PHARMACOLOGICAL AND TOXICOLOGICAL INVESTIGATIONS OF *CRASSOCEPHALUM MANII* MILNE-REDHEAD

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'A thesis submitted in part fulfilment for the degree of Master of Science in the University of Nairobi.'

1980

DECLARATION

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

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(b) This thesis has been submitted for examination with our approvals as University Supervisors.

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ABSTRACT

Pharmacological investigations of the flowers of Crassocephalum mannii Milne-Redhead using the freeze-dried alcoholic extract of the flowers showed that the lethal dose 50% (LD_{50}) in mice was 3078^{+}-42.7 mg/kg of body weight.

On the isolated organ preparations the extract was observed to increase the tone of the isolated guinea pig ileum and rabbit duodenum, and decreased the rate and force of contractions of the isolated perfused rabbit heart. The effects on the intestine were blocked by atropine sulphate and mepyramine maleate while the effects on the heart were blocked by atropine sulphate. These findings indicated the presence in the extract of compound(s) with parasympathomimetic and/or histaminic effects.

Extraction for alkaloids in Crassocephalum mannii flowers revealed the presence of two alkaloids, one of which was detectable with the Dragendorff reagent while the other with the Marquis reagent. The alkaloid solution decreased the tone and amplitude of the isolated rabbit duodenum. The effects of the alkaloid solution thus differed from those of the freeze-dried alcoholic extract when applied on the isolated rabbit duodenum. The alkaloid solution decreased the tone and amplitude of the isolated rabbit duodenum while the freeze-dried alcoholic extract increased the tone of the isolated rabbit duodenum. The different effects due to the alkaloid solution and the alcoholic extract on the

isolated rabbit duodenum were not investigated further and as such further work is required to elucidate the nature of compounds eliciting these varying pharmacological actions.

The isolated perfused livers from the rats which had been fed on ration containing 2.5% of C. mannii flowers and from the rats which had received single intraperitoneal injection of the freeze-dried alcoholic extract of C. mannii flowers showed normal bile outflow but high amounts of sulfobromophthalein in perfusate and delayed sulfobromophthalein excretion in bile.

However, the isolated perfused livers from the rats which had been fed on ration containing 8% of C. mannii flowers showed increased bile outflow and sulfobromophthalein excretion.

In the intact animals the extract seemed to increase the movement of phenol red in the rat intestines when the extract was administered to rats per os but inhibited movement of phenol red in the intestines when the extract was administered intraperitoneally. In the dog the extract caused a drop in blood pressure and this hypotensive effect was adequately blocked by atropine sulphate and to some extent mepyramine maleate.

The feeding of flowers of Crassocephalum mannii Milne-Redhead in high dosages (10, 20 and 40%)to rats for ten days caused acute intoxication and death in some of the rats. The post mortem examination showed enlarged livers and enlarged adrenal glands. The histological

examination of the liver sections revealed massive necrosis of the hepatocytes close to the central vein, haemorrhages and increased number of Kupffer cells.

The feeding of flowers of Crassocephalum mannii in low dosages (2, 4 and 8%) to rats for one hundred days led to a marked reduction in the growth rate and death in some of the rats.

The post mortem examination showed enlarged livers with a dark mottled pattern and pale stained firm livers with granulated surfaces in other rats. The gross changes were most pronounced in the highest dosage group.

The histological examination revealed centrolobular necrosis and degeneration or necrosis of vascular walls with the formation of vascular thrombi. In more protracted cases there were megalocytosis, proliferation of connective tissue and bile duct epithelium in the periportal areas, in addition to the occurrence of centro-lobular necrosis. The severity of the pathological changes were dose-related.

In addition in the cases with the most severe liver changes, kidney changes including tubular degeneration and hyaline casts were observed in the lumen of the tubules.

The extract did not show any bacteriostatic or bactericidal effects on growing Staphylococcus aureus.

During the screening experiments of Crassocephalum mannii some pharmacological and toxicological effects were observed and these effects should be examined in detail in future work on this plant.

INTRODUCTION

Medicinal plants play a considerable role in the treatment of various ailments of the people and their livestock in East Africa. Though the actual statistics of the number of people and livestock involved are lacking, nevertheless herbal specialists exist in various places including the main cities. Considering the fact that the distribution of medical facilities has not extended to all the remote areas of East Africa and that in some cases people in the rural areas do not trust the medical personnel in the treatment of some diseases, the use of traditional healers armed with medicinal plants assumes greater prominence.

The realization of this fact has led to the suggestions that traditional healers be incorporated into the primary health care programmes and that documentation and study centres of medicinal herbs be set up (W.H.O., 1978).

A considerable number of plants in East Africa is reported to be medicinal through folklore. Despite the abundance of these medicinal plants less work has been carried out to determine their biological activities let alone the compounds eliciting these biological activities. Crassocephalum mannii (Hook. f.) Milne-Redhead is one of the many plants

in Kenya that are reputed to be medicinal. It is of widespread distribution in Kenya and various tribes have exploited its medicinal value by using it to cure malaria, as an anthelmintic, a purgative, for dysentery and indigestion (Kokwaro, 1976). Other species of Crassocephalum are of widespread distribution in East Africa and likewise have been used by various tribes in the treatment of various diseases (Kokwaro, 1976, Watt and Breyer-Brandwijk, 1962). The roots, leaves and flowers of members of this genus are reported to contain the active principle(s).

Realizing that people use Crassocephalum mannii and other species in the same genus in the treatment of various ailments and recognizing the fact that nothing is known about the mode of the pharmacological effects it causes and the compounds associated with these effects, this work represents an attempt to establish the pharmacological effects of Crassocephalum mannii Milne-Redhead on isolated organ and intact animal preparations, its toxicological effects in relation to the clinical signs, gross and microscopic pathological changes and the chemical compounds (alkaloids) it contains.

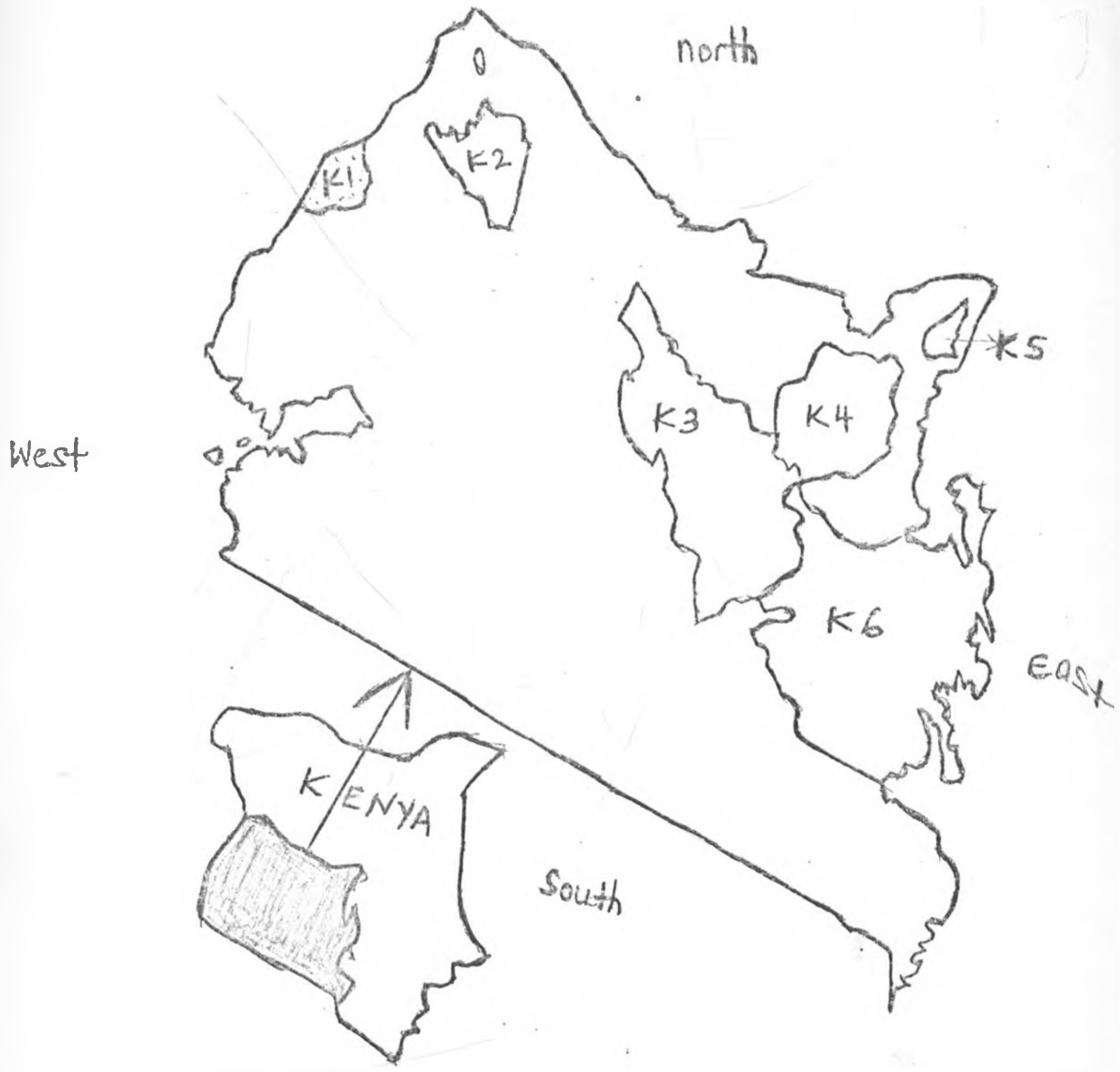

Figure 1: The distribution of Crassocephalum mannii Milne-Redhead in Upland Kenya (in areas K1, K2, K3, K4, K5 and K6)

These areas are:-

K1	(Highlands Elgon) Kenya Elgon above 6000 feet.
K2	(Highlands Cherangani) Cherangani above 6000 feet on east and 7000 feet on west.
K3	(Highlands Aberdares) Aberdare range above 7000 feet on the west. Includes Nyeri and Laikipia.
K4	(Highlands Kenya) Mount Kenya above 6000 feet on the south and east, and 7000 feet on the north and west includes Meru.
K5	(Highlands Nyambeni) Nyambeni hills over 5000 feet.
K6	Machakos

LITERATURE REVIEW

The genus Crassocephalum Moench. belongs to the compositae family or sunflower family the largest of all plant families including perhaps 20,000 species (Benson, 1959).

Several hundred species of the compositae family are indigenous to East Africa while about half a dozen of them possess sufficiently poisonous properties to make them dangerous to livestock (Verdcourt and Trump, 1969). Members of the compositae family known to be poisonous include Ageratum conyzoides, Dichrocephala crysanthemifolia and the Senecio species. Many species of the compositae family including Crassocephalum mannii are also reported through folklore to be medicinal (Watt and Breyer-Brandwijk, 1962; Kokwaro, 1976).

Crassocephalum and Senecio

The genus Crassocephalum Moench. is similar to the genus Senecio L., the difference lies in the flower structures between the two genera (Agnew, 1974). While a lot of information is known about the Senecio species in relation to their toxicity to man and his livestock, and the chemical compounds causing the toxic effects no such information is known about the Crassocephalum species.

Crassocephalum mannii (Hook. f.) Milne-Redhead is synonymous to Senecio multicorymbosus Klatt (Dale and Greenway, 1961).

Distribution of Crassocephalum mannii

The Crassocephalum species are known to occur in East Africa and Agnew (1974) gives the distribution of several species in the Kenya uplands. Crassocephalum mannii has a widespread distribution, the plant is found in scrub and in the wetter parts of Kenya, from altitude of four thousand to eight thousand feet above sea level. It is also known to occur in Rungwe district of Tanzania.

Description of Crassocephalum mannii

Crassocephalum mannii is a much branched, soft-wooded shrub or tree reaching a height of twenty-five feet. The stem is green throughout its length. The leaves are serrate, oblong, elliptic to eighteen inches long and four inches wide. The apex of the leaves is acuminate and the base is cuneate. The influorescence is terminal and measures up to two feet long. The flowers are small, yellow, unpleasantly scented, all in one kind in a head in dense panicled cymes (shown in Fig. 2 and Plate 1).

The plant in Kenya is referred to by different names in different areas because of differences in the languages of the ethnic tribes. The local names for this plant include: Muthariti (Kikuyu;

Mutomboro (Meru): Irangara (Kamba); Lugugutt (Samburu); Mukorombosha (Teita); Gulanguso (Shambaa); Mdaa (Pare and Yergekwa (Marakwet).

Crassocephalum species used by man

Several species of Crassocephalum are used as herbal medicine by man but Crassocephalum montuosum and Crassocephalum rubens are not included in the species which are reported medicinal. The Chagga and Giriama crush and extract fluid from the leaves of Crassocephalum bojeri, when the fluid is drunk it is reported to cure the people from cold, fever and rheumatism (Kokwaro, 1976). Crassocephalum crepidioides appears to have widespread utilization both as a herbal and food plant. In Bukoba and Kakamega districts, the extract obtained from the squeezed leaves is applied to the wounds to promote healing. The dried and powdered leaves are snuffed as a styptic to stop nose bleeding (Kokwaro, 1976).

Watt and Breyer-Brandwijk (1962) reported that the same plant is used in the Shambala snuff to stop nose bleeding. The same authors reported that in West Africa both the whole plant and the semi-succulent leaf being mucilaginous are used for soups and sauces. A decoction of the leaf is used as a lotion for headache and as a mild stomachic. The roots are reported to contain tannin.

In Kakamega and Ukambani, the leaves of Crassocephalum picridifolium are chewed and then the fluid sprayed over the wound to promote healing. Crassocephalum subscandens is used in Tanzania in the treatment of abdominal pains, convulsions, fever, toothache and cancer. The same plant is used as an antidote for irritant poisoning due to Clematis species (Watt and Breyer-Brandwijk, 1962).

The Haya and Kikuyu use Crassocephalum vitellinum in the treatment of a diseased eye. The flower is rubbed into the eye to clear the causative agent of the eye disease, it is also reported to remove tiny particles that get into the eye (Kokwaro, 1976). Watt and Breyer-Brandwijk (1962) on the same plant reported that the Haya use it as a gonorrhoea remedy, for suppurations of the skin and elsewhere and to improve the quality of milk of a lactating woman.

Crassocephalum mannii is reported to be medicinal. The information on the (Nairobi) Herbarium specimen of C. mannii (1961) includes among other things that in Kiambu the roots of the plant are cooked and the fluid obtained, when drunk cures malaria. The roots of the plant are used by the Shambaa and Pare, and in Marakwet as an anthelmintic, a purgative, for dysentery and indigestion (Kokwaro, 1976).

Watt and Breyer-Brandwijk (1962) reported that Senecio multicorymbosus which according to Dale and Greenway is synonymous to Crassocephalum mannii is planted amongst the cassava plantations by farmers in the Rungwe district of Tanzania to repel cassava root-eating rodents. The repellant effect of the plant is attributed to the odour of its flower. The plant is also used extensively to form demarcation of land holdings and as a support to fruiting bananas in Kenya.

The biological effects of Crassocephalum mannii have hitherto not been investigated neither have been the compounds that might be responsible for these effects and the objective of this work is to establish the pharmacological and toxicological effects as well as the compounds (alkaloids) pertaining to Crassocephalum mannii.

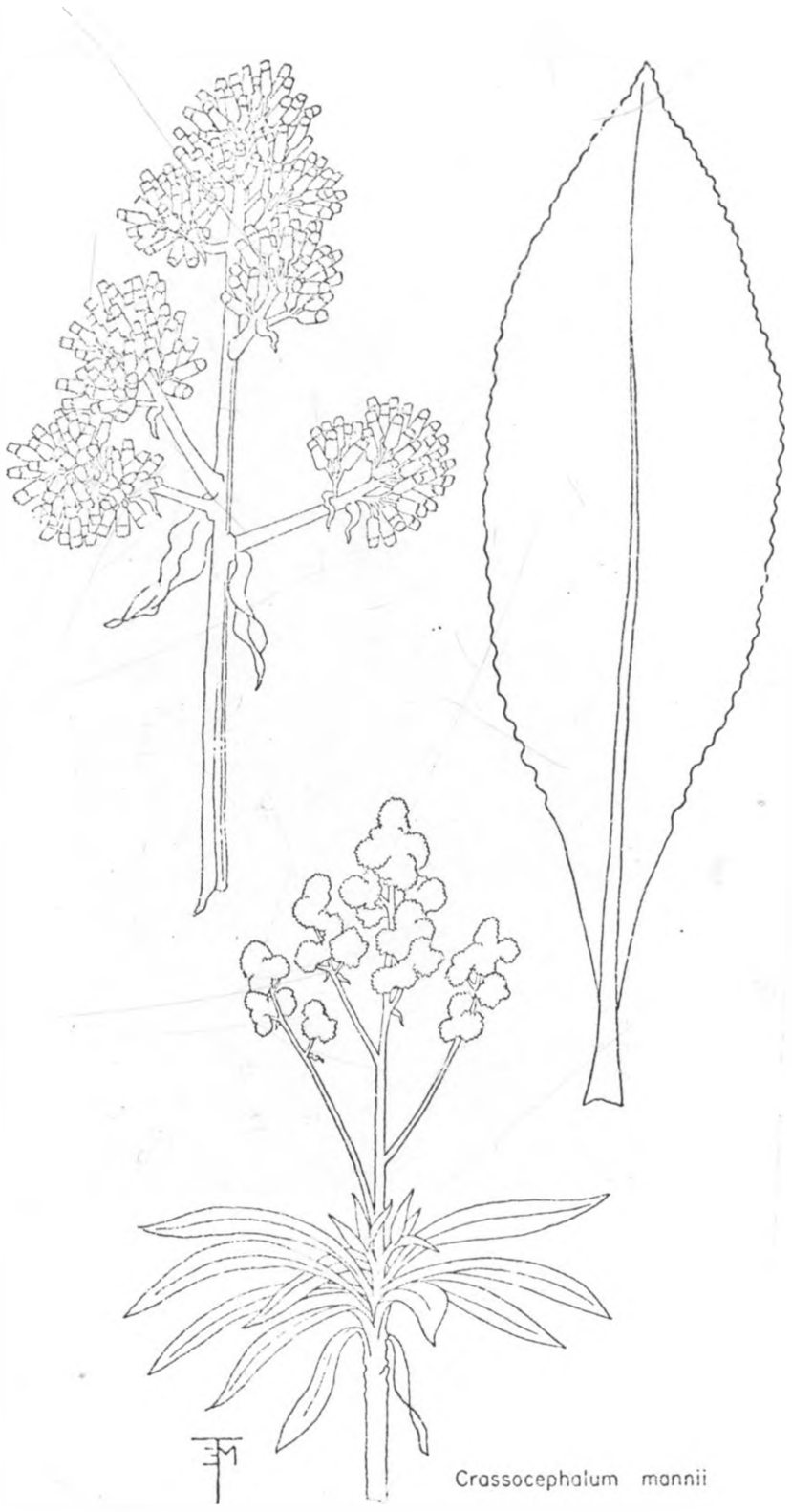

Figure 2: The flowers and leaves of Crassocephalum mannii (Agnew, A.D.Q. (1974). Upland Kenya Wild Flowers, p. 474, Oxford University Press, Ely House, London W.1.).

Plate 1: The shrub Crassocephalum mannii Milne-Redhead (in the background)

MATERIALS AND METHODS

A. Collection of material for investigation

Flowers of Crassocephalum mannii were collected from Muguga, in Kiambu District, Kenya. A sample from these flowers was submitted to the Herbarium in Nairobi where the flowers were identified and confirmed to be those of C. mannii. The flowers were air-dried in a shade for three weeks after which they were removed from the stalks, placed in polythene bags and stored at room temperature.

B. Extraction of pharmacologically active substances from C. mannii flowers

Materials:

(i) For alcoholic extraction

Plant Grinder (Thomas, Arthur H. Thomas, Co., Phila. PA., U S A.)

1 millimetre sieve, spring balance, polythene bags

Round bottom extraction and distillation flasks

Beakers, pipettes, filter funnels

Whatman filter paper No. I

Water bath at 40-50^{o}C

Rotary evaporator (Buchi, app. Nr. 21184)

Vacuum pump (Sargent-Welch Scientific Co.)

Freeze drier (Virtis)

Glass desiccator with knob cover

Activated charcoal, 99.5% methanol,

98% sulphuric acid

Flowers of Crassocephalum mannii

(ii) For alkaloid extraction

Hot box oven (Grundy Equipment Ltd.)

Separating flasks, micro-pipettes

High speed centrifuge (Labofuge II,

John Achelis of Johne)

Centrifuge tubes

20 cm square TLC glass plates

Chromatography tanks (Shandon)

Chromatography viewing cabinet (Chromato-Vue,

Ultra Violet Products Inc., California, U.S.A.)

Silica Gel type 60G Art 7731 (E. Merck,

Darmstadt)

25% ammonia, pure diethyl ether, 99.5%

methanol

10% acetic acid in distilled water

1% ammonium hydroxide in distilled water

2% Pilocarpine nitrate in isotonic saline

Marquis reagent consisting of 1 ml of formal-

dehyde in 10 ml of concentrated sulphuric acid

Dragendorff reagent made out of:

(1) 0.6 g of bismuth subnitrate in 2 ml concentrated hydrochloric acid and 10 ml of distilled water.

