observed in the heart of rats fed both the extract and the non-extracted Catha material. Although these lesions were observed in approximately 30% of rats fed 25 and 50% Catha material, some significance was attached to this finding because of some published work

which shows that several sympathomimetic amines can cause similar lesions.

Results obtained with rabbits were generally in agreement with those obtained with rats in respect to gross and histopathology of various organs. There was no finding to implicate Catha material in malignant growth as has been speculated by some research workers. Degenerative changes (vacuolation, cloudy swelling etc) of varying degree and extent were frequently observed in both rats and rabbits fed Catha material extract or low concentraion of whole Catha material and also in some control rats. Interpretation of degenerative changes is often difficult especially where there is no progressive involvement of the tissue with time. For example, a disturbance in the oxygen or nutrition requirement of the tissue, even for a short time, will often lead to degenerative changes.

The pharmacology of d-norpseudoephedrine closely follows that of l-ephedrine, d-pseudoephedrine and amphetamine and the difference appears to be quantitative.

D-norpseudoephedrine is excreted in urine of the rat, rabbit and man unchanged. Approximately 40% of the drug given orally was recovered in urine of man unchanged within six hours. The drug was detected in urine one hour after oral administration and could still be detected in urine twenty-seven hours later. D-norpseudoephedrine was detected in rat bile and in milk of lactating rats.

The absence of gross and histopathological lesions in suckling young rats, even though d-norpseudoephedrine was detected in milk, is consistent with observation in adult rats fed the pure drug where no significant lesions were found.

The present study shows that d-norpseudoephedrine is relatively unimportant in the overall consideration of Catha edulis toxicosis. Pathological changes observed in rats and rabbits fed Catha material can generally be attributed to the presence of tannins in the material. In particular, the changes in the gastrointestinal tract, the liver and the kidney were simulated by administration of commercial tannins intragastrically. The feeding habits of individuals can influence toxicity of tannins considerably as shown by results of feeding rats with tannic acid incorporated in food and also intragastrically. Even where no lesions were found, Catha material caused a retardation in the rate of growth possibly by interferring with absorption of food from the gastrointestinal tract.

The study suggests the need for additional research to establish whether coagulative necrosis observed in some rats and rabbits fed Catha material is a truly toxic effect.

Since tannins are widespread in the plant kingdom and in some common foodstuffs (e.g. tea, sorghum and vegetables) there appears to be little justification for discrediting Catha material solely on the basis that

tannins were found to cause some lesions. It would be more logical to consider the question of tannins toxity in a wider context as has been done by some workers. The social-economic problems posed by the excessive consumption of Catha material are outside the immediate consideration of this work and accordingly, no attempt is made to discredit Catha material on this basis.

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INTRODUCTION

Catha edulis Forsk, also known as Khat, Miraa or Arabian tea, grows in the Eastern part of the African Continent from Ethiopia to South Africa. The plant is well known because of its central nervous system stimulating effect and is an important item in social intercourses especially at birth, marriage, circumcision and funeral services among many indigenous people of Africa and Arabia. In nearly all cases, it is the shoots that are masticated as a stimulant or infused and the resulting tea drunk as a refreshment. The leaves, shoots and roots have also been used in traditional herbal medicine to treat various ailments.

Among the Moslems of Harar District, Ethiopia, there was a time when no private or religious ceremony took place without the ritual chewing of the shoots, accompanied by much praying and chanting (Margetts 1967). The consequent religious exaltation was then regarded as a gift from heaven. Similarly among the Meru elders in Nyambeni Division of Meru District, the plant is highly revered almost to the point of being considered sacred. Apart from the social importance of this plant, it is becoming increasingly lucrative as a commercial crop.

The euphoric and stimulating virtues of Catha edulis have been known for a long time, The earliest reference to it was in a medical prescription aimed at relieving depression (Neguib Ad-Din, 1237). Another early reference to it was in a Chronicle on Amde Seyon who reigned in Abyssinia,

1314-1344 (Cerulli, 1936). This relates that Sabr Ad-Din, then King of Ifat, before embarking on a holy war against Amda Seyon said, "As for Harade his capital, I shall make it mine and shall plant Khat because the Moslems like it." In 1852, Vaugham, the port Surgeon at Aden noted the keen interst that the Arabs had for Catha edulis material which was brought down from mountain areas, North of Aden on camel back almost daily. He also noted that there existed a controversy as to whether use of Khat was against the Islamic teaching, "thou shall not drink wine or anything intoxicating". It was mostly through his writings that Catha edulis came to be known in Europe.

Since the beginning of the 20th Century, it became apparent that massive consumption of Catha edulis has had serious medico-social repercussions. Control measures were enacted in 1921 and 1939 in what was then British Somaliland and Kenya Colony respectively. However, the first concerted effort to control the indiscriminate use of Miraa was made in 1956, when the United Nations Drug Commission, at the request of the Arab League, considered the problem posed by excessive consumption of Miraa, (Le Bras and Fretillere 1965). In 1957, the same Commission studied a French report concerning the medical, social and economic consequences of the consumption of Catha edulis in Djibouti. The problem of Khat was considered at an opportune time, when Cannabis sativa (bhang) had been discredited and struck off from all Pharmacopoea for not having

any useful medical properties. Shortly afterwards, the United Nations Economic and Social Commission requested World Health Organisation to study the medical aspects of the consumption of Catha edulis. Since then, publications on the effect of chewing Miraa have increased considerably, but not much definitive and authoritative documentation exist about the toxicity of the plant.

Because of this dilemma, many Governments are unsure of their stand with regard to the problem of Catha edulis. In Kenya, for example, the law prohibiting the growing, sale and consumption of Khat has become a silent one and a total embarrassment to the Government. Under the Miraa Prohibitive Ordinance of 1951 (Revised 1962), Laws of Kenya Chapter 339, "Any person who cultivates, sells or uses Miraa without a permit is guilty of an offence and on conviction is liable to a fine of 2,000 shillings or to imprisonment up to one year." Inspite of this, Catha adulis material is being sold openly in many parts of Kenya, including Nairobi City, and many people including Police officers and Members of Parliament use it freely. In particular, the moslems use it in social occassions because their religion forbids the consumption of alcohol.

Catha edulis Forsk belongs to the Celastraceae family of plants. It was first classified by the Swedish physician-botanist, Peter Forskal, (Forskal 1775) who together with Karsten Niebr had been sent by King Frederick V of Denmark on a scientific expedition to the Middle East.

He noted its cultivation along with coffee and reported that the Arabs in Yemen ate the green leaves and ascribed medicinal "virtues" to the plant.

The plant is a glabrous evergreen shrub, 10-20 feet tall but can grow to a height of 80 feet, with a trunk over two feet in circumference at breast height, depending on the climate and soil fertility. The plant grows best at an altitude of 4000 - 8,500 ft. in the warm wet mountainous region of Eastern Africa and Arabia. The bark is fairly thin, smooth, a pale greyish-brown in colour, the crown is pointed, narrowly pyramidal in outline, usually branching all the way up the stem, of which more than one is usually produced. The young leaves are crimson-brown, glossy, becoming dark yellow-green and leatherly with age. Leaves are opposite on compressed twigs, lanceolate or even oval in shape, short stalked and they vary greatly in size ranging from 3 to 12 cm in length by 0.5 to 6.7 cm in breadth. The flowers are small and white. They are produced in axillary cymes from the leaf axils towards the end of the younger branchlets. The fruit is dark brown, oblong 3-locular capsule up to 1 cm long containing 1 to 3 seeds. The seed has a small brown papery wing at the base. The plant is cultivated from cuttings; seeds are sometimes though rarely sewn. The cuttings are planted during heavy rains and take about 3 - 5 years before the first crop is taken.

The trees are pruned to shrub size yearly to keep their height to about 16 feet. During the wet season, the tender shoots are picked about twice a week. Among the Meru people in Nyambeni Division of Meru District, the shoots are first tied in twos to form "Apa"; ten of these "Apas" are then tied together to form a "Bandari". Finally two "Bandaris" are wrapped together with fresh green banana leaves and tied with strips of dry banana fibre. Ten of these wrapped bundles are then tied together to form a bigger bundle, sometimes referred to as a "Kilo".

According to d'Herricourt (1845) the planting of Khat was introduced from Abyssinia into Yemen about 1429 by Sheikh Abou Zerbin. Reference to its use being extended from Yemen to Aden is found in the writings of Arab Abdul Kadir (De Sacy 1826). Its cultivation and use in Ethiopia and South West Arabia is considered to be earlier than that of Coffee.

In Kenya, the plant is now being grown on a commercial scale in Nyambeni Hills on the slopes of Mount Kenya but it is also found growing in a scattered fashion in other parts especially in Central Province, Western Province and Machakos District. Many farmers in the Nyambeni Division of Meru District get more money from the sale of young shoots of Catha edulis than from the coffee crop. It is exported to the neighbouring countries of Zambia, Uganda, Tanzania, Zaire, Sudan, Somalia and the Middle East. It is estimated that the sale of this crop bring in a total of 10 million shillings every year. In 1973, it is estimated that the

crop will earn approximately 20 million shillings in foreign exchange. Because of the tremendous economic gain, the whole question of Catha edulis has become an important political issue.

LITERATURE REVIEW

The stimulant effect of Catha edulis material is well known to those who chew the plant but details of its discovery have been lost in legend.

Fluckinger and Gerock (1887) seem to have been the first to isolate the pharmacologically active substances present in Khat. They isolated a small amount of basic material which they called "Cathine". In subsequent years, other workers (Paul 1887; Mosso, 1891; Beiter 1910; Chevalier, 1911; Stockmann, 1912) also tried to isolate and identify the active principle(s) present in Khat. However, the most significant result was obtained by Wolfes (1930) when he isolated and identified d-norpseudoephedrine. This compound was first isolated from a Chinese herb, Ma huang (meaning yellow drug) by Smith (1928) andsynthesized by Nagai (1928) and later by Pfanz (1955). The same compound has been isolated from a South American tree Maytenus Krukovii (Anonymous).

Von Bruche (1942) considered that the amount of d-norpseudoephedrine present in Catha edulis is not adequate to account for its stimulant activity. A similar view has been expressed by other workers (Paris and

Moyse, 1959; Trellu, 1959). This view has been disputed by other research workers (Hoffman et al. 1955; Alles, Jensen and Fairchild, 1961).

Paris and Moyse (1959) have studied the chemical composition of Catha edulis using chromatographic techniques and they claim to have detected 3 bases. Alles, Fairchild and Jensen (1961) also studied the chemical composition of Catha edulis material using chromatographic techniques and they obtained only one alkaloid, d-norpseudoephedrine. Ristic and Thomas (1962) identified d-norpseudoephedrine but also claimed to have identified l-ephedrine and a third minor alkaloid, which was not identified. Winterfeld and Bernsmann (1960) isolated only one alkaloid, d-norpseudoephedrine, and in addition 17 amino acids. Fairchild and Alles (1967) have shown that d-norpseudoephedrine is approximately 1/10 as potent as amphetamine as a central nervous system stimulant. Amphetamine is the most potent known compound in the phenylalkylamine series. Sheperd, Lader and Rodnight (1968) classifies Catha edulis as a psychotomimetic drug, i.e. a drug which is characterised by its ability to induce disturbance of mood, thought and perception in dosage levels, usually low enough to avoid marked peripheral effects.

Some research workers (Alles, Fairchild and Jensen, 1961) have shown that there is no appreciable difference between the alkaloidal content of fresh Catha leaves and samples that had been dried and preserved. This view has been disputed by some workers. For example, Friebel and

Brilla (1963) claim to have isolated a very unstable alkaloid from the leaves, which is very close in formula to d-norpseudoephedrine but more potent. Leete (1958) has shown that phenylalanine is the precussor in the biogenesis of d-norpseudoephedrine. As yet, the intermediate compounds have not been isolated and the exact sequence of biosynthesis of the alkaloid is also unknown.

Reports of toxicity of Catha plant material are fragmentally and often not well suported by facts. In particular, the central nervous system effects have not been reported objectively. Carothers (1945) described the chronic effect of Catha edulis on two addicts as being mild mania and schizophrenic symptoms. Heisch (1945) describes a fatal case of poisoning with Catha edulis and claims that the symptoms resembled those of strychnine with primary and secondary depression. Peters (1952) claims that excessive chewing of Khat may lead to heavy stupor and that after recovering from stupor, the person is listless, quarrelsome and antagonistic to all forms of authority. According to Peters, "Continued immoderate use of the drug produces a dream world in which the person becomes mentally divorced from reality and suffers a deterioration of charactor. He becomes increasingly apathetic, dull in intellect and unable to concentrate ---------- and there is also a tendency to acquire irresponsible fearlessness". A further claim by Peters is that the person become

mentally and bodily debilitated at about 30–40 years of age and that many of them become sexually impotent. Le Bras and Fretillere (1965) have given a detailed account of the central nervous system effects of Catha edulis based on observations made on 53 human volunteers.

A high rate of periodontal disease among Arabs leaving in Israel was attributed to habitual chewing of the leaves of Catha edulis (Rosenzweig and Smith 1966).

Kamel, Halim and Hussein (1967) have shown that Catha edulis causes abnormalities in chick embryo and therefore lists it as potentially teratogenic in man. Catha edulis contain approximately 14% tannins of the condensed type. The chemistry of tannins is very complex, but it is usual to classify them into two groups: derivatives of flavanol (called condensed tannins) and hydrolysable tannis (the more important group, which are esters of sugars, usually glucose with one or more trihydroxy-benzene carboxylic acid).

Morton (1970) has provided a tentative correlation of plant usage with oesophageal cancer Zones of the world (North China, Transkei, Curacao etc.). On the basis of statistics Morton has speculated on the possible role of tannins (present in tea and wine as a causative agent of oesophageal cancer. Korpassy and Mosonyi (1950) have shown that tannic acid can cause liver cirrhosis and tumors. Le bras and Fretillere (1965) have speculated on the possibility of Catha edulis being a contributing

factor in aetiology of oesophageal cancer, although they do not attribute this to tannins present in the plant material.

On the basis of published work, tannins are undoubtedly of toxicological significance; many of them have been known to cause degenerative changes of varying degree and extent in the liver and kidney when administered parenterally to laboratory animals (Clarke and Rossiter 1943). There are also reports which indicate that, when given orally in large amounts an appreciable amount is absorbed and this cause lesions in the liver and kidney (Boyd, Bereczyky and Godi 1965).

A survey of literature has not revealed any published work with regard to the toxicity of pure d-norpseudoephedrine.

A SURVEY OF MIRAA CHEWING HABITS IN KENYA

Prior to starting the experimental work, a survey was carried out with a view to obtaining a basis for further research and also to confirm or refute certain empirical information quoted freely in literature. Nearly all the important suburbs of Nairobi (Majengo, Eastleigh, Kibera, Kariobangi, Mathare Valley etc) and all the back streets in the centre of Nairobi City, where Catha material is sold were visited. In addition, several remote areas in Kenya, such as Garissa, Isiolo, Nanyuki, Mombasa, Machakos as well as many places in Meru District, were visited. It was considered appropriate to seek the assistance of Miraa dealers in the

course of gathering information. In nearly all cases, the author stayed with the dealers, sometimes for a whole day, interviewing customers. Usually, the customers were promised a free bundle of Catha material on condition that they co-operated fully, This proved to be a wellcome incentive to most customers.

The author preferred interviewing people individually but in a few cases, a small group of people was interviewed and the consesus of opinion, on the questions discussed, recorded. This approach was dictated by circumstances, as often an argument developed when a particular person, being interviewed gave a "biased answer" to the question. In nearly all cases, a questionare was completed.

Several bundles were purchased from different parts of Kenya at different times and their weight determined as soon as possible. From these weighings, the average weight of a bundle or half bundle (bandari) of fresh Catha material was determined. It was decided to determine what percentage of the twigs is normally masticated and how much is discarded. Several people who chew Catha material were invited to peel off the bark from the twigs as they normally do when chewing the material. The percentage of fresh peeled bark (i.e. edible part) as compared to the whole bundle was determined. Similarly the percentage of the woody part, left after peeling off the bark, was determined.

Finally the moisture content of the peeled bark and of the woody portions of Catha edulis twigs was determined by weighing them separately when fresh and later after drying to a constant weight at 100°C.

RESULTS OF THE SURVEY

Information received during the survey with regard to effect of Catha edulis on the central nervous system was basically consistent. Evidence was given that Catha edulis is a central nervous system stimulant. Many people testified that, "it enhances their ability to solve problems by increasing or revealing new ideas". It was claimed that Catha material induces hallucinations the most obvious perceptual disturbance being illusions and euphoria.

From the evidence presented, the effect of Catha material appeared to be biphasic. The first stage is marked by a feeling of satisfaction self-admiration, perhaps overconfidence and on the whole, the power of concentration is greatly enhanced. There is a general tendency to "overdo" whatever one is engaged in. For example, a person doing manual work can work for long hours without feeling tired. The second stage in the consumption of Catha material is characterised by mental fatigue, insomia and a feeling of "self-pity" or "let-down".

A consistent claim was that Catha material cause sleeplessness only in the first few days; thereafter, the disturbance in the pattern of sleep is minimal even after consumption of large amount of Catha material.

It was alleged by a few individuals that excessive chewing of Catha material, without sleep and/or feeding properly could lead to serious chronic disturbance of the central nervous system. A few cases were cited but, on investigation, it was found that these same people either smoked Cannabis sativa (**bhang**) or indulged in excessive drinking of local alcoholic brews. The majority of people interviewed (approximately 70% were moslems who do not consume alcoholic beverages. The author spent much time with people who chew Catha material, observing their behaviour under natural conditions. Contrary to some published reports, the majority of them were generally calm, friendly, withdrawn and tended to avoid noisy places or contraversial topics. The tendency was especially marked in the latter stages, of chewing Catha material.

The amount of Catha material consumed by different individuals varied considerably and in most cases, availability of money appeared to be the major limiting factor. The amount of Catha material consumed by individuals also depend on such factors as: the occassion, the nature of Catha material and what the person is using e.g. water, soft drink, milk etc.

From the survey carried out, it was established that the average consumer of Catha material chews approximately 7.5 "bandaris", per day. The standard deviation show a considerable variation in the quantity consumed by different individuals.

The average weight of a fresh bandari (half bundle) was found to be 41.8 grammes but only 78.7% of the twig is normally masticated (Fig. 1). The average moisture content of the peeled skin is 65.5%. The amount of dry Catha material consumed daily by an average person was found to be 8½.5 grammes.

The majority of people interviewed were of the opinion that Catha material from Kangeeta in Nyambeni Division of Meru District is of superior quality than that from other parts of East Africa. Many of them claimed that they could tell the origin of any Catha material in the market, just by looking at it. However, elders in Nyambeni Division of Meru District dismissed this as "cheap propaganda" by dealers in Catha material and added that the only difference was whether the twigs were from an old shoot or a recently planted one.

There appeared to be no clear-cut and consistent criteria for differentiating material of superior quality from that of low quality. However, as a prorequisite, the shoots had to be tender, fresh and preferably red in colour. Some small red twigs called "Kisa bomu" were

reputed to be more potent than the other types. The criterion for this type of Catha material seemed to be purely on the basis of size. However, some twigs that were being sold as "Kisa bomu" were found to weigh more than other types of Catha material.

The possibility that consumption of Catha material, in large amount, might lead to addiction has been raised by some research workers (Peters 1952). Evidence adduced from several people indicated that those who had been chewing Catha material for a long time had developed a form of "dependence" on it and were generally restless if they failed to get their daily ration. However, if they failed to get Catha material for about 3-4 days, this feeling of uneasiness tended to disappear. There were several people interviewed who had given up chewing Catha material as an expensive, useless, social habit of no real benefit. In all cases, there was no tendency to increase the daily consumption of Catha material; on the contrary the tendency was to reduce the amount consumed daily.

Several people were asked to comment on the effect of Catha material on sexual behaviour. A breakdown of information gained from 200 people interviewed individually and who were very co-operative is presented below:

Definitely stimulated	91 people	(45.5%)
Non-commital	53 "	(26.5%)
No change (i.e. Normal)	31 "	(15.5%)
Definitely inhibited (depressed)	24 "	(12%)

Many people who said that chewing of Catha material caused sexual stimulation were quick to add that this desire is not matched by a corresponding physical ability to indulge in sexual intercourse effectively. Evidence was also given by a few but reliable people that, while urinating, semen tend to ooze out freely (spontaneous ejaculation).

Accurate information on male impotence is difficult to get, because, among many indigenous people of Africa, it is usually regarded as something very shameful. Usually, the wife of an impotent husband is encouraged to have children with other men, to conceal the fact. Using the number of children per family as an index of impotence would therefore give misleading information.

During the survey, the author put forward a suggestion that Miraa might be a teratogen, but this was always refuted. In all areas visited during the survey, there was no indication of a higher incidence of deformities among children born to those chewing Catha edulis material, even though some of the parents had been consuming Catha material for over 30 years.

A few people indicated that extract of Miraa is good for both production and unproductive coughs and generally for colds. It was also alleged that extract of Miraa was of value in the treatment of malaria, diarrhoea and veneral diseases.

A possible source of error in this survey is that the description of the effects of Catha edulis by each individual depended on such factors as the specific interest of the person, his intelligence, perspicacity and verbal descriptive ability.

DISCUSSION ON SURVEY

The controversy over the use of Catha material has been based on its apparent addictive properties and like the Cannabis sativa, the argument has never been conclusive. This is partly due to the fact that the difference between the two types of drug dependence, i.e. habituation and addiction is not definitive. The socio-economic problems created by excessive chewing of Catha material are generally well recognised. A much publicised view possibly on account of its sensational undertone, is that excessive chewing of Catha material has been responsible for many broken marriages supposedly because the husbands become impotent and can no longer satisfy their wives. The economic problems created by excessive chewing of Catha material are, on the average, much more serious than those created by alcoholism. For example an average person consuming 6-8 bandaris of Catha material daily would spend about three hundred shillings a month. This is approximately the salary of an ordinary police constable, with eight years of education, and much more than what the majority of subordinate staff employed by the Government earn.

On the other hand, a person who chews Catha material is likely to be more useful in gainful occupation than one drinking alcohol.

Since 1966, publications on the effect of chewing Catha material have increased considerably but not much definitive and authoritative documentation, with respect to its toxicity in man has been published. This is probably due to the fact that it is difficult to get reliable and accurate information relating to the consumption of Catha material, ever since the Governments enacted legislative measures to control the dealing and consumption of Catha material.

Medical ethics prohibit unnecessary experimentation with human beings. For this reason, toxicological studies are usually carried out using laboratory animals and results extrapolated to predict possible effect in man. There is however no substitute to the actual observation of effect in man and results obtained with laboratory animals are only of relevance if they are confirmed in man. In some cases, the effect cannot be predicted from experiments with laboratory animals. This is true, for example, with drug dependence,

It was in pursuance of this recognition that the present toxicological study was extended to cover a general survey of Catha material consumption among the indigenous people here in Kenya and therefore supplement results obtained with laboratory animals. The result of the survey was expected to form a basis for experimentation with laboratory

animals. Unfortunately, some of the chronic effects attributed to Catha material (e.g. impotence or dependence) could not be confirmed experimentally and at best one can only try to examine the rationality of such allegations, taking into account the basis of this information and the creditability (or personal integrity) of those giving the information.

Deductions and conclusions made from information obtained during the survey can only be regared as valid if an assumption is made that those interviewed were reasonably representative of the entire community of those who chew Catha material here in Kenya. Unfortunately, there is no precise way of testing whether the data is representative or not. It may however be assumed that the majority of those who chew Catha material are found in urban areas, where the material is sold. This assumption may be considered valid on the basis that most people consume fresh Catha material only. An exception may be made in case of those who grow Catha material as for example, farmers in Nyambeni Division of Meru District.

A breakdown of those interviewed show a proportionate representation of people from all walks of life and from all parts of Kenya. For example, the majority of people interviewed were either self-employed or unemployed. Similarly, the Somali and Meru, by far the most important consumers of Catha material, were heavily represented. Furthermore, representation on the basis of age and sex was reasobable. Although the percentage of

young people chewing Catha material was small, many of them were found to be indulging in excessive chewing of the plant material. A large percentage of them were dealers in Miraa, having left school at an early age of about 14-16 years and with no other source of income. In contrast, the women interviewed were not found to be indulging in excessive chewing of Catha material and it appeared as if they were chewing it out of curiosity rather than any strong urge.

Information obtained from the survey will be alluded to frequently in discussion on specific aspects of Catha edulis toxicosis.

Fig. 1. CATHA MATERIAL AS SOLD IN THE MARKET

A. Young twigs showing the tender bark (arrow) that is normally masticated.

B. 2 half-bundles (bandaris). The two were wrapped with green banana leaves to conserve moisture.

C. Two bundles of Catha material twigs.
Each bundle has two bandaris.

D. One big bundle, commonly referred to as a "Kilo".
It has ten bundles similar to one shown in the
picture (right).

EXPERIMENTAL WORK

PART I: CHEMICAL ANALYSIS OF CATHA EDULIS MATERIAL.

INTRODUCTION.

Fresh Catha material (twigs, leaves, roots, leaves etc) was first weighed and then dried in an oven with hot air current at 40-50°C. for approximately two days. The material was then ground to a fine powder (using a Willey mill, made by Arthur H. Thomas Co., Philadelphia, U.S.A.) and stored in polythene plastic bags until required. In all cases, different parts of the plant were examined separately.

ISOLATION OF BASIC COMPOUNDS

MATERIAL AND METHODS.

Approximately 50 grammes ground Catha edulis material was accurately weighed. To this was added approximately 200 ml. acidified water and the mixture boiled for about one hour after which it was simmered at approximately 80°C. for another 3 hours. The aqueous extract was then strained through a muslin cloth.

The residue was taken up again with 100 ml. acidified water and the extraction procedure repeated three times, each time boiling the mixture and leaving it to simmer at 80°C. for about two hours. The combined extract from all four extractions was then filtered using Whatman No. 1 paper. To the filtrate was then added about 10-20 ml. of saturated lead acetate solution and the yellowish amorphous precipitate (mostly tannins and pigments)

removed by centrifuging at 600 rpm for about two hours. Since the volume of the extract was large, the possibility of co-precipitation of alkaloids in the acidified media was considered minimal. After decanting the supernatant liquid, the residue was taken up with 20-30 ml of water and centrifuged. The washing was repeated twice, each time centrifuging the mixture for about 30 minutes. The supernatant liquid fractions were then pooled together. The absence of tannins and pigments in the supernatant liquid was confirmed by adding a few drops of lead acetate solution and observing no precipitation.

Excess of lead ions were removed by adding a few drops of 0.1N sulphuric acid until there was no more precipitation of lead sulphate. The white amorphous mass of precipitated lead sulphate (usually only a small amount) was removed by centrifuging at 600 rpm for about 30 minutes. The supernatant liquid was checked for acidity using broad range pH paper indicator.

The clear detannated extract approximately 250 ml. was transferred to 500 millilitres separating funnel and extracted with equal volume of ether for 15 hours. The extraction procedure was repeated three times but the subsequent extractions were for a shorter duration, Usually 8-10 hours. The combined ether extract, containing acids and neutrals was set aside as the main interest was on the acidified aqueous solution,

which contained alkaloids as salts. The acidified aqueous solution left after the removal of acids and neutrals was made alkaline with either sodium bicarbonate or sodium hydroxide solutions. The alkalinity of the aqueous solution was confirmed by spotting the solution on pH paper indicator using a glass rod which also served to stir the mixture. When the aqueous solution changes from acid to alkaline pH, there is a definite change in colour from light yellow to brownish orange and it is only necessary to confirm this change without determining the pH each time sodium bicarbonate or sodium hydroxide is added.

A volume of ether, equal to that of the aqueous phase was used and the extraction procedure repeated as for acids and neutrals described before. The combined ether extract was washed twice with 2.5 ml, 2% sodium bicarbonate, dried with anhydrous sodium sulphate and after filtration, ether was distilled off and the residue examined for alkaloids.

In the extraction procedure adopted preliminary work had shown that after three extractions, no detectable amount of the alkaloid remained in the aqueous phase. However, as a precautionary measure a fourth extraction was always carried out.

An alternative procedure was to extract the Catha material with 200 ml, 80% ethanol instead of acidified water and to eliminate ethyl alcohol by blowing hot air current over the surface of the combined

extract, contained in a 500 millilitres shallow porcelain dish. The residue, a semi-solid aqueous extract of glue-like consistency, was then taken up in about 200 ml. acidified water. The rest of the extraction procedure is as described before.

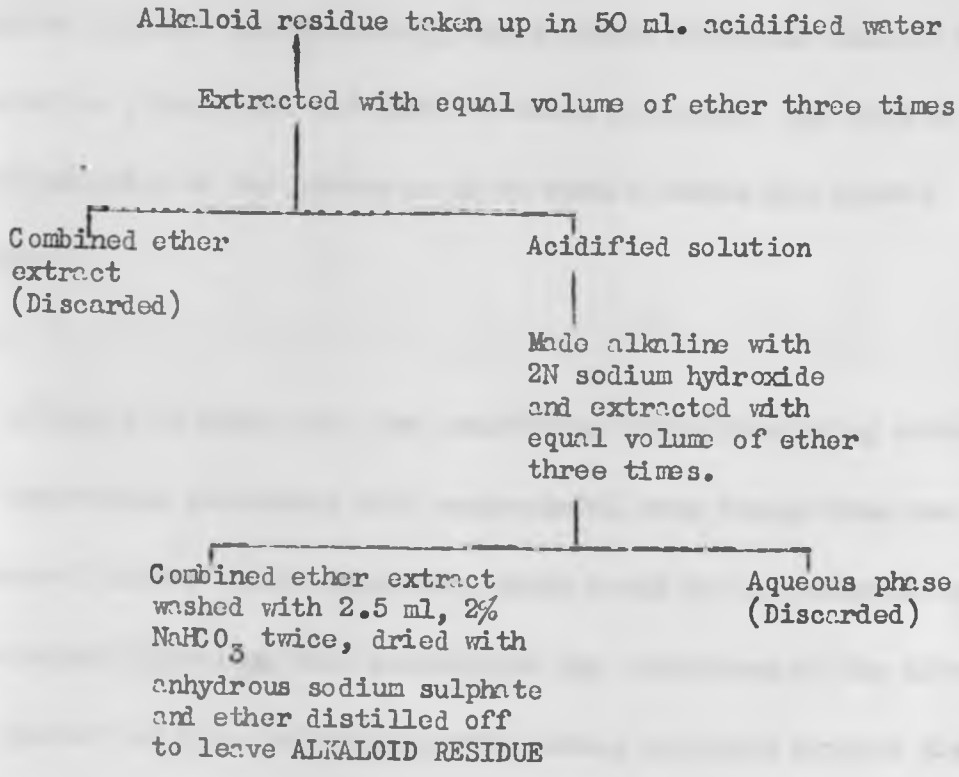
Purification of alkaloid isolated from *Catha* material.

INTRODUCTION

In nearly all cases, solvent-solvent extraction of *Catha* material gave a basic residue which was impure and an attempt was, therefore, made to effect further purification. This was necessary, especially to get a pure sample for infrared and ultraviolet spectra.

MATERIAL AND METHOD

The procedure adopted is outlined below:



The process of purification by solvent-solvent extraction was repeated several times.

Another method used to effect purification of the basic fraction was to add animal charcoal to the aqueous extract of the alkaloid and after

repeated agitation the mixture was filtered to give a clear colourless filtrate.

Purification of the alkaloid by crystallisation was also attempted. The basic residue from approximately 2 kilogrammes dry Catha material was taken up in absolute alcohol and acidified with 1-3 drops of concentrated hydrochloric acid. The mixture was then refluxed at 70-80°C. for 4 hrs. using water-cooled condenser.

The refluxed mixture was transferred to a clean watch glass or evaporating dish, placed over warm plate (about 30-40°C) and alcohol left to evaporate slowly. Alternatively, the refluxed alcoholic mixture was transferred to a test tube and left for about one week. The tube was scratched slightly at the bottom so as to form a centre for crystal formation.

RESULTS

Attempts to remove all the interfering impurities using solvent-solvent extraction procedures were unsuccessful even though this was tried several times. Small impurities which could not be detected by chromatographic technique were responsible for distortion of the ultra-violet spectra and also infrared spectra (using potassium bromide disc or nujol mull) especially in the fingerprint region 800 - 1600 millimicrons. Animal charcoal removed most of the colouring matter in the residue but unfortunately it also removed about 80% of the alkaloid.

When the refluxed alcoholic extract was left at room temperature, about 20°C, for approximately one week, a few crystals were formed and these continued to grow to a reasonable size. Finally, the crystals

were separated from mother liquor and washed repeatedly with small amounts of ice-cold absolute alcohol. The crystals were taken up again in absolute alcohol and the process of recrystallisation repeated twice. Crystals on the watch glass formed easily but these were often found to have trace impurities which appeared to be intimately trapped by the crystals and could not be removed easily by washing with ice-cold alcohol. The isolated alkaloid were examined using various analytical methods.

FACTORS AFFECTING PERCENTAGE RECOVERY OF ALKALOID FROM CATHA MATERIAL

Effect of heat on percentage recovery of the alkaloid.

INTRODUCTION

Extraction of alkaloid from Catha material always involved prolonged and intermittent heating of the neutral or acidified mixture so as to break down cellular wall of plant material and liberate the alkaloid. The present experiment was meant to investigate whether heating of d-norpseudoephedrine in neutral or acidified media lead to appreciable loss of alkaloid content.

MATERIAL AND METHOD

A known amount of d-norpseudoephedrine base, approximately 10 mg. was dissolved in distilled water and made to 10 ml in a standard flask.

The ultraviolet spectrum of the solution was obtained using SP 800 spectrophotometer. The solution was then transferred quantitatively to a small beaker and heated until approximately 80% of the water had been evaporated. The solution was cooled, transferred to a standard flask and made to original volume using distilled water. The ultraviolet spectrum of the solution was then obtained.

The amount of d-norpseudoephedrine present in the solution before and after heating was calculated from the optical density of the major peak at 256.5 nm in the two spectra.

The experiment was repeated using d-norpseudoephedrine hydrochloride and 0.1N sulphuric acid, instead of d-norpseudoephedrine and water respectively.

RESULTS

The results are shown in table 1. Heating the free base in water lead to a small loss of approximately 5%. In contrast, heating the hydrochloride salt of the base did not lead to any appreciable loss.

Most salts of organic bases are not volatile and can withstand prolonged heating even above 100°C. Their melting points are usually above 200°C.

B. Effect of saturating solution with sodium chloride

INTRODUCTION

Saturation of aqueous phase with ionic material (e.g. sodium chloride, ammonium sulphate etc.) often improves the total recovery of

most organic substances, in solvent-solvent extraction procedures. The present experiment was designed to show the effect of the salting out process on percentage recovery of the alkaloid from Catha material.

METHOD AND MATERIAL

Two samples of d-norpseudoephedrine were weighed out accurately and separately and dissolved in distilled water. Each sample was made alkaline with 2 ml, 2N sodium hydroxide solution. One of the sample was then saturated with sodium chloride and the two transferred to 250 ml. separating funnels. They were then extracted with equal volume of diethyl ether for 18 hours, once.

In all cases, the extraction of the alkaloid from the salted and non-salted aqueous phase was carried out in parallel in exactly the same way so as to eliminate all possible variables. In particular, precaution was taken to ensure that the period and method of shaking and duration of extraction were all similar.

The ether extract was washed twice with 2.5 ml, 2% sodium bicarbonate, dried with anhydrous sodium sulphate. The alkaloid was then extracted from ether with 10ml, 0.1N sulphuric acid.

The amount of alkaloid recovered was determined from the UV spectra using the major peak at 256.5 nm. Preliminary experiments had shown good linear relationship between the optical density and the amount of d-norpseudoephedrine in solution between the absorbance values 0.2 to 1.6.

RESULTS.

Result of the recovery experiments are shown in table 2. In all cases, saturating the solution with sodium chloride increased the percentage of alkaloid recovered. Average percentage recovery for salted and non-salted solutions, calculated from six experiments, was 67.8 and 52.5 respectively.