(2) 6 g potassium iodide in 10 ml of distilled water.

These stock solutions were mixed together with 7 ml of concentrated hydrochloric acid and 15 ml of distilled water and then the whole mixture diluted with 400 ml of distilled water. 99.5% methanol and 25% ammonia (Analytical grade) used as solvent for TLC on silica gel in the ratio 200:3

Ground C. mannii flowers

Method:

(i) For alcoholic extraction as described by Harborne, J.B. (1973).

Flowers of Crassocephalum mannii were ground to fine particles and placed in an extraction flask into which 99.5% methanol was added until the flower material was completely submerged. The extraction flask and contents were placed in a water bath which was maintained at 40-50°C. There followed periodical harvesting of the extract fluid. The extraction was stopped at a stage when the extract fluid became colourless. All the extract fluid was pooled, clarified by addition of activated charcoal, mixed, filtered, concentrated on the rotary evaporator at 60°C and freeze dried. The solid material which remained after freeze drying was dark brown, sticky and markedly hygroscopic.

It was stored in a desiccator. This material dissolved in 0.1 N sodium hydroxide, isotonic saline and distilled water.

(ii) For alkaloid extraction as described by Harborne, J.B. (1973) with some modification.

Ten percent acetic acid aqueous solution in 99.5% methanol was added to finely ground C. mannii flowers in an extraction flask until the flower material was completely submerged. It was left to stand for at least four hours and the extract fluid was then removed. The preceding operation was repeated four times. The extract fluid was pooled, clarified by addition of activated charcoal, mixed, filtered and concentrated on the rotary evaporator at 60°C to one quarter of the original volume.

The alkaloids were precipitated by dropwise addition of concentrated ammonium hydroxide to the remaining volume of extract fluid. The alkaloids were collected by centrifugation at 3000 r.p.m. for 10 minutes, washed with 1% ammonium hydroxide and collected again by centrifugation at 3000 r.p.m. for 10 minutes. Diethyl ether was added to the precipitated alkaloids and the ethereal layer was concentrated on the evaporator at 60°C and tested for the presence of alkaloids.

Testing for presence of alkaloids:

Thirty grams of silica gel type 60 G were dissolved in 60 ml of distilled water and spread on five TLC glass plates to make a coating layer of 0.25 mm thickness per plate. The plates were placed in a hotbox oven, dried at 110°C for 1 hour after which they were removed, cooled and stored in a desiccator.

Ten microlitres of the ethereal alkaloid solution, and 10 microlitres of 2% pilocarpine nitrate in isotonic saline (standard control) were chromatographed on the silica gel plates. The plates were placed in a chromatography tank containing a solvent mixture of 99.5% methanol and 25% ammonia in the ratio of 200:3. After 25 minutes in the chromatography tank the plates were removed and placed in the chromatography viewing cabinet. The fluorescence emitted by the alkaloids under UV light was recorded.

The plates were also sprayed with Dragendorff and Marquis reagents and the colour of the alkaloid spots noted. The Rf values for the alkaloids were measured.

C. Determination of lethal dose 50% (LD_{50}) of the freeze-dried alcoholic extract of C. mannii flowers in mice.

Materials:

Plastic cages, wood shavings

Analytical balance (Sartorius), spatula, forceps

25 G ¾ in. needles

1 ml syringes

10 universal bottles

120 ml water bottles

Mice pencils (Unga Ltd. Kenya)

Freeze-dried alcoholic extract of C. mannii flowers

Isotonic sodium chloride

Albino mice

Logarithmic - probability paper

Method:

Four-week old, male albino mice weighing 17-20 g each were obtained from Kabete Veterinary Laboratories. The mice were kept in the animal room at 27-28°C, in groups of ten per cage. The bedding for the mice was wood shavings. The mice were fed on mice pencils and given water ad libitum. They were observed daily for two weeks and any that developed sickness was not used in the LD_{50} determination.

The freeze-dried alcoholic extract of C. mannii flowers was dissolved in isotonic saline and administered to mice intraperitoneally. The control mice were injected isotonic saline intraperitoneally. Dose ranging was done using two mice per dose and the doses used were 50, 100, 200, 400, 800, 1600, 3200 and 4000 mg of extract/kg of body weight. The final doses used in the calculation of lethal dose 50% were 2741, 2839, 2941, 3046, 3155 and 3268 mg of extract/kg of body weight; and ten mice per dose were used. The mice were observed for forty eight hours following intraperitoneal injections and then the percentage mortality per dose was recorded.

The results were plotted on logarithmic-probability paper. The LD_{50} was calculated and its standard error was estimated by the method of Miller and Tainter (1944).

D. Determination of effects of the freeze-dried alcoholic extract and alkaloid fraction of C. mannii flowers on:

(1) Isolated guinea pig ileum

(2) Isolated rabbit intestine

Materials:

Freeze-dried alcoholic extract of C. mannii flowers

Isotonic saline

Kymographic recording set-up (Student Kymograph, Scientific and Research Instruments Ltd.)

Isolated organ bath (C.F. Palmer (London) Ltd.)

Scissors, stunning hammer

Oxygen (95%) and carbon dioxide (5%) mixture

Atropine sulphate 1% in isotonic saline

Mepyramine maleate 0.1% in isotonic saline

Tyrode solution

Adult male guinea pig

Adult male rabbit

Method:

The method used was described by the Staff of the Department of Pharmacology, University of Edinburgh (1970).

(1) An adult male guinea pig was stunned and bled through the carotids. A portion of the ileum was carefully removed, placed in Tyrode solution

maintained at 37°C and allowed to relax. The portion of the ileum was flushed with oxygenated Tyrode solution without applying excessive force. A piece of 1-2 cm length from the ileum was threaded at each end by inserting a needle from the inside of the gut, the lumen of the gut was maintained open. One thread was tied to the end of the oxygenating tube in the organ bath and the other to a lever with a frontal writing point. The preparation was allowed in the bath ten minutes to regain its tone and washed several times during that period maintaining the Tyrode solution at 37°C and aerating it with oxygen and carbon dioxide mixture.

(2) The same procedure was followed in the preparation of the rabbit intestine except that it was a duodenum piece 4-5 cm long which was used

Testing:

The normal intestinal contractions were recorded on the kymograph running at 0.25 mm/sec, this recording served as a baseline for further tests.

(i) 20 mg of freeze-dried alcoholic extract dissolved in isotonic saline were introduced into the organ bath and the effect was recorded on the kymograph.

Further doses of freeze-dried alcoholic extract of C. mannii, that is, 40, 60, and 80 mg in isotonic saline were applied onto the intestine and the effects noted. Each introduction of dose of test material or drug was preceded by washing the piece of intestine 2-3 times so as to remove the tested compounds and re-establish the original tone and movements.

(ii) 0.2 ml of atropine sulphate (1%) in isotonic saline was introduced into the organ bath, allowed to remain for two minutes, then 20 mg of extract were added and the net effect was recorded. The preceding step was repeated with different doses of atropine sulphate and extract, and the overall effect was noted.

(iii) The piece of intestine was washed 2-3 times and 0.2 ml of mepyramine maleate (.1%) in isotonic saline was introduced into the organ bath, allowed to remain for two minutes, then 20 mg of extract were added and the net effect was recorded. The preceding step was repeated with different doses of mepyramine maleate and the extract and the overall effect was noted.

(iv) The alkaloid fraction was tested for its effects on the rabbit duodenum using 0.2 and 0.4 ml of the alkaloid fraction, and the effects were recorded on the kymograph.

E. Determination of effect of the freeze-dried alcoholic extract of C. mannii flowers on the isolated perfused rabbit heart, (Langendorff's preparation).

Materials:

Kymograph with recording set-up (Student Kymograph, Scientific and Research Instruments Ltd.)

Apparatus designed to deliver oxygenated Ringer-Locke Solution at a constant pressure and a constant temperature of 37°C (C.F. Palmer (London) Ltd.)

Scissors, forceps, stunning rod, thread

Oxygen (95%) and carbon dioxide (5%) mixture

Ringer-Locke solution, isotonic saline

Freeze-dried alcoholic extract of C. mannii flowers

Atropine sulphate 100 µg/ml in isotonic saline

Animal: 6-month old, male locally bred rabbit.

Method:

The method followed was described by the Staff of the Department of Pharmacology, University of Edinburgh, (1970).

An adult male rabbit was killed by a blow on the head. The throat of the animal was then cut and when the blood had drained out, the chest was opened. The heart with at least 1 cm of aorta attached was removed as quickly as possible and placed in a dish of Ringer-Locke solution at room temperature of 28^{o}C. The preparation was gently squeezed several times when first placed in the Ringer-Locke solution so as to remove as much blood as possible. The aorta was located and dissected free from its attachment to the pulmonary artery and then was cut just below the point where it divided. The heart was then transferred to the perfusion apparatus where the aorta was tied onto the glass cannula. A small hook was fixed in the tip of the left ventricle and the perfusion was then started. A record was obtained of the force and rate of ventricular contractions. The test materials or the drugs were added to the preparation by injection through the rubber cap into the perfusion fluid. Further doses were given after the preparation had recovered and the rates steadied.

Testing:

(i) The freeze-dried alcoholic extract of *C. mannii* flowers was dissolved in isotonic saline and 4 mg of it were added to the Ringer-Locke solution and a record of its effects on the rate and force of ventricular contractions noted on the kymograph.

(ii) 50 µg of atropine sulphate in isotonic saline were added to the Ringer-Locke solution, this was followed immediately by addition of 4 mg of extract to the Ringer-Locke solution and the effects on the heart recorded on the kymograph.

F. Determination of effects of the freeze-dried alcoholic extract of C. mannii flowers alone and in the presence of atropine, mepyramine, ergotamine and propranolol on blood pressure in the dog.

Equipment:

Mercury manometer with frontal writing point and pressure bottle (Interflon, G. Seringham and Co. Ltd.)

Kymograph and drum (C.F. Palmer, London)

Smoked papers for drum

Operating table (Instruments and Chemicals PVT., Ltd. Ambala)

Analytical balance (Sartorius - Werke GMBH Gottingen)

Arterial cannula PE 2 mm diameter

Venous cannula PE 2 mm diameter

Surgical blades, scissors, cords, clamps, forceps, syringes, needles, cotton wool

Drugs:

The drugs and test material were dissolved in isotonic saline.

Drug	Concentration (mg/ml)
Freeze-dried alcoholic extract of C. mannii flowers	144.00
Ergotamine tartrate	0.12
Propranolol hydrochloride	4.8
Atropine sulphate	1.4
Mepyramine maleate	0.029

Heparinized physiological saline solution for pressure bottle (5,000 I.U. heparin/litre).

Pentobarbitone sodium 60 mg/ml (May and Baker Ltd. Dragenham).

Animal:

A 2-year old, clinically healthy, locally bred male dog.

Method:

The method used was described by the Staff of the Department of Pharmacology, University of Edinburgh, (1970).

Anaesthesia:

The dog was weighed and anaesthetized to a deep surgical plane with pentobarbitone sodium using a calculated dose of 30 mg/kg of body weight given intravenously.

Cannulation of the carotid artery:

A ventral longitudinal midline incision was made through the skin of the neck. Through blunt dissection of the neck muscles, the trachea and one of the carotid arteries were exposed. The carotid artery was separated from the vagus for a distance of about 3 cm. The cephalic end of the exposed artery was occluded with a ligature. A small incision was made between the clamp and ligature, the tip of the cannula was then introduced towards the heart and tied securely in place. The pressure in the bottle was raised and the valve opened for blood pressure recording.

Cannulation of the femoral vein:

The pulse of the femoral artery was palpated as it coursed down the inner surface of the thigh. The over-lying skin was rolled to one side and an incision approximately 4 cm long was made. The femoral vein was located outside the femoral artery and cannulation was made as in the carotid artery.

Testing:

The injections were made in the prescribed sequence utilizing the femoral catheter and were flushed with approximately 5 ml of heparinized physiological saline solution after each injection.

(1) Freeze-dried alcoholic extract of C. mannii flowers in isotonic saline at 12 mg/kg of body weight.

(2) Atropine sulphate in isotonic saline at 0.2 mg/kg of body weight.

(3) Injected concurrently the freeze-dried alcoholic extract of C. mannii flowers at 12 mg and atropine sulphate at 0.2 mg/kg of body weight.

(4) Mepyramine maleate in isotonic saline at 0.0024 mg/kg of body weight.

(5) Injected concurrently the freeze-dried alcoholic extract of C. mannii flowers at 12 mg and mepyramine maleate at 0.0024 mg/kg of body weight.

(6) Ergotamine tartrate in isotonic saline at 0.010 mg/kg of body weight.

(7) Injected the freeze-dried alcoholic extract of C. mannii flowers at 12 mg/kg of body weight after ten minutes.

(8) Propranolol hydrochloride in isotonic saline at 0.4 mg/kg of body weight.

(9) Injected the freeze-dried alcoholic extract of C. mannii flowers at 12 mg/kg of body weight after ten minutes.

G. Determination of effect of the freeze-dried alcoholic extract of C. mannii flowers on gut motility following oral and intraperitoneal routes of extract administration.

Material:

Rat stomach tubes

Meter rule, clock, forceps, scissors,

Pasteur pipettes and rubber teats

Analytical balance (Sartorius-Werke GMBH Gottingen)

Plastic cages

Phenol red, 0.9% sodium chloride in distilled water

Glucose

0.1 N ammonium hydroxide in distilled water

Freeze-dried alcoholic extract of C. mannii flowers dissolved in isotonic saline

Diethyl ether

Animal: 5-month old, male albino rats.

Method:

The method applied was described by Sognen, E. (1965).

Preparation of phenol red solution:

To each millilitre of 0.9% sodium chloride in distilled water, 0.75 mg of phenol red and 5.0 mg of glucose were added. 300 ml of this solution were constituted.

Five-month old, male albino rats were obtained from Kabete Veterinary Laboratories and assigned into groups, each group consisting of four rats. Each rat in each group was ear-marked for observation for a specified period and identification marks were used for this differentiation.

In the first trial the rats were observed for 5, 10, 15 and 20 minutes; and the rats were dosed orally with either 50 or 500 mg of extract/kg of body weight. Each rat that was dosed was followed immediately by an oral dose of phenol red solution at 1 ml/100 g of body weight.

In the second trial the rats were under observation for 30, 60, 90 and 120 minutes. The rats were dosed with the freeze-dried alcoholic extract of C. mannii flowers at either 50 or 500 mg/kg of body weight per os or intraperitoneally. Each rat that was dosed with extract was followed immediately by an oral dose of phenol red solution at 1 ml/100 g of body weight.

The control group of rats were dosed with isotonic saline orally or intraperitoneally; and

each rat was subsequently dosed orally with phenol red solution at 1 ml/100 g of body weight.

At the end of the designated periods the rats were euthanized with diethyl ether. Each rat was opened through the abdomen, the stomach and intestines were exposed. Ligatures were made at the cardia and the pylorus of the ventricle.

The intestines were removed, laid out and opened from the duodenum to the ileo-caecal junction. The lengths of the intestine from the duodenum to the ileo-caecal junction which were covered by phenol red solution were measured. A solution of 0.1 N ammonium hydroxide was spread along the intestinal mucosa to accentuate the reddish colour of phenol red.

The portion of the intestine covered by phenol red at a given time, dose and route was expressed as a percentage of the total length of intestine and the results were submitted to procedures of factorial experiment statistical analysis (Snedecor, G.W. and Cochran, W.G. (1967)).

II. Determination of the ability of rat livers to excrete bile and sulfobromophthalein following pre-treatment of rats with:

(1) Feed containing 2.5 and 8% of C. mannii flowers for eight days.

(2) Single intraperitoneal injections of freeze-dried alcoholic extract of C. mannii flowers at 300 mg/kg of body weight.

Pre-treatment of liver donor rats:

Materials and Method:

Wire-mesh cages, plastic water bottles
Spring balance, polythene bags
Plant Grinder (Thomas, Arthur H. Thomas Co.)
1 millimetre sieve, needles, syringes
Freeze-dried alcoholic extract of C. mannii flowers dissolved in isotonic saline
C. mannii flowers
Commercial mice pellets (East African Importers and Exporters)
Animals: 4-month old, male albino rats weighing approximately 200 g.

Preparation of rations containing 2.5 and 8% of C. mannii flowers:

Dried C. mannii flowers and mice pellets were ground and sieved separately to fine particles of 1 mm circumference and stored in dry polythene bags.

Ration containing 2.5 of C. mannii flowers:

12 g of ground C. mannii flowers were mixed manually with 468 g of ground mice pellets to give a ration mixture weighing 480 g. This ration was fed to four rats for 8 days.

Ration containing 8.0% of C. mannii flowers:

38.4 g of ground C. mannii flowers were mixed manually with 441.6 g of ground mice pellets to form a ration mixture weighing 480 g. This ration was fed to four rats for 8 days.

Four-month old, male albino rats weighing approximately 200 g each were obtained from Kabete Veterinary Laboratories. The rats were divided into four groups, each group consisted of four rats.

The first and second group of rats were fed on rations containing 2.5 and 8% of ground C. mannii flowers respectively for eight days. After eight days, the livers were removed from rats and assessed for sulfobromophthalein and bile excretion. The rats in the third group were injected once intraperitoneally with alcoholic extract of C. mannii flowers at 300 mg/kg of body weight. A day after the injections, the livers were removed from the rats and assessed for bile and sulfobromophthalein excretion.

The rats in the fourth group served as control rats and were fed exclusively on commercial mice pellets. All the rats were given water ad libitum.

The ability of the rat livers to excrete sulfobromophthalein and bile was determined by use of the isolated perfused liver technique.

Isolated perfused liver technique:

The isolated perfused liver technique was applied as described by Mehendale, H.M. (1976) with minor modification.

Conditions:

Water bath temperature of	40°C
Blood temperature	37°C
Room temperature	26-27°C
Oxygen flow	2 litres/minute

Materials:

Apparatus for isolated perfused liver consisted of the following:

Perfusion chamber containing 3 separate units, that is, the upper unit, the middle perfusate dish and the lower oxygenator unit

Perfusion pump (Multifix, Type M 80) for pumping of perfusate

Water pump (B. Braun, Melsungen)

Water bath (Temperor, Techne)

Water tubing and water jacket

Oxygen in tanks

Oxygenator net

Wire-mesh for liver base

Gas washing bottle

Rubber tubings

Bile duct cannula PE-10

Portal vein cannula PE-240

Scissors, forceps, clamps, thread

Needles (gauge 26), syringes

Test tubes and racks, beakers, funnels, measuring cylinders

High speed centrifuge (Labofuge II)

Mixer (Super-mixer, Lab-line Instruments, Inc.)

Clock (Smiths, English Clock Systems)

Spectrophotometer (Spectronic 20)

pH meter (Pye Unicam) for adjusting pH of buffer

Micropipettes of capacity 5, 10, 20 micro-litres

Graph papers

Chemicals:

Sodium sulfobromophthalein (Koch-Light Laboratories) dissolved in distilled water to make 3% solution

Sodium hydroxide 10% in distilled water

0.1 N sodium hydroxide in distilled water to adjust pH

Hydrochloric acid 10% dissolved in distilled water

Bovine albumin fraction V (Sigma Chemicals Ltd.)

Sodium chloride (BDH Chemicals Ltd.)

Heparin 10,000 i.u./ml in saline (BDH Chemicals Ltd.)

Calcium chloride .$2H_2O$ (Merck Darmstadt)

Potassium chloride (Merck Darmstadt)

Magnesium chloride .$6H_2O$ (Merck Darmstadt)

Sodium dihydrogen phosphate (Merck Darmstadt)

Methoxyfluorane (Penthrane, Abbot Laboratories)

Diluent fluid consisting of 100 ml of 0.9% sodium chloride and 5 ml of 10% sodium hydroxide

Perfusion fluid:

Preparation of the perfusion fluid (perfusate)

The following analytical reagents were used to constitute solutions A and B.

Solution A:

19.2 g sodium chloride (NaCl)

0.48 g potassium chloride (KCl)

0.80 g calcium chloride ($CaCl_2.2H_2O$)

0.24 g (hydrated) magnesium chloride ($MgCl_2.6H_2O$)

These compounds were dissolved in distilled water to make 2000 ml of solution and kept in the freezer.

Solution B:

3.0 g sodium bicarbonate ($NaHCO_3$)

0.25 g sodium dihydrogen phosphate ($NaH_2PO.H_2O$)

These reagents were dissolved in distilled water to make 500 ml of solution and kept in the freezer. Solution A and B were collected from the freezer and left to attain room temperature of 26-27^{o}C after which 1.5 g of bovine albumin and 10 ml of solution B were added to 50 ml of solution A. The resulting solution was set at a pH of 7.4 by dropwise addition of 0.1 N sodium hydroxide.

Solution C was prepared as follows:

Blood was collected from adult, healthy rats and heparinized. This blood was filtered and one part of filtered blood was added to 2 parts of albumin/A+B solution of the preceding paragraph. This constituted the perfusion fluid (perfusate) that was used. Each perfusion required 45 ml of the perfusion fluid.

Surgical method:

The rat was anaesthetized with inhalant Penthrane, opened surgically and the liver exposed. The common bile duct was cannulated with PE 10 cannula. 0.15 ml of heparin was injected into the rat intravenously. The portal vein was cannulated with PE 240 cannula which was first filled with the perfusate. The perfusate was pumped through the liver during the rest of the surgical operation. The cannulated liver was removed from the rat, washed in a warm saline bath and the lobes were orientated properly. The liver was connected to the recirculating perfusate in the perfusion apparatus and covered with para film. The operation right from the abdominal incision to restoration of perfusion took about ten minutes. The liver was allowed to stabilize for thirty minutes before starting collection of bile and perfusate samples. This operation was carried out on all test rats including the controls.