EXAMINATION OF ISOLATED BASE(S)

A. Examination by thin layer chromatography (TLC)

MATERIAL AND METHOD

The basic fraction recovered from solvent-solvent extraction was examined with thin layer chromatography (TLC) without first subjecting it to further purification. Preparation of the TLC plates was carried out as described by Stahl (1965). The absorbent on the TLC plates was silica gel (BDH) and the plates after coating and leaving them overnight were activated at 105°C for 30 minutes.

In addition to the basic residue isolated from Catha material, other compounds and in particular phenylalkylamines were also spotted on the plate. These included d-norpseudoephedrine, d-pseudoephedrine, l-ephedrine and amphetamine. D-norpseudoephedrine, and d-pseudoephedrine samples used in the present work were obtained from Ganes Chemical Works (USA). A sample of d-norpseudoephedrine was also received from Dr. Fairchild

of the Veterans Administration Hospital, Long Beach, California (U.S.A.).

Several solvent systems were tried so as to decide on the best one as shown by resolution of phenylalkylamines, included on the plate. The plate was eluted until the solvent front had travelled to approximately 12 cm. It was then dried in air and sprayed with a solution of 0.5% ninhydrin in ethanol. After spraying, the plate was heated in an oven, at 105°C, for approximately 5 minutes.

RESULTS

After spraying the plate with ninhydrin solution and heating it at 105°C for about 5 minutes, the compounds were revealed as bluish-purple spots. Amphetamine could not be revealed with ninhydrin spray. The compounds could not be revealed with Dragendorff reagent (used as spray reagent for most alkaloids) but they were all revealed with iodine vapour.

R_F values are rarely reproducible because this is dependent on several factors such as thickness of the adsorbent layer, temperature and degree of saturation in the development chamber, all of which are difficult to control precisely. However, in all the five solvent systems that were employed, the basic residue from Catha materials had only one compound and this had the same R_F value as d-norpseudoephedrine (table 3). The R_F value for l-ephedrine was similar to that of d-pseudoephedrine, but different from that of d-norpseudoephedrine. The R_F value for

amphetamine as revealed with iodine vapour was close to that of d-norpseudoephedrine in all five solvent systems.

D-norpseudoephedrine was detected in all parts of Catha edulis plant, i.e. leaves, bark, stem, flower, seeds and roots. The alkaloid isolated from fresh Catha material was identical with that from dried Catha material. (Fig. 2).

B. Examination by Gas liquid chromatography (GLC)

MATERIAL AND METHODS

Apparatus Pye Unicam (pye series 104 chromatograph)

Flame ionisation detector

Honeywell Precision integrator

Stainless steel chromatographic column

Hamilton syringe

The basic residue isolated from Catha material was taken up in a known volume of ether solvent, the flask being immersed in an ice bath to minimise loss by evaporation.

In the quantitative estimation of the alkaloid a known amount of Catha material was weighed accurately and the alkaloid extracted as described before. A known weight of synthetic d-norpseudoephedrine was also treated in exactly the same way, particular attention being paid to the aqueous: organic solvent volume ratios and the extration time.

Because of these precautions, it was assumed that the percentage recovery was approximately the same in both extractions.

In all cases, a sample of the material in ether approximately 5 microlitres was injected quickly into the column and the ensuing peak recorded on a moving chart.

The most suitable working conditions for the instrument with respect to column temperature, flow rate of carrier gas (nitrogen) air and hydrogen were determined after some preliminary work. These conditions are given together with results.

The following chemical compounds (free bases) were used for the purpose of comparison: l-ephedrine d-pseudoephedrine, d-norpseudoephedrine and amphetamine. In the quantitative analysis of the base isolated from Catha edulis, replicate analysis (using same volume) indicated good reproducibility. Consequently, it was not considered necessary to include an internal marker.

Finally, the amount of d-norpseudoephedrine recovered from Catha material was determined from the ratio of the peak height, or area under the curve of the standard to that of Catha material sample.

In the particular instrument used (Government Chemists Laboratoris) the vaporising chamber was not working but the Hamilton syringe used was long enough to reach the column.

RESULTS

The following were found to be the most suitable working conditions:

Methyl silicone gum (OV1) 3%, on celite (CQ 100-120 mesh).

Temperature of column 150°C

Temperature of detector 150°C

Flow rate for carrier gas (N_2) 50 ml/min.

Pressure of gases: Hydrogen A: 17 p.s.i

B: 17 p.s.i

Air A: 12 p.s.i

B: 12 p.s.i.

The peaks of all phenylalkylamines and that of basic residue from Catha material were sharp and symmetrical which meant that in the quantitative estimation of the base from Catha material, the peak height could be used to calculate the quantity of recovered alkaloid instead of area under the curve.

Retention values for various phenylkylamine derivatives and also basic Catha extract obtained with above conditions are shown in page 37 and in Fig. 3.

Substance	Retention time (min.)
Amphetamine	1.6 ± 0.1
d-norpseudoephedrine	4.0 ± 0.1
base-from Catha material	4.0 ± 0.1
l-ephedrine	4.7 ± 0.1
d-pseudoephedrine	4.7 ± 0.1

In all cases, only one base was present in Catha edulis material (twigs, flowers, leaves, stem and seeds) and this was identified as d-norpseudoephedrine from the retention time. Even when the working conditions were varied considerably, the retention time for the base isolated from Catha material was always the same as that of d-norpseudoephedrine and different from that of other reference compounds. This confirms results obtained with thin layer chromatography.

Results of quantitative determination of d-norpseudoephedrine in Catha material are shown in table 4.

C. Examination by ultraviolet spectrophotometer.

MATERIAL AND METHOD

Apparatus: SP 800 spectrophotometer, Unicam cuvettes, chart paper

Purified alkaloid crystals were disolved in 0.1N sulphuric acid and UV spectra determined with SP 800 spectrophotometer. Sulphuric acid (0.1N) was used to balance the instrument. Two well matched silica cuvettes (light distance 1cm) were used. The UV spectra of l-ephedrine, d-norpseudoephedrine and amphetamine were obtained for the purpose of comparison.

RESULTS

The spectra of l-ephedrine, d-pseudoephedrine, d-norpseudoephedrine, amphetamine and the alkaloid isolated from Catha edulis (young twigs) are shown in Fig. 4. The major peaks are at 262.5, 256.5 and 250.5 with a flexion at 266, 246, 240.5, and 236 nm. The minima are at 253 and 260 nm. The position of the absorption peaks are given to the nearest 0.5 nanometer. Ultraviolet spectra of the alkaloid isolated from Catha edulis is similar to that of amphetamine, l-ephedrine, d-pseudoephedrine and d-norpseudo-ephedrine. It is therefore not possible to identify the compounds from their ultraviolet spectra.

D. Examination by Infrared spectrophotometer.

MATERIAL AND METHOD

Apparatus: Perkin elmer 237 spectophotometer

Beckmann 00-25 hydraulic press

Vacuum pump

Pestle and mortar

The alkaloid from Catha edulis which was purified by repeated recrystallisation was intimately mixed with potassium bromide and made into a thin transparent disc. The spectrum was then obtained by scanning through the ranges 650-2000 and 1300-4000 nm.

Both potassium bromide and Catha alkaloid had been left in a desiccator for nearly 2 days to ensure that all moisture was removed.

The percentage transmittance was initially adjusted to about 50%. It did not make any difference whether the scanning was done slowly or fast. In addition to the crystalline alkaloid isolated from Catha material, the spectra of the following compounds were also obtained: d-norpseudoephedrine, l-ephedrine, d-pseudoephedrine and amphetamine.

RESULTS:

The spectra of the pure alkaloid isolated from Catha material was identical with that of synthetic d-norpseudoephedrine with all the major peaks in the finger-print region showing. Furthermore, the spectra was different from that of l-ephedrine, amphetamine and d-pseudoephedrine. The major peaks for d-norpseudoephedrine were at the following wave numbers: 635, 700, 760, 830, 855, 920, 960, 1000, 1020, 1040, 2075, 1110, 1135, 1190, 1270, 1330, 1390, 1460, 1590, 1970 cm-1. There was only one other major peak below 2000 cm-and this was a peak at about 3400, probably due to primary amine and hydroxyl groups. The spectra of d-norpseudoephodrine and of the alkaloid isolated from Catha material are shown in fig. 5.

Isolation of steam volatile substances from Catha material

MATERIAL AND METHOD

Approximately 100 g of ground dry material or homogenised fresh material was acidified and steam distilled using the apparatus shown in Fig. 6 (a). The flask containing Catha material was also placed in a heating mantle. The steam volatile substances passed through a water cooled condenser and the distillate was collected into a 125 ml Erlenmeyer flask containing 15 ml, 2% potassium hydroxide. Distillation was allowed to proceed slowly with the end of the condensing tube submerged in potassium hydroxide at all times, until approximately 75 ml of the distillate had been collected.

The residue, left after steam distillation from the acidified material, was cooled and made alkaline using sodium hydroxide. Steam was then passed through the alkaline material as before and the distillate collected in 125 ml Erlenmeyer flask containing 20 ml. 2N sulphuric acid Distillation was continued until approximately 75 ml distillate was collected.

The steam distillate containing volatile bases collected in 2N sulphuric acid was made alkaline with sodium hydroxide and then saturated with sodium chloride. The mixture was transferred to a 250 millilitres separating funnel and extracted with an equal volume of diethyl ether for 8-10 hours. The extraction was repeated three times.

The combined ether extract was examined for bases, using thin layer chromatographic technique, as described before.

Quantitative estimation of cyanide in Catha material.

A fresh bundle of Catha material was weighed accurately and homogenised with 600 ml of 15% ethanol for about 5 minutes at high speed. The homogenate was transferred to a tightly fitting screw-top jar and incubated at 30°C for at least 24 hours. One hundred and fifty millilitres of the homogenate was then transferred to a flask with a long neck and distilled using the apparatus shown in Fig. 6 (b). The distillate was collected in a flask containing 2% potassium hydroxide and this was continued until the volume in the flask was approximately 75 ml. the distillate was transferred to a standard flask and made to 100 ml.

Twenty millilitres of the distillate and 10 ml of the alkaline picrate solution (25g anhydrous sodium carbonate and 5g picric acid per litre) were pipetted into large glass tubes and after mixing thoroughly, the tubes were placed for exactly 5 minutes in a water bath pre-heated at 94°C. The tubes were removed, cooled to room temperature and the optical density determined on a spectrophotometer at 540 nm using a blank which had been distilled in the same manner as the sample.

The amount of cyanide present in twenty millilitres diluted distillate was read from a standard curve, prepared using potassium

cyanide. The amount of cyanide in the original Catha material was then calculated by taking into account all the dilutions. The method has been described in great details by Burn et al. (1970).

RESULTS

Preliminary examination of the steam distillate from the acidified material using benzidine - copper acetate reagent (Vander Watt 1944) showed presence of trace amount of cyanide. The amount of cyanide recovered from Catha material was found to be approximately 2.5 mg per kilogramme of fresh material and therefore of little toxicological significance. For example, only material containing 200 mg per Kg. is considered potentially dangerous to livestock (Garner, 1967).

Spot tests on the steam volatile acids and neutrals with 5% ferric chloride showed a weak violet coloration indicating trace amount of substance(s) with phenolic groups. No quantitative estimation of the phenolic components was undertaken and no other substance was detected in the distillate.

The steam distillate from alkaline medium was found to contain d-norpseudoephedrine; this was considered a suitable method for isolating the alkaloid from Catha material since no other contaminant (e.g. pigments, tannins etc.) was steam distilled from the alkaline media.

DISCUSSION ON CHEMICAL WORK

Present work using chromatographic and spectrophotometric techniques have shown that there is only one alkaloid in all parts of

Catha edulis that were examined and this was identified as d-norpseudo-ephedrine.

An attempt to elucidate the exact nature of chemical compounds present in plant material is often hampered by the possibility that during isolation procedures, slight structural chages might occur. For example, the ester and glycosidic linkages present in several compounds, e.g. tannins, are easily hydrolysed as a result of pH and temperature changes. In nearly all cases, these changes alter the pharmacological (and therefore toxicological) properties of the compound. For example an alteration in the size and polarity of the molecule will often influence the ease with which the compound can pass through cellular membranes. This will, in turn affect the potency and duration (rate of elimation) of effect of the compound. A case in point is the potency of aglycones (e.g. digoxigenin) as compared to the glycosides (e.g. digoxin). Yet another possibility is that some plants when injured, will release enzymes which, in turn, act on the chemical constituents to alter their structure. This might happen when the twigs are plucked from the plant. It is well recognised that when potatoes are injured during harvesting, this type of alteration does take place. Similarly glycosides (e.g. oubain) are often acted upon by enzymes, liberated when plants are injured, to give aglycones. Drying or storage of plant material, under certain unfavourable conditions might also lead to slight structural changes and these

might be accompanied by profound pharmacological and toxicological changes. A case in point is the conversion of coumarin to dicoumarin in spoiled sweet clovers.

It is always difficult to account fully for interferance by other substances when extracting chemical coumpounds from plant material. For example some surfactants widely distributed in plant Kingdom can alter the partition coefficient of the compound being extracted in a variable and unpredictable manner making the quantitative estimation of the compound difficult. A case in point is saponins, which are known to be widely distributed in plants; these are surfactants, and are often responsible for emulsion formation during the solvent-solvent extraction of plant material.

During the survey described earlier, evidence was given by several people that the potency of fresh Catha material was different from the withered material. Other effects associated with withered material included diarrhoea and stomach upsets. It is difficult to quantitate central stimulant effect in experimental animals as well as in man and for this reason it has been difficult to check on possible potency changes (with respect to CNS stimulation) when Catha material is dried. The claim of Friebel and Brilla (1963) that the fresh twigs of Catha edulis contain a compound which is more potent than d-norpseudoephedrine could not be confirmed in the present work. Similarly, the claim of Ristic

and Thomas (1961) that l-ephedrine is present in Catha material could not be confirmed. The resolution of l-ephedrine and d-norpseudoephedrine with both thin layer and gas liquid chromatographic techniques was good and it is therefore possible to state with certainty that l-ephedrine is not present in detectable amounts in Catha material. It is perhaps significant that the claims of Friebel and Brilla (1963) and Ristic and Thomas (1961) have not been confirmed by other research workers.

During the survey, evidence was given that Catha material from Kangeeta region in Nyambeni Hills was more potent than that from other areas in East Africa. It was not the purpose of the present work to investigate the variation of d-norpseudoephedrine content in Catha plants from different geographical regions. However, analysis of Catha material bought from the market, whose origin was stated to be Kangeeta, Kianjahi, Lare and Mutidwa did not reveal any significant difference in alkaloidal content. The concentration of the alkaloid varied considerably, but within the range 100-200 mg per 100 g dry material, even in Catha material obtained from the same region. The variation of chemical compounds in plants on seasonal and geographical basis is a scientifically proved phenomenon even though this was not shown in the case of Catha material. The claim that some Catha material commonly known as, "Kisa bomu" is more potent than others on a weight basis could not be confirmed. During the survey it became clear that the criteria of what could be regarded

as "Kisa bomu" on the basis of size was indefinite and at best presumptive. The problem of determining whether the alkaloid content of "Kisa bomu" was more than in other types of Catha material was therefore hypothetical. However, the content of d-norpseudoephedrine in young twigs which were supposed to be typical, "Kisa bomu" was not different from that found in other twigs.

Several people have remarked that the amount of d-norpseudoephedrine present in the twigs is not adequate to account for pharmacological effect. An average person chewing 7 bandaris of Catha material (approximately 84 g dry material) would consume about 80-160 mg d-norpseudoephedrine. Alles, Fairchild and Jensen (1967) have shown that approximately 30 mg of d-norpseudoephedrine base is adequate to induce euphoria and general CNS stimulation. In the present work, it was estimated that 20-30 mg was adequate to give appreciable central stimulant effect. The author has taken the drug at least four times and recovered it in urine; 25 mg taken in the morning (after drinking milk and no tea or coffee) was adequate to give a feeling of elation. The amount of Catha material required to give an appreciable central stimulant effect will depend, at least in part, on the rate of consumption. This aspect of the problem is considered further in another section.

Table 1 A Effect of heating d-norpseudoephedrine in water on percentage recovery

WEIGHT OF D-NORPSEUDO-EPHEDRINE (mg)*	OPTICAL DENSITY AT 256 nm BEFORE HEATING	AFTER HEATING.	PERCENTAGE RECOVERY AFTER HEATING
8.50	8.87	0.83	96.5
8.50	0.84	0.82	98.2
8.50	0.86	0.81	94.6
8.50	0.85	0.81	94.6
8.50	0.82	0.76	93.0

Average percentage after heating = 94.4

S.D. = 1.8 ± 0.9

* A solution of 42.50mg in 25ml was made and 5ml sample taken.

B. Effect of heating d-norpseudoephadrine hydrochloride in acid media on percentage recovery.

WEIGHT OF D-NORPSEUDO-EPHEDRINE CHLORIDE(mg)	OPTICAL DENSITY AT 256 nm BEFORE HEATING	AFTER HEATING	PERCENTAGE AFTER HEATING
9.26	1.16	1.14	98.3
10.64	1.25	1.23	98.5
6.60	0.94	0.96	102
8.80	1.12	1.12	100
9.20	1.14	1.14	99.2

Average percentage after heating = 99.6

S.D. = 1.4 ± 0.7

Table 2 A. Percentage recovery from solution saturated with sodium chloride

Exp.	Amount of base taken (mg)	Amount of base recovered (mg)	% Recovery
1	10.32	7.8	75.6
2	10.92	7.0	64.1
3	9.4	6.02	64.0
4	4.5	2.97	66.0
5	4.4	3.04	69.1
6	6.6	4.48	69.9

Average % recovery for six experiments = 67.8%

S.D. = 4.0 ± 1.8

B. Percentage from solutions not saturated with sodium chloride.

Exp.	Amount of base taken (mg)	Amount of base recovered (mg)	% Recovery
1	10.32	4.97	48.2
2	10.92	5.4	49.4
3	9.4	5.45	56.0
4	4.5	2.02	44.9
5	4.4	2.6	59.1
6	6.6	3.7	56.1

Average % recovery for six experiments = 52.6%

S.D. = 5.1 ± 2.3

Table 3. R_F Values of phenylalkylamines and basic Catha extract.

Compound	R_F Values Solvent I	II	III	IV	V
D-norpseudoephedrine	0.61	0.31	0.47	0.80	0.62
Basic residue from Catha edulis	0.61	0.31	0.47	0.80	0.62
l-ephedrine	0.53	0.26	0.39	0.75	0.57
d-pseudoephedrine	0.53	0.26	0.39	0.75	0.57

Solvent I: Butanol - acetic acid - water 60:15:25
Solvent II: Butanol saturated with water (upper phase)
Solvent III: Methanol-Ammonia 100:1.5
Solvent IV: Isopropanol - Water - Ammonia 80:15:15
Solvent V: Chloroform - Methanol - Ammonia 85:14:1

Table 4. Quantitative determination of d-norpseudoephedrine in young Catha twigs

Weight of Catha material taken (g)	Weight of base (standard) (mg)	Area under peak(Catha material) (mm^2)	Are under peak(standard.) (mm^2)	Amount of base in(Catha material.) (mg%)
25	25.2	385.0	318	121.2
25	25.2	457.5	318	144.0
50	16.0	693.2	181.8	122.0
25	16.0	370.6	181.8	131.2
20	16.0	252.8	181.8	101.5
25	15.4	384.8	120.6	183.2

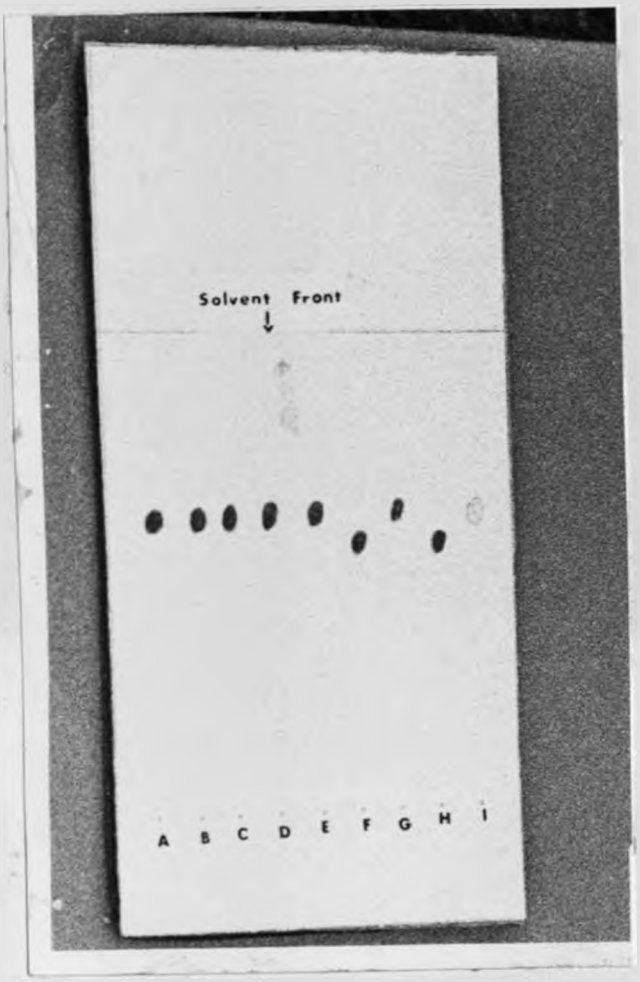


Fig. 2. Thin layer chromatogram of the alkaloid from Catha material and related compounds.

A - Alkaloid from young twigs (fresh material) of Catha edulis

B - Alkaloid from seeds of Catha edulis

C - Alkaloid from leaves of Catha edulis

D - Synthetic d-norpseudoephedrine (From Dr. Fairchild)

E - Alkaloid from young twigs (dried material) of Catha edulis.

F - L-ephedrine (Merck)

G - Alkaloid from roots of Catha edulis

H - D-pseudoephedrine (Ganes Chemical Works, U.S.A.).

I - Amphetamine (revealed with iodine vapour).

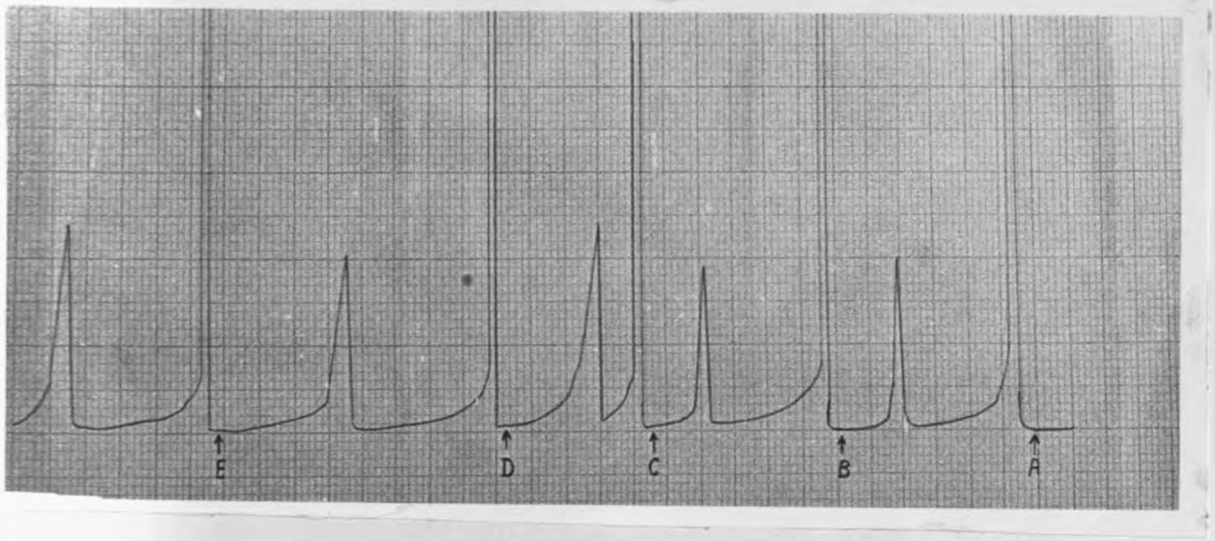


Fig. 3. Gas chromatogram of alkaloid from Catha material and related compounds.

A - Catha edulis alkaloid

B - D-norpseudoephedrine (From Dr. Fairchild)

C - Amphetamine

D - L-ephedrine

E - D-pseudoephedrine

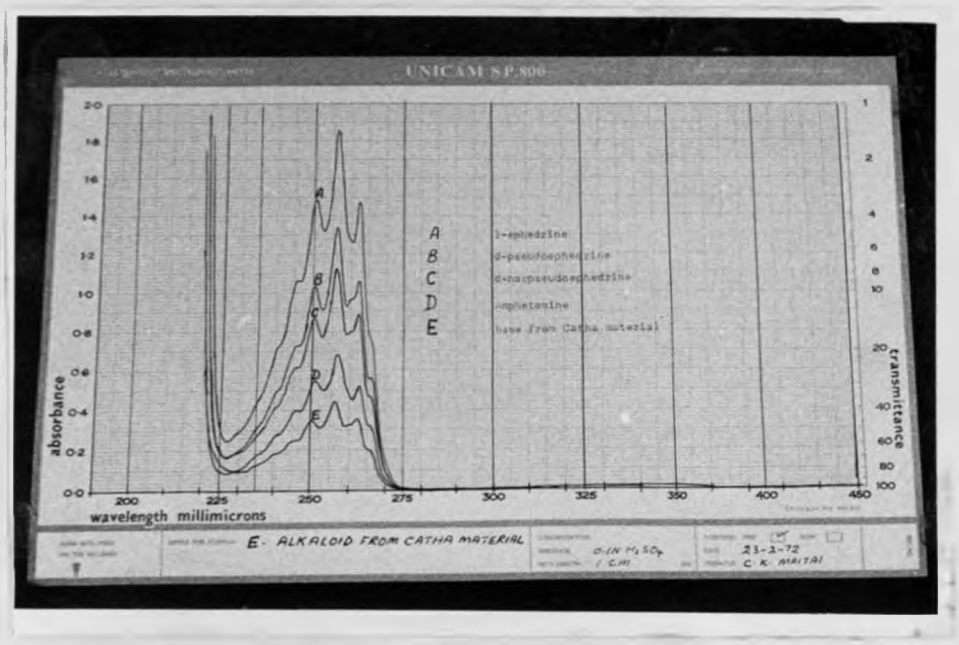


Fig. 4. Ultraviolet spectra of various phenylalkaylamine compounds.

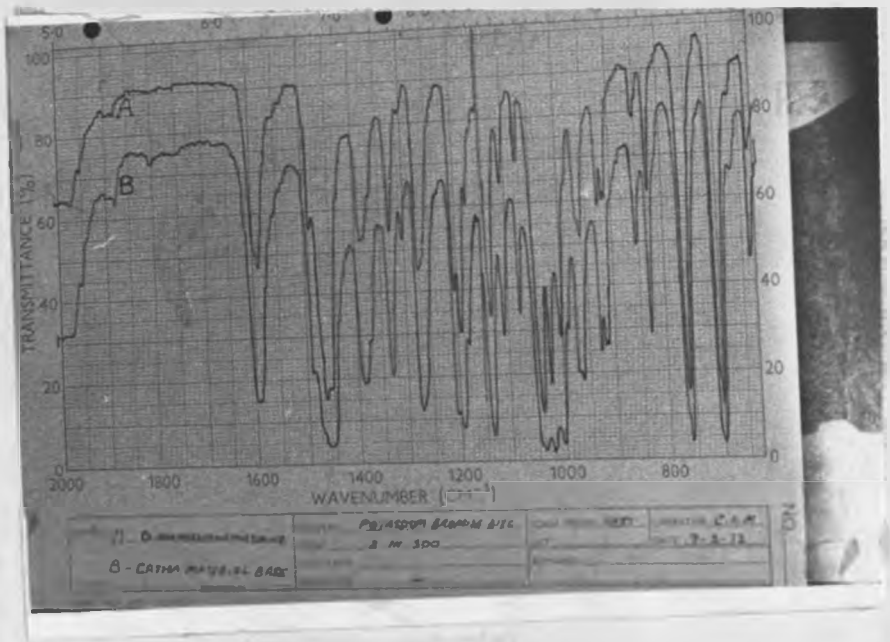


Fig. 5. Infrared spectra of

A - D-norpseudoephedrine

B - Catha material base

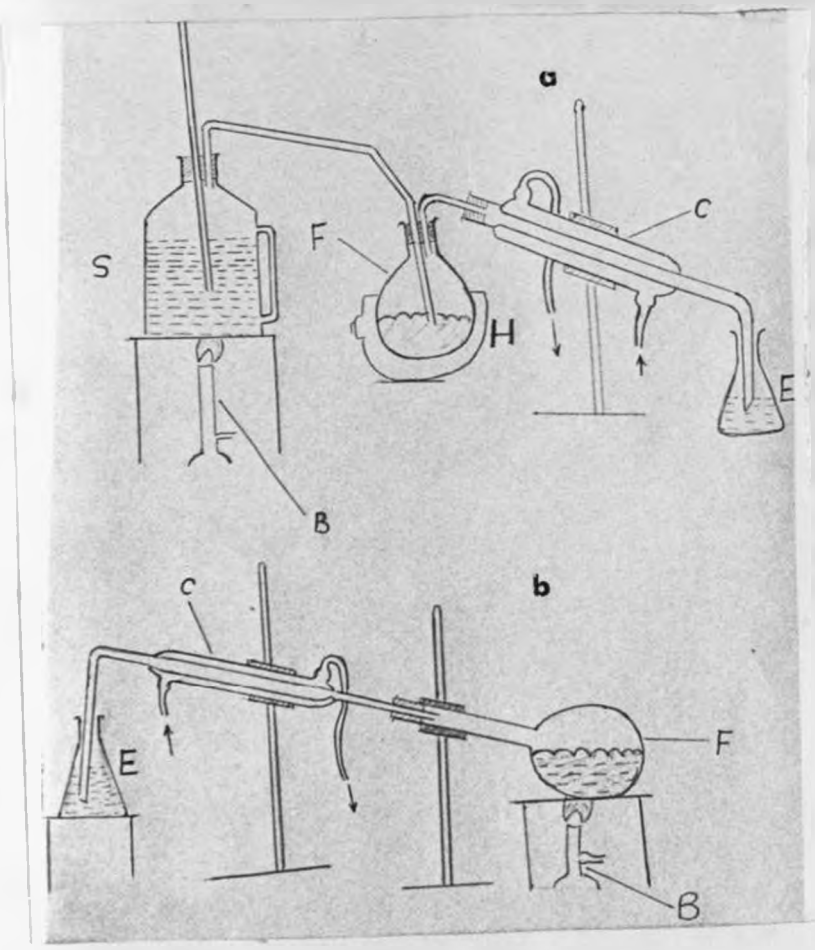


Fig. 6. Steam distillation apparatus

B - Bunsen burner
C - Water-cooled condenser
E - Erlenmeyer flask
F - Flask containing Catha material
H - Heating mantle
S - Steam generator

PART II CATHA EDULIS TOXICOSIS

A. ACUTE AND CHRONIC TOXICITY IN RATS:

INTRODUCTION

Young white albino rats were used as experimental animals to explore the possible harmful effect from *Catha edulis* because large numbers of animals could be maintained under controlled conditions over a long period of time much more easily than would be the case with large animals Since *Catha edulis* toxicosis is essentially a human problem, ruminants were not considered as the results are likely to be greatly influenced by presence of microflora in the rumen, a situation that has no parallel in man. Rabbits were also considered suitable even though it was not easy to keep as many as rats since the cages occupy plenty of room and only one or two rabbits can be kept in each cage.

MATERIAL AND METHODS

Weanling male and female white albino and also black hooded rats approximately 3-4 weeks old were used in the experiment. Young animals are usually more susceptible to the toxic effect of chemicals than old animals, and it is on this basis that the weanling rats were chosen. Two hundred rats (mostly males) were alloted at random and fed on various concentration of *Catha edulis* material, ranging from one to fifty percent incorporated into ground commercial mice pellets. The pellets

were supplied by Kenya Farmers Association stores. The rats were kept in groups of 6-7 and housed in large metal cages provided with coarse-mesh wire bottoms. Male and female rats were kept separately to avoid the inadvertment use of pregnant rats later. A group of rats was kept as control and fed on commercial mice pellets only.

The number of rats in each group, the duration of the experiment and the number of rats that died are shown in table 5.

To the weighed Catha material was added about three times its weight of water and boiled for 3 hours. The mixture was then strained through a muslin cloth, the residue taken up with more water and the extraction procedure repeated three times, each time boiling the mixture for about 1 hour. Preliminary work had shown that very little tannins remained in the residue after the fourth extraction. Similarly, no d-norpseudoephedrine could be detected in the residue after the fourth extraction. Finally the combined aqueous extract was filtered through a Whatman No. 1 paper and the filtrate incorporated into food to make the required percentage. After mixing properly, the moist food was dried in warm air current at 60°C for approximately 24 hours and then stored in large bucket containers. The food was then fed to rats as required.

In some experiments, the Catha material was not extracted but instead, it was incorporated in ground food to make the required percentage. In experiments to determine the effect of Catha material on body weight

gain it was recognised that the large percentage of solid Catha material in food had little or no nutritive value.

Each morning and evening, weighed amounts of fresh food were placed in food containers designed so as to minimize scattering. Fresh water was available at all times.

All the animals were observed for behavioral changes and for symptoms of toxicity. The animals were weighed twice a week.

Whenever an animal looked sick, it was sacrificed and examined by standard necropsy procedure. Usually the animal was left until it was just about to die and then sacrificed by severing the spinal cord. Thin sections from the following organs were preserved in acetate buffered 10% formalin for histological examination: brain, heart, liver, pancreas, kidney, lungs, stomach, duodenum and jejunum. Some sections were also fixed in Carnoys fixative.

Routine sections were stained with haematoxylin and eosin. The following special stains were also used for selected tissues: Best Carmine and Sudan III. The histological and staining procedure followed were as described in the monograph, Manual of Histological staining methods of the Armed Forces Institute of Pathology, Third Edition, 1968, McGraw - Hill Book Co, N.Y.

Rats feeding on high concentration of Catha material were of special significance with regard to acute toxicity. Those feeding on

25 and 50% Catha material were sacrificed frequently for acute toxicity studies and the organs examined for presence of gross and microscopic pathological lesions.

Those rats feeding on low percentage of Catha material were used for chronic toxicity studies.

For groups I, II and III fed 1, 5 and 15% Catha material respectively, organ: body weight ratios, were determined for the liver, kidney, spleen, heart and brain.

RESULTS

Information on the growth rate of rats fed various percentages of Catha material is given in Fig. 7,8,&.9. The actual weights are included in appendix II. The weights of rats at the start of the experiment were between 40 and 70 g for both males and females. Males put on weight much faster than females. Results of weight gain show good consistency, in all cases, after about 4 weeks.

Except for rats feeding on 1%, all other groups showed a slowed growth rate than occurred in the controls. Rats feeding on 1% gained weight at a slightly increased rate than the controls. Those feeding on 5, 10 and 15% were not affected for the first 4 weeks and gained weight consistently well and often more than the controls. After 4 weeks, the average weight gains for the three groups were consistently, lower than for controls. Rats feeding on 25% gained weight at about the same rate

as controls for the first two weeks but thereafter, the weight gain was much less than that of the controls. Rats feeding on 50% showed poor weight gain from the onset of the experiment.

The amount of food consumed by each experimental group is shown in table 6.

Information on organ-body weight ratios for rats fed 1, 5 and 15% Catha material extract, is given in tables 7, 8 and 9. For each experimental group, data from 11 rats is compared with control rats that had been kept under similar conditions (table 10). In all cases, the organ-body weight ratios for experimental rats were not statistically different from those of control rats. The work was restricted to females because males reach maximum body weight at a much later stage; organ weights change considerably with age probably due to atrophy.