Collection of samples:

One millilitre of perfusate was collected at the start of timing (zero time) from the reservoir. The drops of perfusate per minute running out of the liver were also taken at zero time after which sulfobromophthalein was added to the perfusate at

a dose of 12 mg/kg of body weight (0.4 ml of 3% BSP/100 g of rat).

One millilitre of perfusate was collected from the reservoir at specified times of 0, 5, 10, 15, 30, 45, 60, 75, 90 and 120 minutes, this was followed by an immediate addition of 1 ml of perfusate to the recirculating perfusate following each collection of perfusion fluid.

Bile samples were collected at the following times: 15, 30, 45, 60, 75, 90 and 120 minutes.

After 120 minutes of the experiment, the drops of perfusion fluid running out of the liver were counted, the liver was weighed and the perfusate volume remaining in the reservoir was measured. The volume of bile excreted at the various times was recorded.

I. Procedure for preparation of perfusate and bile samples for spectrophotometry readings.

(1) Perfusate samples:

The perfusate samples were centrifuged and the supernatant collected. Five millilitres of 0.9% sodium chloride were placed into each test tube. The readings of sulfobromophthalein in the perfusate supernatant were conducted in both acidic and alkaline environment. 0.2 ml of supernatant of a designated time was placed into each of the two test tubes. This was done for all supernatants collected at the various times. 0.1 ml of 10% sodium hydrochloric acid was added to each test tube in one row while 0.1 ml of 10% hydrochloric acid was added to each test tube in the remaining row. The contents in the test tubes were well shaken before readings in the spectrophotometer were made at 580 nm.

(2) Bile samples:

Five millilitres of diluent fluid were added to each test tube. To each tube 10 microlitres of bile were added making sure that the tube contained bile of only one specified time. The bile sample for zero time was

the bile collected during the thirty minutes of liver stabilization. The test tubes contents were thoroughly mixed and the optical densities were read at 580 nm. Diluent fluid was used as a blank to set absorbance to zero.

J. Preparation of standard curve for sulfobromophthalein (BSP) procedure.

Materials:

Sulfobromophthalein standard stock solution 0.1 mg/ml

Diluent fluid consisting of 100 ml of 0.9 sodium chloride and 5 ml of 10% sodium hydroxide

Spectrophotometer (Spectronic 20) and Mixer (Super mixer, Lab-line Instruments, Inc.)

Graph papers

Method:

Known volumes of diluent fluid were pipetted into cuvets and measured volumes of standard sulfobromophthalein were added to the same cuvets in a pattern shown in Tables Ia and Ib. The total volume of each of these cuvets was 5 ml. The contents of the cuvets were thoroughly mixed and their optical density readings taken at 580 nm. A standard curve was plotted for optical density readings against weight of sulfobromophthalein in micrograms.

TABLE 1a

Data for the standardization curve of sulfobromophthalein (BSP) values ranging from 0-10 micrograms of BSP

Cuvet	Diluent Fluid (µl)	Standard (µl)	Standard (µg)	Optical Density Readings
1	5000	0	0	0.000
2	4990	10	1	0.015
3	4980	20	2	0.030
4	4970	30	3	0.045
5	4960	40	4	0.060
6	4950	50	5	0.070
7	4940	60	6	0.090
8	4930	70	7	0.115
9	4920	80	8	0.125
10	4910	90	9	0.140
11	4900	100	10	0.155

TABLE 1b

Data for the standardization curve of sulfobromophthalein (BSP) values ranging from 0-100 micrograms of BSP

Cuvet	Diluent Fluid (µl)	Standard (µl)	Standard (µg)	Optical Density Readings
1	5000	0	0	0.000
2	4900	100	10	0.195
3	4800	200	20	0.350
4	4700	300	30	0.490
5	4600	400	40	0.620
6	4500	500	50	0.800
7	4400	600	60	0.850
8	4300	700	70	0.975
9	4200	800	80	1.100
10	4100	900	90	1.200
11	4000	1000	100	1.250

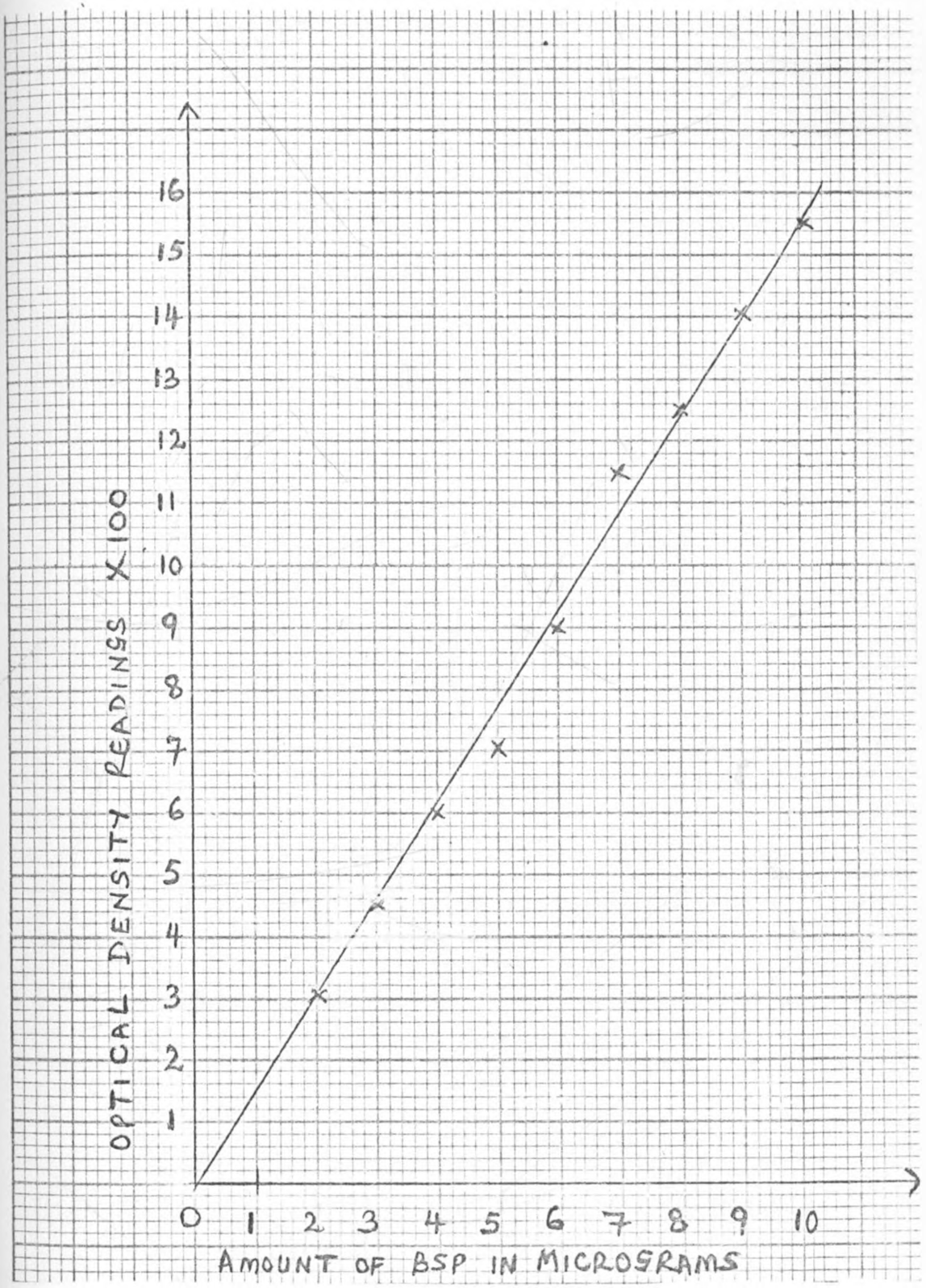

Fig. 3a: Standardization curve for BSP values ranging from zero to ten micrograms (was used where optical density readings of bile or perfusate samples corresponded to values equal to or less than 10 micrograms of BSP).

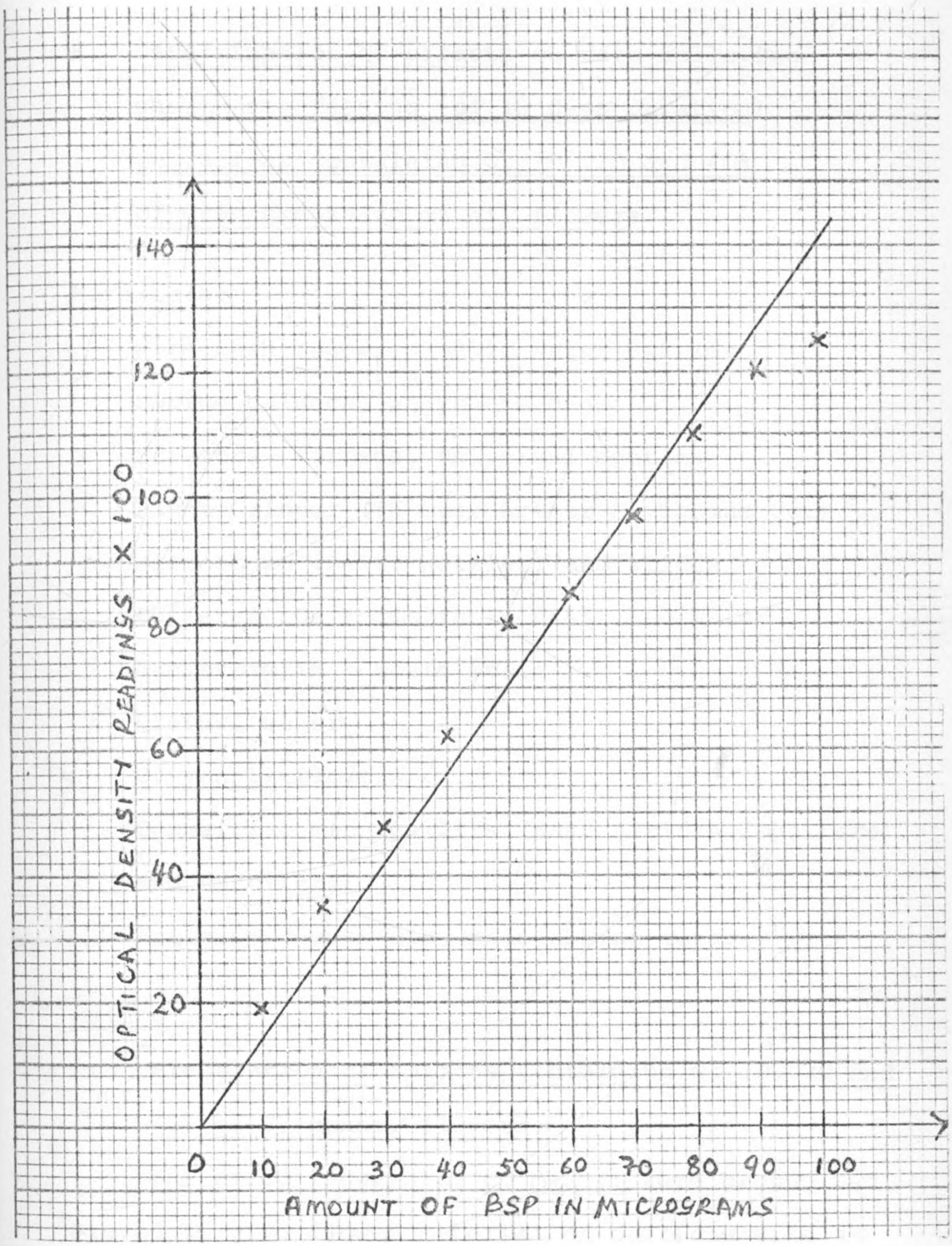

Fig. 3b: Standardization curve for BSP values ranging from zero to one hundred micrograms (was used where optical density readings of bile or perfusate samples corresponded to values greater than 10 micrograms of BSP.

K. Determination of effects on rats following short-term feeding (of 10 days duration) on rations containing 0, 10, 20 and 40% of Crassocephalum mannii flowers.

Materials:

Wire-mesh cages, plastic water bottles

Spring balance, polythene bags

Scissors, forceps, universal bottles

Plant Grinder (Thomas, Arthur H. Thomas Co.)

Microscope (Leitz Wetzlar) and slides

Buffered 10% Formalin in distilled water

Haematoxylin - Eosin stain

Diethyl ether

C. mannii flowers

Commercial mice pellets (East African Importers and Exporters)

Animals: 4-week old, male albino rats.

Method:

Preparation of rations containing 0, 10, 20 and 40% of C. mannii flowers.

The dried flowers of C. mannii were ground and sieved to give flower material of particle size at least 1 mm in circumference. The grinder and sieve were then cleaned of the flower material and used to grind and sieve commercial mice pellets to a particle size of at least 1 mm circumference.

In constituting the rations an allowance was made for each rat to have a daily intake of 15 g of feed (Short, D.J. and Woodnott, D.P., 1969).

(i) Ration containing 0% of C. mannii flowers: 3000g of ground mice pellets were weighed and placed in dry polythene bags. This ration was fed on rats in the control group.

(ii) Ration containing 10% of C. mannii flowers: 300 g of ground C. mannii flowers were mixed manually with 2,700 g of ground mice pellets to give a ration mixture weighing 3,000 g.

(iii) Ration containing 20% of C. mannii flowers: 600 g of ground C. mannii flowers were mixed manually with 2,400 g of ground mice pellets to give a ration mixture weighing 3,000 g.

(iv) Ration containing 40% of C. mannii flowers: 1,200 g of ground C. mannii flowers were mixed manually with 1,800 g of ground mice pellets to give a ration mixture weighing 3,000 g.

Each ration was designed to feed 20 rats of a specified group for 10 days.

Feeding trial:

Four-week old, male albino rats were obtained from Kabete Veterinary Laboratories. The rats were kept in the animal house for observation and

acclimatisation for two weeks. The rats were then assigned to four groups, each group consisted of twenty rats. The rats in each group were accommodated in one cage and were fed on a specified ration. One of the groups acted as a control group and the rats in this group were fed exclusively on ground mice pellets. The remaining three groups were given specified rations containing either 10 or 20 or 40% of ground C. mannii flowers; water was provided ad libitum.

The rats were observed daily for any clinical signs and any rat that died was autopsied. The livers and kidneys were evaluated for histopathological changes. At the end of ten days all the surviving rats were euthanized with ether, autopsied and had their livers and kidneys examined for histopathological changes.

L. Prolonged toxicity testing on rats following long-term feeding (of 100 days duration) on rations containing 0, 2, 4 and 8% of C. mannii flowers.

Materials:

Wire-mesh cages, plastic water bottles

Spring balance, polythene bags

Scissors, forceps, universal bottles

Plant Grinder (Thomas, Arthur H. Thomas Co.)

1 millimetre sieve

Microscope (Leitz Wetzlar) and slides

Buffered 10% formalin in distilled water

Haematoxylin - Eosin stain

Elastin - van Giesson stain

Diethyl ether

C. mannii flowers

Commercial mice pellets (East African Importers and Exporters)

Animals: 4-week old, male albino rats.

Method:

Preparation of rations containing 0, 2, 4 and 8% of C. mannii flowers

The dried flowers of C. mannii were ground and sieved to give flower material of particle size at least 1 mm circumference. The grinder and sieve were then cleaned of the flower material and used

to grind and sieve commercial mice pellets to a particle size of at least 1 mm circumference.

In constituting the rations an allowance was made for each rat to consume 15 g of feed daily (Short, D.J. and Woodnott, D.P., 1969).

(i) Ration containing 0% of C. mannii flowers: 4,200 g of ground mice pellets were weighed and placed in dry polythene bags. This ration was used solely as feed for the control group of rats.

(ii) Ration containing 2% of C. mannii flowers: 84 g of ground C. mannii flowers were mixed manually with 4,116 g of ground commercial mice pellets to give 4,200 g of ration mixture.

(iii) Ration containing 4% of C. mannii flowers: 168 g of ground C. mannii flowers were mixed manually with 4,032 g of ground commercial mice pellets to give 4,200 g of ration mixture.

(iv) Ration containing 8% of C. mannii flowers: 336 g of ground C. mannii flowers were mixed manually with 3,864 g of ground mice pellets to give 4,200 g of ration mixture.

Each of these rations was designed to feed 20 rats of a specified group for fourteen days. These rations were constituted fortnightly.

Feeding trial:

Four-week old, male albino rats were obtained from Kabete Veterinary Laboratories. The rats were kept in the animal house for observation and acclimatisation for two weeks; during this period the rats were fed on commercial mice pellets and given water ad libitum. The rats were then weighed and assigned to four groups, each group consisted of twenty rats. The rats in each group were accommodated in one cage and were fed on a specified ration. The rats in the control group received a ration made solely of ground mice pellets. The other three groups were fed on specified rations containing either 2, 4 or 8% of ground C. mannii flowers. An allowance was made for each rat to receive at least 15 g of feed daily; and weighed fortnightly. Any rat that died during the course of the feeding trial was autopsied and its organs, that is, the liver, kidney, stomach, intestine, spleen and femur were examined for any histopathological changes.

On the 100th day of feeding, all the surviving rats were weighed, euthanized with diethyl ether, autopsied and the organs listed as before were evaluated for any histopathological changes.

Haematoxylin-Eosin stain was used to stain all the organs and Elastin-van Giesson was used to stain some of the liver sections.

The weights of livers of some of the rats that died during the trial and of some of the rats that were euthanized on the 100th day of the experiment were recorded for statistical analysis.

Attention is drawn to the fact that the actual feeding time was 12 weeks due to lack of material. All the experimental animals were, however, euthanized on the 100th day after the start of the experiment.

M. Determination of effect of the freeze-dried alcoholic extract of C. mannii flowers on Staphylococcus aureus (Oxford Heatley NCTC-6571).

Materials:

Freeze-dried alcoholic extract of C. mannii flowers

Terramycin (oxytetracycline hydrochloride, Pfizer) of concentration 0.2 mg/ml dissolved in 0.1 m phosphate buffer at pH 4.5

Isotonic sodium chloride

Medium: Mueller Hinton Agar (Oxoid)

Universal bottles, Sterile Petri dishes

Pasteur pipettes, rubber teats and cork borer.

Method:

The method used was described by Kavanagh, F. (1972).

Twenty one millilitres of Mueller Hinton Agar were poured into a Petri dish to form a "base". After it had solidified 4 ml of "seeded" Mueller Hinton Agar were added onto the base layer. Wells of 10 mm diameter and 200 microlitres capacity each were punched into the medium using a cork borer. The extract of C. mannii flowers

was dissolved in isotonic saline to make solutions of concentrations 0.01, 0.1 and 1 mg/ml. Each well was filled with 100 microlitres of test material of only one and specific concentration. Isotonic saline and terramycin were used as controls.

The Petri dishes and contents were incubated at 37^{o}C for one day. The readings were determined by observation of zones of inhibition of bacterial growth around the wells.

RESULTS

For the determination of lethal dose in 50% (LD_{50}) of the mice following intraperitoneal administration of the freeze-dried alcoholic extract of C. mannii flowers.

The intraperitoneal administration to mice of the various doses of the freeze-dried alcoholic extract of C. mannii flowers was accompanied by clinical signs which included huddling of mice, cyanosis of ears and legs, abnormal gait manifested in an S-curvature when mice were moving and deaths in some mice within 15 minutes of the administration of the extract. The post mortem results of the mice revealed congestion of the peritoneum. The mortality per dose was recorded after 48 hours of observation; these results are shown in Table 2.

The results of percentage mortality per dose were plotted on the log probability paper and the lethal dose in 50% of the mice was read directly from the graph as 3078 mg/kg of body weight (shown in Figure 4). The standard error for the LD_{50} was estimated using the formula:

$$\frac{\text{(Doses 84\% - 16\%)}}{\sqrt{2N}}$$

The lethal doses 84% and 16% were read directly from the graph and found to be 3271.90 and 2886.68 mg/kg of body weight respectively. N which represented

the number of mice contributing to the plotted values lying between 84% and 16% mortality was 40.

Hence the standard error =

$$\frac{(3271.90 - 2888.68)}{\sqrt{2 \times 40}} = 42.84 \text{ mg/kg}$$

The results showed that the lethal dose in 50% (LD_{50}) of the mice following intraperitoneal administration of the freeze-dried alcoholic extract of C. mannii flowers was 3078 ± 42.8 mg/kg of body weight at $28^{o}C$.

Table 2: Percentage mortality per dose following intraperitoneal administration of the freeze-dried alcoholic extract of C. mannii flowers to mice.

Dose mg/kg	Log dose	Mice		Percentage Mortality
		Original Number	Number of dead mice	
2741	3.4379	10	0	0
2839	3.4532	10	1	10
2941	3.4685	10	4	40
3046	3.4837	10	5	50
3155	3.4990	10	6	60
3268	3.5143	10	7	70

All the log doses had a common logarithmic interval of 0.0153.

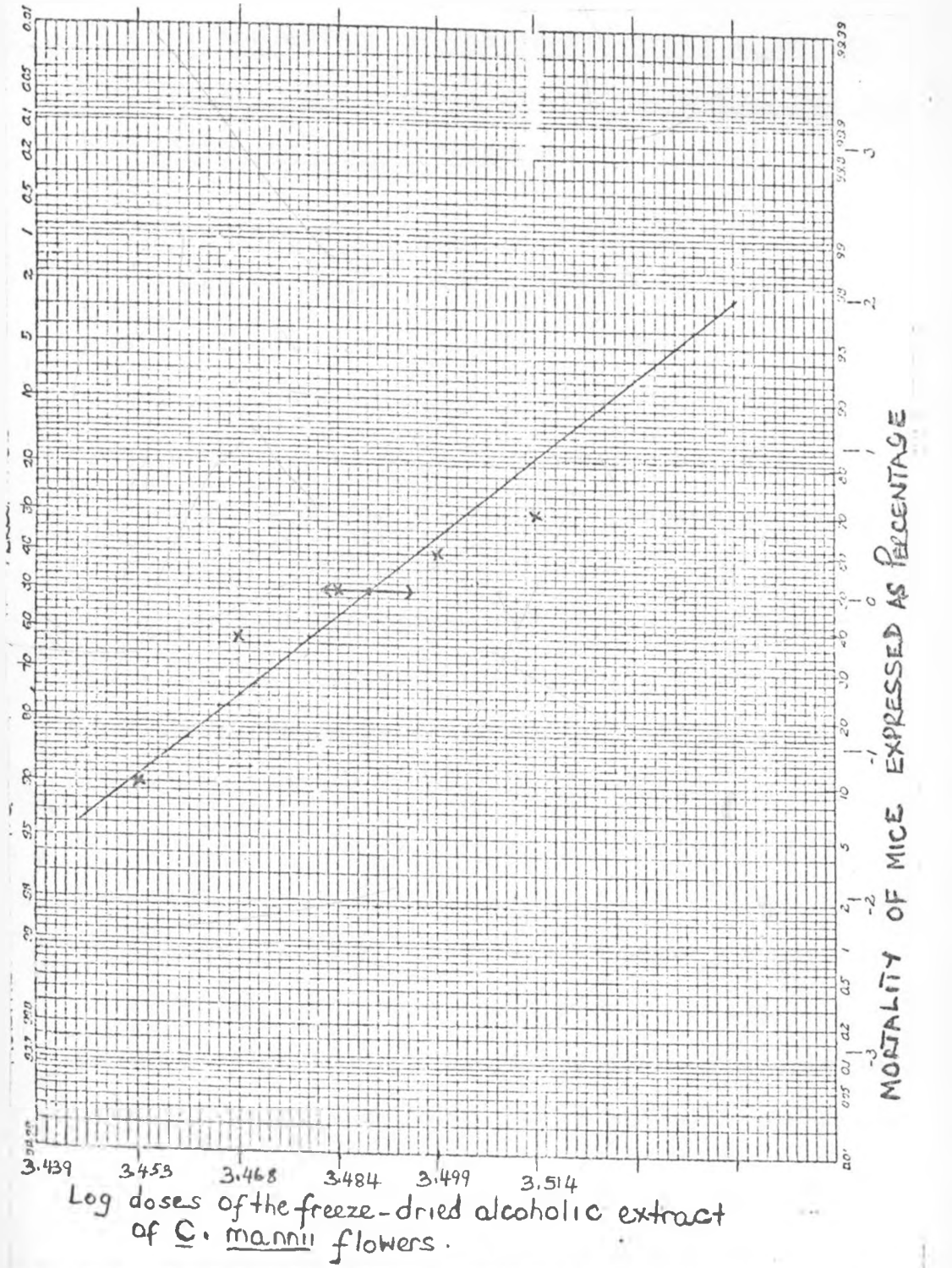

Fig. 4: Logarithmic dose mortality curve for the LD_{50} in mice of the freeze-dried alcoholic extract of C. mannii flowers.

For the effect of the freeze-dried alcoholic extract of C. mannii flowers on the guinea pig ileum and rabbit duodenum.

(i) On guinea pig ileum

After the piece of guinea pig ileum had remained in the organ bath for 10 minutes, a recording of its normal tone was made on the kymograph rotating at 0.25 mm/sec. This was followed by addition of 0.2 ml of isotonic saline to the organ bath; there was no accompanying effect on the tone of the guinea pig ileum. The freeze-dried alcoholic extract of C. mannii flowers had been dissolved in isotonic saline. 20 mg of this extract were added to the organ bath and an accompanying increase in tone of the guinea pig ileum was observed and recorded for 100 seconds on the kymograph. The organ bath was emptied and the ileum washed twice with the Tyrode solution, waited until the ileum had established its original tone. 40 mg of the extract were added to the organ bath and the attendant further increase in tone of the ileum was observed and recorded for 100 seconds on the kymograph. The organ bath was emptied and the ileum washed twice with the Tyrode solution; waited until the tone of the ileum had been re-established. 60 mg of extract were added and the corresponding increase in tone of ileum was observed and recorded (Shown in Figure 5a).

From this testing procedure, it was observed that the freeze-dried alcoholic extract of C. mannii flowers caused an increase in the tone of the guinea pig ileum. The increase in tone was dose-related, that is, an increase in amount of extract added to the ileum was accompanied by a further increase in tone of the guinea pig ileum.

(ii) On rabbit duodenum

After the piece of rabbit duodenum had remained in the organ bath for 10 minutes a recording of its normal tone, rate and amplitude was made on the kymograph rotating at 0.25 mm/sec. The rate was found to be 9 contractions per minute. 0.2 ml of isotonic saline was added to the organbath and was found to have no effect on the tone, rate and amplitude of the duodenum. The freeze-dried alcoholic extract of C. mannii flowers was dissolved in isotonic saline. When 40 mg of this extract were added to the organ bath, there was an immediate increase in tone of the duodenum but the rate and amplitude remained unaffected (rate was still 9 contractions per minute). After 80 seconds of observation, the organ bath was emptied and the duodenum piece washed twice with the Tyrode solution. The duodenum piece was allowed to return to its original tone, after which 80 mg of the extract were added to the organ bath. There was a

further increase in tone but the rate and amplitude of the duodenum contractions remained unchanged. The organ bath was emptied, and the duodenum piece washed twice with the Tyrode solution and allowed to regain its tone. 120 mg of the extract were added to the organ bath, this was accompanied by an increase in tone but the increase in tone was not greater than the one obtained by addition of 80 mg of the extract, the rate and amplitude of the duodenum contractions remained unaffected (shown in Figure 5b).

From this testing procedure it was observed that the freeze-dried alcoholic extract of C. mannii flowers caused an increase in the tone of the rabbit duodenum but the rate and amplitude of the duodenum contractions remained unchanged. The increase in tone was dose-related up to 80 mg of the extract application, addition of greater doses of the extract showed hardly any significant increase in tone of the duodenum.

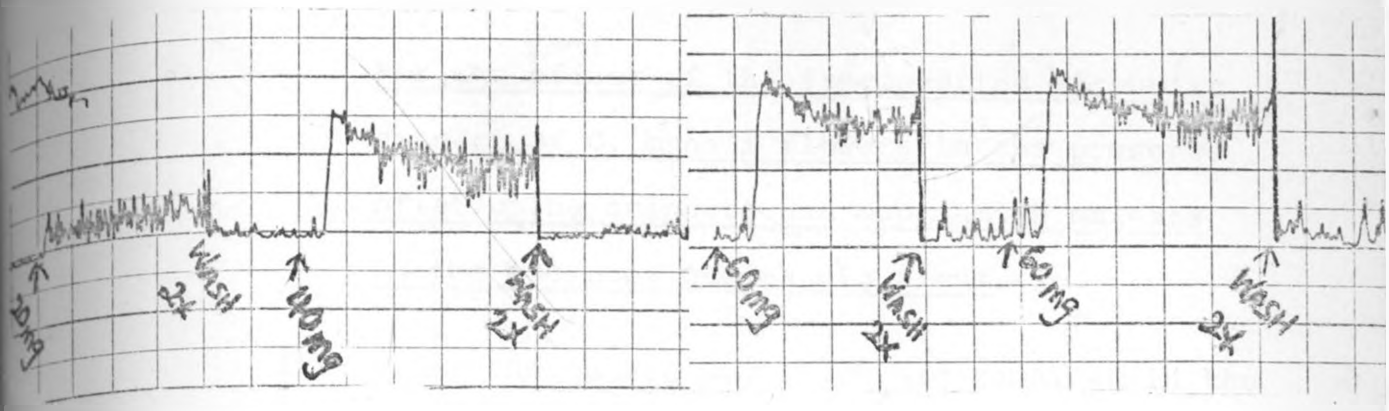

Fig. 5a: Increase in tone of the isolated guinea pig ileum to the various doses of the freeze-dried alcoholic extract of C. mannii flowers.

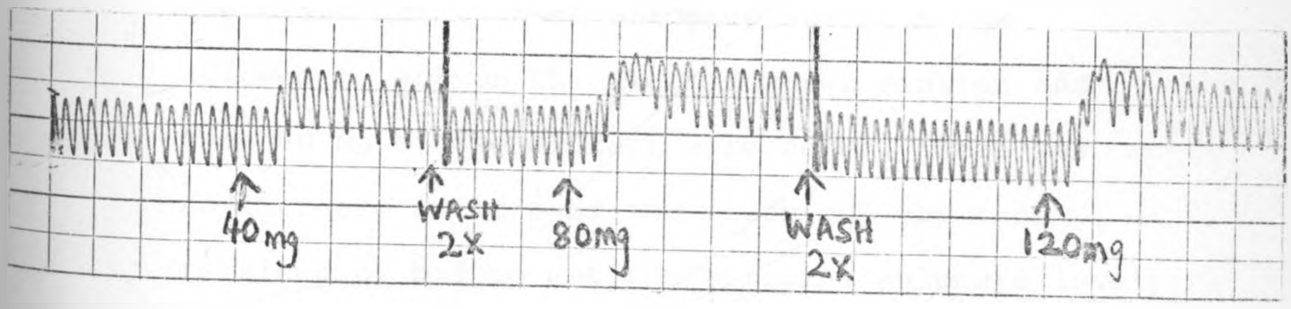

Fig. 5b: Increase in tone of the isolated rabbit duodenum to the various doses of the freeze-dried alcoholic extract of C. mannii flowers.

For the effect of the freeze-dried alcoholic extract of C. mannii flowers in the presence of atropine sulphate and mepyramine maleate on the isolated guinea pig ileum.

(i) After the guinea pig ileum had remained in the organ bath for 10 minutes, a recording of the normal contractions of the guinea pig ileum were made on the kymograph rotating at 0.25 mm/sec.

When 60 mg of the freeze-dried alcoholic extract of C. mannii flowers were added to the organ bath, there was an immediate increase in tone of the guinea pig ileum. After 7 minutes of observation and recording the organ bath was emptied and the piece of ileum was washed twice with the Tyrode solution and allowed to regain its original tone. There was a slight decrease in tone of the ileum following addition of 1 mg. of atropine sulphate in the organ bath. When atropine sulphate was allowed to act on the ileum for two minutes and then 60 mg of the extract were added, there was no increase in tone of the ileum and the tone remained as before (at the atropine sulphate level). (shown in Plate 2a).

From this investigation, it was observed that the freeze-dried alcoholic extract of C. mannii flowers increased the tone of the ileum while

atropine sulphate caused a slight decrease in tone of the ileum. Furthermore, atropine sulphate completely blocked the ability of the extract material to increase the tone of the guinea pig ileum.

(ii) After the guinea pig ileum had remained in the organ bath for 10 minutes a recording of its contractions was made on the kymograph rotating at 0.25 mm/sec. When 20 mg of the freeze-dried alcoholic extract of C. mannii flowers were added there was an immediate increase in the tone of the guineal pig ileum. After 5 minutes of observation and recording of the tone on the kymograph, the organ bath was emptied and the piece of the ileum washed twice with the Tyrode solution and allowed to regain its original tone. When 0.2 mg of mepyramine maleate was added and allowed to act on the ileum, there was no change from the prevailing tone. When mepyramine maleate was allowed to act on the ileum for two minutes and then 20 mg of the extract were introduced into the organ bath, there was no increase in tone of the guinea pig ileum (shown in Plate 2b).

From this investigation, it was observed that the freeze-dried alcoholic extract of C. mannii flowers caused an increase in the tone of the guinea

pig ileum while mepyramine maleate caused no change on the prevailing tone.

Furthermore, mepyramine maleate adequately blocked the ability of the extract to increase the tone of the guinea pig ileum.

Plate 2a: The blocking effect of atropine sulphate on the spasmodic effect of the freeze-dried alcoholic extract of C. mannii flowers on the isolated guinea pig ileum

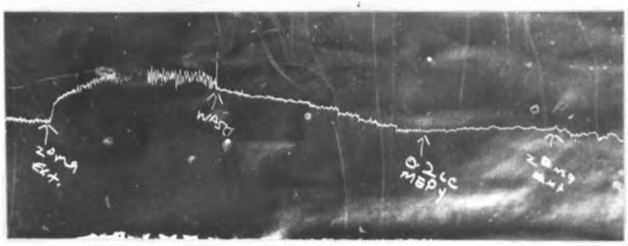

Plate 2b: The blocking effect of mepyramine maleate on the spasmodic effect of the freeze-dried alcoholic extract of C. mannii flowers on the isolated guinea pig ileum

For the effect of the freeze-dried alcoholic extract of C. mannii flowers on the isolated perfused rabbit heart.

After the isolated rabbit heart had been attached onto the cannula of the perfusion apparatus the heart was allowed to stabilize its contractions which were transmitted to a lever with a frontal writing point and recorded on the kymograph rotating at 2.5 mm/sec. The heart rate was found to be 127 contractions per minute. The freeze-dried alcoholic extract of C. mannii flowers was dissolved in isotonic saline. When 0.2 ml of isotonic saline was introduced into the Ringer-Locke solution perfusing the heart there was no alteration in the rate and amplitude of the heart. After 4 mg of the extract had been introduced into the Ringer-Locke solution, upon reaching the heart there was a noticeable decrease in the rate and force of contraction. The heart rate was reduced to 55 contractions per minute (shown in Figure 6a).

From this investigation it was observed that the freeze-dried alcoholic extract of C. mannii flowers reduced the rate and force of contractions of the isolated perfused rabbit heart.

For the effect of the freeze-dried alcoholic extract of C. mannii flowers in the presence of atropine sulphate (1%) on the isolated perfused rabbit heart.

After the isolated rabbit heart had been attached onto the glass cannula of the perfusion apparatus, the heart was allowed to stabilize its contractions which were being transmitted to a lever with a frontal writing point on the kymograph rotating at 2.5 mm/sec. The heart rate was found to be 92 contractions per minute. Addition of 0.2 ml (2 mg) of atropine sulphate to the Ringer Locke solution perfusing the heart did not alter the rate and amplitude of the heart contractions. When 0.2 ml of atropine sulphate was added to the Ringer-Locke solution and was soonafter followed by addition of 4 mg of the extract, the effects of the extract on the heart, that is, a decrease in rate and amplitude were delayed by 38 seconds before they became pronounced (shown in Figure 6b)

From this investigation it was observed that atropine sulphate blocked the effects of the freeze-dried alcoholic extract of C. mannii flowers on the isolated perfused rabbit heart, but the effects of the extract began to show as atropine sulphate levels in the Ringer-Locke solution declined.

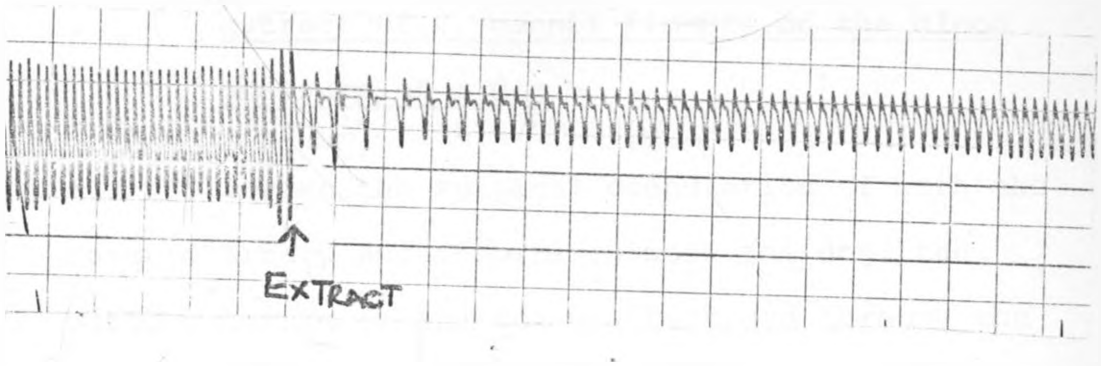

Fig. 6a: Decrease in rate and force of the contractions of the isolated perfused rabbit heart to 4 mg of the freeze-dried alcoholic extract of C. mannii flowers.

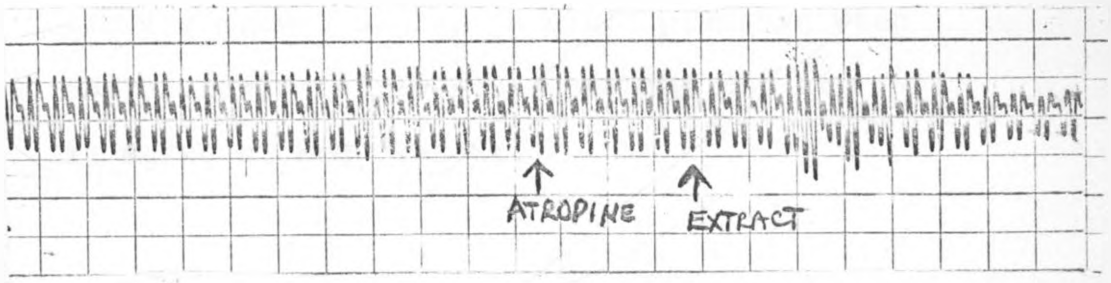

Fig. 6b: The blocking effect of atropine sulphate on the effect of the freeze-dried alcoholic extract of C. mannii flowers on the isolated perfused rabbit heart.

a. For the effect of the freeze-dried alcoholic extract of C. mannii flowers on the blood pressure of a dog.

Following the surgical cannulation of both the carotid artery and femoral vein in the dog, the blood pressure of the dog was recorded through the carotid artery cannula on the kymograph rotating at 2.5 mm/sec. The blood pressure was observed to be 130/127 mm of mercury. The administration on the freeze-dried alcoholic extract of C. mannii flowers to the dog through the femoral vein at 12 mg/kg of body weight caused an immediate drop in blood pressure by 34 mm of mercury but the blood pressure was re-established to the original value within 3.5 minutes (shown in Plate 3a).

From this investigation it was observed that the freeze-dried alcoholic extract of C. mannii flowers caused a drop in blood pressure when administered intravenously to the dog at 12 mg/kg of body weight.

b. For the effect of atropine sulphate alone and in conjunction with the freeze-dried alcoholic extract of C. mannii flowers on the blood pressure of a dog.

Following the surgical cannulation of the carotid artery and femoral vein in the dog, the mean blood pressure as recorded through the carotid artery cannula was 165 mm of mercury. When atropine sulphate was injected into the dog through the femoral vein at 0.2 mg/kg of body weight there was neither a decrease nor an increase in the mean blood pressure. However, when atropine sulphate was injected into the dog at 0.2 mg/kg of body weight and immediately followed by another intravenous injection of the freeze-dried alcoholic extract of C. mannii flower at 12 mg/kg of body weight, there was a slight drop in blood pressure of 3 mm (shown in Plate 3b).

From these investigations it was observed that atropine sulphate injected into the dog at 0.2 mg/kg of body weight did not change the blood pressure but when the same dose of atropine sulphate was injected and followed immediately by the intravenous administration of the freeze-dried alcoholic extract of C. mannii flowers, the hypotensive effect (on blood pressure) of the extract was remarkably blocked.

c. For the effect of mepyramine maleate alone and in conjunction with the freeze-dried alcoholic extract of C. mannii flowers on the blood pressure of a dog.

Following the surgical cannulation of the carotid artery and femoral vein in the dog, the blood pressure as recorded through the carotid artery cannula was 165/162 mm of mercury. When the freeze-dried alcoholic extract of C. mannii flowers was injected into the dog through the femoral vein at 12 mg/kg of body weight, there was an immediate drop in blood pressure by 80 mm of mercury. The blood pressure was re-established to its original value within 3.5 minutes. When mepyramine maleate was injected into the dog through the femoral vein at 2.4 µg/kg of body weight there was neither an increase nor a decrease in the prevailing blood pressure. When the same dose of mepyramine maleate was administered to the dog, and was immediately followed by the intravenous administration of the freeze-dried alcoholic extract of C. mannii flowers at 12 mg/kg of body weight there was an immediate drop in blood pressure by 60 mm of mercury (shown in Plate 3c).

From these investigations it was observed that the freeze-dried alcoholic extract of C. mannii flowers caused a drop in blood pressure when given at least 12 mg/kg of body weight, while mepyramine maleate when administered to the dog at 2.4 µg/kg of body weight did not alter the prevailing blood pressure. Furthermore, when the same dose of mepyramine maleate was injected into the dog and was immediately followed by the intravenous injection of the freeze-dried alcoholic extract of C. mannii flowers at 12 mg/kg of body weight, there was a drop in blood pressure; but the drop was less than the one obtained when the extract was administered alone (60 mm vs 80 mm of mercury).

d. For the effects of ergotamine tartrate alone and in the presence of the freeze-dried alcoholic extract of C. mannii flowers on the blood pressure of a dog.