Control rats were maintained for more than 16 months on the ground mice pellets food and at no time were significant changes or any abnormalities noted in any of the animals except a high incidence of murine pneumonia and some scattered degenerative changes, mostly vacuolation.

Rats fed 1% were essentially normal for the first twelve months except for an assortment of minor microscopic anatomical changes scattered throughout both test and control groups and these were judged to be incidental and unrelated to the feeding of Catha extract. After twelve months some degenerative changes (mostly vacuolation) were noted in the liver and kidney.

In rats fed more than 10% Catha material, the toxicity of the non-extracted whole Catha material was distinctly more than that of the extract. This was especially marked in rats fed 25 and 50% Catha material.

The description of the macroscopic and microscopic lesions observed in different groups is given in the following pages in detail. The specific lesions are shown in Fig. 11 - 26.

GROUP 1: 50% CATHA MATERIAL

CLINICAL OBSERVATIONS:

On the second to third day of the experiment, the rats showed increased motor activity and sensitivity to environmental stimuli. After 5-7 days the rats were generally calm. Food consumption in the first week was low. The decreased food consumption is reflected in the body weight gain, Fig. 7 and 8.

The experiment was continued for over six months and during this period, the rats looked very calm , almost tranquillised, and showed no sign of central nervous system stimulation.

Rats fed 50% non-extracted Catha material died within 10 days of the experiment. Most of them died within 1-2 days of the experiment. Those that did not die in the first 3 days of the experiment showed signs of central nervous system stimulation similar to that shown by rat.

fed the corresponding percentage of the extract. This stimulation was short-lived since all the rats died within 10 days of the experiment.

None of the rats feeding on 50% Catha extract died and they were therefore sacrificed at the rate of 3 per month. During postmortem examination, the following organs were fixed in 10% formalin-acetate buffer for subsequent histological examination: liver, kidney, brain heart, stomach, duodenum, jejunum, pancreas, spleen and lungs.

The various lesions observed in different organs are described in details in the following pages:

GASTROINTESTINAL TRACT

Macroscopic lesions

Rats fed non-extracted Catha material and sacrificed when looking sick had extensive haemorrhage especially in the stomach and duodenum. Similarly, postmortem examination of those rats that died showed extensive haemorrhage in the stomach and duodenum.

Rats fed Catha extract did not show any major lesions but approximately 30% of the rats sacrificed in the first two months showed catarrhal inflammation in the glandular stomach.

Microscopic lesions

Rats sacrificed while looking sick or which had died (see above) showed haemorrhagic ulceration in the lamina propia stomach as well as in the duodenum. The villi in the duodenum looked fragmented with partial

disorganisation of the mucosa.

Rats fed Catha extract showed extensive desquamation and necrosis of the superficial epithelium in the gastrointestinal tract. Several cases of oedema in the submucosa were noted in rats fed Catha extract as well as those fed whole Catha material. These accounted for approximtely 60% of all animals examined.

LIVER

Macroscopic lesions - None was observed in rats fed whole Catha material or the extract.

Microscopic lesions

Rats fed Catha extract did not show any major lesions except localised degenerative changes (e.g. hydropic and fatty vacuolation). Rats sacrificed after feeding on Catha extract for 4 months were showing more degenerative changes than those sacrificed after six months.

Rats fed whole Catha material and which died or were sacrificed after they were looking sick for about 1-2 days were showing extensive necrosis of centrolobular area of hepatic lobes. Those that were sacrificed immediately after they started showing signs of toxicity (e.g. sluggishness) or which did not look sick were showing degenerative changes, mostly vacuolation.

KIDNEY:

Macroscopic lesions

No lesion was observed in rats fed whole Catha material or the extract.

Microscopic lesions

The most consistent finding in rats that died or were looking sick after feeding on 50% whole Catha material was coagulative necrosis of the proximal convoluted tubules and marked vacuolation especially in cortex. Hyaline cast formation and desquamation were also found in more than 80% of the rats.

HEART:

Macroscopic lesions

No lesion was observed in rats fed whole Catha material or the extract.

Microscopic lesions

Localised areas of coagulative necrosis were observed in 26% of the animals fed Catha material. These were found in rats fed Catha extract as well as those fed whole Catha material. Extensive haemorrhage was also observed in about 28% of the animals. There were several cases of fibroblast proliferation replacing the necrotic myocardial fibres.

BRAIN

Macroscopic lesions

Congestion was observed in about 40% of the rats fed either Catha extract or whole material.

Microscopic lesion

In about 32% of the rats, there was congestion and haemorrhage in the meninges. At least 28% of the rats were showing perivascular oedema. There were a few rats showing liquefactive necrosis but these accounted for only 20% of the total experimental rats. These lesions occured with about the same frequency in rats fed Catha extract and those fed non-extracted whole material.

OTHER ORGANS:

No consistent lesions were observed in the spleen, the pancreas and the lungs. Murine infection in the lung was a consistent finding in both control and experimental rats.

GROUP II 25% CATHA MATERIAL:

As in the previous group, some of the rats were fed Catha material extract while others were fed non-extracted whole Catha material and observed for any behavioral change over a period of time or until they died.

RESULTS

Clinical observations

Rats fed the extract and those fed whole material showed signs of

central stimulant effect after about 3 days. In all cases, they exhibited increased motor activity and reacted sharply to external stimuli (e.g. clapping of hands). After about 5-7 days they were generally calm and sluggish. There was also evidence of decreased appetite in those feeding on whole Catha material but not on those feeding on the extract. Many of them started looking sick after about 10 days. Diarrhoea was observed in some of the rats fed whole material but most of them seemed to recover after 1-2 days.

GASTROINTESTINAL TRACT

Macroscopic lesions

Postmortem examination of those that died showed extensive haemorrhage in the stomach and duodenum. No gross lesions were observed in rats fed 25% whole Catha material and which were sacrificed while they were not sick. During postmortem of rats that died or were sacrificed while looking healthy or sick, sections from the following organs were taken and preserved in 10% formalin-acetate buffer for histological examination: liver, kidney, stomach, duodenum, jejunum, brain, heart, spleen, pancreas and lungs.

None of the rats fed 25% Catha extract died or even showed signs of toxicity and they were therefore sacrificed at the rate of 3 per month. During postmortem examination, no gross lesions were observed.

Microscopic lesions

Those rats fed whole Catha material and sacrificed while looking sick or which died had haemorrhagic ulceration in the stomach and duodenum and also general desquamation of the mucosal epithelium. Rats sacrificed while they were looking healthy showed extensive desquamation of mucosal epithelium and shallow ulcers which were not haemorrhagic. Those sacrificed after six months were showing extensive desquamation of epithelia, scattered erosions and localised areas of necrosis similar to those observed in rats fed whole Catha material for a shorter duration of time, e.g. two weeks.

Rats fed 25% Catha extract showed desquamation in the gastrointestinal tract and localised area of necrosis especially in the duodenum. Approximately 50% of the rats were showing oedema in the submucosa. Similarly, several rats fed whole Catha material, oedema was observed in the submucosa. This accounted for approximately 40% of the rats.

LIVER

Macroscopic lesions

None was observed.

Microscopic lesions

Rats fed 25% whole material and sacrificed within 1 - 8 days did not show any lesion. Those that died or were sacrificed after 12 days had necrosis of centrolobular area and extensive vacuolation.

Those rats fed 25% whole material for a long period but which did not die also showed extensive vacuolation and generalised necrosis.

Rats fed 25% Catha extract showed extensive vacuolation but not necrosis.

KIDNEY

Macroscopic lesions

None was observed.

Microscopic lesions

Rats fed non-extracted Catha material and sacrificed when they looked sick were showing extensive coagulative necrosis in the proximal convoluted tubules. There was also extensive desquamation and vacuolation in these tubules and several cases of hyaline cast formation were observed.

Rats fed Catha material extract showed only degenerative changes, mostly vacuolation but no necrosis. There were a few cases of hyaline cast formation but these were not widely distributed.

HEART

Macroscopic lesions

None was observed.

Microscopic lesions

Rats fed whole Catha material and those fed the extract showed hyaline degeneration within the first month. Some of those fed Catha material or extract for more than two months showed localised coagulative

necrosis. This was observed in about 20% of the animals. Usually there was fibroblast proliferation on each end of the necrotic tissue, probably indicating a healing process. There was no correlation between the distribution of necrosis and the vascular system. Haemorrhage was observed in about 25% of rats.

BRAIN

Macroscopic lesions

Congestion was observed in several rats fed Catha extract as well as non-extracted material.

Microscopic lesions

No consistent lesion was observed but a few cases of perivascular oedema (less than 16%) were noted. Congestion and haemorrhage was observed in about 20% of the animals. The lesions occurred with about the same frequency in rats fed non-extracted material and those fed the extract.

GROUP III 15% CATHA MATERIAL

Some rats were fed Catha material extract while others were fed whole Catha material. All animals were observed for behavioral changes and sacrificed at predetermined intervals. In all cases routine post-mortem examination was performed and sections of the following organs were taken and preserved in formalin-acetate buffer for histological examination: Liver, kidney, brain, heart, gastrointestinal tract, spleen, pancreas and lungs.

Clinical observations

Some rats looked stimulated after about 3-5 days but this subsided after about 3 days. This stimulation (as shown by increased motor activity and sensitivity to external stimuli) was much less than that observed with 25 and 50% groups and not all animals were affected. The animals were feeding well throughout the experiment. There was no death that could be attributed to Catha material extract even though the experiment was continued for almost one year.

GASTROINTESTINAL TRACT

Macroscopic lesions

Postmortem examination was done in all rats that were sacrificed at intervals. Some rats fed whole Catha material and sacrificed within the first ten days of the experiment showed mild catarrhal gastroenteritis but those sacrificed after about twenty days did not show these signs.

Rats fed Catha material extract did not show any gross lesions during postmortem examination.

Microscopic lesions

There was extensive desquamation of the glandular mucosa epithelium and localised scattered areas of necrosis in the stomach and duodenum of rats fed whole Catha material. No haemorrhagic ulceration was noted in the gastrointestinal tract. There were some rats showing congestion and oedema in the submucosa and these accounted for approximately 40%.

There was no difference between rats sacrificed after a short period, e.g. one month and those sacrificed about one year later. Extensive desquamation in the GIT and in particular the duodenum region was obseved in all rats fed Catha material extract.

LIVER

Macroscopic lesion: None was observed.

Microscopic lesions:

Rats fed whole Catha material were showing extensive degenerative changes usually characterised by vacuolation and sometimes loss of cellular structure or outline. There was no indication of a progressive involvement of more hepatic cells when the experiment was continued for a longer period and in many cases, those rats sacrificed after about six months were showing less degenerative changes than those sacrificed at the beginning of the experiment.

Rats fed Catha material extract did not show any consistent lesions.

KIDNEY

Macroscopic lesions: None was observed,

Microscopic lesions:

Rats fed whole Catha material showed coagulative necrosis of the proximal convoluted tubules, with hyaline casts formation. Necrosis was observed in approximately 60% of the animals and was present even in those rats sacrificed after two weeks. There did not appear to be a

progressive involvement of the kidney even when the rats were left for up to twelve months.

Rats fed Catha material extract showed some degenerative changes, especially vacuolation of tubular epithelium.

HEART

Macroscopic lesions: None was observed.

Microscopic lesions:

There was coagulative necrosis in about 20% of the rats. There were also several cases of haemorrhages and fibroblast proliferation. These lesions were observed in both groups of rats, i.e. those fed whole Catha material and those fed the extract.

BRAIN:

Macroscopic lesions: None was observed.

Microscopic lesions:

About 20% of the rats were showing congestion in the meninges and at least 16% were showing haemorrhage and perivascular oedema.

GROUP IV 10% CATHA MATERIAL

Rats fed 10% Catha material (whole and extract) were kept for over one year and observed for behavioral changes and any other sign of toxicity. They were sacrificed at the rate of three per month and examined for gross lesions using standard necropsy procedure. As with the previous groups, the following organs were preserved in 10% formalin

acetate buffer for examination: liver, kidney, brain, gastrointestinal tract, heart, spleen, pancreas and lung.

Clinical observations

No definite behavioral change was observed in this group.

GASTROINTESTINAL TRACT

Macroscopic lesions

Postmortem examination revealed no gross lesions except mild catarrhal gastroenteritis in the first month, in less than 50% of the animals fed whole Catha material. Those feeding on Catha material extract did not show any lesions. Congestion was observed in blood vessels of the splanchnic area.

Microscopic lesions:

Rats fed whole Catha material showed mucinous degeneration with extensive desquamation of the superficial epithelium, especially in the duodenum. There were also several localised areas of necrosis in the mucosal epithelium. There was no progressive involvement of the mucosa and some rats sacrificed after two weeks were more affected than some of those sacrificed one year later, possibly indicating a regenerative process.

Rats fed Catha material extract also showed extensive desquamation. A frequent observation in both groups was congestion and oedema in the

submucosa and this was noted in about 40% of the rats.

LIVER

Macroscopic lesions: None was observed.

Microscopic lesions:

There was extensive degenerative changes especially vacuolation. There were also scattered areas of necrosis in centrolobular area of hepatic lobes.

KIDNEY

Macroscopic lesions: None was observed.

Microscopic lesions:

There was extensive vacuolation and desquamation especially in renal cortex. Localised areas of coagulative necrosis were noted in the proximal convoluted tubules in approximately 30% of the rats. Hyaline cast formation was observed in about 20% of the rats.

The observed microscopic lesions did not appear to show a progressive involvement with time. Rats fed Catha extract did not show significant lesions.

HEART

Macroscopic lesions: None was observed.

Microscopic lesions:

Coagulative necrosis was observed in about 10% of the rats. Haemorrhage within the myocardia was also observed in about 10% of the rat

BRAIN:

Macroscopic lesions: None was observed.

Microscopic lesions:

A few cases of perivascular oedema, approximately 20%, and congestion in the meninges (15%) were noted.

OTHER ORGANS:

No significant lesions were observed in the lungs, spleen and pancreas.

GROUP V AND VI: 5 & 1% CATHA MATERIAL

Rats fed 1 and 5% were kept for over 16 months and observed for sighs of toxicity. The rats were sacrificed at a rate of 2 - 3 per month and examined for gross lesions and the following organs preserved in 10% formalin acetate buffer: liver, kidney, heart, stomach, duodenum, jejunum, spleen, pancreas, brain and lungs.

Macroscopic lesions:

No gross lesions were observed in any organ during postmortem examination of rats fed 1 and 5% Catha material. Two cases of cysts formation in the kidney were observed in rats fed 1% Catha material for 16 months. These were considered incidental and no significance was attached to them.

There was no difference between rats fed whole Catha material and those fed the extract.

Microscopic lesions:

GASTROINTESTINAL TRACT:

There was mucinous degeneration with scattered desquamation of superficial epithelium in approximately 40% of rats fed 5%. Those fed 1% had no significant lesions in the GIT, except a few cases of cyst formation within the glandular mucosa. Oedema was noted in the submucosa in less than 20% of the rats fed 1 or 5%.

LIVER:

There were extensive degenerative changes (vacuolation) in almost all rats sacrificed after 8 months. The majority of animals killed after 14 months approximately 80% showed extensive hydropic degeneration.

KIDNEY:

The most consistent finding was vacuolation (fatty and hydropic) and cloudy swelling. There was no progressive involvement with time.

BRAIN:

Congestion in the meninges was observed in about 20% of the animals, mostly those kept for more than 10 months. Perivascular oedema was also observed in approximately 30% of rats kept for more than 12 months. There was no difference between those fed 1 and 5%.

EFFECT OF ANTICHOLINERGIC DRUG ON CATHA EDULIS INDUCED GASTRIC HAEMORRHAGE.

INTRODUCTION

A consistent observation during postmortem examination of rats fed high concentration of whole Catha material was haemorrhagic ulceration in the stomach and duodenum.

Gastrointestinal haemorrhage produced by administration of aspririn in both humans and animals is a well documented effect (Segal 1960). Studies in the rat (Brodie and Chase, 1967) indicate that acid is the causal factor of aspirin induced gastric haemorrhage and acid-inhibiting drugs such as the anticholinergic drugs produces significant reduction in gastric bleeding in rats.

In the present experiment, it was considered likely that if the haemorrhage induced by Catha material in rats is mediated through the back-diffusion of acid through the damaged mucosa, as has been postulated with aspirin, then anticholinergic drugs would produce significant reduction in gastric bleeding in rats.

MATERIAL AND METHOD

Fifteen rats were fed whole Catha material mixed with ground food in the ratio 1:1 into which was incorporated atropine sulphate. To ensure a uniform distribution of atropine, the drug was first dissolved in a small volume of water and then mixed thoroughly with 50% Catha

material. Three concentrations of the drug were fed to rats: 0.25, 0.5 and 1.0 g per Kg of the Catha-food mixture.

Rats were fed atropine-Catha-food mixture and sacrificed whenever they looked sick. In earlier experiments, it had been established that rats fed 50% Catha material died in less than 10 days; many of them died within 1-2 days.

RESULTS:

Incorporation of atropine sulphate in Catha-food mixture did not reduce the incidence or severity of gastric haemorrhage significantly and all animals died within 9 days of the experiment. Most of the rats died in the first 3-5 days except two which survived for 8 days. The two that lived for eight days were feeding on 0.05% atropine.

Postmortem examination of rats that died while feeding on atropine-Catha food mixture revealed extensive haemorrhage of the gastrointestinal tract, similar to that observed on rats fed 50% Catha material.

EFFECT OF FEEDING TANNIC ACID TO RATS

INTRODUCTION

Catha material has a high content of tannins and it was evident that some of the lesions, especially those in the GIT could be attributed at least in part, to tannins. They are readily soluble in water, and a person masticating Catha material will undoubtedly ingest a large quantity of tannins. Because of the astringent effect, tannins might interfere with absorption process and hence influence the growth rate.

MATERIALS AND METHOD

Weanling male and female white albino rats approximately 3-4 weeks old were fed various concentrations of tannic acid (BDH) and their weights determined twice a week. The following concentrations of tannic acid were fed to rats: 1,2.5, 5 and 10%. There were six rats in each group. Tannic acid was dissolved in a small amount of water and mixed thoroughly with ground mice pellets to make the required percentage. Each morning and evening, fresh food was put into containers. Rats were also supplied with water in plastic hygienic bottles. The amount of food eaten by rats was determined three times a week.

Some mature rats, about 2 months old, were given tannic acid solution (1 g) by stomach tube to ensure high concentration in the stomach at once and also to investigate the effect on an empty stomach. After giving the tannic acid solution, rats were returned to the cages and given food and water. Some rats were given tannic acid solution on 2-3 consecutive days.

Rats given various concentrations of tannic acid were sacrificed at intervals and examined by standard necropsy procedure. The following organs were preserved in acetate buffered 10% formalin for histological examination: liver, kidney, heart, brain, stomach, duodenum, ileum, rectum, caecum, spleen, pancreas and lungs. All sick rats were sacrificed and none was left to die naturally.

RESULTS

Information on the growth rate of rats fed various concentrations of tannic acid is shown in graph D. The actual weight gains are included in appendix II. Average weight gains for rats fed 1, 2.5, 5 and 10% tannic acid were less than that for the control.

Macroscopic lesions

No gross lesions were observed during postmortem examination of rats fed various concentrations of tannic acid incorporated in food and there was no death attributable to the feeding of the material.

Rats that were given tannic acid solution by stomach tube showed some consistent changes in the stomach. For rats sacrificed 24 hours after administration of tannic acid the stomach mucosa looked like a white amorphous mass of boiled tissue and there were several cases of haemorrhage, in the stomach and the duodenum region, and catarrhal enteritis in the rest of the intestine.

Microscopic lesions

GASTROINTESTINAL TRACT

There was extensive desquamation of the epithelium in the stomach and duodenum and occasionally in the rest of the GIT and extensive necrosis of glandular mucosa in all rats fed 1, 2.5, 5 and 10% tannic acid incorporated in food. A few cases of erosions in the stomach and duodenum were noted in rats fed 10% tannic acid, especially when sacrificed in the first week.

Rats fed tannic acid by stomach tube showed extensive haemorrhagic ulceration in the stomach and duodenum, generalised necrosis and disorganisation of glandular mucosa. Oedema of submucosa was noted in approximately 60% of the rats fed 5 and 10% tannic acid and approximately 40% of rats fed 1 and 2.5% tannic acid. Oedema of submucosa was also observed in all rats that were given tannic acid intragastrically.

LIVER:

There was generalised necrosis involving nearly all hepatic lobules, in all rats given tannic acid intragastrically. Approximately 40% of rats fed 10% tannic acid were also showing localised necrosis of centrolobular area. Rats fed 5, 2.5 and 1% tannic acid incorporated in food showed degenerative changes, mostly vacuolation.

KIDNEY:

There was coagulative necrosis of the proximal convoluted tubes and extensive desquamation of tubular epithelium in the renal cortex in all rats given tannic acid intragastrically. Those given tannic acid on two or three consecutive days were more affected than those that were given tannic acid once. Haemorrhage in the medulla and cortex of the kidney was observed in approximately 60% of the animals.

BRAIN:

Only a few cases of perivascular oedema and haemorrhage under the meninges (approximately 10%) were observed and these did not appear to be dose-dependent.

HEART:

Haemorrhage was observed in approximately 30% of the rats fed 5 and 10% tannic acid, and in a few rats (less than 20%) fed 1 and 2.5% tannic acid in food. The haemorrhage was not more marked in rats fed tannic acid for a longer period. Approximately 10% of the rats fed 5 and 10% tannic acid and also those given tannic acid intragastrically showed coagulative necrosis.

OTHER ORGANS:

No significant lesions were observed.

EFFECT OF FEEDING D-NORPSEUDOEPHEDRINE TO RATS:

MATERIAL AND METHOD

A group of eight rats in the same cage was fed synthetic d-norpseudoephedrine incorporated in food in the ratio 200mg to 100 g of ground food. Because of the small amount of the pure drug available, the experiment was continued for only 28 days. Rats were observed for any behavioral change and their weights determined twice a week. They were sacrificed at the rate of 1-2 per week and examined by standard necropsy procedures. The following organs were preserved in acetate buffered 10% formalin for histological examination: liver, kidney, brain, heart, stomach, duodenum, ileum, jejunum, caecum, spleen, pancreas and lungs.

RSULTS

Clinical observations

Rats were excessively stimulated in the first 2-5 days as shown by increased motor activity, but after the first week those signs subsided and the rats looked normal. Result of weight gain for rats fed pure drug were inconclusive since the experiment was continued for only 28 days. However, in that short period, the rats had good appetite except for the first 1-3 days. The average weight gain during 28 days of experiment was about the same as that of the controls.

Macroscopic lesions:

Postmortem examination revealed no gross lesions, that could be associated with consumption of drug.

Microscopic lesions:

No lesions were noted in the gastrointestinal tract, the liver, or kidney, except for minor localised degenerative changes which were not considered significant.

Three out of eight rats had localised area of coagulative necrosis in the heart. The lesion was found in rats sacrificed after 1 and 2 weeks.

Congestion and haemorrhage under the meninges was observed in 3 rats.

INVESTIGATION OF POSSIBLE TERATOGENIC EFFECT OF CATHA EDULIS

INTRODUCTION

Kamel et al. (1968) have shown that Catha edulis material causes congenital abnormalities in chick embryo. By implication, it would then appear that Catha material is a potential teratogen in human being. Although it is now generally recognised that results of teratological studies obtained with one species are not even applicable to other species, laboratory animals have continued to be used in experiments designed to predict possible effect in man, for lack of better alternative.

MATERIAL AND METHODS

Fifty six weanling female albino rats (14 in each group) 3-4 weeks old were fed 5,10, 15 and 25% of Catha material extract for more than three months and then mated at intervals with male rats that had been fed a corresponding percentage of the Catha material extract.

Each cage contained two females and a male except a few days before parturition when the females were placed each to a cage. Feeding on Catha material extract was continued up until and after parturition.

After parturition, the young were counted, weighed and examined for any morphological deformities. Some of the young rats from mothers fed 15 and 25% Catha material were then sacrificed by severing the spinal cord and after postmortem examination, the following organs were taken

and preserved in acetate buffered 10% formalin for histological examination: brain, liver, heart, pancreas, kidney, lungs, stomach, duodenum and jejunum.

Nine days later, just before they opened the eyes, more suckling young rats were sacrificed and the stomach content examined for d-norpseudoephedrine. Sucklings selected were those that were actively nursing or had just finished nursing as evidenced by a large curd of milk showing through the translucent abdominal wall. Immediately after removal from its litter, each pup was killed by decapitation and the stomach was excised, placed in a small beaker, and analysed later. No suckling were sacrificed after 9 days to ensure that stomach contents would not be contaminated with food provided to the dam. From day 11 to 14, pups became active enough to find and consume small amounts of solid food. Whenever the sucklings were sacrificed, normal postmortem examination was performed and the following organs of some sucklings preserved in acetate buffered formalin for histological examination: liver, kidney, brain, heart, spleen, pancreas, stomach, duodenum and ileum.

In some cases, the rats were born at night and had suckled the mother the following day. When this happened, the young rats were not weighed as this would have introduced a variable error, but they were examined for morphological deformities.

Many of the rats used in these experiments were mated several times. Approximately 50% of the sucklings were left to grow while still feeding on Catha material.

Milk samples from about 10 sucklings were combined and extracted with 80% ethanol at 60°C for 2 hours. The process of extraction was repeated three times and combined alcoholic extract concentrated at low temperature by blowing hot air current over the surface of the extract contained in a shallow porcelain dish. The concentrated residue was then examined for d-norpseudoephedrine by thin layer chromatography as described in the first part of this work.

RESULTS

The average number of young rats born to dams fed Catha material was not significantly different from the control ones (Table 11). Similarly, the average body weight per pup was not statistically different from that of control pups.

A consistent finding especially in rats fed 15 and 25% Catha material was that some of them, approximately 30% tended to neglect the young ones immediately after birth, leaving them to starve to death. This behaviour was not observed in control rats, or in those feeding on lower concentrations of Catha material.

No consistent abnormalities were observed in the young rats examined immediately after birth or when they were allowed to grow up while feeding on the same percentage of Catha material extract as the

mother. Many of the rats were mated for up to three times and in all cases no consistent morphological deformity was noted. Some of the deformities noted in the experimental as well as control rats were; still-born, abnormally short tail and deformed hind or fore limb. The number of young ones born to rats tend to decrease with age. For the purpose of counting the young rats in both experimental and control rats, only the first litter was considered.

Post morten examination of young rats, one day old did not reveal any gross lesion or abnormality. Similarly, microscopic examination did not show any consistent lesions which could be associated with feeding of Catha material to mothers.

Post morten examination of young rats, nine days old did not reveal any gross lesions. Microscopically, there were some degenerative changes, mostly cloudy swelling in the liver. No lesions were observed in the stomach, duodenum, jejunum, kidney, lungs, spleen and pancreas that could be associated with Catha material.

A trace amount of d-norpseudoephedrine was detected in combined milk obtained from ten sucklings.

Effect of Catha edulis material was also studied in mice. Female mice that had been fed 15 and 25% Catha extract incorporated in ground mice pellets for 3 months were mated at intervals with male mice which had also been fed a corresponding percentage of the Catha extract.

The young mice were examined for morphological deformities immediately after birth and also later when they grew up but unlike with rats, no other information (e.g. litter size, weight of litter, gross and histopathology examination) was considered in detail.

As with rats no consistent morphological deformities was observed in mice.

The present teratological studies may be regarded as preliminary in that no work was done using monkeys or chimpanzees which are close relative of man. However, the experimental data obtained with rats and mice did not justify a further consideration of the problem on a large scale.

ACUTE AND CHRONIC TOXICITY IN RABBITS

INTRODUCTION:

White albino rabbits about one and half months old and weighing approximately 1400g were used as experimental animals to investigate whether the toxic effects of Catha edulis observed in rats could also be reproduced in rabbits. It was also desired to do some of the experiments which could not be easily done using small animals like rats.

MATERIALS AND METHODS

The rabbits used in the present work were obtained from National Public Health Laboratories, Nairobi. Two groups of five New Zealand albino rabbits, approximately one and half month old, were each put in

large metabolic cages and fed ground commercial rabbit pellets (obtained from Kenya Farmers' Association Stores) over a period of approximately two weeks and then 15 and 25% Catha materials extract incorporated in ground pellets. The rabbits were supplied with water in hygienic bottles at all times. Each day, urine was collected over a period of twenty four hours and after making the appropriate dilutions, the total sodium and potassium excretion were determined using clinical flame photometer (Model 150 made by Evans electroselenium Ltd. England.).

In making the dilution, the following procedure was followed:

The volume of urine was determined and diluted to 400 ml if the volume was more than 200 ml., to 200 ml if the volume of urine was more than 50 ml. but less than 200 ml., and 100 ml if the volume of urine was less than 50 ml. One millilitre of the diluted urine was made to 200 ml. in a standard flask using de-ionised water. The diluted urine was then filtered into a test tube and the Na^+ and K^+ concentration read from the scale. The Zero and maximum reading of the photometer were adjusted using a standard solution of sodium and potassium ions. From the reading of sodium and potassium concentration in diluted urine, the total excretion in urine for 24 hours was determined.

Arterial pressure was determined in the intact unanaesthetised state by the ear capsule method (Grant and Rothschild, 1934). A black

ring, approximately 3 mm in diameter was painted on the rubber diaphragm of the capsule. The diaphragm used in the present work was obtained by slitting open "Gossamer" contraceptive sheath and tying the membrane firmly over the capsule with a rubber band.

The diastolic pressure was taken as the point when pressure within the capsule was just sufficient to exclude blood from the ear artery beneath the ring once during every cardiac cycle. The systolic pressure was taken as the point when blood was excluded throughout the cycle.

In all cases, three successive readings of diastolic and systolic pressure were taken and the mean of the readings recorded. If the readings were not consistent, two more readings were taken.

After the control period, rabbits were fed 25% Catha material extract incorporated in ground rabbit pellets and the diastolic and systolic pressures determined every day. The experiment was continued for 4 months. Another group of rabbits was fed 15%, Catha extract but the sodium and potassium output was not determined because it was evident, that the change in output on those fed 25% was very small. Another group of six rabbits was fed ground rabbit pellets and kept as control.

Approximately 1 - 2 weeks before the rabbits were sacrificed, blood samples were taken from experimental and control animals. The blood samples were obtained by making a clean cut in the marginal ear vein and then collecting blood. Attempts to use cardiac puncture technique

was unsuccessful and led to the loss of one experimental and one control rabbit. Blood for haematological tests was collected in bottles containing EDTA to prevent clotting but samples for biochemical experiments were collected in clean bottles with no anticoagulant or preservative.

After doing the haematological and biochemical tests, the experimental and control rabbits were sacrificed by injecting air into the marginal ear vein. The rabbits were examined using the normal necropsy procedures and the following organs preserved in acetate-buffered 10% formalin for subsequent histological examination: liver, kidney, lungs, brain, heart, spleen, pancreas, stomach, duodenum, jejunum, and colon.

RESULTS:

Information on blood pressure changes of rabbits fed 25% Catha material extract is shown in Fig.27 and the actual data is included in appendix II. The mean blood pressure for each rabbit over the control period was calculated. From these values, the mean for all rabbits was determined and this is designated as 100%. Blood pressure changes in experimental rabbits is given as percentage of the mean for all five rabbits.

There was a slight and persistent decrease in blood pressure values when the rabbits were fed Catha material for 4 months. The shift was more marked in the initial stages of the experiment.

The relationship of blood pressure readings obtained by Grants ear capsule method to absolute pressure is uncertain but it is reasonable to expect that changes in the readings reflect corresponding changes in the underlying absolute pressures.

In the first few days of the experiment, there were considerable fluctuations of the blood pressure readings probably due to fear and excitement. Later, after about one week, the readings become much more consistent after the rabbits were used to handling. The readings were more consistent when the rabbits were restrained only loosely in an open-topped box and at least 5 minutes were allowed to elapse between lifting the rabbits from their cages and taking the first reading. Information on total sodium and potassium excretion in urine is presented in Fig. 28 and the actual data is included in Appendix II. Daily average value of sodium and potassium excretion for each rabbit during the control period was calculated and from these values the mean for all rabbits was determined and designated as 100%. Daily excretion of sodium and potassium by experimental rabbits is expressed as percentage of this mean.

There was no significant change in Na^+ and K^+ excretion when rabbits were fed 25% Catha material for approximately 4 months.

Results of haematological and biochemical tests for rabbits fed 15 and 25% Catha material extract and the control are summarised in

table 12 and 13.

There was a considerable variation in the blood cells count as determined in three experiments and it is, therefore, not possible to give the data with great precision. Since the data could not be given with any great precision, the only conclusion that can be drawn is that there is no evidence of an effect on the haematopoietic system since the blood cell count, haemaglobin and the haematocrit values of all experimental animals were comparable to those of controls.

Results of biochemical tests also show that the values for rabbits fed 15 and 25% were comparable to those of controls.

Since the values for transaminase enzymes were not higher than those of controls, it was expected that damage in the organ tissues where these enzymes are concetrated (heart, liver, skeletal muscles) would be minimal.

The plasma glucose concentraion in experimental and control rabbits were also comparable. When d-norpseudoephedrine was administered intravenously to control rabbits and the glucose level determined 30 minutes later, a definite decrease was evident. The control values were not reproduceable and varied considerably from one day to another, but in all cases the plasma glucose before the administration of d-norpseudoephedrine was more than that obtained 30 minutes after administration of the drug.

Variation in blood glucose following administration of Catha material or d-norpseudoephedrine to rabbits was of special interest since according to "glucostatic theory of hunger" (Glass 1968) appetite can be regulated by the concentration of glucose in blood. D-norpseudoephedrine was shown to be an appetite depressant, at least in the first few days of administration.

Rabbits fed 15 and 25% Catha extract for six months did not show any gross lesions during post-mortem examination.

MICROSCOPIC LESIONS.

GASTROINTESTINAL TRACT:

Rabbits fed 25% Catha extract showed desquamation of epithelium, especially in the duodenum region and part of ileum. Necrotising gastroenteritis was also observed but there was no haemorrhagic ulceration.

Rabbits fed 15% Catha extract showed only desquamation especially in the stomach and duodenum but no ulcerations.

LIVER:

The most consistent finding was extensive vacuolation in both groups of rabbits. Fatty infiltration was very marked and appeared to involve almost the entire organ.

Rabbits fed 25% Catha extract showed centrolobular necrosis.

Rabbits fed 15% Catha extract did not show necrosis but there was degenerative changes characterised by cloudy swelling and fatty infiltra-

KIDNEY:

Rabbits fed 25% Catha extract showed coagulative necrosis in both renal cortex and medulla. Hyaline cast formation and desquamation were noted in proximal convoluted tubules.

Rabbits fed 15% extract also showed coagulative necrosis and extensive vacuolation in proximal convoluted tubules. Degenerative changes (hydropic, fatty infiltration, cloudy swelling, etc) appeared to be widespread in all rabbits. Hyaline cast formation and desquamation of the tubules were also observed but these were not as widespread as in rabbits fed 25%.

HEART:

Coagulative necrosis was observed in 2 rabbits fed 25% Catha extract and 1 rabbit fed 15% extract. The infarct were small and localised and did not appear to be associated with the vascular system.

Haemorrhage was observed in 3 rabbits fed 25% Catha extract.

No siginificant lesions were observed in the other organs (brain, spleen, pancreas, etc.).

ELIMINATION OF D-NORPSEUDOEPHEDRINE FROM THE BODY

INTRODUCTION

In order to account for the effects of Catha material fully, it was considered necessary to determine the time-course of excretion of the active principle in the body.

The most important route for elimination of many drugs is through the kidney. Other routes of drug elimination include the biliary tract, exocrine glands, mammary glands and exhalation through lungs (for volatile anaesthetics). It is easier to study the elimination of drugs in urine than in bile because of the ease of obtaining urine. Furthermore, drugs excreted through the biliary tract into the gastrointestinal tract are often reabsorbed into the enterohepatic circulation making the quantititive study difficult or impossible. A further limitation in studying elimination of drugs through the biliary tract is that where a drug is given orally, it is impossible to say with certainty whether the quantity recovered in the GIT is the unabsorbed drug or the one excreted in bile. It is however possible to cannulate the bile duct, under anaesthesia and collect the bile thus overcoming these problems.