Following the surgical cannulation of the carotid artery and femoral vein in the dog, the blood pressure as recorded through the carotid artery cannula was 170/167 mm of mercury. When ergotamine tartrate was administered to the dog through the femoral vein at 0.01 mg/kg of body weight there was a rise in systolic and diastolic pressuresto 180/178 mm of mercury.

When the freeze-dried alcoholic extract of C. mannii flowers was administered to the dog at 12 mg/kg of body weight after waiting for 10 minutes for the effects to ergotamine tartrate to stabilize, there was an immediate drop in blood pressure by 60 mm of mercury (shown in Plate 3d).

From these investigations it was observed that ergotamine tartrate caused a rise in blood pressure. When ergotamine tartrate was allowed to act on the circulatory system for up to 10 minutes, and the freeze-dried alcoholic extract of C. mannii flowers administered soonafter, the hypotensive effect of the extract remained unaltered.

e. For the effect of propranolol hydrochloride alone and in the presence of the freeze-dried alcoholic extract of C. mannii flowers on the blood pressure of a dog.

Following the surgical cannulation of the carotid artery and femoral vein of the dog, the blood pressure of the dog as recorded from the carotid artery cannula was 165/162 mm of mercury. When propranolol hydrochloride was administered to the dog through the femoral vein at 400 µg/kg of body weight there was a decrease in systolic pressure and an increase in diastolic pressure, the mean blood pressure, however, remained the same.

When the freeze-dried extract of C. mannii flowers was injected into the dog at 12 mg/kg of body weight after allowing propranolol hydrochloride to act on the circulatory system of the dog for 10 minutes, there was a drop in blood pressure by 40 mm of mercury.

From these investigations it was observed that propranolol hydrochloride caused an increase in diastolic pressure and a decrease in systolic pressure without changing the mean blood pressure. It was also observed that the freeze-dried alcoholic extract of C. mannii flowers caused a drop in blood

pressure in spite of the presence of propranolol hydrochloride in the circulatory system.

From the investigations of the effects of the freeze-dried alcoholic extract of C. mannii flowers on the blood pressure in the dog when given alone and in the presence of mepyramine maleate, atropine sulphate, ergotamine tartrate and propranolol hydrochloride, it was observed that the extract caused a drop in blood pressure and this hypotensive effect was adequately blocked by atropine sulphate; the presence of mepyramine maleate in the circulatory system did not allow the extract to cause a drop in blood pressure up to the original level, though there was a convincing drop in blood pressure.

The presence of ergotamine tartrate and propranolol hydrochloride in the circulatory system did not influence the hypotensive effect of the extract.

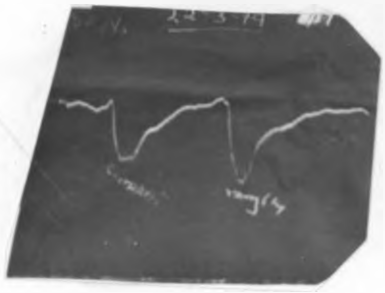

Plate 3a: The drop in blood pressure following the intravenous administration of the freeze-dried alcoholic extract of C. mannii flowers at 12 mg/kg of body weight

Plate 3b: The blocking effect of atropine sulphate on the hypotensive effect due to the freeze-dried alcoholic extract of C. mannii flowers on the blood pressure in the dog

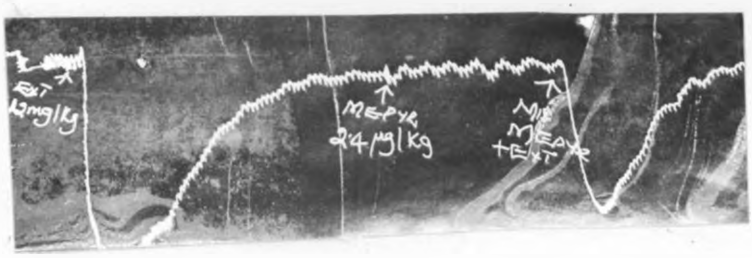

Plate 3c: The drop in blood pressure due to the freeze-dried alcoholic extract of C. mannii flowers and the partial blockade of the drop in pressure by mepyramine maleate (in the dog)

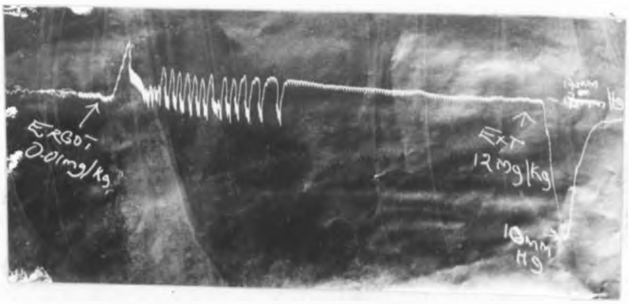

Plate 3d: The rise in blood pressure in the dog due to ergotamine tartrate and the accompanying drop in blood pressure following intravenous administration of the freeze-dried alcoholic extract of C. mannii flowers

For the effect of the freeze-dried alcoholic extract of C. mannii flowers on movement of phenol red in the small intestines of rats.

Following the oral administration of the freeze-dired alcoholic extract of C. mannii flowers at 50 and 500 mg/kg of body weight to rats and the subsequent oral dosing of the same rats with phenol red solution at 1 ml/100g of body weight, the lengths of the small intestines of rats that were covered by phenol red at the given times of 5, 10, 15 and 20 minutes were expressed as percentages of the total lengths of the small intestines of the rats under observation (shown in Table 3a).

From the eye inspection of the means of the various treatment combinations of dose and time of the afore-mentioned data (shown in Table 3b), the overall impression was that phenol red covered more distance at each given time in those rats that had been dosed with the alcoholic extract when compared to the control rats. However, the results were not analyzed by statistical calculations due to lack of enough data (replications).

Table 3a: The percentage of each small intestine that had been covered by phenol red following the oral administration to rats of the freeze-dried alcoholic extract of C. mannii flowers and phenol red solution.

Dose mg/kg	Replication	Time in minutes			
		5	10	15	20
0	1	44.8	54.4	63.4	53.4
	2	45.0	62.5	64.4	62.1
50	1	56.7	58.5	60.2	80.2
	2	59.5	60.5	93.6	86.5
500	1	47.1	74.3	77.6	100.0
	2	63.4	67.1	45.8	70.6

Table 3b: The mean percentages of the small intestines that had been covered by phenol red following the oral administration to rats of the freeze-dried alcoholic extract of C. mannii flowers and phenol red solution (for dose and time)

Dose mg/kg	Time in minutes			
	5	10	15	20
0	44.9	58.5	63.9	57.8
50	58.1	59.5	76.9	83.4
500	55.3	70.7	61.7	85.3

For the effect of the freeze-dried alcoholic extract of C. mannii flowers in relation to the dose, route of administration and time of observation on movement of phenol red solution in the small intestines of rats.

Following the administration of the freeze-dried alcoholic extract of C. mannii flowers to rats at either 50 or 500 mg/kg of body weight per os or intraperitoneally and the subsequent oral dosing of the same rats with phenol red solution at 1 ml/100 g of body weight, the lengths of the small intestines of rats that were covered by phenol red solution at the given times of 30, 60, 90 and 120 minutes were expressed as percentages of the total lengths of the small intestines of the rats under observation (shown in Table 4a).

From the eye inspection of the means of the various treatment combinations of dose, route and time of the afore-mentioned data (shown in Table 4b), the overall impression was that that at each given time phenol red covered less distance in the small intestines of those rats which were dosed with the extract intraperitoneally. Through the intraperitoneal route the high dose of the extract was observed to have inhibited movement of phenol red more than the low dose of the extract. It also seemed that movement of phenol red

was not affected substantially when the extract was administered orally when compared to the control rats.

The results were however not analysed by statistical calculations due to lack of enough data (replications).

Table 4a: The percentage of each small intestine that had been covered by phenol red solution following the oral or intraperitoneal administration to rats of the freeze-dried alcoholic extract of C. mannii flowers.

Dose mg/kg	Route	Replication	Time in minutes			
			30	60	90	120
0	Oral (p.o.)	1	85.0	86.5	100.0	100.0
		2	63.1	72.5	87.5	82.5
	Intraperi-	1	67.5	87.2	90.7	100.0
	toneal (i.p.)	2	70.6	74.4	93.2	95.0
50	Oral (p.o.)	1	76.3	95.5	100.0	100.0
		2	80.3	81.4	81.2	88.1
	Intraperi-	1	60.0	81.6	90.0	66.7
	toneal (i.p.)	2	24.6	58.4	59.1	86.1
500	Oral (p.o.)	1	81.3	81.1	100.0	100.0
		2	79.2	85.2	86.1	87.5
	Intraperi-	1	40.0	51.4	31.6	69.9
	toneal (i.p.)	2	31.5	18.9	39.8	46.8

Table 4b: The mean percentages of the small intestines that had been covered by phenol red at various doses and times following oral and intraperitoneal administration of the freeze-dried alcoholic extract of C. mannii flowers to the rats.

Dose mg/kg	Route	Time in minutes			
		30	60	90	120
0	Oral (p.o)	74.1	79.5	93.8	91.3
	Intraperitoneal (i.p.)	69.1	80.8	92.0	97.5
50	Oral (p.o.)	78.3	88.5	90.6	97.5
	Intraperitoneal (i.p.)	42.3	70.0	74.6	76.4
500	Oral (p.o.)	80.3	83.2	93.1	93.8
	Intraperitoneal (i.p.)	35.8	35.1	35.7	58.4

For the excretion of sulfobromophthalein (BSP) and bile in the isolated perfused livers of the rats which had been pre-fed on rations containing 2.5 and 8% of ground C. mannii flowers and of the rats which had received single intraperitoneal injections of the freeze-dried alcoholic extract of C. mannii flowers at 300 mg/kg of body weight.

Sulfobromophthalein (BSP) excretion

Tables 5a, b, c and d give the optical density readings for the BSP contained in 10 microlitres of bile that had been excreted by the isolated perfused livers from the specified rats under experimentation for each given period. These values were converted into micrograms of BSP using the BSP standard curves of Figures 3a and 3b.

The total weight of BSP excreted at each given time was calculated by multiplying the volume of bile excreted within that period by the amount of BSP (in micrograms) contained in 10 microlitres of the same bile sample.

These values were used to compute the cumulative percentage of BSP that had been excreted in bile by the isolated perfused livers at various times (shown in Table 6).

From this investigation the percentage of BSP excreted in bile by the 120th minute of the perfusion was 97% for the isolated perfused livers of the rats which had been pre-fed on ration containing 8% of C. mannii flowers, 90% for the control rats, 74% for the rats which had been pre-fed on ration containing 2.5% of C. mannii flowers and 71% for the rats which had received single intraperitoneal injection of the freeze-dried alcoholic extract of C. mannii flowers at 300 mg/kg of body weight.

Sulfobromophthalein (BSP) excretion rate

Table 7 shows the BSP excretion rates in µg of BSP/g of liver/min. These values were obtained by dividing the amount of BSP that had been excreted by the isolated perfused livers with the period of BSP excretion (120 minutes) and the weights of the isolated perfused livers. The same table gives the mean BSP excretion rates for the various groups. The mean BSP excretion group rates were tested using the Duncan's Multiple Range test and the rates did not show any significant differences between and amongst themselves at 5% probability level but the BSP excretion rate was highest in the isolated perfused livers of the rats which had been pre-fed on ration containing 8% of C. mannii flowers and lowest in the livers of

the rats which had received single intraperitoneal injections of the freeze-dried alcoholic extract of C. mannii flowers at 300 mg/kg of body weight.

Sulfobromophthalein (BSP) in perfusate

Tables 8a, b, c and d show the optical density readings for BSP contained in 0.2 ml of each perfusate supernatant of each given time, the perfusate had been withdrawn from the perfusion fluid of the isolated livers of the rats under experimentation. The optical density readings were converted into the corresponding weights of BSP using the BSP standardization curves of Figures 3a and 3b.

The total amount of BSP present at each given time in the circulating perfusion was calculated by multiplying the volume of the perfusion fluid in circulation (30 ml) with the amount of BSP contained in 0.2 ml of each perfusate supernatant; the mean values obtained were then expressed as percentages of the original amount of BSP that was added to the perfusion fluid.

Table 9 gives the mean percentage of BSP remaining in the perfusion fluid of the isolated livers of the rats under experimentation at specified times.

From these investigations it was observed that by the 45th minute of the perfusion trials, the mean percentage of BSP remaining in the perfusate was 6.3% for the control rat livers, 6.2% for the livers of the rats which had been pre-fed on ration containing 8% of ground C. mannii flowers, 21.8% for the livers from the rats which had been pre-fed on ration containing 2.5% of ground C. mannii flowers and 10.8% for the livers from the rats which had received single intraperitoneal injections of the freeze-dried alcoholic extract of C. mannii flowers at 300 mg/kg of body weight.

Bile excretion

Tables 10a, b, c and d give the amount of bile that was excreted by the isolated perfused livers of the rats under experimentation.

Table 11 gives the bile excretion rates for the test and control rat livers. These rates were obtained by dividing the total volume of bile collected with the period of bile collection (120 minutes) and the weights of the isolated perfused livers. The same table gives the mean bile excretion rates for the test and control rat livers.

By using the Duncan's Multiple Range test it was observed that at 5% probability level the bile excretion rate of 1.25 µl/g of liver/min. for the rats which were fed on ration containing 8% of C. mannii flowers was significant and different

from those of the remaining test and control groups of rats, it was observed that there was increased bile excretion in the rats which had been pre-fed on ration containing 8% of C. mannii flowers.

The excretion rates of bile in the remaining test and control groups were not significant and were not different from each other though the rats which were injected with the freeze-dried alcoholic extract of C. mannii flowers at 300 mg/kg of body weight displayed the least excretion rate of 0.38 µl/g of liver/minute.

Table 5a: Optical density readings for sulfobromophthalein contained in 10 microlitres of each bile sample excreted at various times of the experiment from the isolated perfused livers of the control rats.

Bile sample from liver No.	Time in minutes							
	0	15	30	45	60	75	90	120
1	0.00	0.23	1.59	1.10	0.46	0.18	0.09	0.03
2	0.01	0.01	0.55	0.89	0.71	0.51	0.31	0.15
3	0.00	0.00	1.32	1.81	0.65	0.19	0.07	0.03
4	0.00	0.04	1.98	1.86	0.75	0.24	0.10	0.04

Table 5b: Optical density readings for sulfobromophthalein contained in 10 microlitres of each bile sample excreted at various times of the experiment from the isolated perfused livers of the rats which had received single intraperitoneal injections of the freeze-dried alcoholic extract of C. mannii flowers at 300 mg/kg of body weight.

Bile sample from liver No.	Time in minutes							
	0	15	30	45	60	75	90	120
1	0.00	0.00	0.98	1.89	1.60	1.02	0.65	0.30
2	0.00	0.02	1.33	1.94	1.00	0.31	0.10	0.05
3	0.00	0.06	1.34	1.33	0.88	0.44	0.22	0.12
4	0.00	0.01	0.22	0.52	0.81	1.19	1.25	0.77

Table 5c: Optical density readings for sulfobromophthalein contained in 10 microlitres of each bile sample excreted at various times of the experiment from the isolated perfused livers of the rats which had been prefed on ration containing 8% of C. mannii flowers for 8 days.

Bile sample from liver No.	Time in minutes							
	0	15	30	45	60	75	90	120
1	0.00	0.22	1.00	0.60	0.22	0.10	0.07	0.03
2	0.00	0.16	0.95	0.48	0.18	0.08	0.03	0.02
3	0.00	0.25	1.10	0.66	0.29	0.14	0.06	0.03
4	0.00	0.18	1.70	0.90	0.27	0.12	0.05	0.03

Table 5d: Optical density readings for sulfobromophthalein contained in 10 microlitres of each bile sample excreted at various times of the experiment from the isolated perfused livers of the rats which had been prefed on ration containing 2.5% of C. mannii flowers for 8 days.

Bile sample from liver No.	Time in minutes							
	0	15	30	45	60	75	90	120
1	0.00	0.03	1.50	2.00	1.70	0.90	0.48	0.17
2	0.00	0.35	1.50	1.00	0.52	0.24	0.14	0.10
3	0.00	0.01	0.85	1.60	1.20	1.10	0.66	0.58
4	0.00	0.41	1.20	0.90	0.60	0.30	0.18	0.12

Table 6: Cumulative percentages of sulfobromophthalein (BSP) that was excreted in bile by the control and test rat livers (each value was based on 4 observations).

Group	Time in minutes							
	0	15	30	45	60	75	90	120
Control	0	1.4	33.7	67.3	79.3	84.0	89.1	90.4
300 mg/kg	0	0.3	19.2	42.4	57.0	64.1	67.4	70.7
2.5% C. mannii	0	2.0	25.1	46.6	57.2	65.7	69.1	73.6
8.0% C. mannii	0	9.4	53.0	82.8	90.4	93.9	95.4	96.9

Table 7: Sulfobromophthalein (BSP) excretion rates in µg/g of liver/minute for the control and test rat livers.

Liver No.	Groups			
	Control	300mg/kg	2.5% C. mannii	8% C. mannii
1	3.32	1.83	2.29	2.49
2	1.34	2.54	2.95	3.91
3	2.66	2.53	2.22	2.16
4	3.05	0.77	2.60	2.38
Mean	2.59 ± 0.88	1.92 ± 0.83	2.52 ± 0.33	2.74 ± 0.80

Table 8a: Optical density readings for sulfobromophthalein contained in 0.2 ml of each perfusate supernatant (of various times) that had been withdrawn from the perfusion fluid of the isolated perfused livers of the control rats.

Perfusate for Liver No.	Time in minutes									
	0	5	10	15	30	45	60	75	90	120
1	0.00	0.27	0.20	0.13	0.06	0.02	0.01	0.01	0.01	0.01
2	0.00	0.19	0.15	0.11	0.06	0.03	0.01	0.01	0.01	0.01
3	0.00	0.27	0.14	0.14	0.10	0.02	0.01	0.00	0.00	0.00
4	0.00	0.79	0.35	0.18	0.05	0.02	0.01	0.01	0.01	0.00

Table 8b: Optical density readings for sulfobromophthalein contained in 0.2 ml of each perfusate supernatant (of various times) that had been withdrawn from the perfusion fluid of the isolated perfused livers of the rats which had received single intraperitoneal injections of the freeze-dried alcoholic extract of C. mannii flowers.

Perfusate for liver No.	Time in minutes									
	0	5	10	15	30	45	60	75	90	120
1	0.00	0.12	0.08	0.04	0.04	0.03	0.04	0.03	0.03	0.02
2	0.00	0.29	0.21	0.16	0.08	0.01	0.01	0.01	0.01	0.03
3	0.00	0.22	0.13	0.10	0.03	0.01	0.01	0.00	0.01	0.01
4	0.00	0.25	0.24	0.20	0.11	0.05	0.06	0.04	0.04	0.05

Table 8c: Optical density readings for sulfobromophthalein contained in 0.2 ml of each perfusate supernatant (of various times) that had been withdrawn from the perfusion fluid that was perfusing the isolated livers from the rats that had been prefed on ration containing 8% of C. mannii flowers for 8 days.

Perfusate for liver No.	Time in minutes									
	0	5	10	15	30	45	60	75	90	120
1	0.00	0.15	0.10	0.07	0.03	0.01	0.01	0.01	0.01	0.01
2	0.00	0.19	0.12	0.07	0.03	0.01	0.02	0.01	0.01	0.01
3	0.00	0.17	0.15	0.09	0.04	0.02	0.02	0.01	0.01	0.01
4	0.00	0.22	0.14	0.10	0.04	0.01	0.02	0.01	0.01	0.01

Table 8d: Optical density readings for sulfobromophthalein contained in 0.2 ml of each perfusate supernatant (of various times) that had been withdrawn from the perfusion fluid of the isolated perfused livers from the rats that had been prefed on ration containing 2.5% C. mannii flowers for 8 days.

Perfusate for Liver No.	Time in minutes									
	0	5	10	15	30	45	60	75	90	120
1	0.00	0.31	0.22	0.19	0.09	0.05	0.02	0.02	0.01	0.01
2	0.00	0.21	0.16	0.13	0.07	0.05	0.03	0.02	0.02	0.01
3	0.00	0.29	0.25	0.22	0.16	0.11	0.08	0.06	0.06	0.04
4	0.00	0.22	0.15	0.10	0.06	0.04	0.03	0.02	0.02	0.01

Table 9: Percentage of sulfobromophthalein (BSP) remaining in the perfusate of the control and test rat livers (each value was based on 4 observations).

Group	Time in minutes							
	0	15	30	45	60	75	90	120
Control	100	43.7	15.3	6.3	2.2	2.0	1.2	0.8
300 mg/kg	100	42.9	21.2	10.8	9.0	6.0	7.7	9.0
2.5% C. mannii	100	59.3	34.0	21.8	14.0	10.4	9.4	6.3
8% C. mannii	100	38.9	16.8	6.2	8.4	5.1	4.6	3.1

Table 10a: Microlitres of bile collected at various times of the experiment on the isolated perfused livers of the control rats.