In the present work, the urine of rats and rabbits fed Catha material and also human volunteers who had consumed Catha material was examined for d-norpseudoephedrine or its metabolite(s). Bile ducts of rats were also cannulated under anaesthesia, using the method of Cox and Wright (1959) and bile collected over a period of approximately 6-8 hours.

Collection of Urine and bile.

MATERIAL AND METHODS

Six male rats feeding on 15% Catha material were put in metabolic cages and urine collected over a period of 24 hours. The combined urine

from three cages was filtered to remove small food particles. Control urine from rats fed ground pellets was also collected.

A rat was anaesthetized using urethane (ethyl carbamate) and the bile duct cannulated. The method is described in detail by Cox and Wright (1959). The femoral vein was also cannulated for the purpose of giving drug and also normal saline to prevent dehydration.

Control bile was collected for about 2 hours. Approximately 5 mg of the alkaloid was then given through the femoral vein and the bile collected over a period of 6-8 hours. Normal saline was administered to rats occasionally to ensure that there was no dehydration.

Rabbits fed 25% Catha material incorporated in ground pellets were kept in metabolic cages and urine collected over a 24 - hour period. As with rats, control urine was first collected from rabbits feeding on pellets and used for comparative purposes.

Several people known to be casual chewers of Catha edulis material were requested to abstain from consuming it for about 24 hours and to submit control urine collected towards the end of this period. They were then given about four bundles of fresh Catha material and requested to submit urine samples collected over a period of 12 hours (overnight) as they continued to chew the tender twigs of Catha materials. These people were known to the author very well and were considered reliable.

Preliminary experiments showed that the "control urine" samples submitted always contained a single alkaloid which was identified as

d-norpseudoephedrine. The volunteers who gave these control urine samples insisted that they had not eaten any Catha material during the 24 - hours control period as requested. Under the circumstances, no quantitative work was possible and it was decided to give pure drug (d-norpseudoephedrine hydrochloride) to human volunteers who were not chewers of Catha edulis. Approximately 30 mg d-norpseudoephedrine hydrochloride was accurately weighed and dissolved in a small volume of water. The mixture was then given to each of 3 healthy male volunteers who had only drunk milk in the morning. Just before taking the drug, each volunteer gave a sample of urine to serve as a control. The volunteers were encouraged to drink water so as to promote frequent voiding of urine.

Urine samples were collected in different containers as follows:

Sample	1:	After ½	-1 hour
"	2:	" 1	-2 hours
"	3:	" 3	-4 hours
"	4:	" 4	-8 hours
"	5:	" 8	-12 hours
"	6:	" 12	-15 hours
"	7:	" 15	-24 hours

Examination of urine and bile for d-norpseudoephedrine and metabolites.

MATERIAL AND METHODS

To the urine or bile samples was added 1 - 2 ml saturated lead acetate solution and centrifuged to remove any precipitate. The precipitate was washed twice and the combined supernatant liquid was saturated with

sodium chloride at 60°C. The mixture was cooled, transferred to a separating funnel and extracted with solvent ether three times.

The combined ether extract was washed with 2 ml, 2.5% sodium bicarbonate twice and dried with anhydrous sodium sulphate. The dried ether was distilled and the residue examined for bases by thin layer chromatography as described for Catha material in the first part of this work.

The amount of drug present in each sample of urine collected was found to be small and often difficult to estimate accurately. It was therefore decided to combine several urine samples for the purpose of determining the amount excreted quantitatively. Quantitative estimation of d-norpseudoephedrine in urine was done in the same way as described for Catha material using gas liquid chromatography as described in the first part of this work.

RESULTS

D-norpseudoephedrine was detected unchanged in urine and bile from rats, urine from rabbits and urine from people who had consumed fresh Catha material. Similarly, when d-norpseudoephedrine hydrochloride was given orally to human volunteers, it was recovered unchanged in urine. The drug was detected in human urine obtained within the first hour and also in all other samples taken up to 27 hours later when the experiment was discontinued. Approximately 40% of the ingested drug was recovered

in urine unchanged within the first six hours of the experiment.

Hydrolysing urine samples by heating under reflux in boiling water bath for 1 hour (after acidifying with 2N HCl) before subjecting it to solvent-solvent extraction procedure did not give different results. This then indicated that conjugation is not an important metabolic pathway for d-norpseudoephedrine.

DISCUSSION ON CATHA EDULIS TOXICOSIS IN RATS AND RABBITS

Results of body weight gain averages of rats fed various concentration of Catha material extract indicate a definite retardation in growth rate for rats fed concentration of Catha material higher than 5%. Food consumption averages by individual rats in the different groups were not significantly different except for those feeding on 50%. The anorexic effect of Catha material could partly be due to the gastrointestinal irritant effect of tannins and partly due to the centrally mediated effects of d-norpseudoephedrine.

The majority of drugs used to treat obesity are CNS stimulants and it is now generally accepted that the central stimulant effect of these drugs is inextricably interwoven with the anorexic effect (Modell and Hussar 1965). At the beginning of the experiment, the mean food consumption was low probably because of detestable palatability of Catha extract. Both d-norpseudoephedrine and tannins have a bitter taste.

Rats feeding on 15, 25 and 50% showed signs of central stimulant effect only in the first few days; thereafter they looked very calm. Their appetite after the first few days, as shown by food intake, was basically unaltered.

Since the amount of food eaten by rats feeding on 10, 15 and 25% is not very much different from that eaten by the control rats, the observed retardation in growth may be attributed to decreased absorption from the gastrointestinal tract and/or defective food utilization due to the impairment of the function of a vital organ, as for example liver.

Results of histopathology of various organs from rats fed high concentration of Catha material extract (including 50% for up to six months) show that there are no major lesions which could account for defective utilization of food. Only minor degenerative changes were found in the liver and kidney.

The mean value for haematologic and blood biochemical analysis for rabbits fed 15% and 25% Catha material for four months were essentially normal and comparable to values for the controls. The fluctuations in blood cells count was such that minor changes in the haematopoetic system would have been difficult to detect. This flunctuation has been observed by other research workers. Considering that it is possible to destroy up to 80% of the liver cells and maintain the animal

alive then the possible contribution of minor degenerative changes observed in rabbits and rats fed Catha material extract to defective food utilization appear to be small.

Results of organ-body weight ratios, for several organs, indicate no significant difference from the controls. Although it is difficult to interpret the mechanism by which the weights of various organs are altered or indeed to determine whether the shift in weight is a truly toxic manifestation, it is generally recognised that organ weight shifts can be good indices in toxicological studies. Unfortunately such factors as age and methods of sacrificing animals make the interpretation difficult (Pfeiffer and Muller 1967).

By far the most important and consistent histopathological lesions observed in rats fed various concentration of Catha material were those localised in the gastrointestinal tract. With high concentration of whole Catha material and sometimes Catha extract, there was ulcerative gastritis, duodenitis and in very few cases enteritis. Animals fed concentration of Catha material less than 10%, showed catarrhal gastritis and duodenitis. There was extensive desquamation of the epithelia especially in the stomach and duodenum.

The extensive involvement of the stomach and duodenum where the pH is acidic seemed to suggest that the lesions were, at least in part, caused by tannins present in Catha material. Rats fed pure d-norpseudoephedrine did not show similar changes in the gastrointestinal tract and

direct contribution from other known chemical constituents of Catha material was considered minimal.

In the stomach and duodenum, the acid pH favours the precipitant action of tannic acid. However, as the pH of the intestinal contents become progressively alkaline, the precipitant action of tannic acid is less marked. Most tannic acids are easily decomposed in alkaline media into less toxic compounds with little or no astringent effect. For example, the commercial tannic acid is readily decomposed to gallic acid; the latter has no astringent effect and is readily absorbed and oxidised in the body. In nearly all cases, tannic acid seem to lose its astringent effect before reaching the posterior end of the intestinal tract.

Rats fed 25 or 50% Catha material and sacrificed as soon as they started looking sick were always found to have haemorrhagic gastritis and duodenitis. These lesions were also observed in rats that died. Those rats that died or were sacrificed after they had been sick for 1-2 days showed centrolobular necrosis and coagulative necrosis in the liver and kidney respectively. In contrast, rats sacrificed as soon as they showed definite signs of toxicity had haemorrhagic gastritis and duodenitis but no necrosis in the liver or kidney. These findings seemed to suggest that death as a result of feeding Catha material to rats was not due to lesions in the kidney or liver but rather to haemorrhagic lesions localised in the gastrointestinal tract.

Feeding rats with atropine at the same time as Catha material did not make much difference to the toxicity of the latter; in particular the haemorrhagic ulceraton in the gastrointestinal tract were not altered. This probably indicate that acid secretion play a minor role in production of ulcers in rats fed Catha material.

When rats were fed 1, 2.5, 5 and 10% tannic acid incorporated in ground mice pellet, no death was observed even though there was marked retardation in growth with even the lowest concentration. Rats given 1 g tannic acid in solution (using stomach tube) for 2-3 consecutive days revealed extensive haemorrhagic ulceration in the stomach during postmortem examination. The stomach was covered with greyish amorphous mass of tissue. Microscopic examination of the liver and kidney showed the presence of centrolobular necrosis and coagulative necrosis in the proximal convoluted tubules respectively.

An average rat eating a minimum of 15 grammes 10% tannic acid-food mixture would consume approximately 1.5 g tannic acid daily. This is much more than the amount of tannic acid given orally to rats which were found to have haemorrhage in the GIT and centrolobular necrosis. A possible explanation of the differences is that tannic acid mixed intimately with food does not have the same effective contact, in terms of area, as the tannic acid given intragastrically. This situation is analogous with chewing Catha material before eating.

Another consistent finding in rats fed high concentration of Catha material was oedema especially in the submucosa of the gastrointestinal tract. For example in rats fed 50% Catha material extract, approximately 60% of the animals were showing oedema. Perivascular oedema in brain was also found but this accounted for less than 30% of the cases.

Oedema is a condition in which there is excessive amount of fluid in the intercellular spaces of the body cavities. The aetiology of oedema is poorly understood and may be due to several factors such as change in colloid density, osmotic pressure, blood pressure, capillary permeability and the nervous system. Since oedema was observed in rats fed tannic acid but not those fed d-norpseudoephedrine it appears that the effect might be attributed directly to the tannins present in Catha material. Tannins are known to alter the permeability of cell membranes, an effect which could easily cause oedema.

There was no definite and consistent lesions in the brain, although a few cases of perivascular oedema, congestion and haemorrhage in the meninges were observed. Most chemical compounds do not pass readily into the brain because of the blood brain barrier and it is only when this barrier is impared that substances pass freely into the brain.

Blood pressure of rabbits fed 15 and 25% Catha material over a period of ninety days showed a slight decrease especially in the early

stages of the experiment. A decrease in blood pressure is usually considered to have little toxicological significance as compared to a rise in blood pressure. The regulation of blood pressure is mainly dependent on the vasomotor centre. For example, sectioning of the hind brain below the level of these cells lead to generalised vasodilation which is manifested as a decrease in blood pressure. A general depression of the CNS, would also lead to an appreciable decrease in blood pressure. The effect of Catha material is biphasic (i.e. an initial stimulation followed by depression) and the slight decrease in blood pressure observed especially in the initial stages might be a direct consequence of reduced vasomotor tone. There were several rats showing localised necrosis in the heart but these accounted for less than 30% of the total number even with 50% Catha material. Some significance was attached to this finding because of some report which implicate sympathomimetic amines in cardiac lesion formation.

Although Catha material has been shown to cause deformities in chick embryo (Kamel et al. 1968), results obtained in the present work using rats and mice did not indicate any possible teratogenic effect. The chick embryo is a unique system since material is retained after injection and only metabolised slowly. The present teratological studies are therefore preliminary since no work was done using monkeys or chimpanzees which are close relatives of man. However, further

studies could not be justified since no indication of a teratogenic effect in rats and mice was evident.

Results of Catha edulis toxicosis in rats and rabbits will be referred to later in the general discussion.

Table 5 Result of feeding rats on various concentrations of Catha material

Group	% Catha Material	Number of rats fed Catha Material		Maximum duration of experiment (Months)	Number of animals dead
I	50%	Catha extract	20	6 months	Catha extract None Whole Catha material-All
		whole Catha material	20		
II	25%	Catha extract	21	6 months	Catha extract-None Whole Catha material-9
		whole Catha material	20		
III	15%	Catha extract	14	12 months	None
		Whole Catha material	14		
IV	10%	Catha extract	14	16 months	None
		whole Catha material	14		
V	5%	Catha extract	14	16 months	None
		whole Catha material	14		
VI	1%	Catha extract	21	16 months	None
		whole Catha material	14		

Table 6.

AMOUNT OF FOOD (CATHA EXTRACT) CONSUMED BY MALE RATS DAILY *

(g/rat/day)

Week	Control	1%	5%	10%	15%	25%	50%
1	8±1	7±2	8±2	7±2	5±2	5±2	4±2
2	10±2	9±2	10±1	9±2	8±2	9±2	7±2
3	13±2	12±1	13±1	11±2	13±2	12±1	10±2
4	14±1	15±2	13±2	12±1	14±2	14±2	12±2
5	17±1	14±2	15±2	14±2	14±2	16±1	13±2
6	17±2	16±1	16±2	17±2	18±1	17±2	15±2
7	18±1	16±2	15±2	15±2	20±2	16±2	14±1
8	21±2	19±2	19±2	19±1	18±1	19±1	16±2
9	18±2	17±2	18±2	20±2	20±2	19±2	14±2
10	19±2	17±2	19±2	18±1	21±2	18±2	15±2

* Average of 3 days.

Table 7. RATS FED 1% CATHA EXTRACT FOR 16 MONTHS

ORGAN WEIGHT AS % OF BODY WEIGHT

RAT NO.	HEART	LIVER	SPLEEN	KIDNEY	BRAIN
1	0.36	4.45	0.32	0.81	1.62*
2	0.41	4.12	0.36	0.79	0.92
3	0.48	4.47	0.52	0.66	0.77
4	0.34	3.66	0.35	0.83	0.85
5	0.37	4.98	0.22	0.87	1.02
6	0.49	4.17	0.33	0.93	0.97
7	0.34	3.49	0.28	0.77	0.92
8	0.36	4.07	0.58	0.78	1.01
9	0.43	3.39	0.23	0.76	0.91
10	0.44	3.91	0.40	0.90	0.01
11	0.31	4.60	0.32	1.09*	1.17
12	0.37	4.17	0.54	0.66	0.99
AVERAGE	0.39	4.12	0.37	0.80	0.96

Table 8. RATS FED 5% CATHA MATERIAL EXTRACT

ORGAN WEIGHTS AS % OF BODY WEIGHT

RAT NUMBER	HEART	LIVER	SPLEEN	KIDNEY	BRAIN
1	0.41	4.06	0.68	0.74	0.84
2	0.39	4.13	0.35	0.71	0.97
3	0.38	4.10	0.43	0.76	0.97
4	0.36	4.24	0.48	0.82	1.05
5	0.37	4.30	0.42	0.42	0.97
6	0.30	4.25	0.40	0.70	0.94
7	0.35	4.28	0.42	0.75	0.83
8	0.44	4.18	0.43	0.72	0.86
9	0.33	4.32	0.54	0.82	0.97
10	0.35	4.16	0.40	0.74	0.92
11	0.34	4.30	0.41	0.76	0.92
12	0.31	4.24	0.42	0.81	0.94
Average	0.36	4.21	0.45	0.76	0.93

* abnormally high.

Table 9. RATS FED 15% CATHA MATERIAL EXTRACT FOR 12 MONTHS
ORGAN WEIGHTS AS PERCENTAGE OF
BODY WEIGHT

RAT NO	LIVER	SPLEEN	KIDNEY	HEART	BRAIN
1	4.4	0.31	0.67	0.42	1.0
2	4.6	0.33	0.89	0.35	1.03
3	4.3	0.39	0.88	0.38	0.98
4	4.2	0.34	0.86	0.37	1.00
5	4.26	0.39	0.71	0.36	0.98
6	4.28	0.33	0.87	0.36	0.98
7	4.23	0.34	0.68	0.37	1.01
8	4.31	0.36	0.82	0.37	0.95
AVERAGE	4.32	0.35	0.80	0.37	0.99

Table 10. CONTROL RATS SACRIFICED AFTER 16 MONTHS
ORGAN WEIGHTS AS PERCENTAGE OF BODY WEIGHT

RAT NO	LIVER	SPLEEN	KIDNEY	HEART	BRAIN
1	4.76	0.45	0.77	0.36	0.90
2	4.30	0.40	0.71	0.35	0.83
3	3.76	0.75	0.69	0.44	0.73
4	3.69	0.39	0.79	0.36	0.76
5	4.10	0.35	0.71	0.43	0.83
6	5.25	0.51	0.95	0.95	0.95
7	4.81	0.39	0.66	0.53	1.10
8	4.32	0.61	0.74	0.34	1.18
9	4.2	0.45	0.77	0.41	0.83
10	5.16	0.47	0.95	0.56	0.99
11	4.62	0.43	0.76	0.37	0.86
AVERAGE	4.28	0.47	0.77	0.38	0.91

Table 11. EFFECT OF FEEDING CATHA MATERIAL TO RATS ON NUMBER AND AVERAGE WEIGHT OF YOUNG RATS AT BIRTH

CATHA EXTRACT (1)	NUMBER OF YOUNG RATS (10 LITTERS)	AVERAGE NUMBER PER LITTER	TOTAL WEIGHT OF YOUNG RATS AT BIRTH (g)	AVERAGE WEIGHT PER YOUNG RAT (g)
Control	70	7.0	406	5.8
25	68	6.8	415	6.1
15	72	7.2	418	5.8
10	65	6.5	403	6.2
5	70	7.0	413	5.9
1	78	7.8	437	5.6

TABLE 12 RESULTS OF HAEMATOLOGY OF BLOOD FROM RABBITS FED CATHA MATERIAL (25, 15%) and CONTROLS FOR SIX MONTHS.

% Catha Material		PCV	HB	REC	WBC	MCV	MCHC
		(%)	(%w/v)	($x10^6$)	(10^3)	(%)	(%)
Control: Rabbit	1.	35	11.8	5.27	6.1	61	33.7
	2.	38	12.7	4.65	3.6	82	33.5
	3.	42	13.7	6.07	6.3	70	32.6
	4.	35	11.5	5.23	5.8	67	32.8
	5.	37	11.5	5.20	8.7	71.2	31.1
Mean		37.4	12.2	5.28	6.1	70	32.7
25% Catha Material. Rabbit	1.	26	12.6	6.00	7.0	45.5	35
	2.	34	11.0	4.98	3.9	68.2	32.4
	3.	35	10.9	5.29	5.0	61.2	31.0
	4.	37	12.5	5.58	5.8	66.5	33.7
	5.	38	12.3	6.20	9.2	62.1	32.5
Mean		36	11.8	5.6	6.4	60.7	32.9
15% Catha Material. Rabbit	1.	35	10.5	5.13	6.2	68.5	30
	2.	35	11.2	4.45	3.7	78.5	32
	3.	32	10.8	4.89	7.8	65.5	34.7
	4.	39	12.4	6.17	6.0	63	31.8
	5.	37	11.5	5.20	8.7	71.2	31.1
Mean		35.6	11.3	5.17	6.5	69.3	31.9

Key:
PCV - packed cell Volume
HB - haemoglobin
RBC - red blood cells
WBC - white blood cells
MCV - mean corpuscular volume
MCHC - Mean corpuscular haemoglobin concentration

Table 13. RESULTS OF BIOCHEMICAL ANALYSIS OF BLOOD FROM RABBIT FED CATHA MATERIAL EXTRACT

Control		Alkaline Phosphate	SGOT	Glucose	CPK
Rabbit No.	1	1.75	125	83.5	110
"	2	4.15	150	76.0	129.5
"	3	1.75	130	131.0	129.5
"	4	1.20	120	102.7	120
"	5	1.90	110	108.5	119
Mean		2.15	127	100.3	121.6
25% Catha extract					
Rabbit No.	1	1.40	110	80	110
"	2	2.85	125	103	81.5
"	3	3.15	95	151	123
"	4	2.30	64	105	139
"	5	1.43	42	119	179
Mean		2.23	87.2	111.6	126.5
15% Catha extract					
Rabbit No.	1	2.70	5	138.5	126
"	2	2.30	39	126	141
"	3	4.40	7	123	152
"	4	1.25	123	147	110
"	5	1.75	70	80	105
Mean		2.48	48.8	122.9	126.8

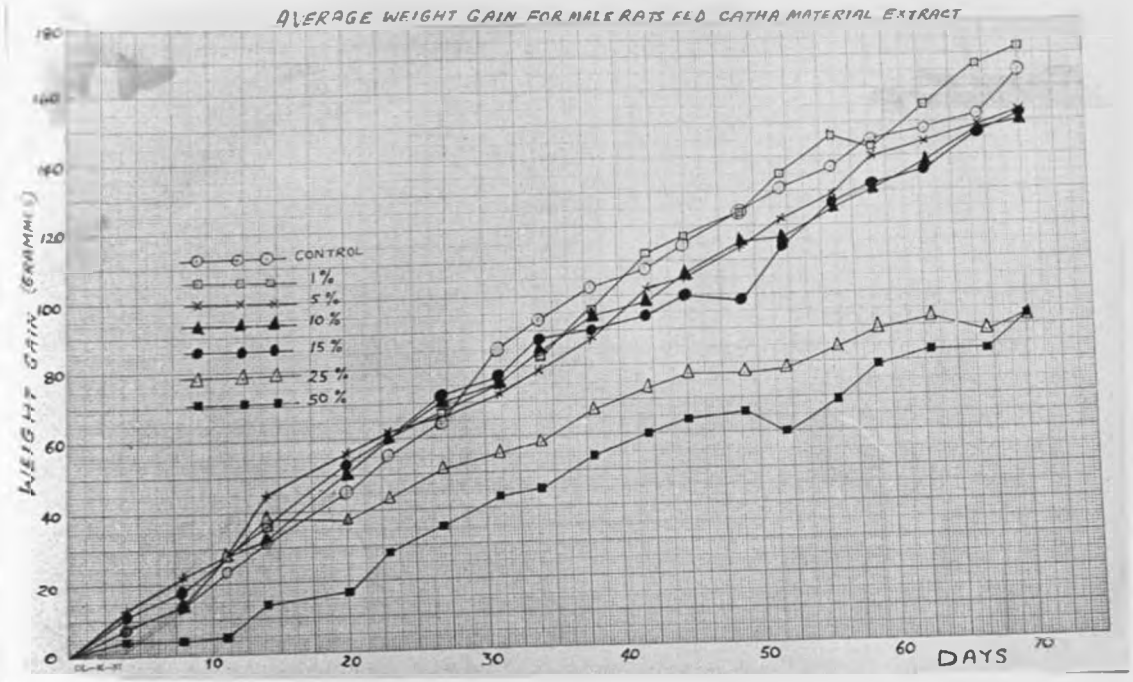


Fig. 7. Weight gain for male rats fed Catha material extract.

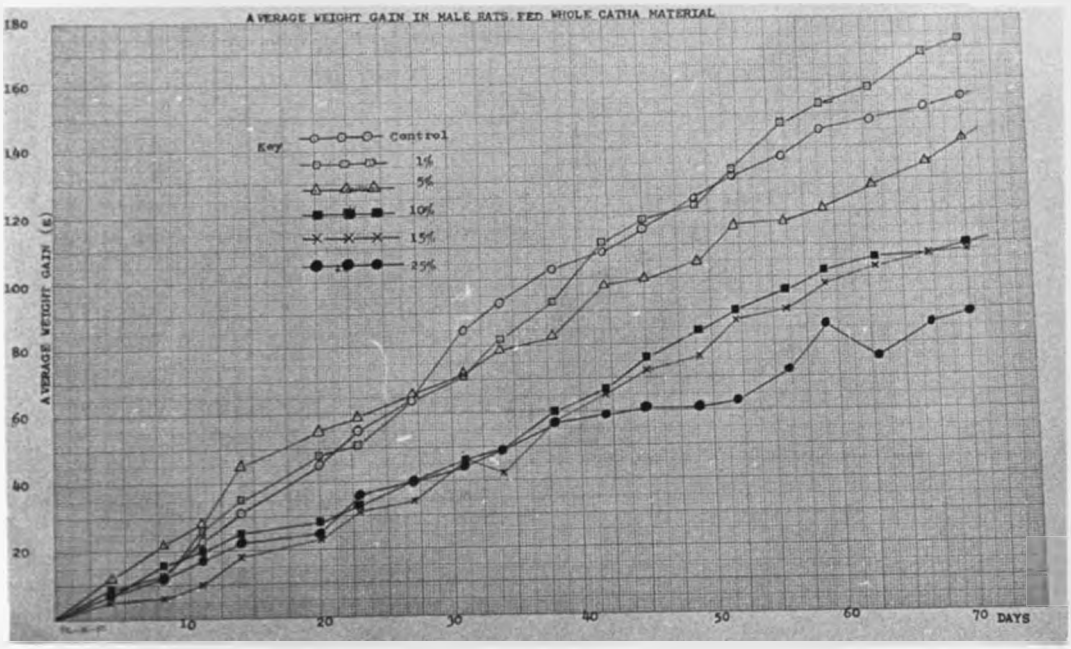


Fig. 8. Weight gain for male rats fed whole Catha material.

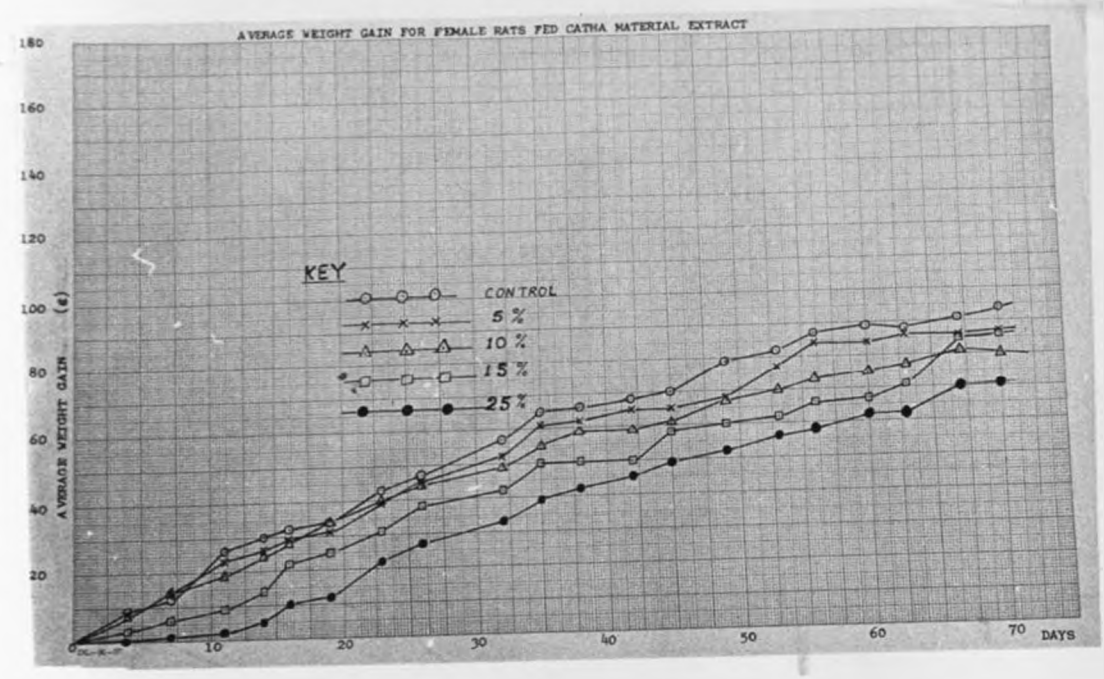


Fig. 9. Weight gain for female rats fed Catha material extract.

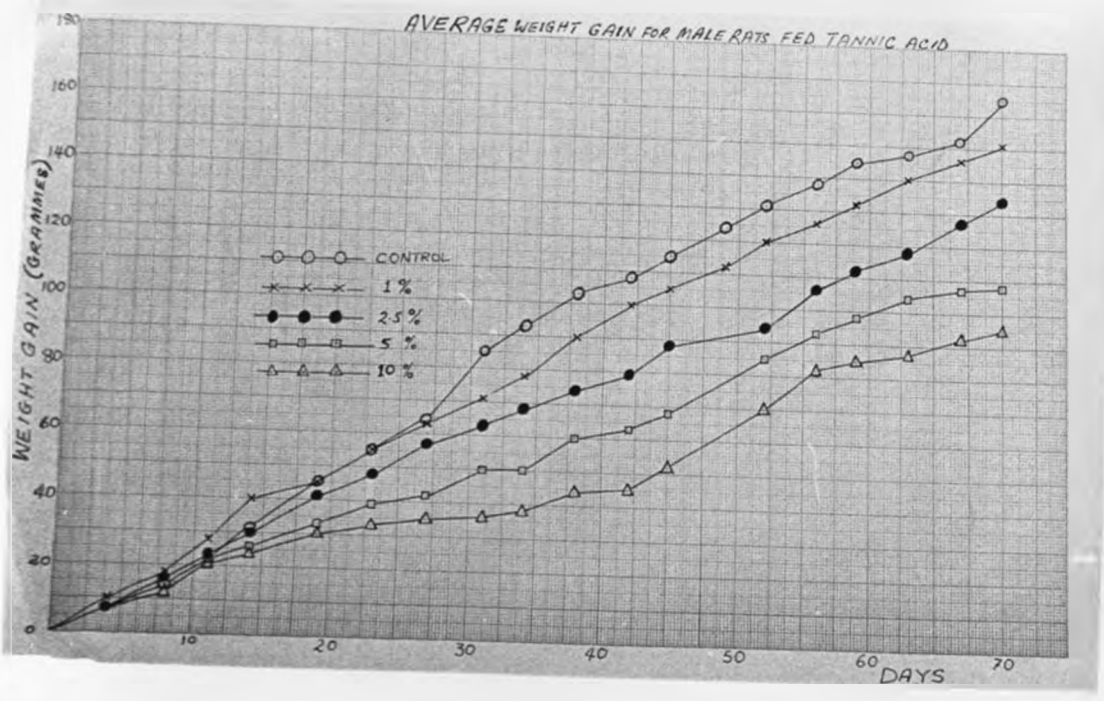


Fig. 10. Weight gain for male rats fed tannic acid.

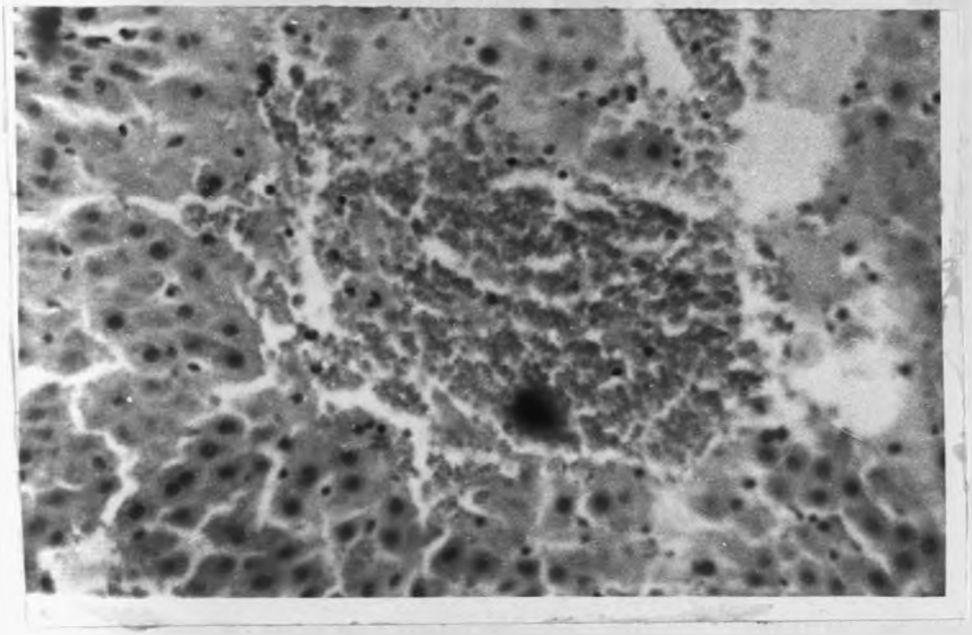


Fig. 11. Necrosis of hepatic cells and haemorrhage in the centrolobular area of liver of a rat fed 50% whole Catha material for 6 days. X 156.

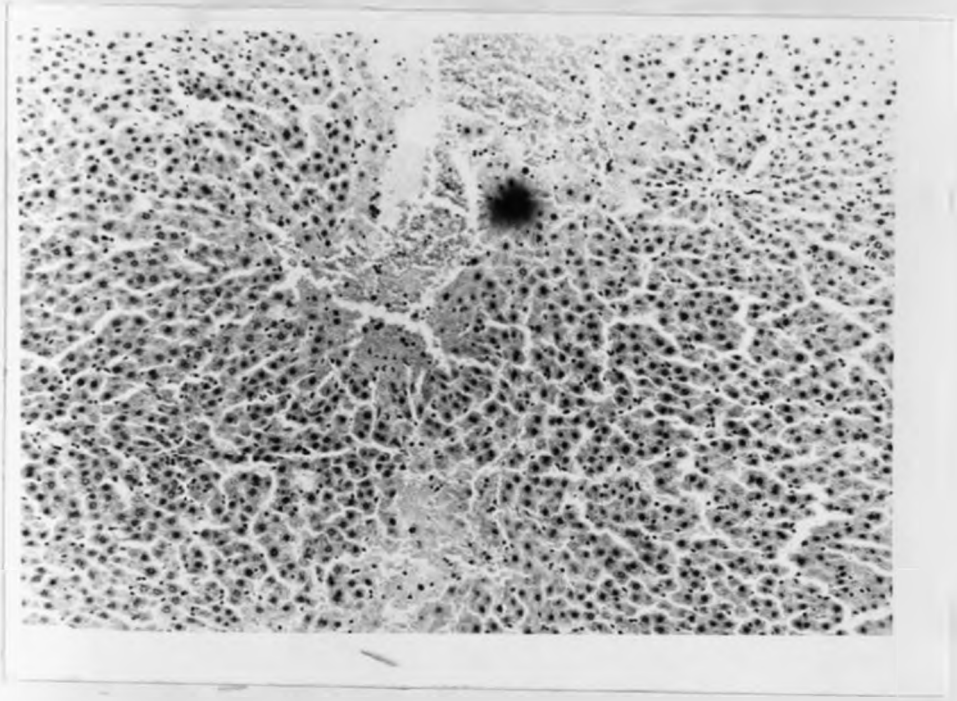


Fig. 12. Necrosis and haemorrhage in the centrolobular are of liver of a rat fed 25% whole Catha material for 3 weeks. X 63.

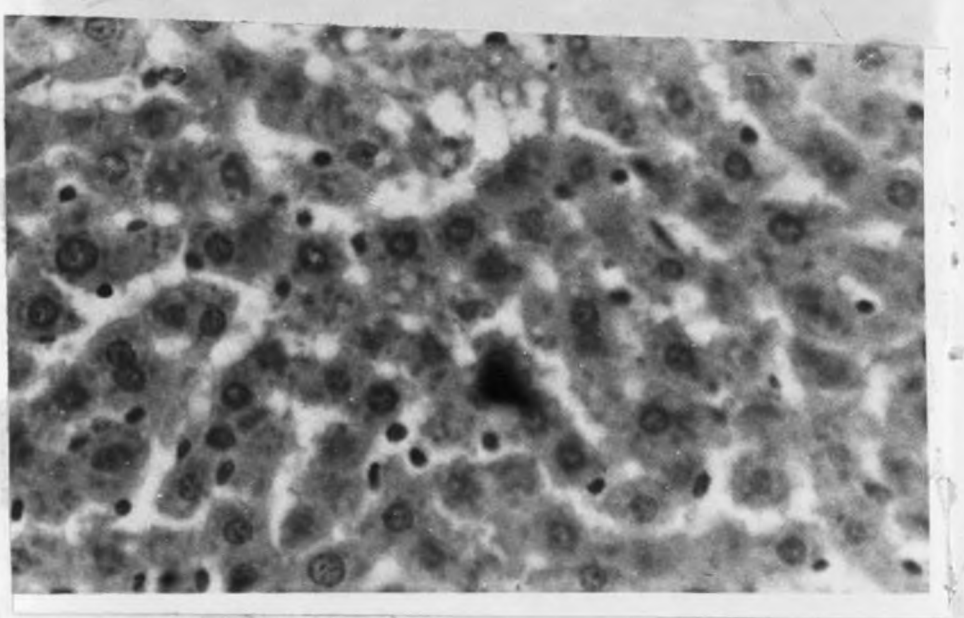


Fig. 13. Extensive vacuolation in hepatic cells of rat fed 10% whole Catha material for 4 months. X 391.

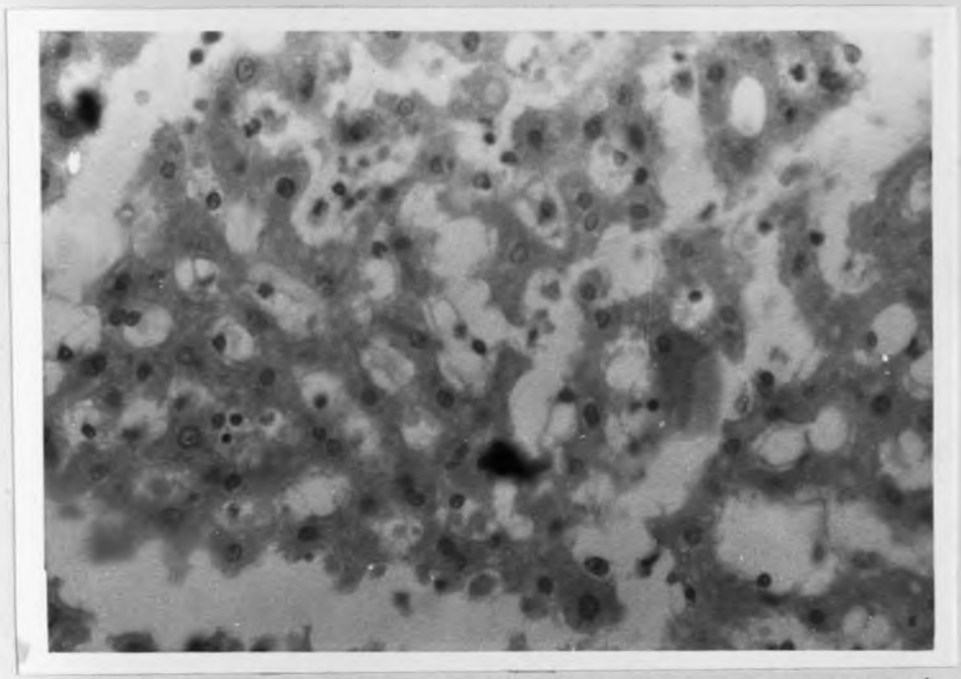


Fig. 14. Extensive vacuolation in hepatic cells of a rat fed 5% whole Catha material for six months. X 391.

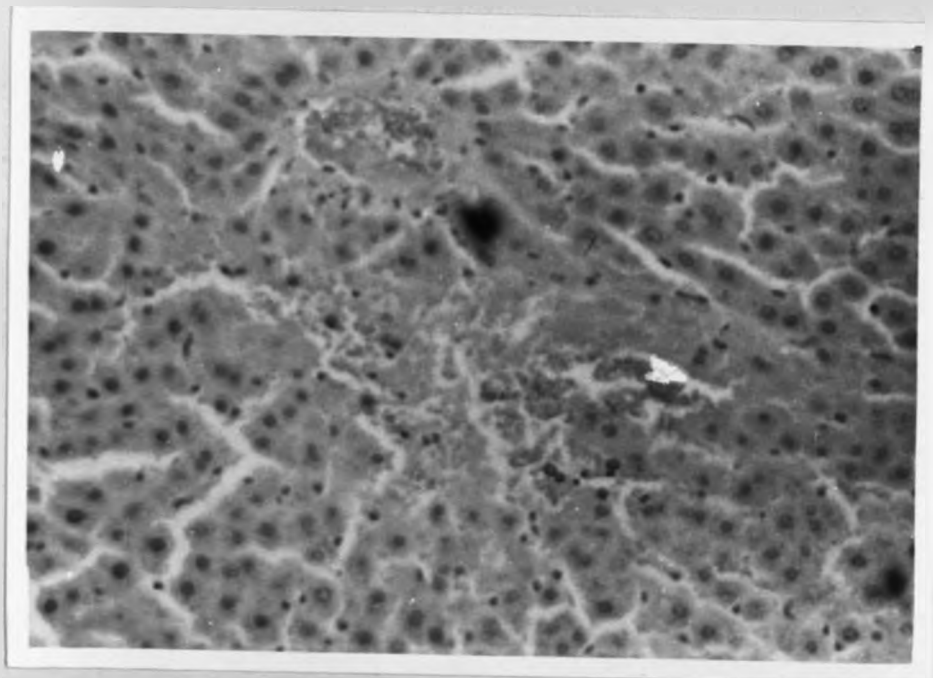


Fig. 15. Necrosis in the centrolobular area of a liver of a rat fed tannic acid intragastrically on 3 consecutive days X 156.

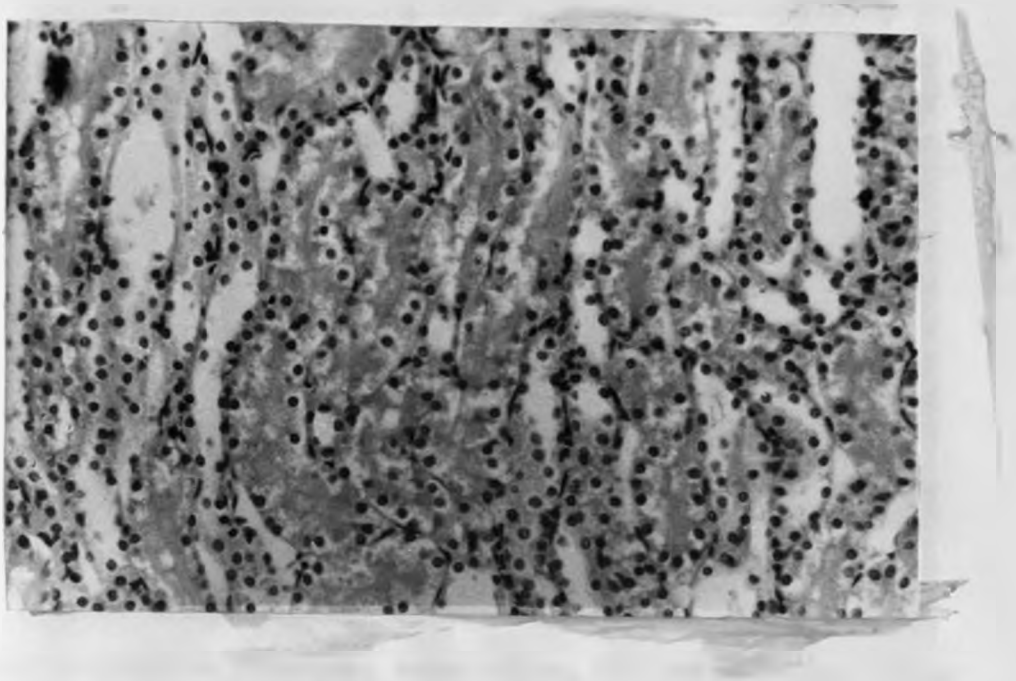


Fig. 16. Coagulative necrosis and vacuolation in the renal proximal convoluted tubules in rat fed 50% whole Catha material for 3 days. X 156.

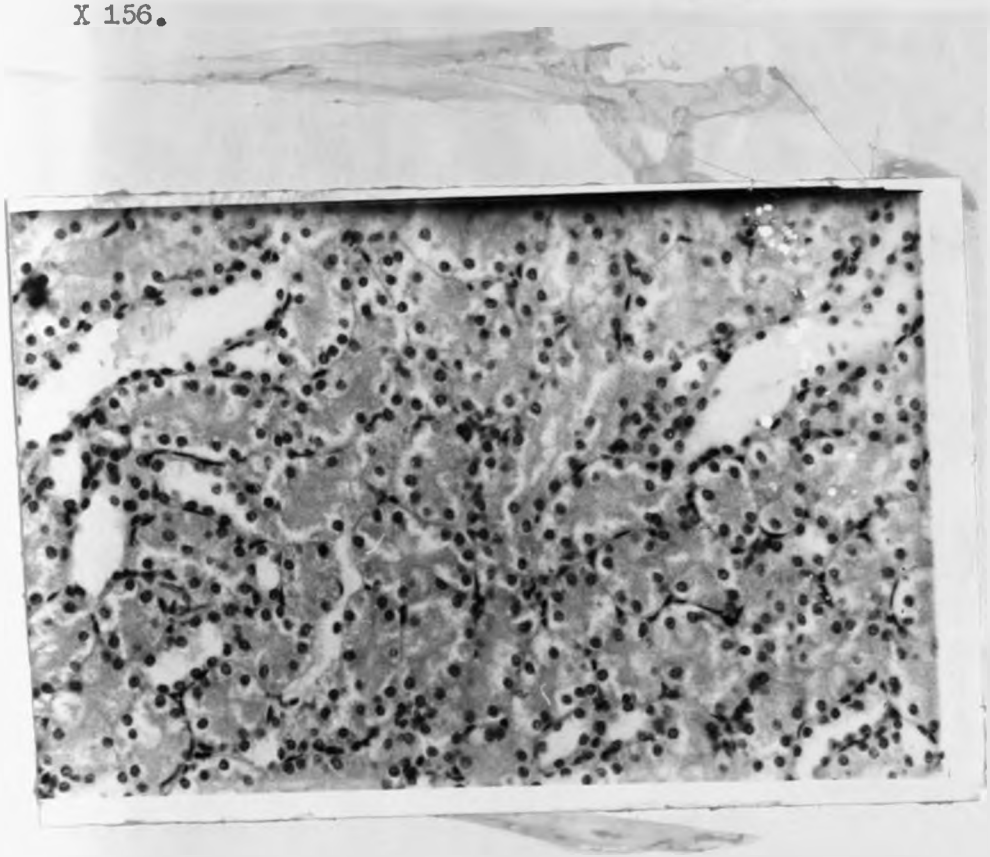


Fig. 17. Extensive Coagulative necrosis and vacuolation in renal proximal convoluted tubules in rat fed 25% whole Catha material for 14 days. X 156

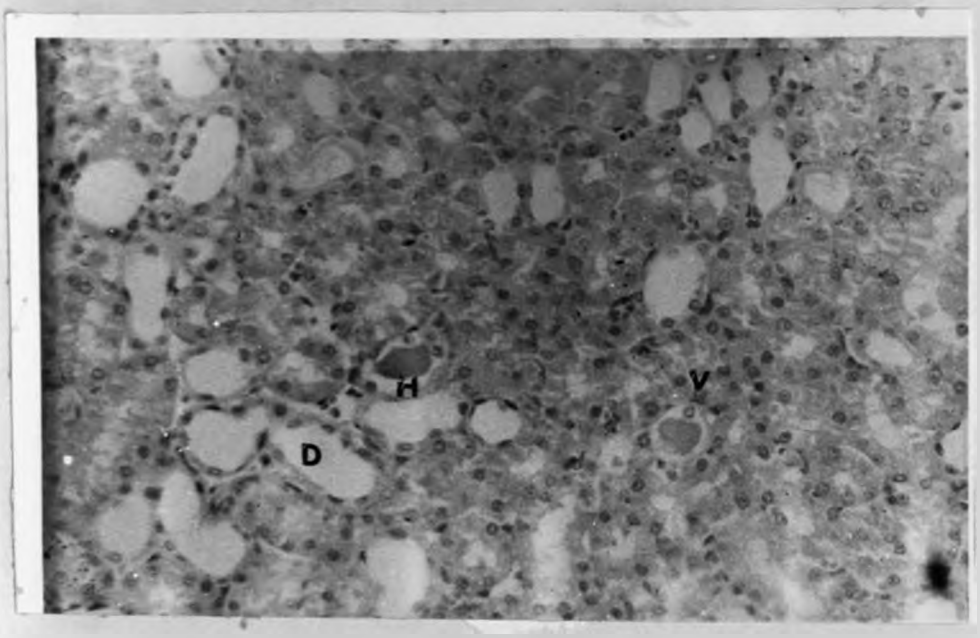


Fig. 18. Coagulative necrosis, vacuolation, (V) desquamation (D) and hyaline cast formation (H) in renal proximal convoluted tubules in rat fed 25% whole Catha material for 18 days. X 156.

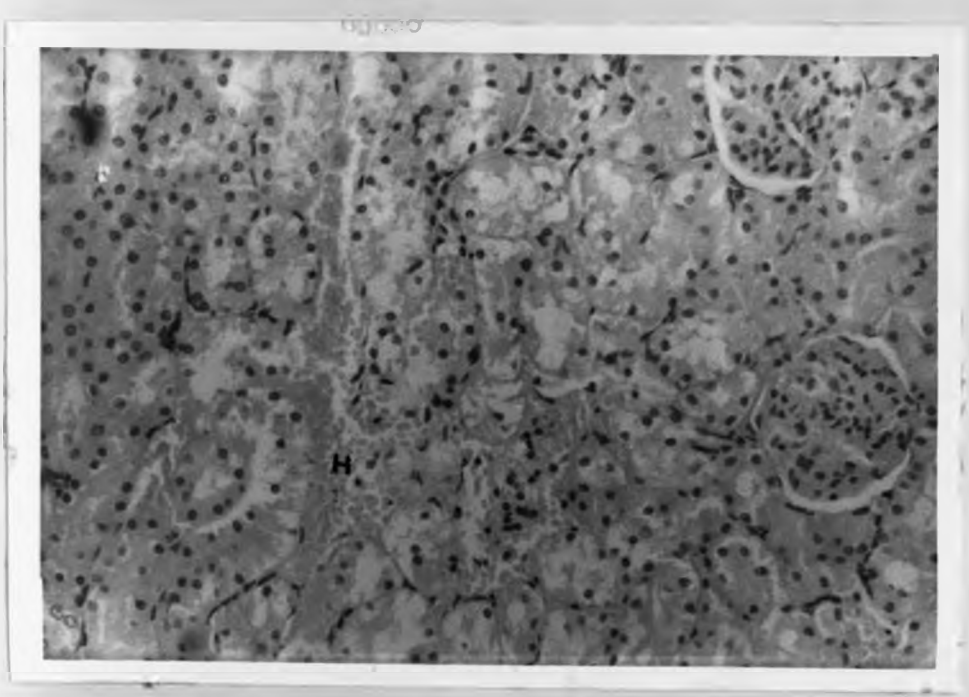


Fig. 19. Coagulative necrosis and haemorrhage (H) in renal cortex in rat fed 15% whole Catha material for two months. X 156.

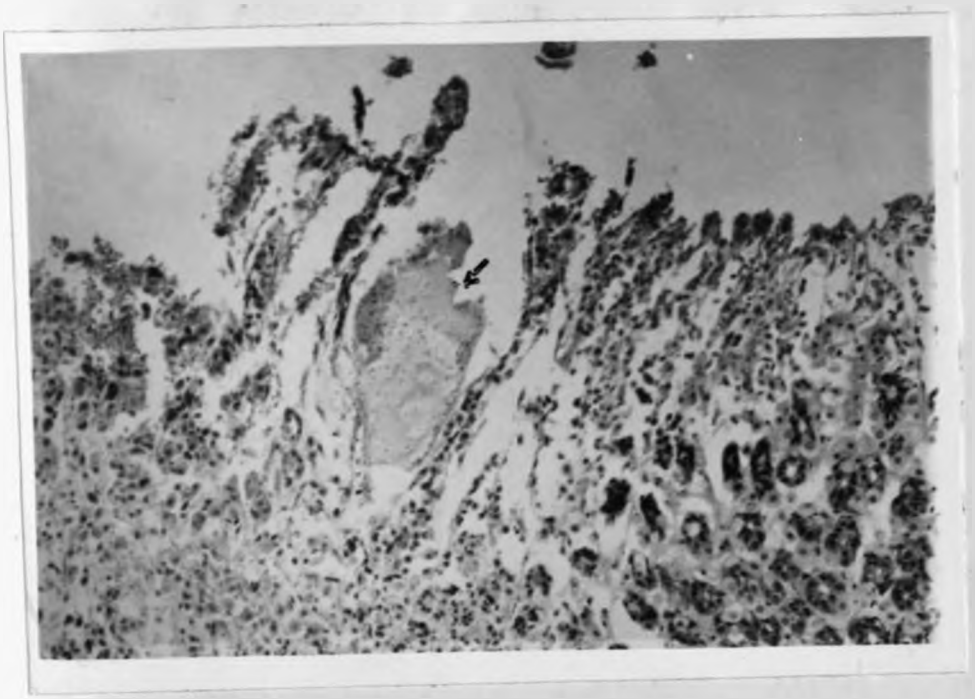


Fig. 20. Ulcerative gastritis in a rat fed 50% whole Catha material for 2 days. (arrow) X 156.

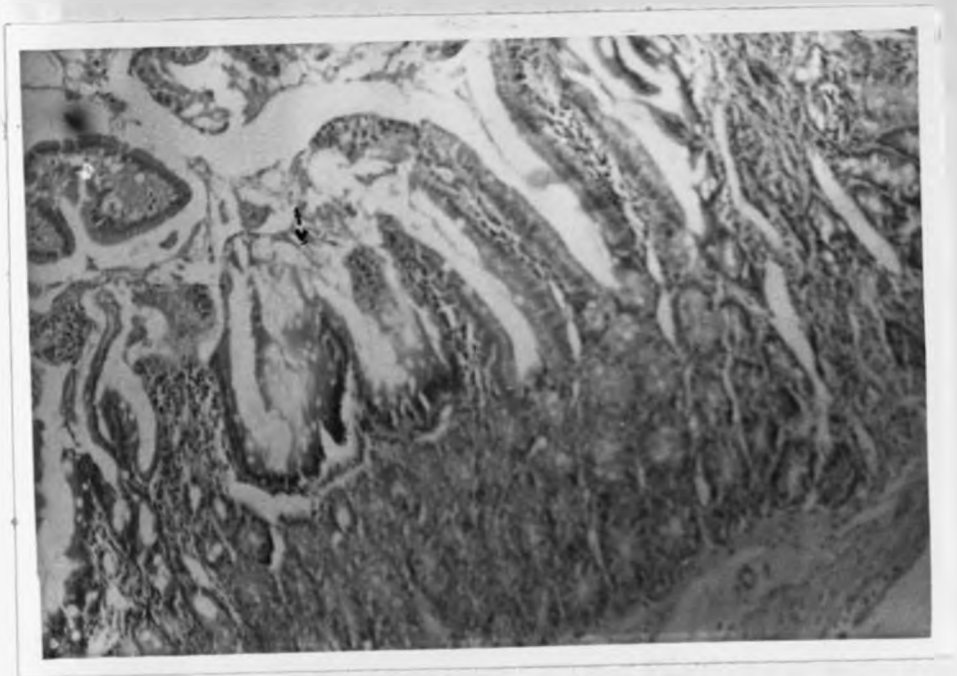


Fig. 21. Ulcerative duodenitis in a rat fed 25% whole Catha material for 2 days (arrow) X 156.

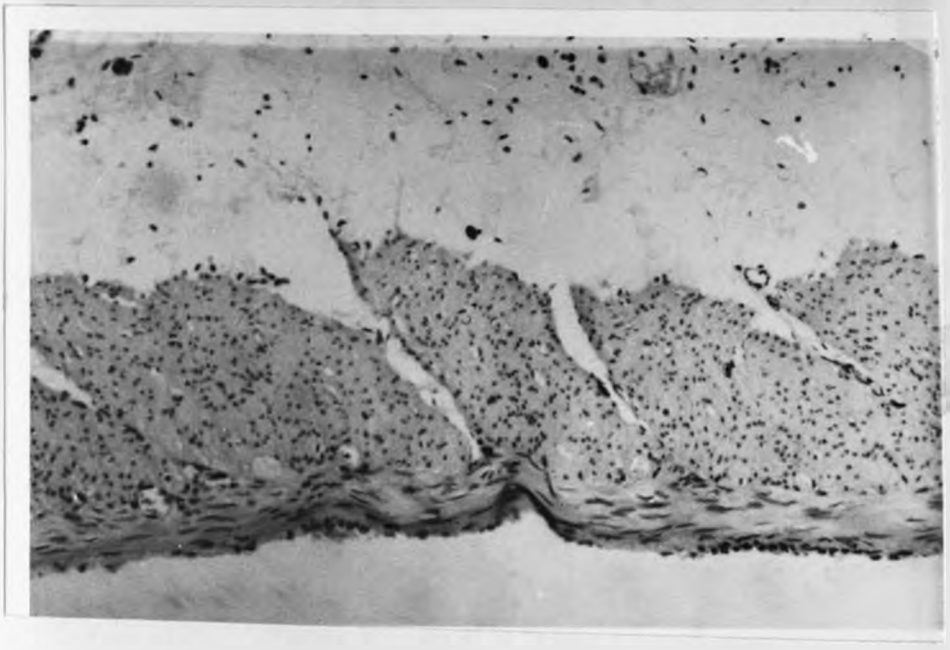


Fig. 22. Oedema in submucosa of a rat fed 25% Catha extract for 2 months. X 156.

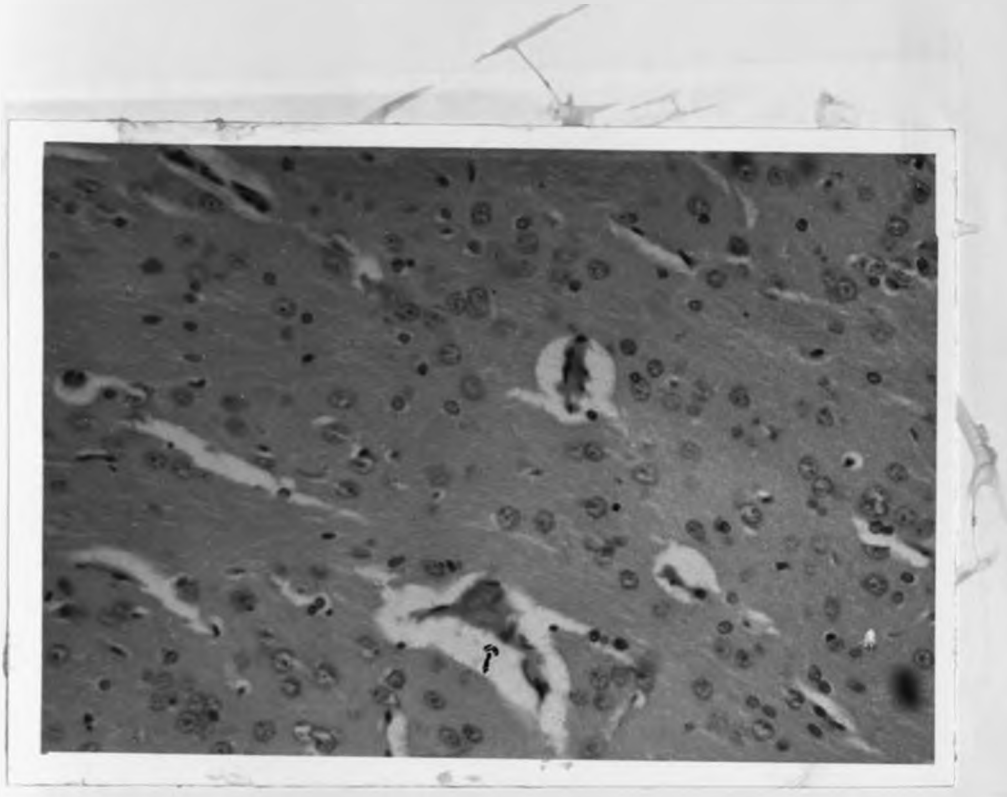


Fig. 23. Perivascular oedema in the brain of a rat fed 25% *Catha* extract for 2 months (arrow) X 391.

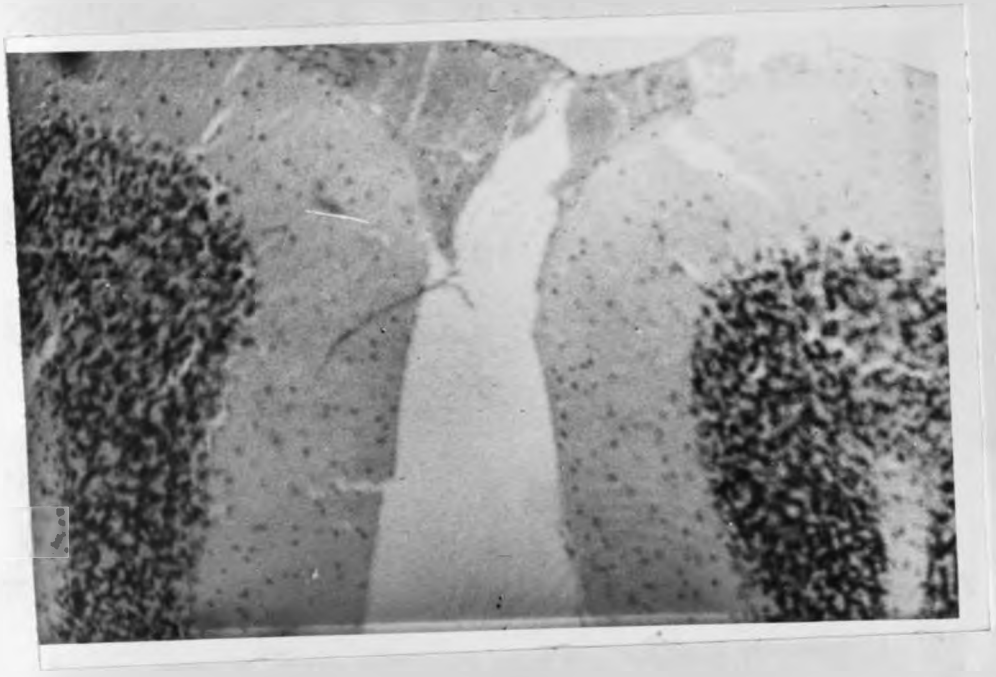


Fig. 24. Congestion in the meninges of a rat fed 25% Catha extract for 3 months. X 391.

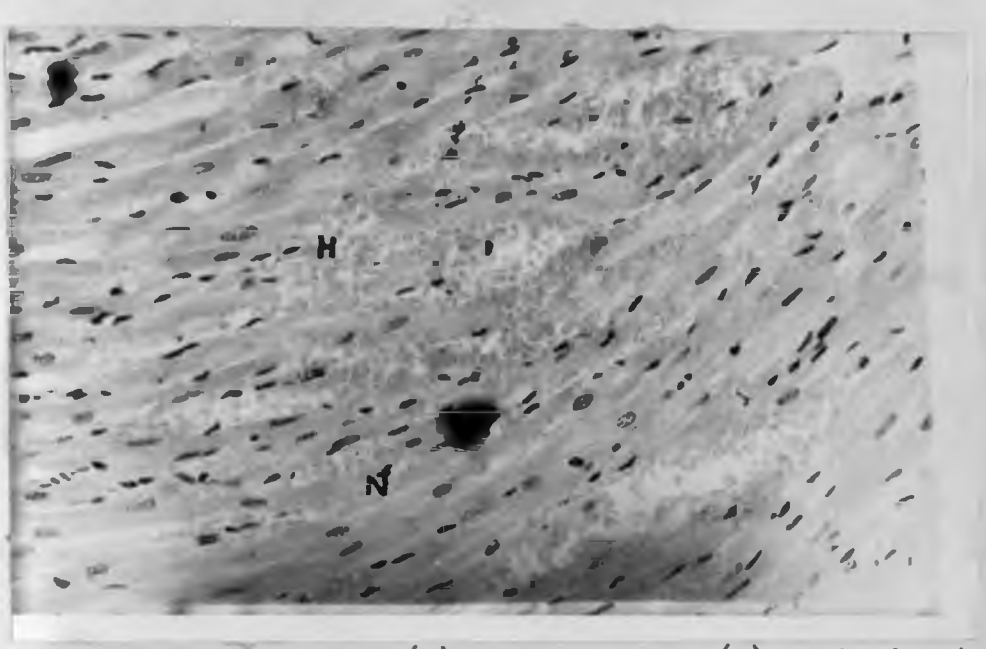


Fig. 25. Coagulative necrosis (N) and hemorrhage (H) in the heart muscle of a rat fed 25% Catha extract for three months. X 156.

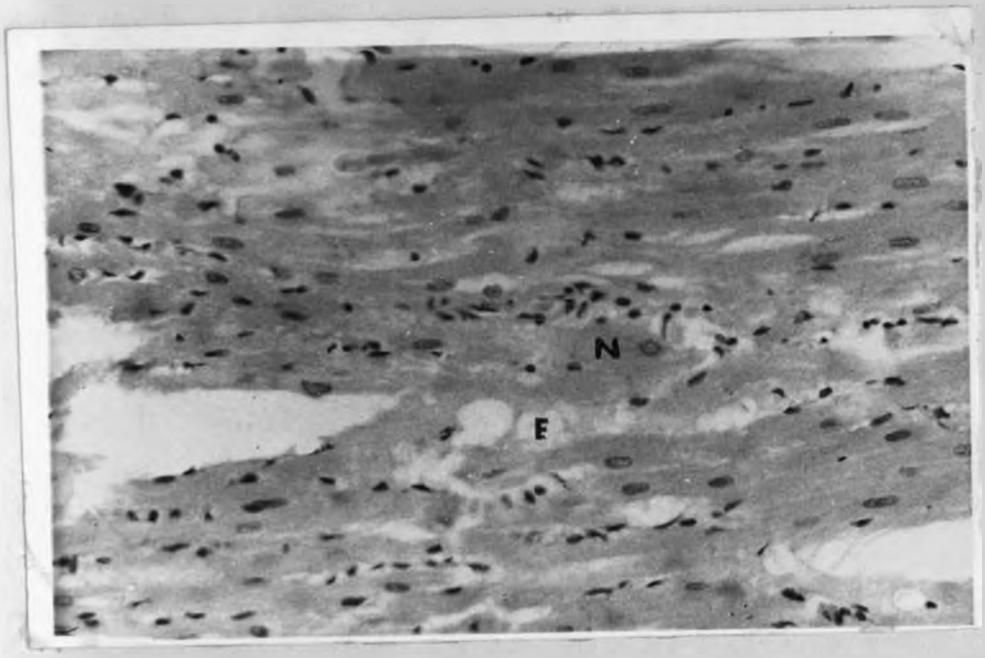


Fig. 26. Coagulative necrosis (N) and oedema (E) in heart muscle of a rat fed 15% Catha extract for 4 month. X 156.

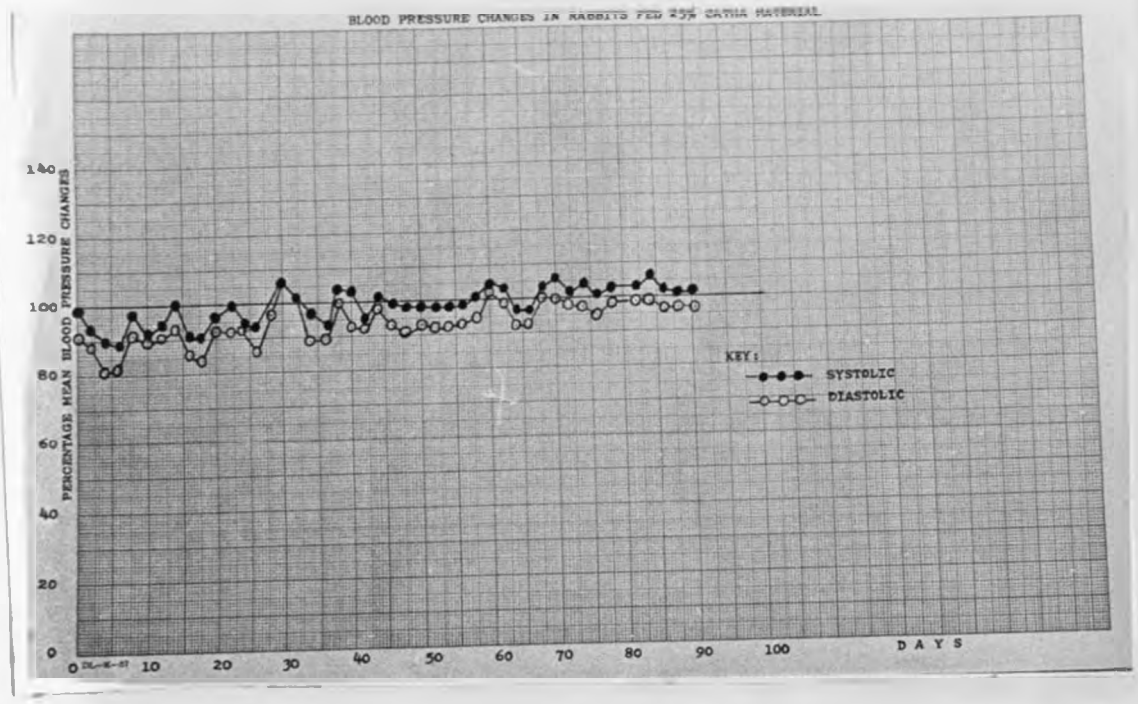


Fig. 27. Blood pressure changes in rabbits fed 25% Catha extract for more than ninety days.

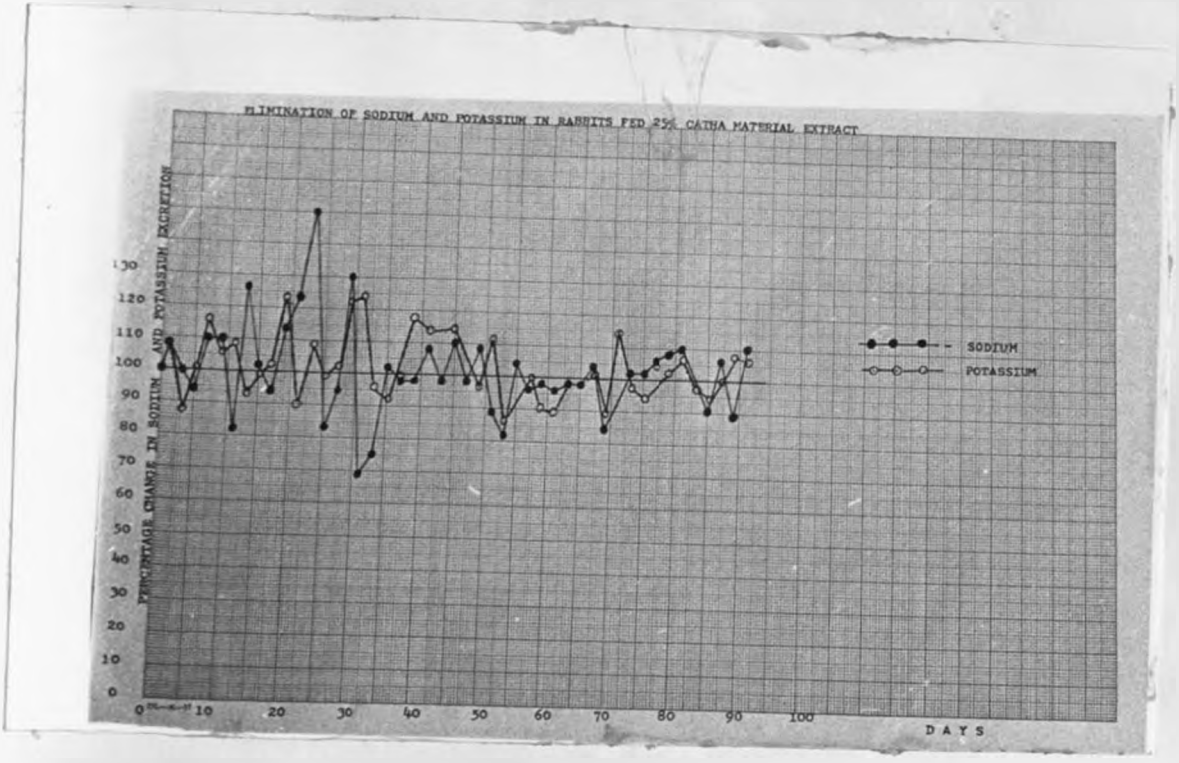


Fig. 28. Elimination of sodium and potassium in rabbits fed 25% Catha extract for more than ninety days.