Liver No.	Time in minutes								Total
	0	15	30	45	60	75	90	120	
1	0	83	101	87	78	82	74	148	653
2	0	62	70	68	55	45	45	85	430
3	0	95	101	120	95	101	110	205	827
4	0	85	111	101	88	83	78	152	698

Table 10b: Microlitres of bile collected at various times of the experiment on the isolated perfused livers of rats which had received single intraperitoneal injections of the freeze-dried alcoholic extract of C. mannii flowers at 300 mg/kg of body weight.

Liver No.	Time in minutes								Total
	0	15	30	45	60	75	90	120	
1	0	43	43	45	44	40	35	100	350
2	0	71	94	95	74	90	70	130	624
3	0	90	110	75	53	50	40	46	464
4	0	43	35	83	53	25	14	20	273

Table 10c: Microlitres of bile collected at various times of the experiment on isolated perfused livers of rats which had been pre-fed on ration containing 8% of C. mannii flowers for 8 days.

Liver No.	Time in minutes								Total
	0	15	30	45	60	75	90	120	
1	0	100	86	100	95	95	82	172	730
2	0	270	168	164	148	144	148	274	1316
3	0	90	86	90	74	80	71	145	636
4	0	94	94	162	63	80	82	176	751

Table 10d: Microlitres of bile collected at various times of the experiment on isolated perfused livers of rats which had been prefed on ration containing 2.5% of C. mannii flowers for 8 days.

Liver No.	Time in minutes								Total
	0	15	30	45	60	75	90	120	
1	0	58	50	53	21	58	30	150	420
2	0	76	72	82	73	78	80	172	634
3	0	47	55	50	42	38	33	40	305
4	0	89	85	80	72	73	45	50	494

Table 11: Bile excretion rates in µl/g of liver/ minute for the control and test rat livers.

Liver Number	Groups			
	Control	300mg/kg	2.5% C. mannii	5% C.mannii
1	0.78	0.31	0.44	1.22
2	0.48	0.52	0.88	2.19
3	0.69	0.49	0.36	0.88
4	0.58	0.19	0.46	0.70
Mean	0.63 ± 0.13	0.38 ± 0.16	0.54 ± 0.23	1.25 ± 0.6

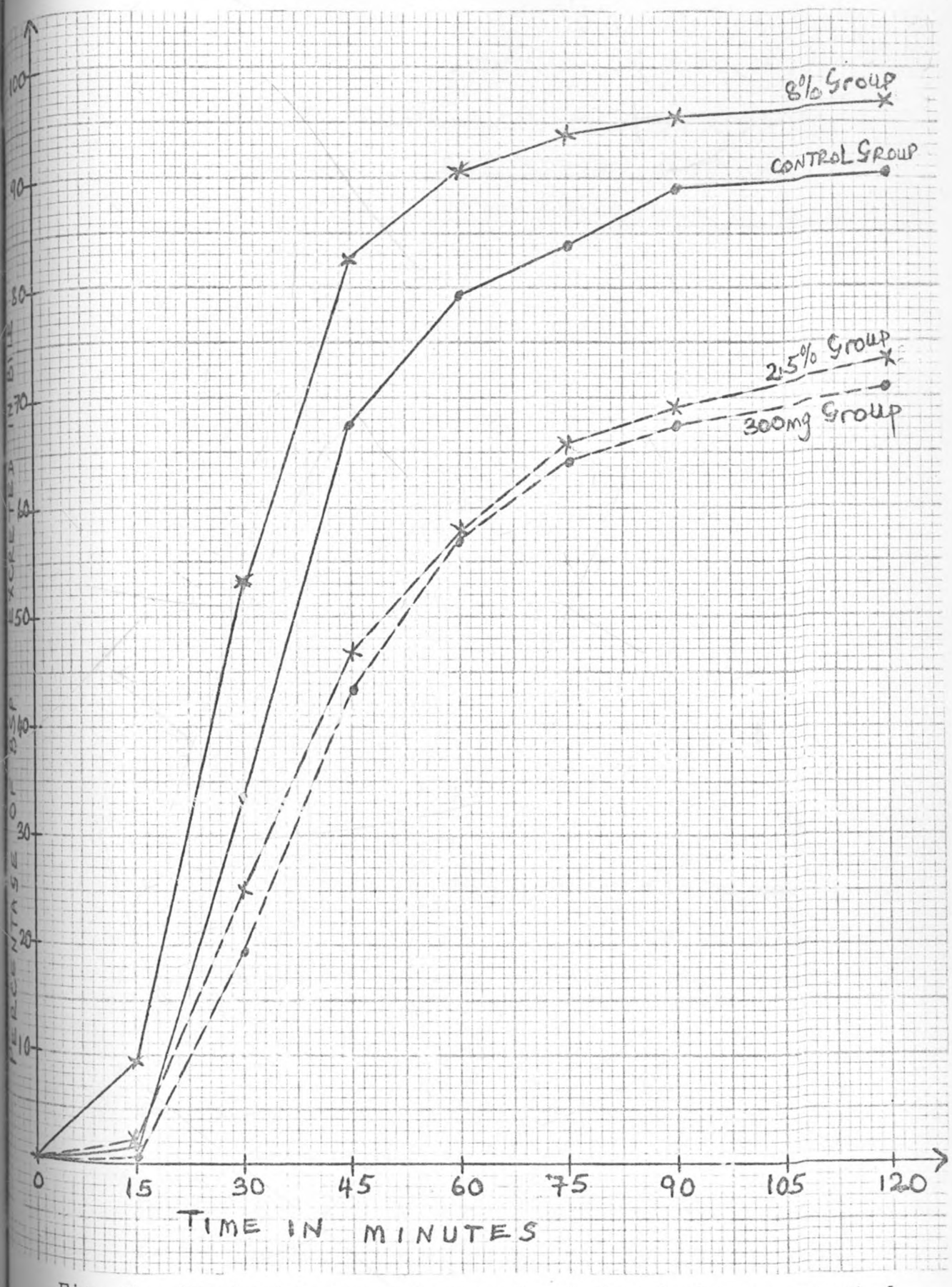

Fig. 7: Cumulative percentages of BSP that had been excreted in bile for the control and test rat livers at various times

For the effects on rats following short-term feeding (of 10 days duration) on rations containing 10, 20 and 40% of ground C. mannii flowers.

When the rats were fed on rations containing 10, 20 and 40% of ground C. mannii flowers for ten days duration acute intoxication and some deaths were observed in all the groups except the control group.

During the course of feeding, the rats developed diarrhoea, inappetance and restlessness more so in the groups feeding on rations containing 40% and 20% of ground C. mannii flowers.

Three deaths were observed in the group of rats feeding on ration containing 40% of C. mannii flowers on the fifth day of the feeding trial, these deaths were followed by three more on the sixth day of the feeding trial. By the tenth day of the experiment 10 rats had died in the highest dose group.

Two deaths were observed in the group of rats feeding on ration containing 20% of C. mannii flowers on the sixth day of the feeding trial and two more had died in this group by the tenth day of the feeding trial.

One rat died on the seventh day of the feeding trial in the group of rats feeding on ration containing 10% of C. mannii flowers, there were no further deaths of rats in this group by the tenth day of the experiment.

From these observations by the tenth day of the feeding trial 50% of the rats in the highest dose group had died while 20% and 5% died in the intermediate and lowest dose groups and no death occurred in the control group.

The post mortem examination for all the rats which fed on some plant flowers revealed congested livers and enlarged adrenal glands.

The histological examination of four liver sections (from different rats) per group showed marked haemorrhage and centrolobular necrosis of hepatocytes in all the rats that had fed on rations containing 40% and 20% of ground C. mannii flowers. Two liver sections from the rats that had been fed on ration containing 10% of ground C. mannii flowers showed coagulative necrosis of the hepatocytes close to the central vein; reduced haemorrhage was also observed in all the liver sections as compared to the livers from the other groups.

The histological evaluation of four kidney sections (from different rats) per group of rats did not reveal any pathological changes.

The rats in the control group did not show any gross and histopathological changes.

For the effects on rats following prolonged toxicity feeding of 100 days duration) on rations containing 2, 4 and 8% of ground C. mannii flowers.

When the rats were fed on rations containing 2, 4 and 8% of ground C. mannii flowers for one hundred days, intoxication and some deaths were observed in all the test groups during the course of the feeding trial.

During the first two weeks of the feeding trial the test rats developed diarrhoea, later on however, the faeces were of a firmer consistency. After four weeks of the feeding trial inappetance and inactivity prevailed in the rats feeding on ration containing 8% of ground C. mannii flowers.

The first two deaths were observed in the group of rats feeding on ration containing 8% of C. mannii flowers on the 21st day of the feeding trial, two more deaths were observed on the 22nd day of the feeding trial and by the 100th day of the feeding trial only two rats out of the original twenty were still alive in this group.

One death was observed in the group of rats feeding on ration containing 4% of C. mannii flowers on the 28th day of the feeding trial, three more rats had died by the 42nd day of the trial and by the 100th day of the feeding trial eight rats out of the original twenty were still alive in this group.

The first and only observed death in the group of (20) rats feeding on ration containing 2% of C. mannii flowers occurred on the 43rd day of feeding trial.

From these investigations it was observed that by the 100th day of the feeding trial of rats on rations containing 2, 4 and 8% of ground C. mannii flowers, 90% of the rats in the highest dose group (8%) had died followed by 40% of the rats in the intermediate dose group (4%) and 5% of the rats in the lowest dose group (2%).

There were no observed clinical signs and deaths in the control group of rats.

Body weight

All the test and control groups of rats had similar mean weights (by F-test) at the start of the feeding trial but all the test rats, that is, those rats feeding on some plant material showed reduced weight gains compared to the control rats during the course of the feeding trial; and the least

weight gain was manifested in the group of rats which were fed on ration containing 8% of ground C. mannii flowers (shown in Tables 12a, and 12b, and Figures 8a, 8b, 8c and 8d).

The gross changes

The post mortem examination on all the test rats which died within six weeks of the feeding trial showed enlarged congested livers with dark brown areas uniformly distributed with normal brown colouration of the livers. However, the post mortem examination of all the test rats that died after six weeks of the feeding trial and the test rats which were euthanized on 100th day of the feeding trial revealed enlarged, granulated livers the surfaces of which felt uneven and corrugated to the finger. In all these cases the edges of the livers remained as sharp as those of the control rats.

Liver weight

By examination of the mean ratios of the liver weight to body weight for the control rats and the rats which were fed on rations containing ground C. mannii flowers (treated rats) using the Least Significant Difference (LSD) test, it was realized that the difference between the two mean ratios was significant at 5% probability level. The mean ratio

of the liver weight to body weight was greater for the treated rats than for the control rats. The eye inspection of the livers on post mortem examination had shown that the livers from the treated rats were bigger than those from the control rats (shown in Table 13b).

By examination of the mean ratios of the liver weight to body weight for all the groups of rats using the Duncan's Multiple Range test it was found that the difference between the mean ratio for the group of rats which were fed on ration containing 8% of C. mannii flowers and the mean ratio for either of the remaining groups was significant at 5% probability level. In relation to body weight, the rats which were fed on ration containing 8% of C. mannii flowers had bigger livers than the livers from any of the remaining groups (Table 13c).

The difference between the ratios for the group of rats which were fed on ration containing 2% of ground C. mannii flowers and for either the control rats or the group of rats which were fed on ration containing 4% of C. mannii flowers was significant at 5% probability level. In relation to body weight, the livers from the rats of the group that was fed on ration containing 2% of C. mannii flowers were bigger than those from the rats which were fed on ration containing 4% of C. mannii flowers.

The difference between the mean ratios of the liver weight to body weight in the rats that were fed on ration containing 4% of ground C. mannii flowers and the control was not significant at 5% probability level. In relation to body weight, the livers from the rats which were fed on ration containing 4% of ground C. mannii flowers, though bigger than livers from the control rats did not show significant enlargement from the control at 5% probability level (see Table 13c).

Other Organs

All the test rats showed splenomegaly and the adrenal glands retained their normal size and shape (in contrast to their increased size in the rats of the short-term feeding trial).

The other organs examined, that is, the kidney, stomach, intestine, heart, lung, femur and brain did not show any changes. The rats in the control group did not show any gross changes on post mortem evaluation.

The Histological changes

Of the organ sections examined for any possible histopathological changes, the liver displayed the most dose-related pathology; the liver sections showed marked histopathological changes in the highest dose group of rats followed by the intermediate and lowest dose groups respectively.

The liver sections from the highest dose group of rats showed centro-lobular necrosis of the hepatocytes, some hepatocytes showed vacuolation while others had lost their cell boundaries and had pyknotic nuclei. The vascular walls of the central veins were degenerated, homogenous and had lost the muscular and endothelial nuclei. In some cases, thrombus material was attached to the necrotic wall of the blood vessel. The vascular changes were most prominent in the portal tract. There were haemorrhages around the degenerated or necrotic vessels and at the periphery of the necrotic hepatocytes.

Inflammatory cells, that is, macrophages, neutrophils and lymphocytes were abundant in the necrotic areas around the central vein and the triad. The Kupffer cells were increased in size and number.

There was proliferation of the bile ducts and abundance of fibroblasts and collagen fibres in areas around the triad in some rats. There were also hepatocytes with giant nuclei and prominent nucleoli adjacent to the necrosis. From these histopathological changes, it was observed that the liver tissue was partly destroyed and some connective tissue proliferation was initiated. The liver sections from the intermediate dose group displayed similar histopathological changes to those observed

in the highest dose group but were less extensive in comparison to those in the highest dose group.

The liver sections from the rats in the lowest dose group displayed small necroses and haemorrhages around the central veins and triads, and in some of these areas there were fibroblasts and collagen fibres. The liver sections from the rats of this group showed the least histopathological changes.

The livers from rats in the control group did not show any histopathological changes. All the five kidney sections from rats of the highest dose group showed proximal tubular necrosis, vacuolation of tubular epithelium and some haemorrhages in the medulla. Two of the kidney sections showed hyaline casts.

The kidney sections (5 each) from rats of the intermediate and the lowest dose groups did not show any histopathological changes and so did those from the control group.

The spleen sections (5 each) from the rats in the highest, intermediate and lowest dose groups showed congestion; the spleen sections from the rats in the control group did not.

The other organs sections, that is, the stomach, intestine and femur for the three test and one control groups (5 sections/group) did not show any histological changes.

Table 12a: Mean weights in grams for the groups of rats which were fed on rations containing various percentages of ground C. mannii flowers.

Time in Weeks	Percentage of C. mannii flowers in the rations			
	0	2	4	8
0	65.5±18.50	68.3±20.20	67.9±23.20	68.6±20.21
2	98.2±26.48	91.3±22.01	85.7±25.64	74.2±22.50
4	138.2±34.07	114.8±29.71	106.6±28.69	85.9±19.12
6	154.6±30.12	124.4±32.32	118.7±35.79	89.9±20.20
8	173.3±34.70	146.1±35.85	133.0±42.03	93.3±24.76
10	178.7±34.66	150.1±34.97	138.3±41.77	92.3±22.68
12	206.1±39.05	167.8±40.74	148.6±46.41	73.0± 2.83

Table 12b: Number of surviving rats per group when the rats were fed on rations containing various percentages of ground C. mannii flowers.

Time in Weeks	Percentage of C. mannii flowers in the rations			
	0	2	4	8
0	20	20	20	20
2	20	20	20	20
4	20	20	19	16
6	20	20	16	15
8	20	19	12	10
10	20	19	12	3
12	20	19	12	2

Table 13a: Ratios of liver weight to body weight expressed as percentages for the rats which were fed on rations containing various percentages of ground C. mannii flowers.

Rat Number	Percentage of C. mannii flowers in the rat rations			
	0	2	4	8
1	3.64	4.62	4.44	6.37
2	3.84	5.45	4.13	6.91
3	4.28	4.86	4.50	6.68
4	3.55	4.67	4.02	6.41

Table 13b: Mean ratios of liver weight to body weight expressed as percentages for the control rats and the rats which were fed on rations containing various percentages of ground C. mannii flowers.

Group	Mean ratio expressed as percentage
Control	3.83 ± 0.33
Treated	5.25 ± 1.06

Table 13c: Mean ratios of liver weight to body weight expressed as percentages for the rats which were fed on rations containing various percentages of ground C. mannii flowers.

Percentage of C. mannii flowers in rat rations	Mean ratio expressed as percentage
0	3.83 ± 0.33
2	4.90 ± 0.38
4	4.27 ± 0.23
8	6.59 ± 0.25

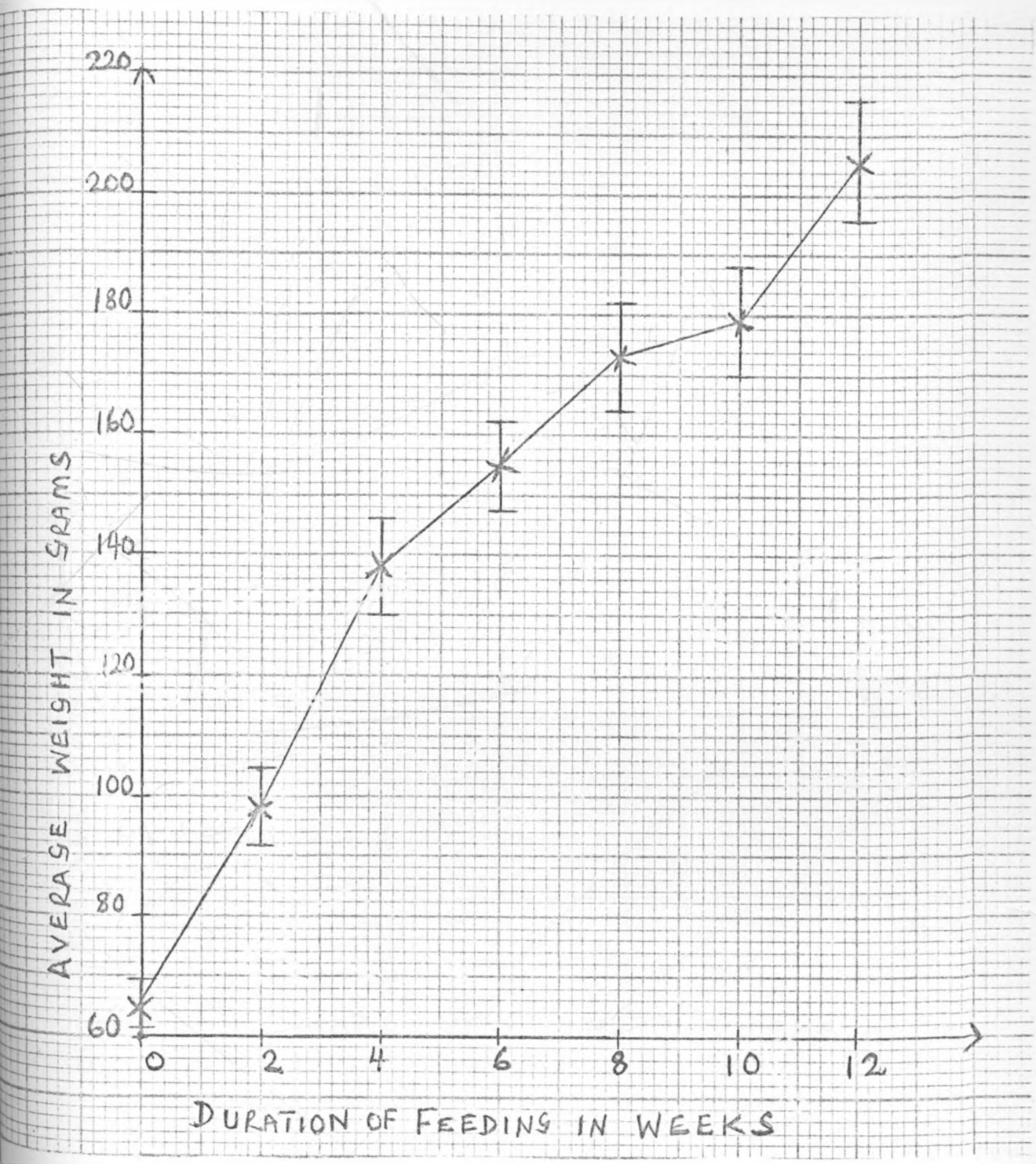

Fig. 8a: Growth curve for the control rats which were fed on ground mice pellets.