PART III THE PHARMACOLOGY OF CATHA EDULIS.

INTRODUCTION

Chemical work on Catha edulis using chromatographic (TLC and GLC) and spectrophotometric (IR) techniques has shown that only one alkaloid is present in the material in appreciable amount. This does not eliminate the possibility of others being present in trace amounts below the limit of detection. Indeed, a consideration of biogenesis of d-norpseudoephedrine, shows that other basic compounds could be present. For example, methylation of primary amino and hydroxyl groups occur readily in living tissue of both animals and plants. Methylation of primary amino group in d-norpseudoephedrine would give d-pseudo-ephedrine and after isomerisation, l-ephedrine. None of these two alkaloids, whose chemical structure closely parallels that of d-norpseudoephedrine, was detected in Catha material.

Tannins which are present in Catha material are not readily absorbed unless consumed in large amounts and their systemic (pharmacological) effects when given orally with food, is not appreciable.

The other chemical compounds isolated from Catha material (amino acids, ascorbic acid, mineral elements and choline base) are present in very small amounts and since they are widely distributed in food of animal and plant origin, their possible contribution to the pharmacology of Catha material can be considered minimal.

There is therefore, some justification in assuming that the effect of Catha material on physiological systems (e.g. CNS and cardiovascular system) can almost wholly be attributed to d-norpseudoephedrine. Accordingly, the present study on the pharmacology of Catha material is focussed mainly on this alkaloid. Furthermore, the purpose of the present study was to provide pharmacological data which relates to toxicity of Catha material. For example, the effect of d-norpseudoephedrine on the smooth musculature of the GIT might influence the motility and hence the effective contact time between the tannins and the mucosal epithelium. This variation in contact time could have a profound bearing on the consequent toxicity of Catha material in the gastrointestinal tract.

A literature survey has revealed that published work on the pharmacology of ephedrine and isomers is confusing and often contradictory, For example, both ephedrine and d-pseudoephedrine have been shown to cause bronchodilation and bronchoconstriction (Aviado, Wnuk and De Beer 1959). The two compounds, have also been shown to have positive and negative ionotropic effects.

In the study of d-norpseudoephedrine, both compounds have been included for comparison purposes because they are structurally related to d-norpseudoephedrine.

1. DETERMINATION OF LD50 FOR D-NORPSEUDOEPHEDRINE IN MICE.

INTRODUCTION

The Median Lethal Dose (LD50) is an important parameter in acute toxicity studies of any drug. A determination of Median Lethal Dose and Median Effective Dose (ED50) gives the therapeutic index which is a measure of safety margin and hence indicates the usefulness of a drug.

Determination of LD50 is easy because it is based on all-or-none response. In contrast, the ED50 is often difficult to determine because the desired effect is usually non-specific or not easy to measure. For example, the dose of d-norpseudoephedrine required to give a certain level of CNS stimulation would be difficult to determine.

MATERIAL AND METHODS.

A group of 24 male albino mice (4 litters) were obtained from National Public Health Laboratories, Nairobi, and divided at random into 4 groups of six. They were injected intraperitoneally with a graded dose of d-norpseudoephedrine and returned to the cage (six in a cage) at room temperature, approximately 25°C. The mice were observed over a period of 10 hours (8.00 a.m. - 6.00 p.m.) and then left overnight in the animal house.

RESULTS

Preliminary experiments using groups of 2-3 mice had shown a

considerable variation in the lethal dose; some were dying when given 125 mg/kg while others did not die even with 180 mg/kg. There was also an indication of appreciable difference in lethal dose between those mice that were kept in a group and those that were kept singly in individual cages. This is similar to the effect first described by Chance (1946) for amphetamine and which has been confirmed by other research workers.

Results of the experiment are shown below. The method that has found much application in the determination of LD50 is that of Litchfield and Wilcoxon (1949). Using this method, the LD50 for d-norpseudoephedrine in mice was found to be 178 mg/kg.

	GROUP I	GROUP II	GROUP III	GROUP IV
Dose of d-norpseudoephedrine hydrochloride given (mg/kg mouse)	100	150	200	250
Number dying within 10 hr.	0	1	4	6
Number dying within 22 hr.	0	2	5	6
Percentage dying within 22 hr.	0	33.3	83	100

2. EFFECT OF D-NORPSEUDOEPHEDRINE ON CENTRAL NERVOUS SYSTEM.

A. ANTAGONISM OF PENTO BARBITONE-INDUCED NARCOSIS.

INTRODUCTION

The effect of chewing Catha edulis, on the central nervous system is one of stimulation and this was confirmed by virtually all people interviewed during the survey described in the first part of this thesis. Central nervous system stimulation may be manifested in different forms but in nearly all cases, the behavioral change is evident.

Results of comparative studies of the relative potency of various CNS stimulants are often contradictory because they tend to be influenced by many other variables. For example results obtained when the animals are aggregated together deviate considerably from those obtained when the animals are placed each in single cages (Chance, 1946). Similarly, small variations in ambient temperatures have been known to affect results (Askew, 1962). This finding might be of particular relevance to the study of behavioral changes resulting from sonsumption of Catha material in social occassions.

One of the earliest methods employed in studies of central nervous system stimulants is based upon the reduction they produce in the duration of anaesthesia (Trevan, 1939). A major objection to this method is the variable depth of anaesthesia resulting from administration

of a fixed dose of anaesthetic to a group of mice. There is really no precise method of quantitising the degree of anaesthesia other than that based on loss of sensation and reflexes. Despite this short coming, the method has been used extensively to show, in a qualitative manner the CNS stimulating effect of various chemical compounds. In the present experiment, attempt was made to see how effective d-norpseudoephedrine is in antagonising the pentobarbitone-induced narcosis.

MATERIAL AND METHODS.

Male albino white mice from National Public Health Laboratories, Kenya, were used in the experiment. They were alloted at random into four groups of eight. The weight distribution in all groups was not different by more than 20% from the mean value for all the mice. The mice were anaesthetised with an appropriate dose of pentobarbitone which had been determined in preliminary experiments. The dose chosen was that which gave anaesthesia lasting more than 15 minutes but less than 40 minutes in about 80% of the animals. Similarly, two doses of d-norpseudoephedrine were used and these were approximately one quarter and one half the LD_{50} as determined in the first part of this work.

Immediately after induction of narcosis (as shown by loss of righting reflex), d-norpseudoephedrine was administered and the animals observed until the return of righting reflexes. All drugs were administered intra-peritoneally. For the purpose of comparison, the effect of bemegride on pentobarbitone induced narcosis was also studied.

RESULT

Result of the experiment is shown in table 14. The reduction in duration of anaesthesia for experimental mice given d-norpseudoephedrine was not statistically significant even though there was an apparent reduction in duration of anaesthesia, especially in group III, given 100 mg/kg of the drug.

3. EFFECT OF D-NORPSEUDOEPHEDRINE ON SMOOTH MUSCLES

A. ISOLATED RABBIT INTESTINE

INTRODUCTION

Chen, Wu and Henriksen (1929) studied and compared the pharmacological activity of 24 compounds chemically related to ephedrine, including 6 optical isomers of ephedrine and attempted to furnish an explanation of differences in their pharmacological activity on the basis of their structure and stereoisomerism. From this study, they concluded that d-norpseudoephedrine does not give any appreciable effect on the isolated guinea pig intestine. Other research workers have tended to imply that all ephedrine isomers inhibit the motility of isolated intestine. In the present experiment, the effect of d-norpseudoephedrine on isolated rabbit ileum was investigated.

MATERIAL AND METHODS

The rabbit was killed by a blow to the head, and the abdomen opened immediately. A few pieces of ileum were cut and washed with warm Tyrode solution. The ileum was then set up in an organ bath containing oxygenated Tyrode solution and thermostatically controlled at 37°C (Fig. 29). The contractions (motility) of the isolated intestine were recorded on a rotating smoked drum (kymograph). After the contractions had become uniform, d-norpseudoephedrine was added into the 30 ml. organ bath and the recording continued for about 3 minutes or

until the observed effect had reached a maximum. The effect of amphetamine, d-pseudoephedrine and l-ephedrine on the isolated intestine was also observed.

RESULTS.

Typical responses of d-norpseudoephedrine, amphetamine d-pseudo-ephedrine and l-ephedrine are as shown on Fig. 30.

Attempt to photograph the tracings on the original furnished smoked paper gave poor contrast even though attempt was made to smoke the drum as evenly as possible. To overcome this problem, the tracings were transfered on to a white opaque paper and photographed.

In all cases, the four drugs had the same effect i.e. decreasing the motility and tone of the intestine. There was no indication of tachyphylaxis when the drugs were given repeatedly, provided the preparation was washed properly and allowed to rest for an adequate period.

B. EFFECT OF D-NORPSEUDOEPHEDRINE ON ISOLATED RAT UTERUS

INTRODUCTION

Chen, Wu and Henriksen (1929) studied the effect of ephedrine and several isomers on isolated guinea pig uteri and concluded that the drugs, which also included d-norpseudoephedrine caused increased contraction of the uteri.

The responses of the uterine muscle to various adrenergic drugs vary with species, phase of sexual cycle, state of gestation and the dose given. For example epinephrine the "prototype" adrenergic drug contracts strips of pregnant or non-pregnant human uterus *in vitro* in any effective concentraion, but *in situ* the effect depend on the stage of uterus cycle. The same drug relaxes the non-pregnant uterus and contracts the pregnant isolated cat uterus. In contrast ephedrine relaxes human uterus *in vivo* and has been used to relieve pain of dysmenorrhea. Published results by other workers also show that experimental data on the uterus of one species can not be extraporated to predict probable effect in another species.

In the present work, an attempt was made to obtain strips of human uterus so as to study the effect of d-norpseudoephedrine but it was unsuccessful. The drug was therefore tested on the isolated rat uterus, which is the most commonly used species in testing the effect of drugs on the uterus.

MATERIAL AND METHODS

A non-pregnant rat, about two months old, was used to provide a uterine horn. The animal had been given a small dose of stilboesterol (approximately 0.5 mg intramuscularly) in hope of increasing sensitivity of the uterus.

The rat was killed with a blow on the back of the head and the abdomen opened immediately. One uterine horn was dissected and a piece, approximately 2 cm, set up in the same way as the isolated intestine (Fig. 29). The bathing solution was a modified Ringer-Locke solution (de Jalons 1945) which differed from ordinary Ringer-Locke solution in containing only half the normal amount of glucose and one quarter the usual amount of calcium chloride. Thus 10 litres contain 90g of NaCl, 42g of KCl, 0.6g of $CaCl_2$, 5g of glucose and 5g of $NaHCO_3$. The effect of reducing calcium chloride is to cut down on the spontaneous activity of the uterus.

RESULTS.

The effect of d-norpseudoephedrine on the isolated rat uterus was to relax the preparation (Fig. 31). Attempt to photograph the original furnished smoked paper gave poor contrast. Consequently, the tracing was transferred on to a translucent white paper and phtographed.

The response of the uterus to l-ephedrine, d-pseudoephedrine and amphetamine were qualitatively similar to that of d-norpseudoephedrine.

C. ISOLATED TRACHEA OF GUINEA PIG.

INTRODUCTION

Both l-ephedrine and d-pseudoephedrine are used as brochiodilators in asthma therapy but d-norpseudoephedrine is not. Although the latter is considered ineffective in asthma therapy (Canes Chemical Works), the

author could find no reference to experimental work in which it has been discredited. Information received from those who chew the tender twigs of Catha material indicate that it is of some value in relieving the distressing symptoms of asthma, and it was considered likely that this effect might, in part, be due to d-norpseudoephedrine.

Jameson (1962) has discussed the relative merits of various methods used in the estimation of pharmacologically active compounds on smooth musculature of the respiratory tract. By using a simple volume displacement method, he was able to screen and assay various drugs on the isolated whole trachea. This method has been used to compare the effect of l-ephedrine, d-pseudoephedrine and d-norpseudoephedrine.

MATERIAL AND METHOD

The guinea pig was killed by a blow to the head, the trachea removed immediately and placed in oxygenated, warm Tyrode solution. The whole trachea, usually 4-5 cm long, was dissected free of extraneous tissue and set as shown in Fig. 32. One end was tied tightly around the tapering end of the capillary tubing, while the other was tied to the short end of a U-shaped tube. A syringe filled with Tyrode solution and connected to the U-tube was used to flush the system through until all air bubbles were eliminated. The trachea was then adjusted so as to remove the slackness without stretching it unduly. The temperature of the organ bath was thermostatically controlled at 37°C and Tyrode

solution aerated with 95% oxygen and 5% carbon dioxide. After about 20 minutes, the level of Tyrode in the capillary tube was adjusted to zero mark. The drug was then added into the organ bath (30ml capacity) and the level of Tyrode in the capillary read after every 30 seconds. A fall of 15mm corresponded to a volume decrease of $2x10^{-2}$cc. It was therefore possible to detect a change in volume of $4x10^{-4}$cc.

RESULTS.

Preliminary work had shown that there was diminution in response to repeated dose of l-ephedrine and d-pseudoephedrine. Consequently the quantitative responses of both drugs could not be compared on the same preparation.

In all cases, the maximum response was obtained after 5-6 minutes; thereafter, the change was minimal. Results of the experiment are given in table 15. The recorded readings are those observed after 5-6 minutes. A positive sign indicate a rise while a negative one indicate a fall in the level of manometer.

D. EFFECT OF D-NORPSEUDOEPHEDRINE ON THE SMOOTH MUSCLE OF THE EYE.

INTRODUCTION

The effect of d-norpseudoephedrine on the smooth musculature of the eye was investigated by Chen, Wu and Henriksen (1929) using rabbits. They observed that the drug causes mydriasis. The effect has been confirmed in human beings by other workers (Alles, Fairchild and Jensen,

1961; Le bras and Fretillere, 1965).

Chen et al (1929) attempted a comparative study of 0.05 M solutions of hydrochlorides of d-norpseudoephedrine, l-ephedrine and d-pseudo-ephedrine and observed that the first two were equipotent and more than the last one. On the basis of structural consideration, and in view of the results obtained with isolated intestine and trachea this finding can not be justified from purely theoretical consideration. It was therefore decided to repeat the experiment so as to confirm or refute these findings. In addition to the three drugs, amphetamine was also included.

MATERIAL AND METHODS

The left eye of white albino New Zealand rabbits was irrigated with standard solutions of the four drugs. The effect on the eye was observed until the maximum effect was obtained. Usually, pupil diameter was determined at intervals of 5-10 minutes so as to follow the progressive development of the effect. In all cases, comparison was made with the control right eye.

RESULTS

Results of the experiment are shown in table 16. In all cases, d-pseudoephedrine gave the least response, while amphetamine gave the greatest response. There was a difference in the onset of effect and time to maximum response between the four drugs tested. The maximum

response was manifested after a very short time in the case of amphetamine while it took a long time for the maximum effect of d-norpseudoephedrine hydrochloride to be manifested. The difference in the onset and time to maximum effect of the drugs is probably related to their solubility in water. In all four drugs, reflex to light was not abolished.

EFFECT OF D-NORPSEUDOEPHEDRINE ON THE CARDIOVASCULAR SYSTEM.

A. Effect on blood pressure of anaesthetised rats

INTRODUCTION

Chen Wu and Henriksen (1929) studied the effect of d-norpseudoephedrine in cardiovascular system using the pithed cat method and observed that it causes a rise in blood pressure, which is less than that caused by an equimolecular dose of l-ephedrine but more than that of d-pseudoephedrine. Chen et al. (1929) makes no reference to tachyphylaxis, the well known phenomenon associated with ephedrine isomers. The author is not aware of any other published work in which the three drugs have been compared with respect to their effect on blood pressure. In the present experiment the effects of the three drugs, on the blood pressure, were compared using anaesthetised rats.

MATERIAL AND METHODS

Male albino rats were anesthetised with urethane (ethyl carbamate). The jugular vein was cannulated for the purpose of administering the

drug. The carotid artery was also cannulated and connected to a recorder (Beckmann type R). As a precaution, to stop possible clotting of blood, the latter cannula was filled with heparinised saline. Before giving the drug, a normal blood pressure tracing was obtained. After giving the drug, normal saline was also injected through the system to push the drug and the response was recorded.

RESULTS:

The effect of administering d-norpseudoephedrine was to cause a rise in blood pressure. When 1 mg of the compound was administered, a rise of 20-40 mm Hg was recorded. Repeated administration of the drug caused a diminution in response such that after about 4 doses of d-norpseudoephedrine, given at 10-15 minutes intervals, little or no response could be elicited by administration of further doses. The diminution in pressor response with repeated doses of d-norpseudoephedrine examplifies the phenomenon of tachyphylaxis.

Because of the phenomenon of tachyphylaxis, no valid comparison between various ephedrine isomers could be made on the same rat. However, there was no appreciable difference in pressor responses of l-ephedrine, d-pseudoephedrine and d-norpseudoephedrine when the experiment was repeated using equimolecular doses of the drugs on different rats. In all cases, a transient rise of 20-40 mm Hg in mean blood pressure was recorded when approximately 1 mg of each drug was

given. The sharp rise in blood pressure lasted approximately 90 sec. and this was followed by a steep and later a slow fall to a value slightly less than the starting one.

B. Effect of d-norpseudoephedrine on isolated heart preparation.

INTRODUCTION

A survey of literature has revealed some inconsistency and uncertainty with regard to the effect of ephedrine and d-pseudoephedrine on the heart. Most text bookscategorically state that ephedrine has a positive inotropic and chronotropic effect on the heart. However there are several reports in which negative inotropic effect or a dual effect on myocardial force of contraction was elicited (Aviado 1957; consistently,). No reference to the effect of d-norpseudoephedrine on isolated heart was encountered during a literature survey.

In the present work, the effect of l-ephedrine, d-pseudoephedrine and d-norpseudoephedrine has been investigated using the classical Langendorrf heart preparation

MATERIAL AND METHODS

The rabbit was killed with a blow at the back of the neck, and the heart removed immediately and placed in a dish of Tyrode solution at room temperature. The heart was squeezed several times so as to remove as much blood as possible.

After dissecting the aorta free, the heart was transferred to the perfusion apparatus (Fig 33) where the aorta was tied onto the glass cannula and perfused with Tyrode solution at 37°C. A balloon was inserted into the heart and then filled with Tyrode solution through a short glass tubing attached to a needle. This was then connected to recorder (Beckmann Type R).

The perfusion pressure was adjusted to 50-60 mm Hg. A normal trace was always obtained before administering the drug through the rubber cap into the perfusion fluid.

RESULTS

It is of course difficult to state with certainty the concentration of drug reaching the heart, since the perfusion fluid is flowing continuously through the heart, However, provided the rate of injection and the perfusion pressure are constant, the concentration of the drug would very likely attain the same maximum concentration thus giving reproducible results. It took about 15 seconds before the drug reached the heart and the effect passed off in a few minutes as the drug was washed through.

Typical heart response to d-norpseudoephedrine, and l-ephedrine are shown in Fig. 34. Epinepherine was given at the end of the experiment to check on the normal response of the heart. Several doses of ephedrine and the related compounds were tried and in all cases the qualitative response was the same, i.e. a negative inotropic effect with no obvious

change in chronotropic effect. There was no indication of tachyphylaxis when the three drugs were administered repeatedly. Hearts obtained from reserpinised rabbits gave similar responses and attempt to block the response with either alpha or beta adrenolytic drugs or antihistaminics was unsuccessful.

C. Effect of d-norpseudoephedrine on a vascular bed:

Rat hind quarter.

INTRODUCTION

The two important components of the cardiovascular system which account for changes in the blood pressure of an intact animal are the changes affecting the heart (inotropic and chronotropic effects) and those affecting the peripheral blood vessles.

For most drugs, the resultant changes in blood pressure closely parallel those on peripheral blood vessels. For example, isoproterenol has a pronounced positive inotropic and chronotropic effect but because of the vasodilation effect the net result when administered to an intact animal is to cause a fall in blood pressure.

In the present work, d-norpseudoephedrine was found to give a negative ionotropic effect but the net effect when administered to anaesthetised rats was to cause a rise in blood pressure. It was therefore postulated that the compound would have a vasoconstrictor

effect if tested on a suitable vascular bed. Among the vascular beds that have been used to study the interaction of vasoactive drugs with the smooth muscle of peripheral vessels are the following: perfused rat hinquarters (Fastier and Smirk, 1943), perfused rat tail (Wade and Beilin, 1970), and the central ear artery of the rabbit (Burn 1952).

An ideal vascular resistance model should be easy to set up, show stable resistance over a reasonable period of time and should be sensitive to vasoactive substances with reproducible responses. Of the many vascular beds considered, that of the rat hindquarter appeared to be the most suitable especially because of the ease of setting up the experiment and the simplicity of the apparatus required.

A survey of literature showed that the effect of d-norpseudoephedrine on the peripheral blood vessels had not been studied and accordingly, the rat hindquarters was used to study the effect of this drug.

MATERIAL AND METHODS

The rat was killed with a blow to the head and the abdomen opened so as to allow the cannulation of abdominal aorta. The rat was then cut into two and the fore part discarded, leaving the hindquarters. The hindquarters was then perfused at a constant pressure of 60 mm Hg using a set-up similar to the Langendorff preparation (Fig. 35). The perfusion rate was determined by collecting and measuring the volume of perfusate over a one-minute interval. For control purposes, the system was

considered stabilised when five consecutive readings were consistent. The drug was then injected into the rubber cap and the change in flow rate determined. A change in flow rate was indicated by a change in the volume of perfusate collected and in perfusion pressure due to a corresponding change in the peripheral vessels resistance. For example a decrease in volume of perfusate accompanied by an increase in perfusion pressure indicated a vasoconstrictor effect.

RESULTS:

When d-norpseudoephedrine was given, the volume of perfusate collected over a one-minute period decreased appreciably and this was accompanied by an increase in perfusion pressure (table 17). Tachyphylaxis was evident after a very short period.

Results obtained with l-ephedrine and pseudoephedrine were qualitatively similar to that obtained with d-norpseudoepherine.

The normal response of the preparations were checked at the end of each experiment by administering adrenaline (a vasoconstrictor) and isoproterenol (a vasodilator).

DISCUSSION ON PHARMACOLOGY OF CATHA EDULIS.

Results obtained in the present work and those of other research workers (Alles, Fairchild and Jensen 1961) show that the pharmacology of the active principle of Catha edulis closely follow that of indirectly acting adrenergic drugs e.g. ephedrine and amphetamine. The difference is merely quantitative.

Like ephedrine and amphetamine, d-norpseudoephedrine has been shown to inhibit monoamine oxidase enzyme (Eldin 1968). Development of tachyphylaxis in anaesthetised rats and in rat hindquarters preparation suggest that it might be acting indirectly, possibly by depleting catecholamines from the storage sites. D-norpseudoephedrine relaxed rat uterus and this is probably a direct effect since the uterus is very sparsely innervated by the adrenergic nerves. Similarly, the effect on the intestine, where no tachyphylaxis was observed is probably a direct effect on the smooth muscle not mediated through the transmitter substance.

The effect of d-norpseudoephedrine on the isolated guinea pig trachea indicate that it is much less effective than ephedrine and would probably be of little value in astham therapy. The benefitial effect of d-norpseudoephedrine as claimed by asthmatics and several people who chew Miraa might be a consequence of the euphoric properties of the drug. There are several instances where the useful effect associated with a drug is only incidental and unrelated to the actual disorder or disease. A case in point is the initial success of iproniazid in tuberculosis therapy. This drug was though to cure tuberculosis but later the improvement in the clinical picture was shown to be due to its central nervous system effect.

Effect of drugs on the central nervous system is difficult to interpret because the functioning of the central nervous system is poorly understood. Furthermore, most CNS stimulants show no obvious structural similarity and it is possible that they effect the CNS by different mechanisms and possibly by acting at different brain centres.

Pentobarbitone induced narcosis lead to marked depression of the respiratory centre and this is often the cause of death in case of barbiturate poisoning. Phenylalkylamines are not considered to be analeptics and their value in antagonising barbiturate induced respiratory depression is insignificant.

Effect of d-norpseudoephedrine on the isolated heart preparation is difficult to interpret. However, it is unlikely that given orally, the concentration of the drug in blood will attain an appreciable level required to give a depressant effect on the heart of an intact animal, similar to that observed on the isolated heart preparation.

The significance of results obtained in the pharmacological study of d-norpseudoephedrine will be referred to in the general discussion on Catha edulis toxicosis.

Table 14 EFFECT OF D-NORPSEUDOEPHEDRINE ON PENTOBARBITONE INDUCED NARCOSIS IN MICE

	Group I (Control)	Group II	Group III	Group IV
Number of mice	8	8	8	8
Dose of pentobarbitone (mg/kg)	100	100	100	100
D-norpseudoephedrine hydrochloride	-	50	100	-
Benegride (mg/kg)	-	-	-	25
Duration of anaesthesia (Min) Mouse - 1	12	15	8	10
" - 2	14	17	13	11
" - 3	20	23	23	16
" - 4	23	30	24	21
" - 5	31	31	24	23
" - 6	46	37	37	27
" - 7	55	57	41	35
" - 8	died	died	43	37
Average duration for anaesthesia (min)	28.7	30.0	26.6	22.5
S.D.	15.1	13.2	11.9	9.5
S.EM	6.2	5.4	4.5	3.6

TABLE 15. Effect of 1-ephedrine, d-pseudoephedrine and d-norpseudoephedrine on Guinea pig trachea.

DRUG	CONCENTRATION IN ORGAN BATH (mg/ml)	RESPONSE (mm)	MEAN (mm)
D-norpseudoephedrine	0.5	0,+0.5,+0.8,+1.4,+1.5	+0.84
	1.0	0,+0.5,+1.2,+2.2,+4.5,+5.6	+2.3
D-pseudoephedrine hydrochloride	0.5	-2.0,-2.5,-2.8,-3.0,-3.0,-3.5	-2.8
	1.0	-4.5,-4.8,-5.0,-5.4,-5.6,-6.0	-5.2
1-ephedrin hydrochloride	0.5	-3.2,-3.6,-4.0,-4.4,-5.8.	-4.2
	1.0	-6.0,7.6,-8.0,-8.4,-9.0,-9.0.	-8.0

TABLE 16 EFFECT OF D-NORPSEUDOEPHEDRINE, D-PSEUDOEPHEDRINE, L-EPHEDRINE AND AMPHETAMINE ON THE RABBIT EYE

DRUG	MOLARITY OF SOLUTION	MAXIMUM PUPIL DIAMETER OF LEFT EYE	TIME TO MAXIMUM EFFECT	PUPIL DIAMETER OF CONTROL RIGHT EYE
Amphetamine	0.022	10 mm	12 minutes	6 mm
l-ephedrine	0.050	9 mm	15 minutes	6 mm
pseudoephedrine	0.050	7 mm	20-30 minutes	6 mm
d-norpseudoephedrine	0.050	9 mm	40-60 minutes	6 mm

TABLE 17. RESULTS OF PERFUSING RAT HINDQUARTER

DRUG GIVEN	TIME (Min)	VOLUME OF PERFUSATE COLLECTED ml/min.	PERFUSIN PRESSURE	CHANGE IN PERFUSION PRESSURE (mm/Hg)
Control	1	20.5	60mm	-
	2	20.0	60	-
	3	20.2	60	-
	4	20.2	60	-
D-norpseudoe-phedrine (10mg)	5	20.2	60	-
	6	16.5	68	8
	7	12.2	74	14
	8	12.5	74	14
	9	13.2	72	12
	10	14.2	70	10
	11	14.5	70	10
	12	16.0	68	8
	13	16.5	68	8
	14	16.0	68	8
left to perfuse for 5 minutes without collecting the perfusate.				
	20	15.5	69	1
	21	15.0	70	2
D-norpseudoe-phedrine (10mg)	22	15.5	70	2
	23	15.0	70	2
	24	14.5	70	2
	25	13.2	72	4
	26	14.0	70	2
	27	14.8	70	2
	28	14.5	70	2
left to perfuse for 5 minutes without collecting the perfusate				
	34	15.0	69	
	35	14.8	69	
	36	15.0	69	
D-norpseudoe-phedrine(10mg)	37	14.6	70	0
	38	14.4	70	0
	39	14.0	70	0
	40	14.6	69	-1
	41	14.6	69	-1
	42	14.4	69	-1

O.B. - Organ-bath

W - Water at 37°C

S.D. - Smo ed drum

M - Motor

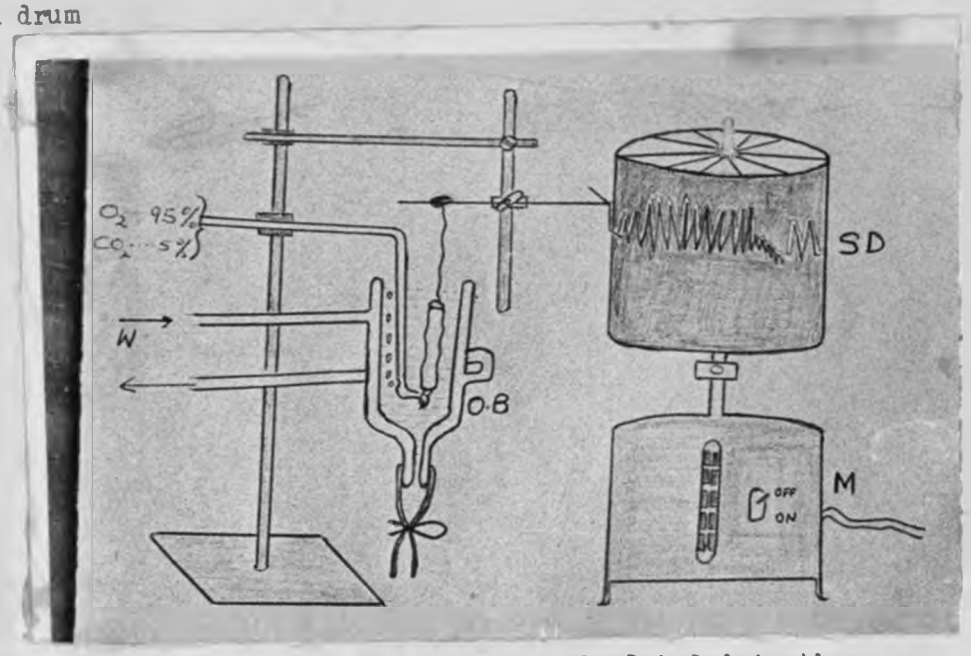


Fig. 29. Apparatus for studying effect of drug on isolated intestine or uterus.

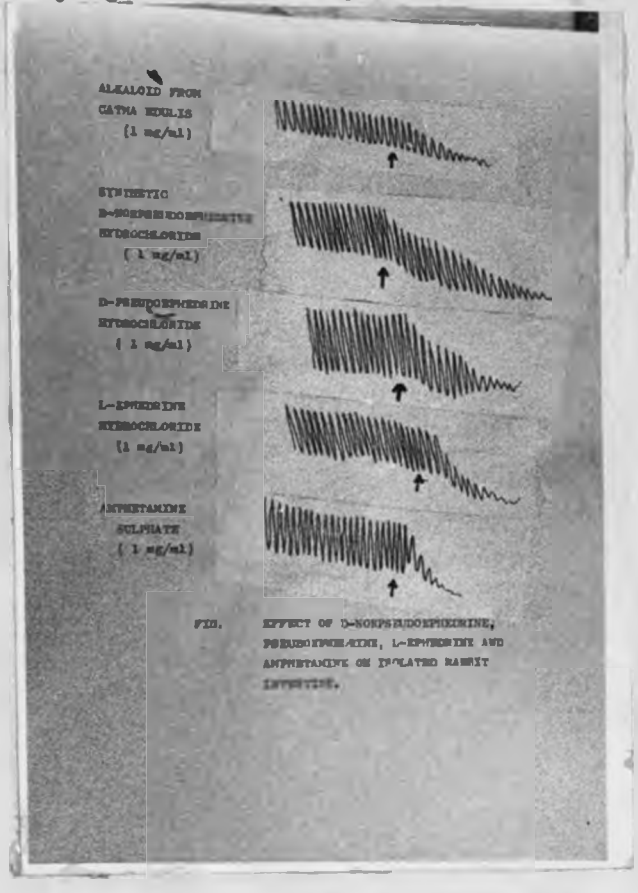


Fig. 30. Effect of phenylalkylamine drugs on isolated rabbit intestine.

A d-norpseudoephedrine
B l-ephedrine
C d-pseudoephedrine

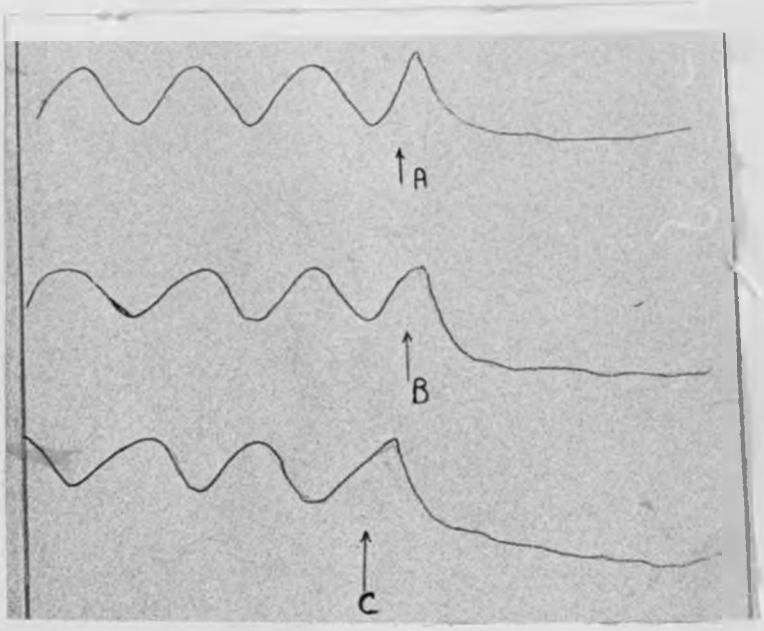


Fig. 31. Effect of d-norpseudoephedrine and related drugs on isolated rat uterus.

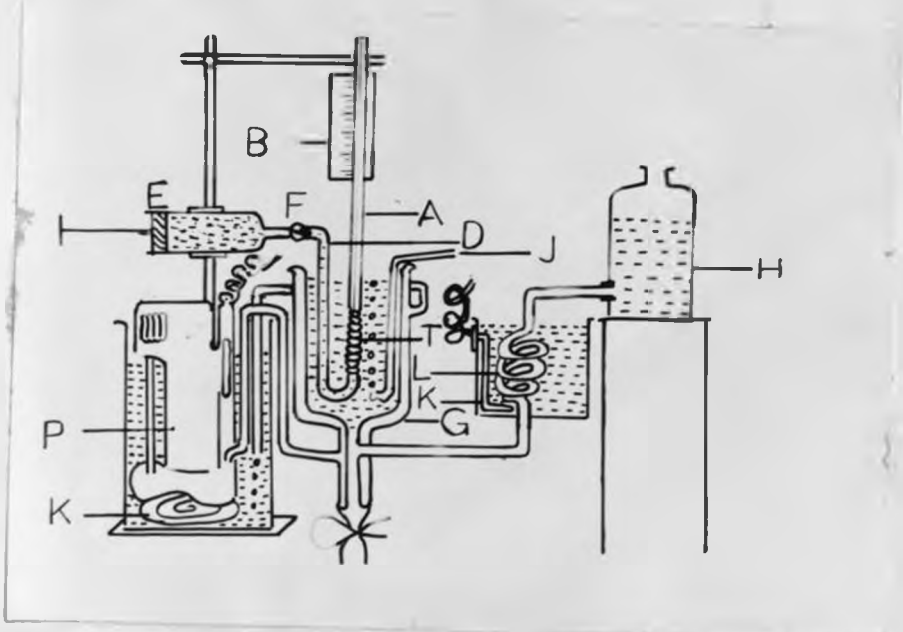


Fig. 32. Apparatus for studying effect of drugs on intact guinea pig trache

A - Capillary tube
B - Scale
D - U - tube
E - Syringe
F - Two way tap
G - Organ bath
H - Container for Tyrode solution.
J - Inlet for Oxygen
K - Heater
L - Glass coils
P - Thermostat
T - Trachea

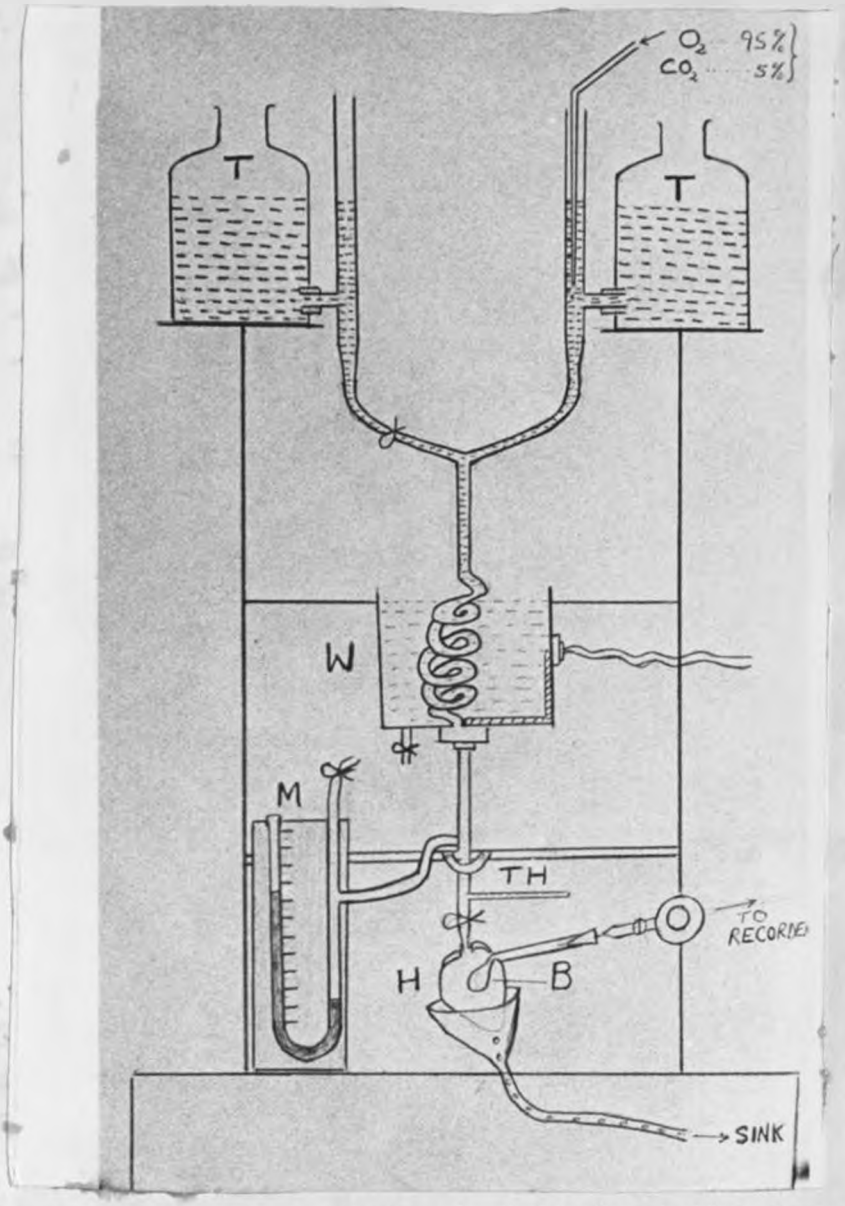


Fig. 33. - Apparatus for perfusing isolated heart with drugs

B - Baloon filled with Tyrode solution.

H - Heart

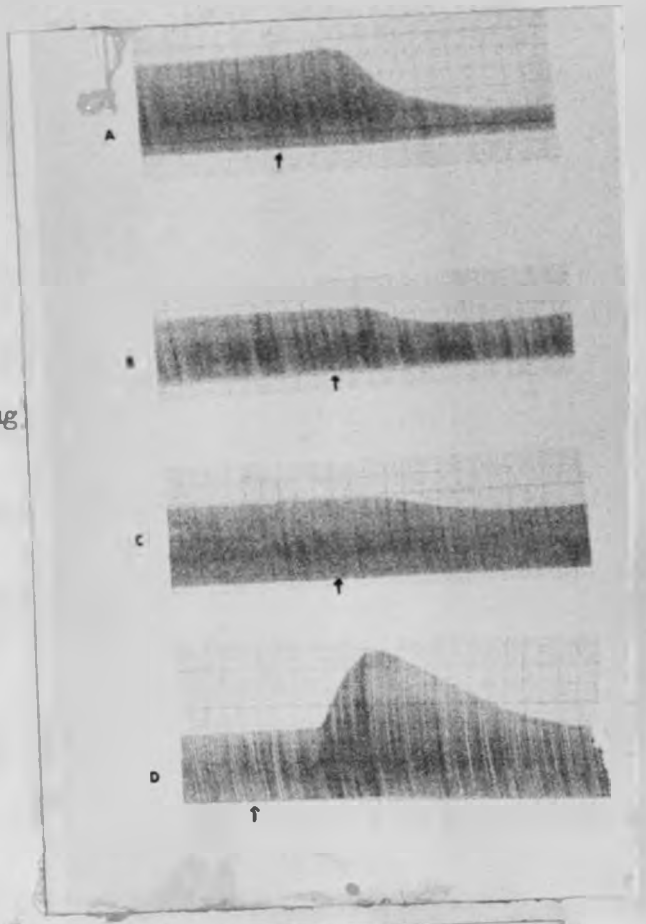
M - Manometor

T - Tyrode solution

TH - Thermometer

W - Water bath, thermostatically controlled at 37°C.

Fig. 34.



Effect of perfusing rabbit heart with drugs.

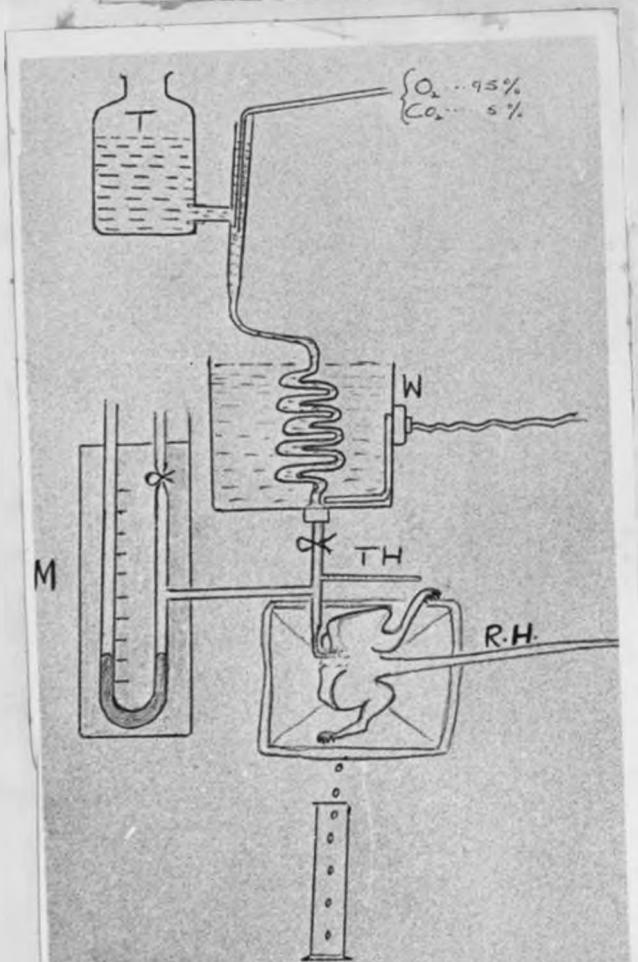
A - L - ephedrine (1mg)

B - D - norpseudoephedrine (1mg (from Catha material)

C - D - pseudoephedrine (1mg)

D - Epinephrine (10 microgrammes)

Fig. 35.



Apparatus for perfusing rat hind quarters

M - Manometer

R.H. - Rat hindquarters

T - Tyrode Solution

T.H. - Thermometer

W - Water bath thermostatically controlled at 37°C.

GENERAL DISCUSSION ON CATHA EDULIS TOXICOSIS

A survey of literature revealed that the most important constituents of Catha material had been identified and many of them estimated quantitatively. It was therefore not the purpose of the present work to undertake an exhaustive chemical analysis of Catha material as this would have served no useful purpose. For example, the nature and amount of tannins present in Catha material is well documented (El. Sissi et al, 1966; Alles, Fairchild and Jensen, 1961) and research by these workers were consistent and in agreement with each other.

In the present work, preliminary experiments to confirm results as reported in literature, were often done but as there was no basis for questioning these results (the author could just as well have quoted literature without confirming them), these confirmatory tests have not been reported here to avoid redundancy. It was, however, evident that a controversy existed as to whether there was more than one basic compound (alkaloid) in Catha material.

Most central stimulants are basic compounds and this might explain why research on Catha material has been directed mostly towards basic constituents. The author could find no reference to published work in which gas liquid chromatographic (GLC) or infrared spectrophotometric techniques have been used to investigate basic constituents present in Catha material and accordingly, these techniques were employed.

In all cases, only d-norpseudoephedrine was detected and this was present in all parts of the plant examined.

Leete (1958) has shown that phenylalanine is the precussor for d-norpseudoephedrine. The exact sequence in the biogenesis of d-norpseudoephedrine was not determined but from purely theoretical considerations, it would appear that there is close similarity between the biogenesis of this compound and catecholamines. A possible pathway in the biogenesis of d-norpseudoephedrine, together with the accepted pathway for the biogenesis of catecholamine, is presented in Fig. 36. The first two steps, i.e. decarboxylation of the alpha carbon and subsequent hydroxylation of beta carbon atoms are easy to justify but the last step methylation of alpha carbon is difficult to justify because it is easier to methylate the amino group directly rather than the carbon atom. Methylation of the amino radical of d-norpseudoephedrine would give d-pseudoepherine, a stereoisomer of l-ephedrine. Both l-ephedrine and d-pseudoephedrine cannot be resolved by chromatographic techniques and it is possible to mistake one for the other. The claim of Ristic and Thomas (1961) is infact not unrealistic, even though this could not be confirmed in the present work, because methylation is a common occurrence in living tissue.

Among the chemical compounds isolated from Catha material are the following: d-norpseudoephedrine, tannins, polyphenols, sugars, mineral

elements, choline, ascorbic acid and several amino acids. Of these compounds, the amino acids, ascorbic acid, sugars, choline base and mineral elements are probably of little toxicological significance since they are widely distributed in food of plant and animal origin. For example, dosages of choline salt, as large as 10g, can be taken orally by man without obvious pharmacodynamic response. The concentration of choline in Catha material is approximately 0.05%.

Tannins are produced in plants from low molecular weight polyphenols by a process of oxidative condensation. These substances, many of them with complex molecular structures, apparently serve to protect the plant from potential infection by tanning the invading parasites (virus, fungi etc.). The chemical and physical properties of tannins have been reviewed by White (1958). Classification of the phlobaphene producing tannins, such as those present in Catha material is difficult (Russel, 1935). The chemical and pharmacological properties of the tannins has been found to vary substantially, possibly due to certain changes occurring in the course of isolation.

Most of the published literature on toxicity of tannic acid date back to the period 1925 and 1945 when the drug was extensively used in the treatment of burns (Barnes and Rossiter, 1943; Cameron, Milton and Allen, 1943; Clarke and Rossiter 1943). At that time, hepatoxicity was frequently observed in people treated for burns with tannic acids;

the most characteristic lesion being centrilobular necrosis. Handler and Baker (1944) fed diets containing 1 and 2.5% tannic acid to rats and found no necrotic lesions in the liver and kidney. They concluded that the astringent action of tannic acid interferes so much with absorption from the digestive mucosa that poisoning by this route is quite unlikely and is even difficult to produce experimentally. Korpassy, Horva and Koltay (1951) showed that tannins can generally be readily absorbed from the gastrointestinal tract leading to liver and adrenal damage. Their work did not attract much attention and is rarely quoted. Since then, tannic acid has continued to be used in diagnostic radiology in barium sulphate formulations to improve the contrast. Toxicity or tannic acid came into the limelight again in early 1960s. Lucke, Hodge and Patt (1963) described a fatal case of liver necrosis in which tannic acid contained in a barium sulphate diagnostic enema was the only known causative agent. Similar cases have also been reported by Mcalister (1963). These reports prompted Boyd, Bereczky and Godi (1965) to investigate the possibility that tannic acid in higher doses might be absorbed from gastrointestinal tract. They administered tannic acid intragastrically and observed severe necrosis in the liver and kidney. Unlike earlier workers, they did not incorporate tanic acid in food.

In the present work, necrosis of the liver and kidney similar to that described by Handler and Baker (1944) and Boyd, Bereczky and

Godi (1965) have been observed in rats fed very high concentration of whole Catha material (but not extract) or tannic acid solution intragastrically. These lesions were observed in rats which had haemorrhagic ulcerations in the stomach and intestine (especially duodenum). The cause of haemorrhage was probably due to an irritative gastritis and duodenitis with superficial epithelia desquamation which by deepening would lead to haemorrhage from disrupted subepithelial capillaries. It appears that tannins must first impair the integrity of the mucosal epithelium before they can be absorbed in adequate concentration to cause lesions in the liver and kidney. This can only occur with high concentration and even then only after the necrotized tissue has been desquamated. Results obtained in the present work are consistent with this expectation in that those rats which died suddenly or were sacrificed immediately they showed definite signs of toxicity had haemorrhagic gastroenteritis and yet the effect in the liver and kidney was minimal, except for some localised degenerative changes. Degenerative changes often represent the initial response of a cell to presence of toxic substance and is the first step toward necrosis. If the substance is not rapidly eliminated and acts for a longer period or the concentration is increased, necrosis will be the end result. Unlike necrosis, degenerative changes are often reversible if the toxic material is removed. Small amounts of tannins are known to cause mild irritation of the gastrointestinal tract and this is usually manifested as inappetence, hypermotility

of the intestine, and hypersecretion of the glands. During the survey, several people gave evidence of pressing motion (liquid stool) following the chewing of the first bundle of Catha material. Excessive chewing of Catha material was said to cause constipation. Since large amount of tannic acid is known to cause constipation (hence its use in treatment of diarrhoea) the constipating effect of Catha material is probably due to tannins. The possible contribution of other substances present in Catha material, though in a small way can not be ruled out. For example, the effect could also be centrally mediated. Central nervous system depression observed in the latter stage of chewing Catha material could lead to decreased motility and secretion in the GIT.

Tannins usually precipitate proteins of secretions and ingesta, thus forming a protective film on the gastrointestinal mucosal surface. Consequently, the secretory activity of mucosa and transudation of fluids is retarded and this might lead to cyst formation, in the secretory glands. The protective layer of precipitated proteins is not permanent and is easily lost, thus exposing epithelium to the precipitant effect of tannins.

Mild irritation in the gastrointestinal tract cause hypersecretion of glands and if the effect is persistent, the glands, might rupture. Microscopic examination of the GIT would then reveal extensive desquamation. This was a consistent finding in rats fed Catha material.

In the case of Catha material, the effect on motility of GIT and the secretion of glands is difficult to predict as this depends on the amount consumed and even more important on the effective contact (with respect to time and area) between the material and the mucosal surface. For example, the astringent effect of tannins is made less effective by admixture of the extract with water, soft drink and in particular milk which has a slight demulcent effect. A decrease in motility will, in turn increase the contact time between Catha material and mucosal furface (hence enhancing the astringent effect of tannins) while increased motility will tend to promote quick passage through the GIT.

In the present work, it was considered that the raw material might have an abrasive action, especially in view of the fact that haemorrhage was mostly at the primary site of contact. However, boiling the material in water for 8 hours to break down cell wall and hence soften it made no difference to its toxicity. Tannins are readily extracted with water and since the extraction procedure was repeated several times, it is reasonable to assume that all the tannins were effectively extracted. Even when the extraction was carried out using ethyl alcohol or ethyl acetate, the toxicity of the extract when incorporated in food was not significantly different from that of aqueous extract. It is therefore

difficult to account for the difference in toxicity of whole Catha material and the extract. However, from the public health point of view, only the toxicity of the extract would be of any interest since human beings do not consume whole Catha material.

Oedema in the submucosa was a consistent finding in rats fed Catha material. Oedema is a condition in which there is excessive accumulation of fluid within the intercellular spaces of the body cavities. The entire system of exchange of nutrients, oxygen and metabolites between the blood and tissue involves the passage of these substances through a semipermeable membrane (the endothelial wall) and this is possible because of the difference in osmotic pressure of tissue fluids and blood plasma. Under normal circumstances, the blood pressure is the limiting factor on the amount of fluid which can enter the blood plasma; it is the difference in blood pressure which accomplishes the exchange of fluids through the endothelial wall.

Results obtained in the present work show that there is no serious chronic disturbance of cardiovascular system, and in particular the blood pressure, as a result of feeding rabbits on Catha material. When the material was fed to rabbits for about 4 months, there was only a slight fall in blood pressure of rabbits in the initial stages as determined by Grant's ear capsule. The results are in agreement with those of Le bras and Fretillere (1965).

Another possible cause of oedema is variation in osmotic pressure of blood plasma as might occur if there is loss of protein as is likely to happen with extensive haemorrhage. It is known that blood proteins can be lost as a result of renal injury as is the case in glomelurar nephritis which permits protein to escape through glomeluri into urine. Most cases of oedema were found in those rats showing haemorrhagic gastritis and duodenitis regardless of whether there were lesions in other organs. Boyd, Bereczky and Godi (1965) have shown that tannins cause loss of blood proteins and they were of the opinion that this might be as a result of slowly bleeding gastric ulcers.

The majority of gastric ulcers observed were shallow affecting only the glandular mucosa. Very rarely was the muscularis mucosae affected. Small erosions often serve as a primary focus from which chronic ulcers develop but the vast majority of erosions heal with no trace. Healing of an erosion is rapid for proliferation of cells in the remaining stumps of glands can rapidly make good the deficit. More important perhaps is the possibility that these erosions might serve as primary sites for infection. Lymphocytic proliferation was occassionally observed in rats fed high concentration of Catha material. The aetiology of gastric ulcers in man has been considered in detail by Truelove and Reynel (1963) and there are suggestions that gastritis might be a precursor to carcinoma of the stomach.

Both liver and kidney are primary targets in many types of chemical poisoning because they are well supplied with blood. A consistent finding in rats fed 50% Catha material extract was haemorrhage in the liver and kidney. In a few rats fed low concentration of Catha material, bloody urine was voided but only for a short period. A few rats voiding bloody urine were sacrificed and in all cases, microscopic examination of the important organs revealed some haemorrhage in the liver and kidney, possibly as a result of increased capillary permeability; an effect known to be caused by tannins.

There were several rats showing localised necrosis in the heart but these accounted for less than 30% of the total number. A number of mecanisms have been postulated to explain the pathogenesis of cardiac lesions caused by sympathomimetic amines. Some workers (Rona et al. 1959) have sought to explain the development of these lesions through cardiovascular actions of the drugs. Raab, (1960;) suggested that lesions occur when amines interfere with the process of energy production and utilization at cellular level. Yet another theory is that sympathomimetic amines may impair the function of myocardium through a myogenic mechanism manifested as a reduction in myocardial contractile force (Rosenblum 1965). Although both l-ephedrine and d-norpseudoephedrine gave a negative ionotropic effect when perfused on isolated heart preparation it is unlikely that given orally the

given orally the concentration in blood would be adequate to give this effect. The individual theories advanced by various research workers to explain cardiac lesions do not exclude the possibility of a pluricausal origin for the lesions. The distribution of cardiac lesion in rats fed Catha material and also in those fed d-norpseudoephedrine was variable and no association with vascular supply could be demonstrated. Drugs known to act mostly on the alpha-adrenergic receptors (e.g. methoxamine) have been shown to cause cardiac lesions. Since the adrenergic receptors in the heart are mostly of the beta type, it is possible that such drugs cause the lesions indirectly by affecting important physiological components e.g. venous return to the heart as a result of change in peripheral resistance in vascular beds. Le Bras and Fretillere (1965) could find no significant changes in blood pressure and heart rate in 48 out of 53 human volunteers chewing Catha material for 5 hours. After about 24 hours the blood pressure was found to be slightly lower than initially. Alles, Fairchild and Jensen (1961) noted a slight increase of 10-16 mm in systolic and 6-12 in diastolic pressure with a change of 4-6 pulses per min. in a person who had consumed detannated Catha extract. However, they noted that these values are not definitely outside normal variations.

Continuous administration of several chemical compounds often lead to marked increase of enzymes involved in the metabolism of the same as

well as other related and unrelated substances (Corney, Davison, Gastel and Burn, 1960). This finding has important toxicological implication since it is conceivable that a chronically administered compound may stimulate the microsomal liver enzymes to such a degree that it is metabolised and detoxified rapidly. Consequently, certain toxicity experiments may fail to demonstrate potentially important organ changes because the test compound is quickly eliminated or detoxicated in the body and has no chance of producing a sustained toxic effect. Possible tolerance to toxicity of tannic acid has been observed by Boyd, Bereczky and Godi (1965). In the present work, it was noted that rats fed Catha material tended to show symptoms of toxicity initially but this improved later. In particular the central stimulant effect was pronounced in the first week but later this effect was not observed. The modification of effect of chemical substances by enzyme induction may be viewed as a form of tolerance. It is now well accepted that tolerance is a common phenomenon in non-opiates psychotropic drugs.

Attempt to relate the phenomenon of tolerance and dependence to physiological and biochemical processes has been made but the exact relationship of these processes to the integrated function of the nervous system is far from clear and it is therefore difficult to say which processes are causally related and which ones are consequent to the

changes (Russel, 1969). For example the relationship of brain biogenic amines (catecholamines and indoleamines) to behavioural disturbance has generally been recognised although no quantitative relationship has been established. In general much of the behavioral work remain empirical with no clear biological framework of drug action.

Although the chick embryo has continued to be used in teratogenic studies, the accuracy and relevance of this technique, in predicting possible effect in man, has been questioned (Walker, 1967). The extensive use of chick embryo in teratological studies appear to be due to the fact that it is inexpensive, available in large numbers and easy to manipulate. However, results obtained are of uncertain relevance in man and at best, it is only a coarse screening method. It is now generally accepted that teratogenic studies should be conducted in mammals, preferably monkeys and chimpanzees, because of their close relationship to man. The cost of monkeys and chimpanzees is prohibitive and most research workers have had to make do with laboratory animals e.g. rabbits, rats and mice. Whatever animal is used, it must be appreciated that no other experimental animal parallels the teratogenic response observed in humans and in all cases man must be the ultimate test species.

In the present work, in which mice and rats were used, there was no indication of a teratogenic effect which could be attributed to feeding on *Catha* material.

During the survey, the author could find no evidence to suggest a higher incidence of deformities in children born to parents known to be chewers of Catha material. In the case of mild teratogen, several thousands women would have to be exposed to the chemical compound in order to detect a significant increase over normal background level of human spontaneous malformations caused by such factors as the cosmic rays radiation and radioactive fallout. If the compound interferes with foetus formation at an early stage (e.g. during implantation) the effect might never become evident as pregnancy is most difficult to diagnose in the first few days. In any case, moral and social considerations outside the province of this thesis prevent the use of human subjects in teratological studies. It is estimated in the general population that there is an approximately 20 per cent periconceptional loss (i.e. pregnancies that do not terminate in live birth). This figure cannot be accurately established because of the difficulty in proving early pregnancy (Mellin 1962). Of the 20 per cent periconceptional loss, 16.8 per cent were malformed. Carr(1963) has shown that approximately 20 per cent specimens obtained from spontaneous abortions, mostly during first trimester showed major chromosomal abnormalities. This compares with 2 per cent for live births including babies that have survived for at least 28 days after birth. It is well recognised that the body has a general

tendency of rejecting deformed foetus in the early stages of pregnancy and consequently examining newborn for deformities does not always give correct information as regard potential teratogenic effect of a compound.

One of the most important aspects of placental function is the ability of the organ to transfer material to foetus against a concentration gradient. Recently, it has been recognised that placenta has an active and selective role in transfer mechanisms and very few substances cross by a simple diffusion process. Many of them are transferred against an electrical gradient (Ginsburg, 1971). However, most compounds (e.g. sedatives, tranquillises) are likely to affect the foetus indirectly by causing a disturbance in the maternal physiological systems e.g. cardiovascular, respiratory, endocrine and central nervous system rather than acting directly on the foetus. This will in turn depend on the final concentration of drug in blood. Since no gross or microscopic lesions were observed in young rats born to rats feeding on Catha material, it can be assumed that the disturbance in maternal physiological system is not very much.

Passage of drugs into the milk of lactating mammals has been considered in detail by Rasmussen (1966). In all cases, it is the non-protein bound, unionised fractions of weak acids and bases which diffuse across glandular epithelium. A consideration of Henderson-Hasselbach equation indicate that the pH of milk, pH of plasma and the pKa

of drugs are the important determinants of the amount of drug passing into milk. Under normal circumstances, the pH of milk and of plasma are constant and the pka of the drug is the only variable. In general, basic drugs are found present in milk in slightly higher concentration than in plasma. Results obtained in the present work are consistent with this expectation.

The central stimulant activities of Catha edulis material are of primary pharmacological interest in connection with its usage. A study of Catha material without a consideration of this aspect would therefore be incomplete. The central stimulant activity of the material is almost wholly attributed to d-norpseudoephedrine. Some workers have claimed that the amount of d-norpseudoephedrine present in Catha material is not adequate to account for central stimulant effect and have speculated on the presence of other substances as yet not identified. Results obtained in the present work using gas liquid chromatographic and spectrophotometric techniques shows that there are no other basic compounds as has been claimed by some workers. This is in agreement with results of Alles, Fairchild and Jensen (1961) who used thin layer chromatographic technique and found only one basic compound, d-norpseudoephedrine. They also showed that the motor stimulant effect in mice of detannated extract of Catha material could be accounted for by the amount of d-norpseudoephedrine present.

In the present work, no quantitative evaluation of the central stimulant activity of Catha material was undertaken because of instrument limitation but it was established that 25-35 mg of d-norpseudoephedrine hydrochloride is adequate to give a definite central stimulant effect. This amount would be present in 1-2 bundles of Catha material, depending on the size of the bundle.

Stimulant drugs like amphetamine are useful for combating fatigue in normal subjects and for inducing transient euphoria but they are relatively unimportant as antidepressants as compared to monoamine oxidase inhibitors (nialamide, iproniazid isocarboxazid, phenelzine, tranylcypromine) and tricyclic compounds (imipramine, amitriptyline), possibly because the effect is short-lived making it necessary to ive the drug frequently. The widespread abuse of stimulant drugs, coupled with their limited clinical application makes their extensive use in therapy undesirable. For this reason, the use of d-norpseudoephedrine as a stimulant is not likely to find much clinical application in future. The Pharmacy and Poison Ordinance (1962) Chapter 244 of Laws of Kenya lists alkaloids from Ephedra species as part I poisons which means that only those authorised under the Ordinance can possess them for any purpose. Since d-norpseudoephedrine is found in one of the Ephedra species, ma huang (Ephedra sinica), it is therefore according to Laws of Kenya a part I poison, regardless of anything contained in the

Miraa Prohibitive Ordinance of 1951.

Although there was no tendency among those chewing Catha material to increase their daily consumption, it does not follow that the same would be true of a pure drug. The astringent effect of Catha material is probably a limiting factor in the amount that can be consumed within a short period.

Present work using smooth muscle preparations, has shown that ephedrine is much more effective in relaxing smooth muscles than d-norpseudoephedrine. In particular, d-norpseudoephedrine had little or no effect on the guinea pig trachea preparation and in some cases it had a constricting effect. This shows that it would be of little use in asthma therapy, as compared to ephedrine.

Twenty five grammes of ephedrine and amphetamine salts cost approximately 10 and 70 shillings respectively (E.T. Monks Chemists, Nairobi). In contrast, a similar quantity of d-norpseudoephedrine, if isolated from Catha material would cost at least 1,000 shillings and possibly as much as 2,000 shillings. If d-norpseudoephedrine is produced synthetically, the cost would probably be comparable to that of ephedrine and amphetamine. From an economic point of view it would not be feasible to extract d-norpseudoephedrine from Catha edulis on a commercial scale.

It is reasonable to assume that the astringent effect of tannins will interfere with absorption of d-norpseudoephedrine from the

gastrointestinal tract, to an extent which is difficult to predict. Furthermore, since d-norpseudoephedrine is readily eliminated from the body, the final blood concentration will depend on the rate at which it is being consumed. If the material is consumed at a slow rate (it is usual for Miraa chewers to eat only 1 bundle per hour) a dynamic equilibrium will be reached at a low blood concentration; the amount absorbed will then be only adequate to offset that being eliminated. Under such circumstances, the central stimulant effect might not be appreciable, even though the person might continue to chew material continuously for a long time. These factors might explain the considerable variation in the amount needed to give the required effect in different individuals and even in the same individual at different times. A further source of variation is that the weight of bundles varies considerably, some being approximately three times as heavy as others. During the survey, many people were reluctant to say precisely how much Catha material (bundles) they considered adequate for an appreciable central stimulant effect.

Le bras and Fretillere (1965) have recorded observations on 53 human volunteers chewing Catha material and they noted two distinct phases. The first phase lasting 1-2 hours is dominated by a feeling of mirth, familiarity, excitement, optimism, stimulation of the memory, ease of speech and later a psychic illusion in which the person overestimates his capability to solve problems. The second phase is marked by

depression, usually manifested as insomnia, anorexia and finally by profound sleep.

Information obtained during the survey described in the first part of this thesis confirmed that Catha material induces hallucinations, the most perceptual disturbace being illusions and euphoria. Several people claimed to remember things which happened many years back, a situation analogous with that encountered with LSD. After the initial central stimulant effect, many people lapse into a compensatory phase, the first part of which is characterised by mental fatigue, uneasiness and a feeling of "let-down." The exact sequence of these effects, with respect to time, were not precisely described. It was evident that in the majority of cases, those who indulge in excessive chewing of Catha material rarely have deep sleep and this inevitably creates a vicious cycle in which the person becomes dependent on the effect of Catha material for sustained mental clarity. Many people who chew Catha material complain of "general weakness" if they do not get their daily ration. In this respect, chewing of Catha material leads to some type of dependence.

According to the Expert committee on Addiction-producing Drugs of World Health Organization, drug dependence is defined as " a state of discomfort produced by withdrawal of a drug from a subject who has been chronically or repeatedly exposed to it and alleviated by renewed

administration of that drug or another with similar pharmacological action (Eddy, Halbach, Isbell and Jeevers, 1965)". The discomfort may consist of a non-specific and ill-defined disatisfaction giving rise to a desire (ranging from a mild wish to intensive craving) for the perceived effect of the drug. As yet, the physiological and biochemical basis of drug dependence are poorly understood. The cellular processes examined so far, do not permit identification of the fundamental alterations related to development of either psychological or physical dependence. Of the drugs that are known to cause dependence, only the general depressants (opiates, barbiturates and minor tranquillisers) give rise to clearly recognisable withdrawal syndrome both in man and in experimental animals. Withdrawal of stimulants (amphetamines etc.) is said to give rise only to fatigue and depression in man (Kalant, 1966) and to negligible behavioral modification in other species (Kosman and Unna 1968).

Information gathered during the survey indicate a possible tolerance to the subjective effects on mood and appetite but this apparently does not lead to an increase in the amount taken each day, possibly because of gastrointestinal discomfort associated with consumption of large amount of Catha material. It appeared that the only consideration was the availability of money. In fact, several cases were encountered where the

individuals had reduced their consumption of Catha material considerably or had given up the habit completely. People addicted to common notorious drugs (amphetamine, heroine, morphine and cocaine) rarely give up the habit voluntarily once they are "hooked" and in nearly all cases, there is a tendency to increase the dose. On this basis, the dependence on Catha material appear to be "habituative" rather than "addictive".

Rats fed high concentration of Catha material were generally stimulated in the first 2-4 days of the feeding experiment. Almost about the same time, the rats had poor appetite as shown by the amount of food consumed. Both effects were only evident in the first week of the experiment. The anorexigenic and behavioral modifications were also evident in the first week of experiment in rats fed d-norpseudoephedrine. It was thought that the anorexigenic effect was due to detestable palatability of the material. However, there are reports of experimental rats developing tolerance to the effect of amphetamine on operant behavior and on anorexigenic effect within 7-30 days of the experiment (Schuster, Dockens and Wood, 1966). This tolerance has also been reported in man after 2 weeks (Rosenberg, Wolbach, Miner and Isbell, 1963). Since the pharmacology of d-norpseudoephedrine closely parallels that of d-amphetamine, it is possible that observed effects in rats fed Catha

material represent a true form of tolerance.

The quasi-quantitative parameters adopted in assessing tolerance make it difficult for meaningful evaluation of the phenomenon. Even where quantitative measures have been used (e.g. weight loss, body temperature, food and water consumption, number of struggle responses and convulsions) results are difficult to interpret. Objective investigation of drug dependence has also been hampered by the " a priori" moral implication inherent in such words as "addiction" which makes it difficult to use purely descriptive concepts divested of moral stigma.

It has often been alleged that excessive chewing of Catha material causes male impotence. In Isiolo District, in the Northern part of Kenya, women had petitioned the administration to ban Catha edulis material on the basis that it was ruining their marriage by causing male impotence. As with any other sensational news, this incidence was given wide coverage by the press and consequently it has become an important milestone in the continuing controversy over excessive chewing of Catha material. During the survey, the author repeatedly invited Somali men to comment on the significance of this incident. In the majority of cases, the explanation was that the women were, in essence, objecting to their husbands being away, sometimes for days on end.

Among many indigenous people of Africa, male impotence is usually regarded as something very shameful and is often ridiculed. Consequently,

there is a deliberate attempt to conceal male impotence by encouraging the wife to have children with other men. Because of this practice, information on male impotence is not readily available and certainly very few people will volunteer to give the information freely if it concerns them or an immediate relative. The problem becomes manifold when one considers that among the same people, the wife is usually free to have children with other men, even when the husband is capable of getting children; in all cases the children belong to the husband regardless of their paternal origin.

Results of the survey show that approximately 46% of the people interviewed thought that Catha edulis is a sexual stimulant. This figure compares with 26% who were non-committal, 16% who thought it had no appreciable effect, and 12% who thought it was a sexual depressant. However, most of those who claimed that it was a sexual stimulant, on being questioned further, admitted that the feeling of sexual stimulation was not marched with a corresponding ability to indulge effectively in sexual play.

It was also stated that this feeling was only experienced in the first few hours after consuming Catha material. Evidence was also given by a few but very reliable people that, when urinating semen tend to ooze out freely and uncontrollably (i.e. spontaneous ejaculation).