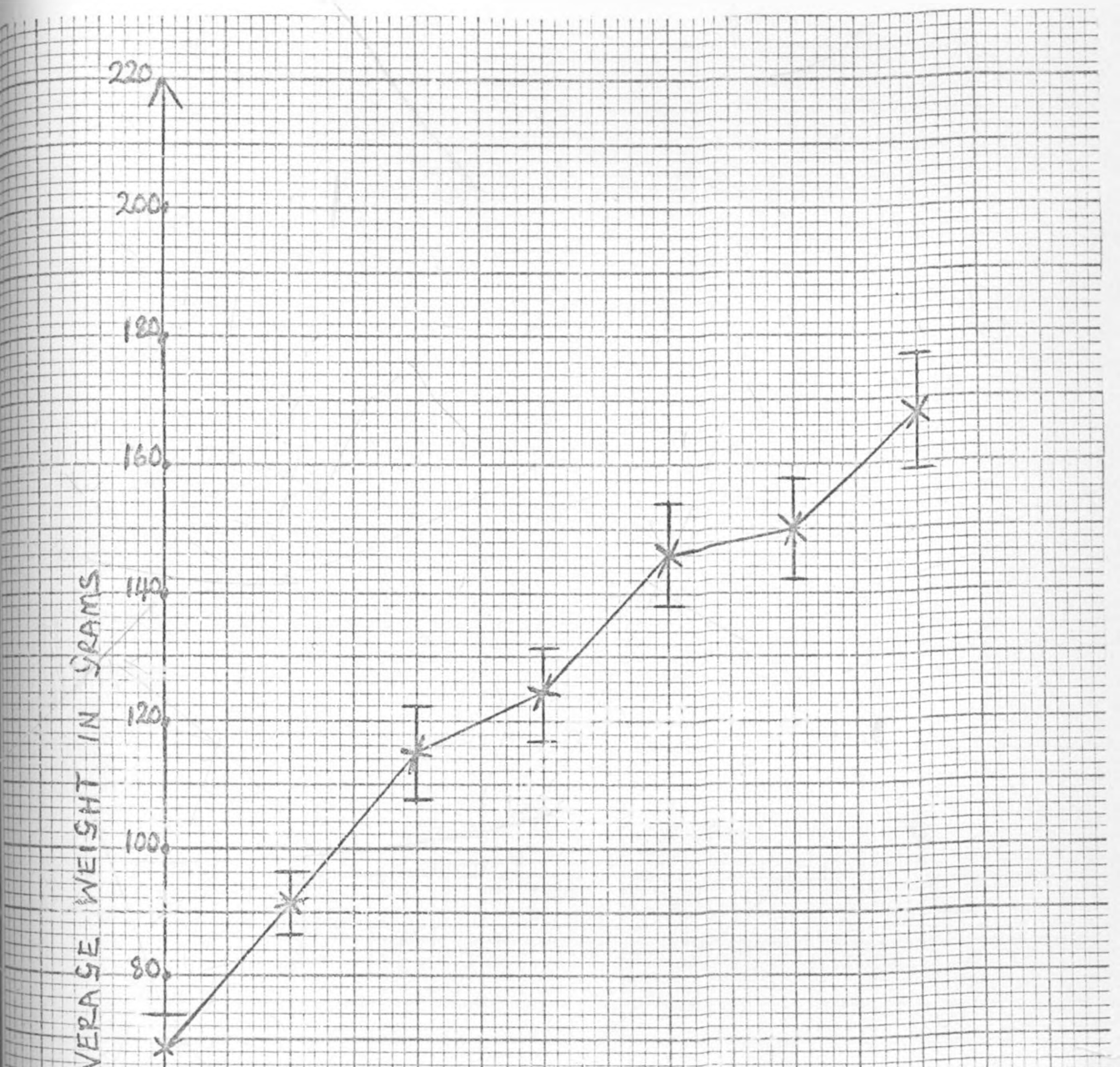

Fig. 8b: Growth curve for the test rats which were fed on ration containing 2.5% of C. mannii flowers.

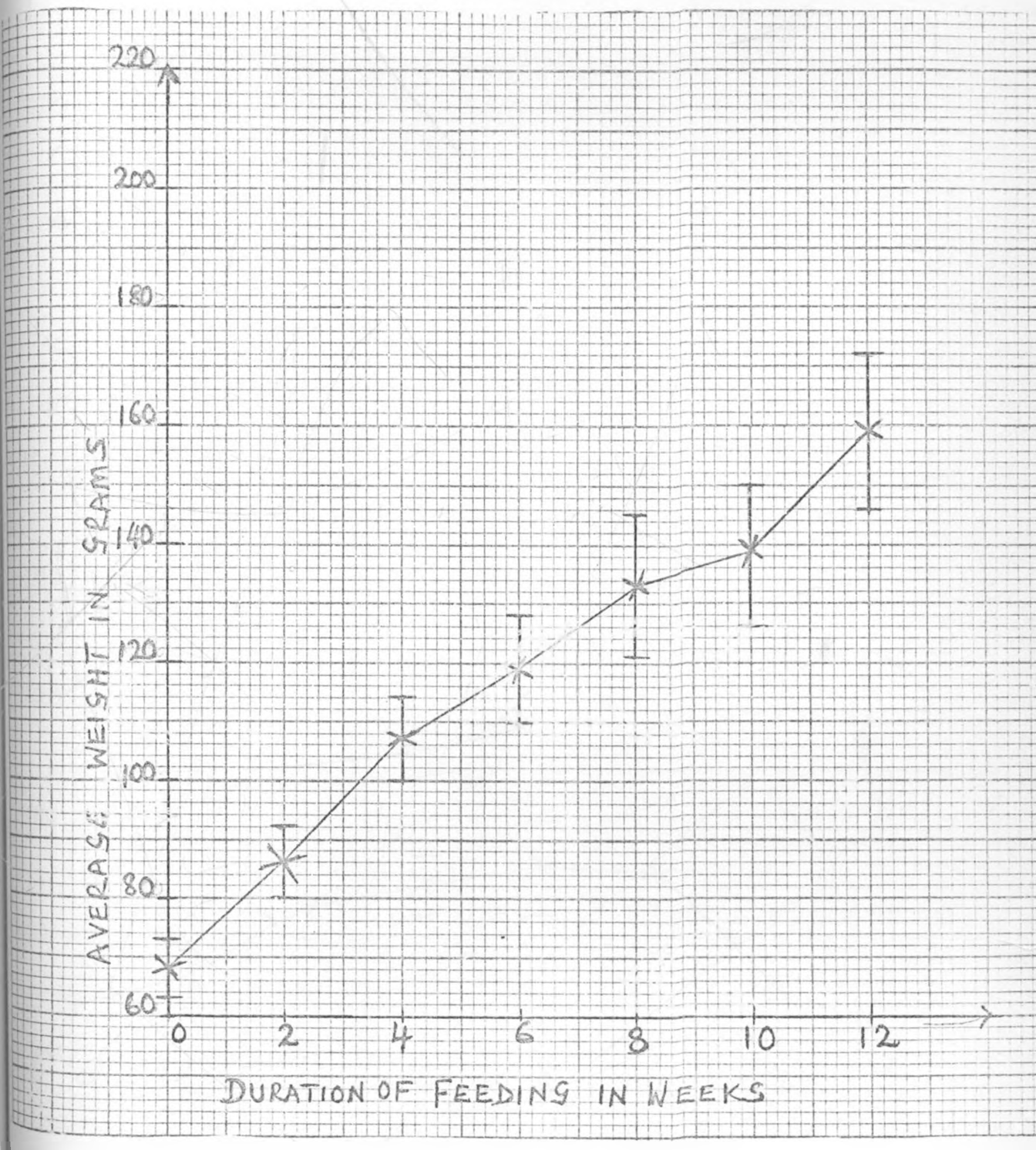

Fig. 8c: Growth curve for the test rats which were fed on ration containing 4% of C. mannii flowers.

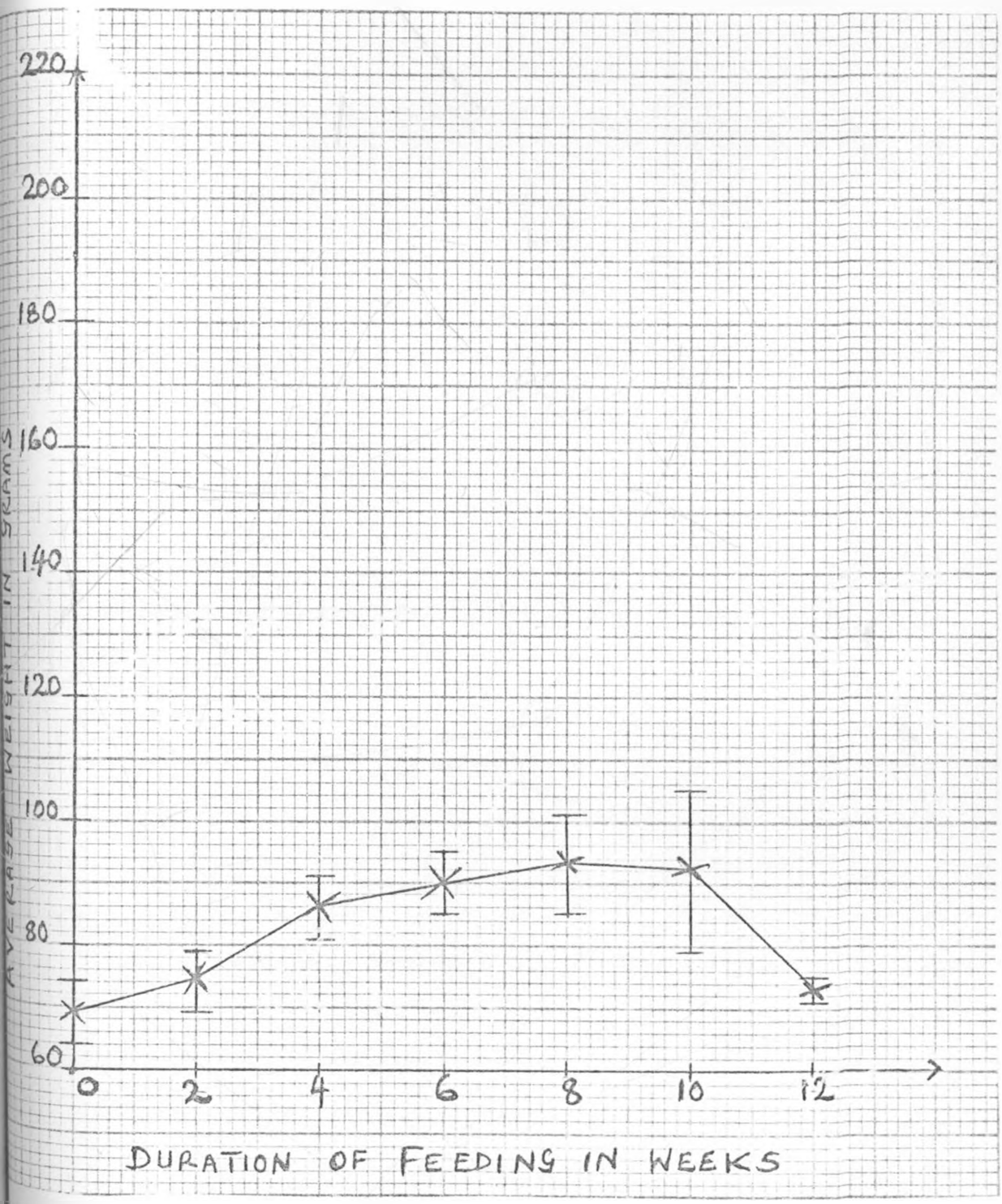

Fig. 8d: Growth curve for the test rats which were fed on ration containing 8% of C. mannii flowers.

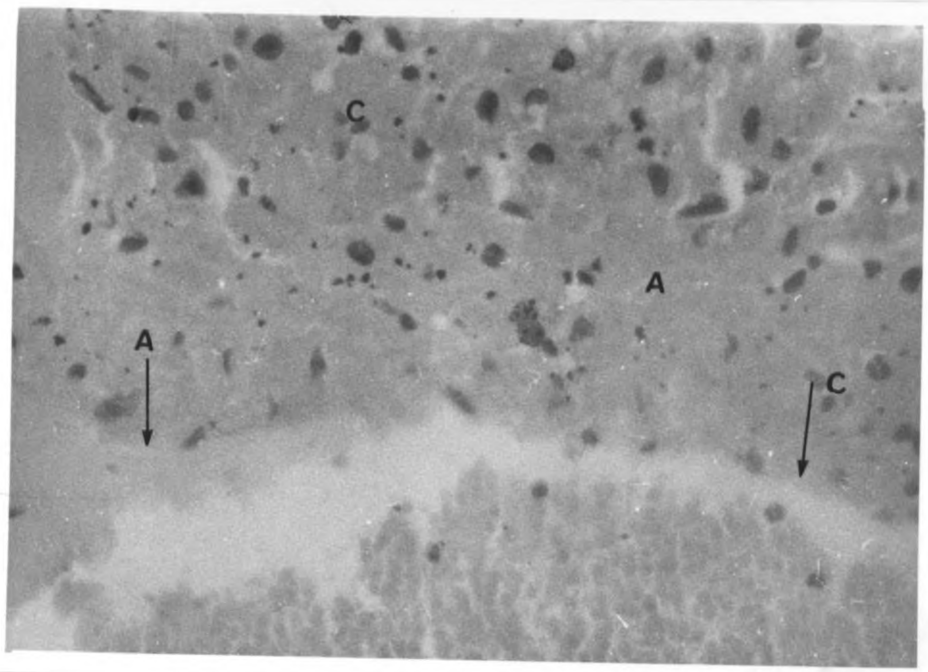

Plate 4a: Necrosis of the hepatocytes (A), fragments of nuclear material of the degenerated hepatocytes (C) and destroyed wall of the central vein (↓) in the rat that was fed on ration containing 40% of C. mannii flowers (Haematoxylin Eosin x 500).

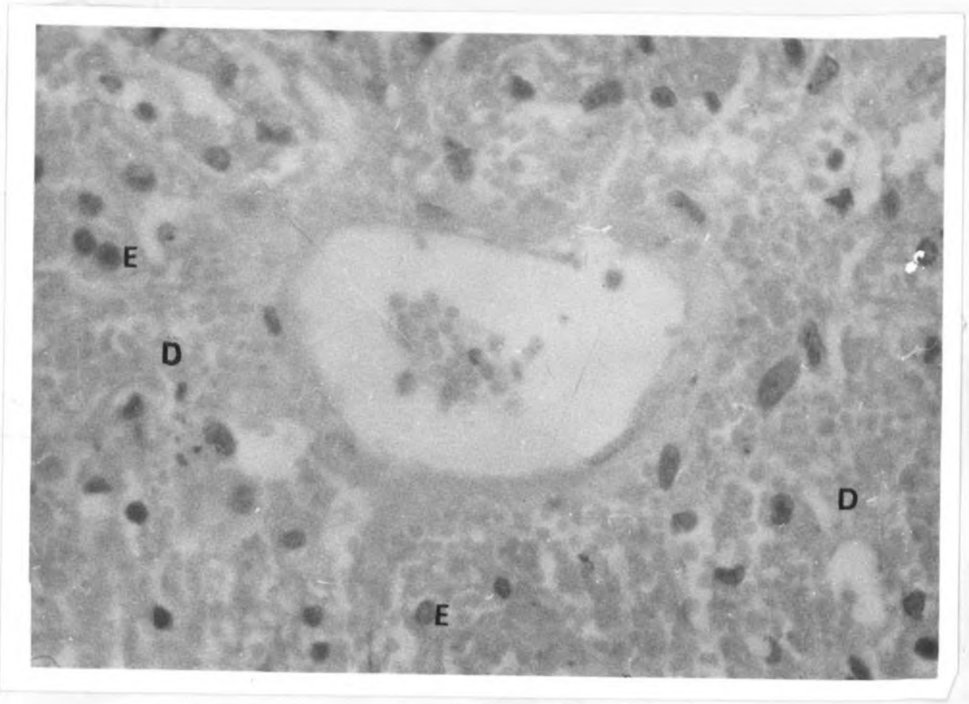

Plate 4b: Haemorrhages in the liver parenchyma (D) and dark nuclei of the hepatocytes (E) in a rat that was fed on ration containing 40% of C. mannii flowers (Haematoxylin Eosin x 500)

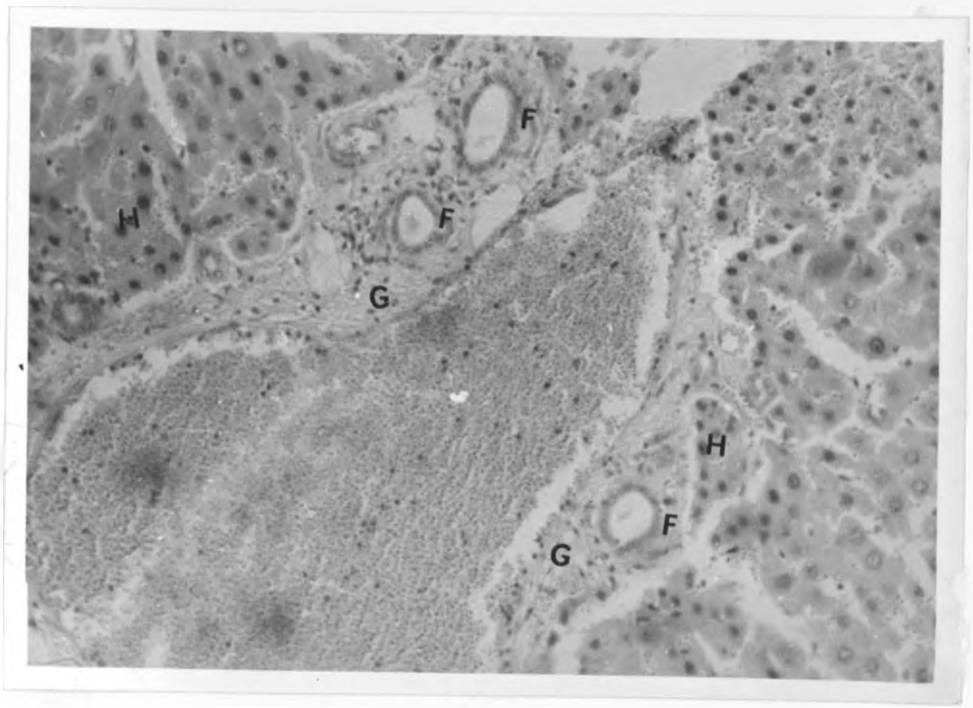

Plate 4c: Bile duct proliferation (F) and connective tissue formation around the portal (G) and hepatocytes with dark nuclei (H) in a rat that was fed on ration containing 8% of C. mannii flowers (Haematoxylin Eosin x 312)

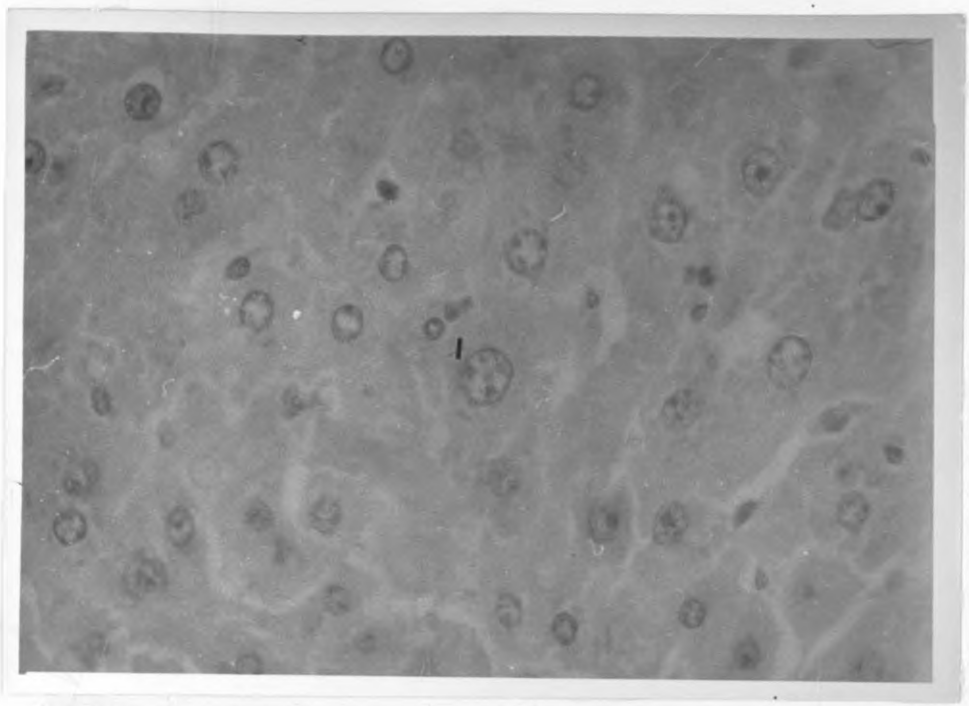

Plate 4d: Hepatocyte with a giant nucleus (↓) in a rat that was fed on ration containing 8% of C. mannii flowers (Haematoxylin Eosin x 500)

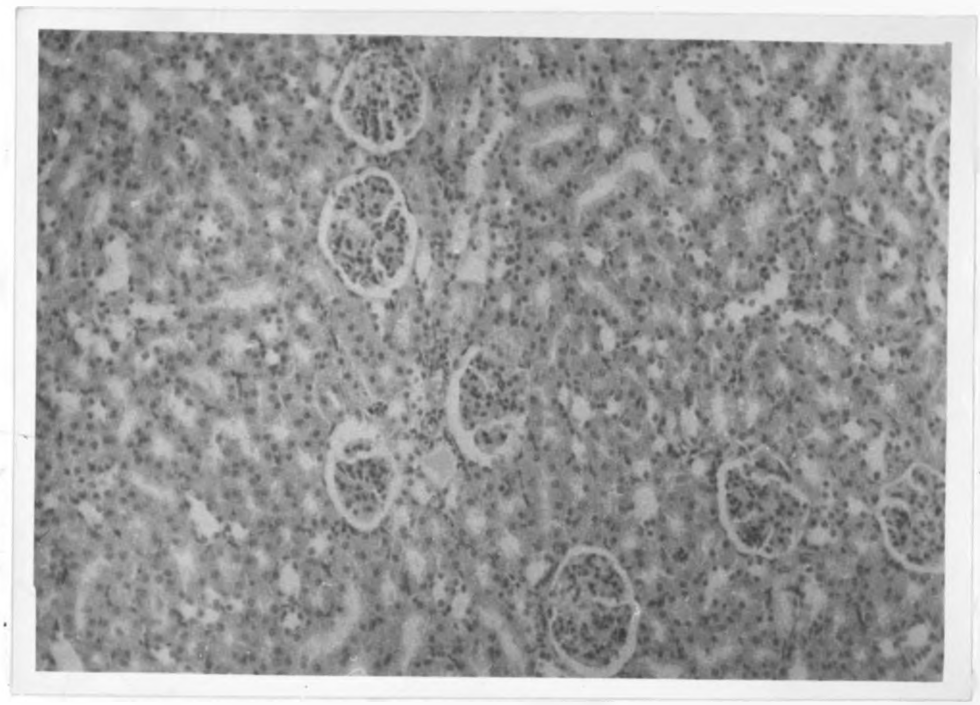

Plate 4e: Normal kidney parenchyma in a rat that was fed on ground mice pellets (Haematoxylin Eosin x 156)