From the information gained during the survey, the effect of Catha material on the sexual motivation appear to be biphasic and this is in

agreement with the observations of Lebras and Pretillere (1965). There is obviously no clear cut difference between the first phase (stimulation) and the second phase (depression) as most people will continue chewing Catha material until just before going to bed. The second phase is probably a consequence of general central nervous system depression which affects all levels of the CNS and hence the autonomic nervous system as a result of depression of the integrating centres in the medulla and midbrain (more specifically the hypothalamus).

The penis becomes erect as a result of increased activity in the branches of the sacral parasymphathetic nerves (the nervi erigentes) which innervate the arteries of the erectile tissue. These tissue are dilated and the rate of blood flow into the cavities of the erectile tissue increases. The erectile tissue within the penis consist of three portions: the two corpora cavernosa which occupy each side of the shaft and the spongiosa which surrounds the urethra and forms the glans at the free end of the penis. The erectile tissue consist of a sponge-like net-work of vascular spaces, supplied by thick muscular walled arteries and drained by the thin walled veins. All three erectile tissues continue into the perineum (region between the scrotum and the anus) to form the root of the penis.

Sympathetic nerves supplying the epididymis, vas deferens, seminal vesicles and the prostate are responsible for ejaculation. This is as

a result of rhythmic contractions of striated muscles which compress the urethra at the root of the penis. The sympathetic reflexes initiated ejaculations also result in constriction of the arteries supplying the erectile tissue, the blood drains away and consequently the erectile tissue return to a flaccid state.

The first phase of sexual stimulation, characterised by spontaneous ejaculation is probably due to increased adrenergic stimulation of the penis, an effect which can be attributed directly or indirectly to d-norpseudoephedrine. The increase in autonomic "tone" of the adrenergic nerves may be a direct consequence of CNS stimulation rather than an independent peripheral effect. One must consider that in man, many psychological and sociological factors play a dominant role in reinforcing or inhibiting both the sympathetic and the parasympathetic divisions of the autonomic nervous system. Furthermore, since there is reciprocal innervation of the two divisions in most peripheral organs, the net result might be difficult to predict.

Male impotence is usually taken to mean "failure of erection" and this can occur as a result of damage or inhibition of the parasympathetic innervation of the erectile tissue to the penis. Impairment of the sympathetic innervation of the penis would lead to a failure in ejaculation. In the case of central nervous system depression, the direct peripheral stimulation by autonomic nerves might be unimportant.

It is common knowledge that most people do not like to have sexual intercourse when they are mentally and/or physically tired.

Le bras and Fretillere (1965) state that the first phase following the chewing of Catha material is often painful and sexual act is often accompanied by abnormal ejaculation - precocious or retarded and without orgasm. To keep off testicular pains, most people use cloves.In the course of gathering information, the author encountered several people using cloves, together with Catha material, and this had the effect of disguising the astringent effect of the latter. Other people were using some nuts, known in Swahili as, "Kungu manga" and these were supposed to delay ejaculation during sexual intercourse.

Most people spend a lot of time chewing Catha material and it is reasonable to assume that by the time they go to bed, they are already in the second phase, characterised by mental fatique and insomnia, and hence unable to indulge in sexual play effectively.

This is probably what happens with amphetamine, a drug whose pharmacology closely resembles that of d-norpseudoephedrine and which is known to cause impotence. Most people gave evidence that they would normally fall asleep shortly after stopping chewing Catha material. Several people who use amphetamine (e.g. students) have been known to suffer from mental fatigue suddenly, for example at the middle of an examination, and this might be the same effect described by people who chew Catha material for several hours.

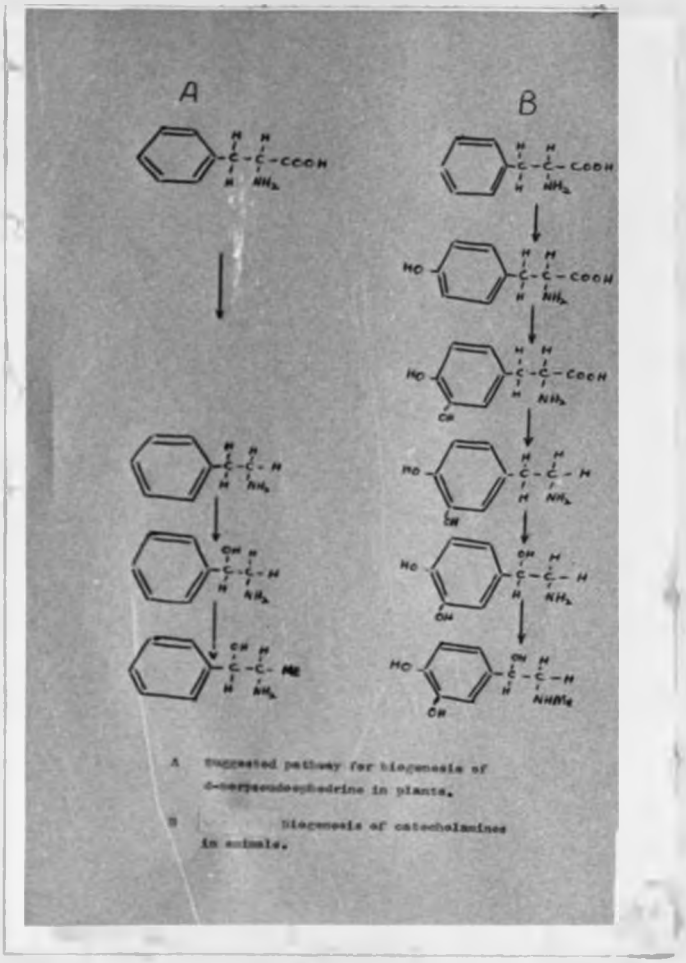


Fig. 36. Proposed biogenesis pathway for d-norpseudoephedrine in plants.

SUMMARY

Chemical analysis of Catha material (twigs, leaves flowers, roots, seeds and stem) has shown that only one alkaloid is present and this was identified as d-norpseudoephedrine using spectrophotometric and chromatographic techniques. The same compound was present in fresh and dried Catha material. The claim of Ristic and Thomas(1961) that l-ephedrine is present in Catha material could not be confirmed. Similarly the claim by Friebel and Brilla (1963) that there is another labile but more potent alkaloid in fresh Catha material could not be confirmed. Trace amount of cyanide, possibly present as cyanogenetic glycoside was detected in Catha material but this was considered to be of little toxicological significance.

The toxicity of Catha material can almost wholly be attributed to the tannins present in the material and possible contribution by other substances was considered minimal. In particular, haemorrhagic gastritis and duodenitis observed in rats fed 25 and 50% whole Catha material was attributed to tannins. Centrolobular and coagulative necrosis in the liver and kidney respectively were also attributed to tannins absorbed after the integrity of the mucosal epithelia had been impaired. Oedema was also found in more than 50% of rats fed 25 and 50% Catha material and this was also thought to be due to tannins. Coagulative necrosis was observed in about 30% of rats fed Catha material and also d-norpseudoephedrine. Some significance was attached to this finding because of

some published reports which tend to implicate sympathomimetic amines in cardiac lesions formation. Rats fed Catha material extract were not very much affected. Desquamation of the GIT and vacuolation in the liver and kidney were commonly encountered in rats fed more than 5% Catha material extract.

Results of toxicity studies using rabbits were generally in agreement with those obtained with rats.

The pharmacology of d-norpseudoephedrine closely parallels that of indirectly acting adrenergic drugs, e.g. ephedrine and amphetamine and the difference is merely quantitative. The widespread abuse of stimulant drugs coupled with their limited therapeutic application makes their extensive use to induce euphoria undersirable. According to the Pharmacy and Poison Ordinance (1962), Chapter 244, Laws of Kenya, d-norpseudoephedrine is a part I poison since it is an alkaloid of Ephedra species. Accordingly, only those authorised under the Ordinance can possess the pure drug for any purpose.

From an economic point of view, it would notbe feasible to extract d-norpseudoephedrine from Catha edulis on a commercial scale and in particular since the drug can be produced more cheaply, synthetically, if required.

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APPENDIX I: ADDITIONAL INFORMATION OBTAINED DURING THE SURVEY OF MIRAA CHEWING HABITS IN KENYA

A. A BREAKDOWN OF ETHNIC GROUPING OF 500 PEOPLE INTERVIEWED INDIVIDUALLY

Meru	201	Indians	4
Somali	130	Bajun	2
Boran	55	Gala	1
Swahili	25	Comorians	1
Arabs	22	Samburu	1
Sudanese	15	Giriama	3
Kamba	15	American (Peace Corps)	1
Kikuyu	10	Nazrui	2
Turkana	7	Pokomo	1
Tanzanians	4		

B. OCCUPATION OF PEOPLE INTERVIEWED

The people interviewed were from all walks of life and they included the following categories:

1. Poor unemployed individuals
2. Dealers in Miraa (retailers or wholesalers)
3. Self employed people e.g. farmers, butchers
4. Very rich people, some owning many commercial vehicles (tankers) and business premises.
5. Watchmen and Securicor guards
6. Drivers of heavy commercial vehicles
7. Policemen
8. Men in armed forces (army, navy and air force)
9. Prostitutes living in the suburbs of Nairobi
10. Local actors and Television personalities
11. People employed in the Government and the private sector.

C. DISTRIBUTION PATTERN OF AGE GROUPS OF 200 INDIVIDUALS INTERVIEWED:

AGE GROUP: (Years)	NUMBER OF PEOPLE:	PERCENTAGE:
10 - 20	15	7.5
21 - 30	53	26.5
31 - 40	55	27.5
41 - 50	32	16.0
51 - 60	31	15.5
61 - 70	14	7.0
71 - 80	4	2.0
81 - 90	3	1.5
91 - 100	1	0.5

Males = 189
Females = 11

D. NUMBER OF YEARS DURING WHICH INDIVIDUALS (200) HAD BEEN CHEWING MIRAA

DURATION OF CONSUMPTION OF CATHA MATERIAL. (Years)	NUMBER OF PEOPLE	% OF PEOPLE
1 - 5	24	12
6 - 10	33	16.5
11 - 15	34	17
16 - 20	41	20.5
21 - 25	11	5.5
26 - 30	20	10
31 - 35	12	6
36 - 40	13	6.5
41 - 45	8	4
46 - 50	4	2
OVER 50	9	4.5

E. APPROXIMATE AMOUNT OF CATHA EDULIS MATERIAL CONSUMED DAILY BY INDIVIDUALS.

NUMBER OF BANDARI*	NUMBER OF PEOPLE:
1 - 2	26
2.5 - 3	8
3.5 - 4	36
4.5 - 5	10
5.5 - 6	36
6.5 - 7	7
7.5 - 8	12
8.5 - 9	1
9.5 - 10	32
10.5 - 11	7
11.5 - 12	4
OVER 12	29

* Where the daily consumption was stated to be, for example, 4 - 5 bandaris, the recorded value is 4.5

Average consumption for 200 people = 7.5 bandaris

S.D. = 4.5

F. WEIGHT OF BANDARIS OBTAINED FROM DIFFERENT PARTS OF KENYA AT DIFFERENT TIMES*

No.	(g)	No.	(g)	No.	(g)	No.	(g)	No.	(g)
1	19	36	124	72	25	106	35	142	37
2	21	37	90	73	24	107	36	143	46
3	27	38	54	74	21	108	23	144	48
4	21	40	34	75	47	109	27	145	34
5	27	41	70	76	66	100	21	146	31
6	22	42	45	77	31	112	20	147	40
7	18	43	48	78	37	113	31	148	50
8	32	44	22	79	27	114	27	149	54
9	35	45	25	80	75	115	37	150	31
10	30	46	66	81	31	116	25	151	34
11	21	47	103	82	17	117	26	152	52
12	71	48	90	83	37	118	56	153	40
13	38	49	46	84	54	119	18	154	36
14	27	50	70	85	36	120	18	155	33
15	55	51	41	86	27	121	49	156	23
16	26	52	26	87	44	122	19	157	22
17	52	53	43	88	56	123	54	158	22
18	30	54	97	89	36	124	22	159	40
19	40	55	42	90	46	125	63	160	18
20	29	56	45	91	34	126	29	161	27
21	53	57	80	92	36	127	45	162	17
22	82	58	54	93	63	128	25	163	23
23	105	59	61	94	30	129	29	164	26
24	108	60	57	95	23	130	21	165	26
25	95	61	35	96	22	131	19		
26	84	62	84	97	25	132	26		
27	79	63	30	98	22	133	45		
28	42	64	33	99	25	134	34		
29	90	65	83	100	37	135	26		
30	42	66	91	100	36	136	40		
31	45	67	90	101	39	137	35		
32	25	68	31	102	24	138	46		
33	86	69	20	103	30	139	35		
34	90	70	18	104	16	140	33		
35	25	71	33	105	33	141	30		

Average weight of bandari = 41.8 ± g.

S.D. = 22.3

* To the nearest unit.

G. DETERMINATION OF PERCENTAGE OF THE CATH8 MATERIAL MASTICATED

WEIGHT OF WHOLE BANDARI	WEIGHT OF PEELED SKIN (g)	WEIGHT OF STICKS (g)	% BANDARI MASTICATED (Peeled Skin)*
20.2	16.7	3.5	83
18.4	16.0	2.4	88
32.8	27.2	5.6	82
24.7	20.2	4.5	80
23.8	20.2	3.6	84
21.0	18.0	3	85
46.7	35.6	11.1	75
65.6	46.5	19.1	71
31.2	25.4	5.8	81
37.1	29.8	7.8	80
26.7	21.8	4.9	80
75.4	57.5	17.9	76
31.4	24.7	6.7	79
17.4	13.9	3.4	81
37.3	31.6	5.7	85
54.3	41.2	13.1	76
36.2	28.0	8.2	77
26.9	22.6	4.3	83
43.8	30.7	13.1	69
56.2	41	14.3	74
36.5	28.6	7.9	79
46.2	34.6	11.6	75
33.9	27.0	6.9	79
36.2	26.2	10	72
62.7	45.5	17.2	72
24.9	19.3	5.6	77
22.7	18.7	4.0	81
25.8	21.4	4.4	82
37.0	27.8	9.2	75
22.0	19.4	2.8	88
27.7	21.4	6.3	76
Total 10640	8216	243.3	2442
Average 34.32	26.5	7.8	78.7%

* To the nearest unit

H. MOISTURE CONTENT IN FRESH CATHA MATERIAL

WEIGHT OF PEELED BARK FROM 20 BANDARIS

BEFORE DRYING (g)	AFTER DRYING (g)	LOSS IN WEIGHT (g)	% MOISTURE CONTENT
325	125	200	61.5
215	77	138	64.0
267	78	189	70.8
225	70	155	69.0
276	96	180	65.2
223	78	145	65.0
248	88	160	64.5
190	67	123	64.7

Average Moisture Content = 65.5%
S.D. = 2.75

WEIGHT OF WOODY PART FROM 20 BANDARIS

BEFORE DRYING (g)	AFTER DRYING (g)	LOSS IN WT. (g)	% MOISTURE CONTENT
82	28	54	65.9
47	20	27	57.5
50	18	32	64.0
80	25	55	69.0
65	22	43	66.2
41	16	25	61.0
55	25	30	54.5
65	27	38	58.5

Average Moisture Content = 62.7
S.D. = 4.73

APPENDIX II: ADDITIONAL DATA ON CATHA EDULIS TOXICOSIS IN RATS AND RABBITS

A. AVERAGE WEIGHT GAIN OF MALE RATS FED VARIOUS PERCENTAGE OF DRY CATHA MATERIAL EXTRACT:

DAY	WEIGHT GAIN PER RAT* (AVERAGE OF SIX RATS) (grammes)							
	Control	1%	5%	10%	15%	20%	25%	50%
0	0	0	0	0	0	0	0	0
4	7	8	13	2	1		11	4
8	14	14	22	14	13		19	4
11	23	28	27	27	28		27	5
14	31	36	45	32	36		38	14
19	-	-	-	-	-			
20	45	53	56	50	53		37	17
23	55	60	62	60	60		43	28
27	64	67	66	70	72		51	35
31	85	75	72	75	77		55	43
34	93	83	79	84	88		58	45
38	103	96	88	94	90		67	54
42	108	112	102	98	94		73	60
45	115	117	105	106	100		77	64
49	124	124	114	115	98		77	66
52	131	135	122	116	114		78	60
56	137	146	129	125	126		84	69
59	145	143	140	130	131		89	79
63	148	155	141	138	136		92	83
67	152	167	149	148	147		88	83
70	165	172	153	150	152		92	93

* To the nearest unit

B. AVERAGE WEIGHT GAIN FOR MALE RATS FED VARIOUS PERCENTAGE OF NON-EXTRACTED DRY CATH MATERIAL

DAY	WEIGHT GAIN PER RAT (AVERAGE OF SIX RATS)						
	Control	1%	5%	10%	15%	25%	50%*
0	0	0	0	0	0	0	0
4	7	9	12	6	5	7	
8	14	13	22	16	6	12	
11	23	26	28	20	10	17	
14	31	35	45	25	18	22	
19	-						
20	45	48	55	28	23	25	
23	55	51	59	33	31	36	
27	64	64	66	40	34	40	
31	85	71	72	45	46	44	
34	93	82	79	49	42	49	
38	103	93	83	60	57	57	
42	108	111	98	67	65	59	
45	115	118	100	76	72	61	
49	124	122	105	84	76	61	
52	131	133	116	90	87	63	
56	137	147	117	96	90	72	
59	145	153	121	102	98	86	
63	148	158	128	106	103	76	
67	152	169	135	106	107	86	
70	155	173	142	110	108	89	

* All of them died within the first ten days of experiment.

C AVERAGE WEIGHT GAIN FOR FEMALE RATS FED VARIOUS PERCENTAGES OF CATHA EDULIS EXTRACT.

DAY	WEIGHT PER RAT (AVERAGE OF 6 RATS)				
	Control	5%	10%	15%	25%
0	0	0	0	0	0
4	9	8	8	3	-1
7	12	15	14	6	1
11	26	23	19	9	2
14	30	26	24	14	5
16	32	29	28	22	10
19	34	31	34	25	12
23	43	39	40	31	22
26	47	45	44	38	27
32	57	52	49	42	33
35	65	61	55	50	39
38	66	62	59	50	42
42	68	65	59	50	45
45	70	65	61	58	49
49	77	73	67	60	52
53	82	78	70	62	56
56	87	84	73	66	58
60	89	84	75	67	62
63	83	86	77	71	62
67	91	86	77	71	62
70	94	91	80	86	71

D. AVERAGE WEIGHT GAIN OF MALE RATS FED VARIOUS PERCENTAGES OF TANNIC ACIDS (GRAMMES)

DAY	CONTROL	1%	2.5%	5%	10%
4	7	10	7	6	7
8	14	18	16	14	12
11	23	28	23	22	21
14	31	40	30	26	24
19	45	45	41	33	30
23	55	55	48	39	33
27	64	63	57	42	35
31	85	71	63	50	36
34	93	78	68	50	38
38	103	90	74	60	44
42	108	100	79	63	45
45	115	105	88	68	52
49	124	112	-	-	-
52	131	120	94	85	70
56	137	126	106	93	82
59	145	132	112	98	85
63	148	140	118	104	87
67	152	146	127	107	92
70	165	151	134	108	95
73	168	153	137	109	97

E. BLOOD PRESSURE CHANGES IN RABBITS FED 25% CATHA MATERIAL

(mm Hg)

DAY	RABBIT 1		RABBIT 2		RABBIT 3		RABBIT 4		RABBIT 5			
	D	S	D	S	D	S	D	S	D	S		
1	60	70	57	72	66	74	60	66	58	76		
3	64	66	68	84	58	68	59	78	56	72		
5	62	70	72	82	62	78	61	78	60	80		
7	70	78	58	74	48	66	48	66	60	78		
9	60	76	59	76	59	68	49	68	59	66		
11	50	64	58	74	62	76	55	76	59	70		
13	62	70	60	80	58	80	50	66	68	78		
15	60	72	59	76	52	68	52	66	60	80		
17	65	80	59	78	60	76	68	78	59	64		
19	68	74	60	76	62	74	60	76	60	78		
21	60	82	65	84	65	80	49	68	58	76	MEAN	(%)
											D	S
MEAN	61.9	72.9	61.4	77.8	59.3	73.4	55.5	71.4	59.7	74.4	100	100
Feeding experiment starts here												
23	60	74	57	76	46	68	49	70	60	78	91.5	98.9
25	58	70	51	68	50	66	50	68	54	74	88.7	93.5
27	62	78	49	64	41	60	45	62	50	68	80.4	89.7
29	49	66	48	66	48	64	49	66	49	66	81.5	86.6
31	60	74	50	68	50	70	52	68	60	80	91.3	97.3
33	68	68	49	66	49	66	52	70	58	66	89.3	90.9
35	59	72	50	70	50	68	50	64	60	76	90.3	94.5
37	50	68	62	78	59	78	48	68	58	78	92.9	100
39	58	64	50	66	51	68	46	64	51	71	85.6	90
41	50	56	49	68	50	70	50	70	50	70	83.5	90.3
43	66	70	56	72	48	64	48	66	58	76	92.6	96.8

DAY	RABBIT 1		RABBIT 2		RABBIT 3		RABBIT 4		RABBIT 5		MEAN %	
	D	S	D	S	D	S	D	S	D	S	D	S
45	50	68	60	78	59	76	50	70	54	76	91.6	99.4
47	66	72	49	64	60	78	52	68	50	68	92.9	94.6
49	50	66	50	70	50	66	46	64	60	78	85.9	93.0
51	58	72	59	78	60	74	60	82	50	66	96.3	100.5
53	60	78	60	76	68	78	68	84	60	80	106	107
55	64	76	62	78	66	84	62	80	50	68	102	101.6
57	50	70	48	68	58	76	50	68	58	78	88.6	97.3
59	48	64	50	56	48	68	62	80	58	76	89.3	93.0
61	58	72	54	72	62	80	60	78	63	82	99.7	103.8
63	40	58	64	80	60	80	64	80	64	84	98	103.2
65	60	66	48	66	50	68	58	76	56	74	91.3	94.6
67	62	72	60	80	52	74	62	80	54	70	97.3	101.6
69	58	78	58	76	50	66	52	70	58	76	92.6	98.9
71	54	72	48	66	56	74	54	74	58	74	90.6	97.3
73	64	80	49	68	54	72	60	78	50	66	93.0	98.4
75	58	76	42	60	50	68	64	80	60	78	91.9	97.8
77	47	68	52	70	60	82	60	80	54	62	91.9	97.8
79	48	66	56	76	62	78	54	70	56	74	92.6	98.4
81	52	70	56	72	58	78	58	76	58	76,	94.6	100.5
83	64	80	60	80	58	76	60	78	62	74	102	104.9
85	60	80	63	80	65	72	50	66	64	82	98.3	102.7
87	62	78	52	68	52	70	52	68	54	74	91.3	96.7
89	50	70	50	66	60	78	60	78	52	68	91.3	97.3
91	52	68	54	70	68	86	62	80	62	78	100	103.2
93	60	80	56	74	62	84	60	76	60	78	100	106.0
95	62	76	56	72	58	76	62	78	54	72	98	101.1
97	60	80	64	82	60	78	49	68	58	76	97.6	103.8
99	54	72	58	76	62	80	52	70	54	72	94	100
101	56	72	60	80	68	84	56	74	52	70	98	102.7
103	54	76	56	74	64	80	58	76	62	80	98.6	77.2
105	64	80	62	78	58	76	52	72	58	76	98.7	103.2
107	60	80	58	76	60	80	56	78	60	80	98.6	106.5
109	56	74	56	76	62	78	60	78	50	72	95.3	102.2
111	60	76	60	78	58	76	56	74	54	72	96.6	101.6
113	56	74	54	72	58	78	58	76	56	74	94.6	101.0
115	56	76	60	80	60	78	54	72	52	72	95.3	102.2

F. ELIMINATION OF POTASSIUM IN URINE OF RABBITS FED 25% CATHA MATERIAL

(Meq)

DAY	RABBIT 1	RABBIT 2	RABBIT 3	RABBIT 4	RABBIT 5		
1	12.0	13.4	13.8	19.0	17.6		
2	11.8	13.0	-	12.5	14.6		
3	14.4	16.0	16.4	16.8	13.6		
4	15.0	17.2	25.0	13.0	15.4		
5	17.6	20.0	13.2	11.7	14.5		
6	14.0	21.3	14.0	10.0	-		
7	20.0	14.8	12.0	12.5	15.0		
8	19.0	-	18.0	13.0	15.8		
9	21.6	17.5	11.6	20	23.0		
10						MEAN (Meq)	(%)
Mean	16.1	16.7	15.4	14.3	16.2	15.7	100
	Feeding experiment starts here						
1	20.0	17.5	14.0	19.5	14.8	17.5	109.5
3	12.6	17.6	12.8	14.5	10.6	13.6	86.6
5	14.8	20.0	15.2	-	13.0	15.8	100.1
7	20.0	19.2	14.8	11.0	26.0	18.2	115.9
9	11.6	13.6	12.4	22.0	23.0	16.5	105.1
11	13.2	14.8	-	19.0	19.0	21.0	108.3
13	13.2	18.8	11.6	14.0	14.4	14.4	91.7
15	14.0	13.0	14.0	19.6	17.5	15.6	99.4
17	13.5	17.5	16.0	14.5	18.6	16.0	101.9
19	18.6	21.2	18.0	20.0	-	19.4	123.6
21	11.6	17.2	-	12.6	15.0	14.1	89.8
23	15.2	18.5	14.0	17.6	19.6	17.0	108.3
25	14.0	14.0	15.5	14.0	20.0	15.5	98.7
27	15.6	16.8	14.0	14.0	19.6	16.0	101.9
29	23.2	14.8	17.5	16.0	30.0	20.3	122.3
31	17.5	17.2	21.0	22.0	20.0	19.5	124.2
33	11.5	14.0	18.0	14.0	17.5	15.0	95.5
35	15.0	16.8	11.5	12.0	16.8	14.4	91.7

Potassium Elimination Contd.

DAY	RABBIT 1	RABBIT 2	RABBIT 3	RABBIT 4	RABBIT 5	(Mean Cont.) Meq	(%)
37	13.5	19.6	13.5	16.0	15.6	15.6	99.4
39	21.0	18.0	19.0	-	16.0	18.5	117.8
41	16.4	20.0	14.5	16.0	14.5	16.3	103.8
43	13.6	14.4	17.5	14.8	-	15.1	96.2
45	17.5	16.8	20.0	21.0	14.0	17.9	114.0
47	20.0	14.0	18.0	15.0	14.8	16.4	104.4
49	13.5	14.8	17.6	12.5	16.5	15.0	95.5
51	22.0	18.0	13.6	16.8	16.4	17.4	110.8
53	11.5	12.8	17.5	14.0	-	13.4	85.3
55	15.5	14.0	14.0	19.5	18.0	16.2	103.2
57	16.2	14.8	15.6	17.6	15.5	15.7	100
59	14.6	11.5	14.5	16.0	14.0	14.1	89.8
61	17.5	13.2	13.6	14.0	11.5	14.0	89.2
63	13.5	15.6	15.6	18.0	14.5	15.4	98.1
65	16.0	17.5	13.5	16.6	14.0	15.5	98.7
67	17.5	11.0	17.5	17.5	15.6	15.9	101.3
69	10.5	14.0	16.0	16.4	12.5	13.9	88.5
71	14.8	19.0	23.0	11.5	21.0	17.9	114.0
73	13.6	10.0	17.3	-	15.5	14.1	96.9
75	14.0	18.5	12.0	14.8	14.0	14.7	93.6
77	17.5	15.5	14.0	19.5	14.8	16.3	103.8
79	21.0	13.5	19.0	10.5	16.0	16.0	101.9
81	19.6	16.0	18.0	15.0	14.5	16.6	105.7
83	12.1	-	14.5	17.5	16.8	15.2	96.8
85	20.0	11.8	11.5	16.8	16.0	14.8	94.2
87	19.0	14.5	14.0	17.5	12.5	15.6	99.4
89	14.0	15.6	21.0	15.5	18.0	16.8	107.0
91	16.8	18.0	15.5	11.5	21.0	16.6	105.7

G. ELIMINATION OF SODIUM IN URINE OF RABBITS FED 25% CATH. MATERIAL

(Meq)

DAY	RABBIT 1	RABBIT 2	RABBIT 3	RABBIT 4	RABBIT 5	MEAN (Meq.	(%)
1	5.0	6.8	5.2	2.7	6.0		
2	6.0	6.4	-	7.25	4.0		
3	5.6	8.0	4.0	2.6	3.5		
4	3.9	3.4	4.75	5.0	-		
5	5.0	4.4	6.6	6.4	2.8		
6	5.0	4.4	6.6	6.4	-		
7	4.4	4.0	7.8	6.0	8.0		
8	7.2	-	5.0	5.6	4.0		
9	4.4	5.0	7.2	3.5	6.4		
10	4.0	4.0	4.5	3.0	6.9		
Mean	5.22	4.25	5.16	4.74	5.15	(4.90)	100
	Feeding experiment starts hear						
1	3.3	3.0	7.2	4.8	8.3	(5.3)	108.2
3	7.5	5.1	4.3	4.6	3.0	(4.9)	100.0
5	3.2	4.0	5.4	-	5.6	(4.6)	93.9
7	6.8	6.0	3.2	6.8	4.0	(5.4)	110.2
9	4.0	7.2	3.7	5.6	6.7	(5.4)	110.2
11	3.6	4.0	-	4.8	3.6	(4.0)	81.6
13	5.0	4.6	2.8	15.0	5.8	(6.2)	126.5
15	6.2	3.5	4.8	6.7	4.0	(5.0)	102
17	4.8	4.0	4.4	6.4	3.0	(4.6)	93.9
19	6.8	6.3	6.0	3.2	-	(5.6)	114.3
21	9.2	5.2	-	6.0	4.0	(6.1)	124.5
23	5.6	15.0	6.8	2.8	8.0	(7.4)	151.0
25	5.6	3.2	6.0	3.0	2.8	(4.1)	83.7
27	4.4	5.6	3.0	4.8	5.0	(4.6)	93.9
29	6.3	11.0	7.2	3.0	4.5	(6.4)	130.6
31	3.2	2.8	5.7	2.2	3.0	3.4)	69.4
33	4.8	4.0	3.0	4.0	2.8	(3.7)	75.5

Sodium Elimination Contd.

DAY	RABBIT 1	RABBIT 2	RABBIT 3	RABBIT 4	RABBIT 5	MEAN (Meq)	MEAN (%)
35	5.0	8.0	5.6	2.6	3.6	(5.0)	102.0
37	6.0	5.6	1.8	6.0	4.8	(4.8)	97.9
39	3.8	4.8	5.2	-	5.6	(4.8)	97.9
41	4.0	9.5	5.0	4.6	3.6	(5.3)	108.2
43	5.3	3.6	3.3	7.2	-	(4.8)	97.9
45	4.9	4.0	7.8	5.2	4.8	(5.4)	110.2
47	5.0	6.0	3.6	4.4	5.0	(4.8)	97.9
49	6.6	3.0	4.0	5.6	7.2	(5.3)	108.2
51	3.0	4.0	6.0	3.6	4.5	(4.2)	89.4
53	2.5	6.8	3.0	3.6	-	(4.0)	81.6
55	5.6	4.4	6.4	6.0	3.0	(5.1)	104.1
57	4.4	4.0	5.6	4.8	4.6	(4.7)	95.9
59	3.8	6.0	4.0	5.6	4.8	(4.8)	97.9
61	4.4	3.6	6.0	4.0	5.4	(4.7)	95.9
63	6.0	3.0	4.8	5.6	4.4	(4.8)	97.9
65	5.0	4.0	8.0	3.0	4.0	(4.8)	97.9
67	5.0	7.2	4.0	3.6	5.6	(5.1)	104.1
69	4.4	4.0	4.4	5.0	2.8	(4.1	83.7
71	3.6	4.5	4.8	5.6	4.2	(4.5)	91.8
73	3.2	6.0	7.2	-	3.6	(5.0)	102.0
75	4.0	4.0	4.8	5.6	6.4	(5.0)	102.0
77	7.2	6.4	3.6	5.0	3.6	(5.2)	106.1
79	5.6	5.6	4.8	5.4	5.0	(5.3)	108.2
81	4.4	3.6	5.0	6.0	8.0	(5.4)	110.2
83	8.0	-	3.1	4.8	4.0	(4.9)	100.0
85	4.4	4.0	5.6	4.0	4.2	(4.4)	89.8
87	4.8	6.2	4.4	6.6	3.8	(5.2)	106.1
89	3.0	6.0	4.5	3.6	4.0	(4.2)	89.4
90	5.6	7.2	4.8	5.0	4.4	(5.4)	110.2

APPENDIX III. CHAPTER 339

THE MIRAA PROHIBITION ORDINANCE (REV. 1962)

Commencement: 29th September, 1951

An Ordinance to prohibit the sale, cultivation, use and possession of miraa in certain areas

1. This Ordinance may be cited as the Miraa Prohibition Ordinance, and shall apply to the areas specified in the Schedule to this Ordinance.

2. In this Ordinance, except where the context otherwise requires -

"miraa" means the shrub Catha edulis, and includes any tree, plant, leaf, stem, shoot or any derivative or alkaloid thereof.

3. (1) Any person who-

(a) cultivates or plants miraa, or being the owner of occupier of any land, allows, miraa to grow thereon;

(b) not being a permit holder, sells or purchases miraa; or

(c) not being a permit holder, consumes, uses or is in posession of miraa,

shall be guilty of an offence and, on conviction therefor by a subordinate court of the first or second class, liable to a fine not exceeding two thousand shillings or to imprisonment for a term not exceeding twelve months.

(2) Any permit holder who sells, purchases, consumes, uses or possesses miraa otherwise than in accordance with the conditions of the permit issued to him in that behalf, or who delivers or parts with possession of any miraa to any person other than a permit holder authorized to purchase or possess miraa, shall be guilty of the offence and, on

conviction therefor by a subordinate court of the first or second class, liable to a fine not exceeding two thousand shillings or to imprisonment for a term not exceeding twelve months

(3) Where a court convicts any person of an offence under subsection (1) or subsection (2) of this section, it may order that any miraa found in the possession of the offender, or in or upon any land owned or occupied by such person, be destroyed and, in addition, the court may order that the cost of such destruction shall be paid by the offender and in default of payment thereof that the offender be imprisoned for a term or, as the case may be, for a further term not exceeding one month.

4. (1) The Provincial Commissioner may issue a permit, subject to such conditions, to be specified in the permit, as he may see fit to impose, to any African or Somali resident in the areas specified in the Schedule to this Ordinance authorizing such person to sell, purchase, consume, use or possess miraa.

(2) No permit issued under the provisions of subsection (1) of this section shall authorize any permit holder to sell, deliver or part with possession of miraa to any person other than a permit holder authorized to purchase or possess miraa.

(3) The Provincial Commissioner may at any time cancel any permit issued under this Ordinance or amend any condition thereof.

5. In any case where any miraa is found on any premises or in or upon or growing on any land or on or in any vehicle or animal, the following persons shall, for the purposes of this Ordinace, be deemed to be in possession of such miraa-

(a) in relation to any premises or land, the owner of such premises or land and every person occupying any part thereof or any room therein, or having access thereto;

(b) in relation to any vehicle, the owner, driver and any person travelling with or in or on such vehicle;

(c) in relation to any animal, the owner, any person having or appearing to have control thereof and any person travelling with or riding upon such animal,

unless, in any such case, any such person proves to the satisfaction of the court that he did not know, nor had he any reason to believe, that such miraa was on or in such premises or in or upon or growing on any such land or in or on such vehicle or animal.

6. All offences under this Ordinance shall be cognizable to the police.

SCHEDULE (s.4)

1. The northern Province.

2. The area situate within a radius of ten miles of the office of the District Commissioner, Isiolo.

3. That portion of the Meru Land Unit lying to the north of the Isiolo-Garba-Tulla Road and that portion thereof lying within one mile of such road to the south thereof.

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