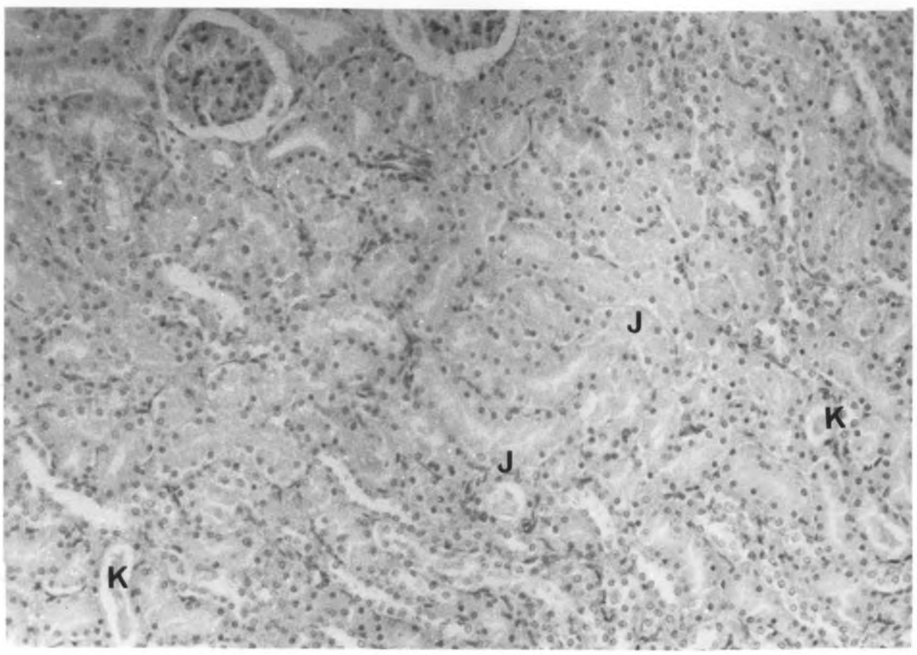

Plate 4f: Degenerated tubular epithelial cells (J) and hyaline casts in the tubular lumen (K) of the kidney of a rat that was fed on ration containing 3% of C. marnii flowers (Heamato-xylin Eosin x 156)

For the effect of the freeze-dried alcoholic extract of C. mannii flowers on the growth of Staphylococcus aureus (Oxford Heatley NCTC-6571).

Following the incubation of Staphylococcus aureus on Mueller Hinton Agar medium at 37°C for one day, it was observed that there was inhibition of bacterial growth around the well containing 0.02 µg of terramycin. The diameter for the zone of inhibition of bacterial growth was 14.7 mm. However, there were no observed zones of inhibition of bacterial growth around the wells containing 0.2 ml of isotonic saline and 1, 10 and 100 µg of the freeze-dried alcoholic extract of C. mannii flowers.

From this investigation, it was observed that at 1, 10 and 100 µg the freeze-dried alcoholic extract of C. mannii flowers did not inhibit the growth of Staphylococcus aureus.

For the alkaloid extraction

Following the dissolution of the precipitated alkaloids in diethyl ether and the subsequent concentration of the ethereal solution, the ethereal solution was found to contain two different alkaloids by Thin Layer Chromatography (TLC) technique.

One of the alkaloids showed an orange-brown colouration when sprayed with the Dragendorff reagent but displayed no colouration with the Marquis reagent. The other remaining alkaloid in the ethereal solution showed a brown colouration when sprayed with the Marquis reagent but displayed no colouration with the Dragendorff reagent.

Pilocarpine which was used as a standard, reference alkaloid showed an orange-brown colouration when sprayed with the Dragendorff reagent but displayed no colouration with the Marquis reagent.

The results indicated that at least two alkaloids were present in the flowers of C. mannii.

Table 14: Characteristics of pilocarpine and the two alkaloids of the ethereal solution on TLC; a mixture of 99.5% methanol and concentrated ammonia in the ratio of 200:3 was the developing solvent used.

Alkaloid	Rf x 100	Behaviour in UV light	Positive reagent for detection
Pilocarpine	75	invisible	Dragendorff reagent
Alkaloid (1)	59	invisible	Dragendorff reagent
Alkaloid (2)	74	blue	Marquis reagent

Plate 5: Alkaloid spots of one of the alkaloids extracted from C. mannii flowers, was spotted with Marquis reagent(L)

For the effect of the alkaloid solution on the isolated rabbit duodenum.

After the duodenum piece had remained in the organ bath for 10 minutes, a recording of its tone, rate and amplitude was made on the kymograph rotating at 0.25 mm/sec. The rate of the duodenal contractions was found to be 11 contractions per minute. The alkaloid solution had been obtained from the alkaloid precipitate that had been dissolved in diethyl ether, and the resulting solution then concentrated on the rotary evaporator. 0.2 ml of this solution was added to the organ bath, there was an immediate decrease in tone and amplitude of the duodenum contractions. After 80 seconds of observation and recording on the kymograph, the organ bath was emptied and duodenum piece washed twice with the Tyrode solution. When the duodenum had re-established its normal tone, rate and amplitude, 0.4 ml of alkaloid solution was added to the organ bath. There was an immediate and further decrease in tone and amplitude of the duodenum contactions (shown in Figures 9a and 9b).

From this investigation, it was observed that the alkaloids present in the alkaloid solution decreased the resting tone of the duodenum and reduced the amplitude as well; and the greater (concentration) amount of the alkaloids eliminated completely the amplitude of the duodenum contractions.

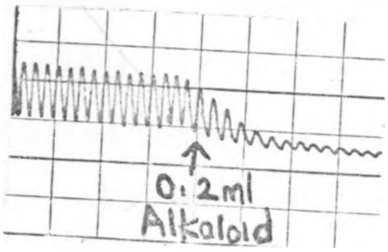

Fig. 9a: Decrease in tone and rate of the isolated rabbit duodenum contractions to 0.2 ml of the alkaloid solution

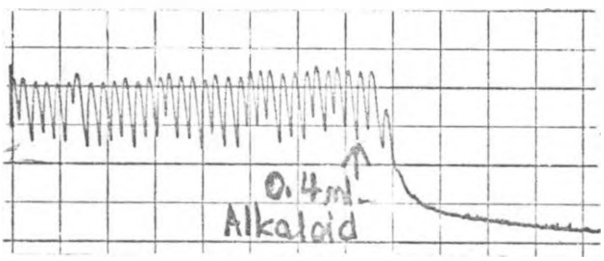

Fig. 9b: Decrease in tone and rate of the isolated rabbit duodenum contractions to 0.4 ml of the alkaloid solution

DISCUSSION

The plant Crassocephalum mannii Milne-Redhead has hitherto not been investigated for its biological activities in spite of its usage in the treatment of malaria, as an anthelmintic, a purgative, for dysentery and indigestion by various tribes in Kenya. In the course of this work, the plant Crassocephalum mannii was investigated with the purpose of examining whether it has any specific biological activities when applied to isolated organs and intact animals and if possible relate the effects to specified compounds within the plant. The results revealed some pharmacological and toxicological effects as well as the presence of at least two alkaloids in C. mannii flowers.

The freeze-dried alcoholic extract of C. mannii flowers increased the tone of the isolated guinea pig ileum and rabbit duodenum. The increase in tone was viewed as a spasmodic effect. Atropine sulphate and mepyramine maleate though individually had no effect on the guinea pig ileum blocked the expected spasmodic effect of the extract. These findings indicated that the spasmodic effect of the extract was governed by either cholinergic or histaminic mechanisms.

The freeze-dried alcoholic extract of C. mannii flowers decreased the force and rate of contractions of the isolated perfused rabbit heart and these effects of the extract were blocked by atropine sulphate. This was perhaps possible if atropine sulphate occupied the same receptors as the extract on the heart. The action of the extract on the heart seemed to be similar to that of parasympathomimetic drugs like acetylcholine and thereby indicated a cholinergic mode of action.

The freeze-dried alcoholic extract of C. mannii flowers caused a drop in blood pressure but the blood pressure returned to its original value within 3.5 minutes. Turner (1972) suggested that if a test substance causes a fall in blood pressure briefly after being injected it may have muscarinic activity like acetylcholine. it may have a vasodilator activity or it may have a cardiodepressant activity. The same author suggested that if a test substance causes a fall in blood pressure enduring beyond two minutes, the substance may have ganglion blocking activity or possible tranquilizing (ataractic) activity. Atropine sulphate though alone had no effect on the blood pressure was able to block the expected hypotensive effect of the extract on blood pressure. Atropine sulphate is a parasympatholytic drug and is specific in blocking the muscarinic effects of the parasympathomimetic drugs. The fact that atropine sulphate blocked the expected hypotensive effect of

the extract was yet another indication of the possible presence of a parasympathomimetic compound in the freeze-dried alcoholic extract of C. mannii flowers.

Mepyramine maleate had no effect on blood pressure but did not allow the drop in blood pressure to reach the level caused by the extract alone when injected concurrently with the extract. The less drop in blood pressure indicated that mepyramine maleate was able to block to some extent the hypotensive effect of the extract. Mepyramine maleate was able to block to some extent the hypotensive effect of the extract if there was a histaminic compound in the extract or due to the local anaesthetic properties known to pertain to antihistaminic drugs (like mepyramine maleate) which would make the cardiovascular system less responsive to the hypotensive effect of the extract.

The cardiovascular system is innervated by the sympathetic and parasympathetic branches of the autonomic nervous system. The sympathetic nerves release noradrenaline and some adrenaline while the parasympathetic nerves release acetylcholine. The cardiovascular system has alpha and beta receptors in the blood vessels while the heart has beta receptors. Stimulation of the alpha receptors by noradrenaline causes vasoconstriction and a rise in blood pressure but stimulation of the beta receptors by isoprenaline causes an increase in the rate and force of contractions of the heart, vasodilation and

a drop in blood pressure. Stimulation of the parasympathetic nerves causes a decrease in the rate and force of contractions of the heart, vasodilation and a drop in blood pressure.

Ergotamine tartrate caused a rise in blood pressure as it initially stimulated the alpha receptors thereby causing vasoconstriction. However, in higher dosage ergotamine tartrate blocks the alpha receptors, leaving the beta receptors under the influence of adrenaline which would prompt vasodilation which in turn would cause a decline in blood pressure. The subsequent administration of the freeze-dried alcoholic extract of C. mannii flowers to ergotamine tartrate caused a drop in blood pressure; this was perhaps possible if the extract stimulated the beta receptors or cholinergic receptors in the cardiovascular system. The action of the extract on the isolated perfused rabbit heart seemed to indicate a cholinergic mode of action other than stimulation of the beta receptors of the heart and as such there was less likelihood of a sympathomimetic mode of action of the extract.

Propranolol hydrochloride caused no change on the resting mean blood pressure. Propranolol hydrochloride blocks the beta receptors of the cardiovascular system thereby leaving the alpha receptors under the influence of noradrenaline, prompting vasoconstriction and a rise in blood pressure. The subsequent administration of the freeze-dried alcoholic

extract of C. mannii flowers to propranolol hydrochloride however caused a drop in blood pressure; this indicated that the extract did not stimulate the alpha receptors of the cardiovascular system and as such there was less likelihood that it had a sympathomimetic mode of action.

The fall in blood pressure due to the extract in the presence of ergotamine tartarate and propranolol hydrochloride in the cardiovascular system indicated that the extract did not act on the alpha and beta receptors of the cardiovascular system and as such there was less likelihood of a sympathomimetic mode of action by the extract on the blood pressure.

The freeze-dried alcoholic extract of C. mannii flowers seemed to increase the movement of phenol red when the extract was administered to the rats per os. As the extract was observed to cause an increase in tone of the isolated guinea pig ileum and rabbit duodenum a similar effect might have been elicited in the intestines of the living rats. The observed increase in tone and the presumed increase in the rate of contractions of the intestine were responsible for the increase in movement of phenol red along the intestines.

The intraperitoneal route of extract administration to the rats resulted in inhibition of movement of phenol red along the intestinal tract and this effect was more pronounced at the highest dose of the extract. The explanation for this observation might have been one of these:-

(i) that the active principle(s) in the extract caused the tonic effect as was seen in the isolated guinea pig ileum and rabbit duodenum when in direct contact with the intestinal mucosa. However, in the case of the intraperitoneal route, absorption through the peritoneum and extract biotransformation in the body tissues might have resulted into metabolites with inhibitory effect on the intestinal movements.

(ii) that irritation of the peritoneum might have provoked a nervous reflex whose effect was to decrease the intestinal movements.

(iii) that the alkaloids which were observed to decrease the tone and amplitude of contractions of the rabbit duodenum, their presence in the extract and contact with the intestine might have inhibited movement of intestinal contents.

However these experiments involving the determination of the effect of the alcoholic extract using different doses, times and routes on movement of phenol red along the small intestines in the rats ought to be repeated with enough data to justify by statistical calculations the results obtained during the pilot trials. The results of the pilot trials seemed to indicate that the alcoholic extract increased movement of phenol red in the small intestines of the rats when the extract was administered per os but inhibited movement of phenol red when the extract was administered intraperitoneally.

The results of the acute toxicity showed that the lethal dose 50% (LD_{50}) in mice following the intraperitoneal administration of the freeze-dried alcoholic extract of C. mannii flowers was 3078^{+} 42.8 mg/kg of body weight. According to standard toxicological classification of toxicity the alcoholic extract of C. mannii flowers should be characterized as slightly toxic. The test mice died within 3-15 minutes after the extract administration with cyanosis as the most outstanding clinical sign suggesting that the cause of death might have been failure of the cardiovascular or respiratory systems. However, there was no difficulty in breathing in the mice that died.

Crassocephalum mannii was also tested for toxic effects on the liver using the sulfobromophthalein (BSP) test. The sulfobromophthalein (BSP) test is used to evaluate the overall liver function. BSP is removed from the circulation of intact animals largely by the liver, taken up by the hepatocytes, conjugated with glulathione to form the BSP conjugate which is then excreted in bile without further change. Direct hepato-toxicity is manifested in BSP retention in circulation and delayed BSP excretion in bile. The normal value of BSP in circulation for human beings is 0-5% BSP retention 45 minutes after BSP administration.

However, (in 45 minutes) the retention of BSP from 20-40% in circulation indicates slight impairment of liver function, from 50-80% moderate damage and above 90% very severe damage (Goodale, 1964).

In the investigations of Crassocephalum mannii only the sub-toxic doses of the extract and flowers were used to determine the effects on the rats livers using the isolated perfused liver technique. The perfusate flow to and out of the livers was kept within constant ranges to maintain steady flow of BSP to the livers.

The isolated perfused livers from the rats which had received single intraperitoneal injections of the extract (300 mg/kg of body weight) and from the rats which had been fed on a ration containing 2.5% of C. mannii flowers for 8 days showed normal bile outflow but the BSP excretion in bile was delayed and the circulating levels of sulfobromophthalein in the perfusate were high. The retention of high amounts of BSP in the perfusate and the delay of BSP excretion in bile were possibly due to the toxic effect of the extract on the liver interfering with either cellular uptake or conjugation of BSP to glulathione.

The alcoholic extract of C. mannii flowers and the low dosage (2.5%) of C. mannii flowers in the ration seem to have caused a slight toxic effect on the rat livers.

The isolated perfused livers from the rats which had been fed on ration containing 8% of C. mannii flowers for 8 days showed increased bile outflow, increased BSP excretion in bile and low levels of BSP in perfusate. Mehendale (1979) reported that some compounds which enhance their own biotransformation, increased the bile outflow and the same researcher cited that phenobarbitone has been observed to increase bile flow and BSP excretion due to enhanced biotransformation of the same compound.

The high dosage of 8.0% C. mannii flowers in the ration did not reveal any toxic effects on the liver using the retention and excretion of BSP parameters, instead it increased bile flow and BSP excretion, the increase in BSP excretion was possibly related to the increase in bile flow. The cause of the increase in bile flow was not determined.

The feeding of high amounts of ground C. mannii flowers (at 10, 20 and 40%) incorporated in the rations caused diarrhoea, inappetance, restlessness in all the rats and death in some of the rats. The gross changes were in the livers which were congested and mottled in colour, and in the adrenal glands which were enlarged. The adrenal glands were enlarged possibly due to stress. The microscopic changes in the liver included centro-lobular necrosis, haemorrhages and increase in the number of Kupffer cells. The gross

and histological changes of the liver indicated that the toxic principle(s) in Crassocephalum mannii affected the liver more than any other organ.

The prolonged feeding of low amounts of ground C. mannii flowers (at 2, 4 and 8%) incorporated into the rations caused diarrhoea, retarded growth and deaths in some of the rats.

The gross changes revealed enlarged and mottled livers in some rats while in others the livers were firm, light in colour and granulated. The spleens were also enlarged.

The microscopic changes in the liver consisted of centro-lobular necrosis, haemorrhages, bile duct proliferation, giant hepatocytes (megalocytosis), destruction and/or necrosis of the walls of the central veins and periportal vessels, thrombi in the blood vessels and connective tissue formation around the portal tract. The spleens showed congestion while the kidneys showed tubular degeneration and hyaline casts. The prolonged toxicity feeding of C. mannii flowers to rats revealed marked gross and histological changes in the liver which indicated that the toxic principle(s) in Crassocephalum mannii affected the liver more than any other organ.

The gross and microscopic changes in the rats following both the short term and prolonged toxicity feeding on rations containing ground C. mannii flowers showed that the liver was the organ that was markedly affected by the toxic compound(s) contained

The centro-lobular necroses indicated that the toxic principle(s) were in high concentration in the area around the central veins. This was possible since most of the drug metabolizing enzymes (the mixed function oxidases) have been determined to occur in great amounts in the hepatocytes close to the central vein (Brodie, 1973). The centro-lobular necroses also indicated that the parent compound might not have been toxic but through biotransformation by the drug metabolizing enzymes, toxic compound(s) resulted, causing great damage to the hepatocytes close to the central vein and to the walls of the central veins. The haemorrhages that were observed were possibly the direct outcome of the effects of the toxic compounds on the walls of the blood vessels. The bile duct proliferation was perhaps in a response to irritation caused by the toxic compound(s) and connective tissue formation in the periportal areas was a response to tissue destruction. Hooper (1978) reported that bile duct proliferation and portal fibrosis are common non-specific features of hepatic disease.

The megalocytosis that was observed was probably initiated to compensate for the hepatocytes that had been destroyed.

Hooper (1978) reviewed the comparative pathology of pyrrolizidine alkaloid poisoning. The author mentioned that on the liver the pyrrolizidine alkaloids exert their effects on tissues by either causing

necrosis, inhibiting mitosis and thereby causing megalocytosis, or acting directly on the blood vessels resulting in oedema and vascular disease. The specific types of liver lesions mentioned included acute necrosis, parenchymal megalocytosis and veno-occlusive disease. Other changes included were cytoplasmic invaginations, cytoplasmic inclusion globules, portal fibrosis, bile duct proliferation, regeneration nodules and/or tumours.

During the short term and prolonged toxicity feeding of C. mannii flowers to rats acute necrosis, megalocytosis and destruction of the walls of the central veins and portal vessels were observed. Other changes observed were haemorrhages, thrombi in blood vessels, connective tissue formation around the portal tract (beginning of portal fibrosis) and bile duct proliferation. However the cytoplasmic inclusion globules, regeneration nodules and/or tumours were not observed. Whether tumours would have been produced in chronic toxicity testing, remains to be investigated. The lesions described for pyrrolizidine alkaloids on the liver by Hooper (1978) seemed to tally with the lesions recorded following the short term and prolonged toxicity feeding of C. mannii flowers to rats during the course of investigating the biological effects of C. mannii. The possible presence of pyrrolizidine alkaloids in Crassocephalum mannii needs to be investigated.

Other Crassocephalum species have been used in folk medicine by various tribes in East Africa. The leaves of Crassocephalum crepidioides S. Moore, however, are also used in West Africa to make soups and sauces. Identical chemical compounds have been found in plants of related ethnobotany. Identical phytochemical compounds may also be found in various parts of the same plant. The short term and prolonged toxicity feeding of rats on Crassocephalum mannii flowers caused liver damage to rats. The need to investigate those species of Crassocephalum and more so those that are used for food by human beings might be worthwhile.

Two distinct alkaloid spots with different Rf values were extracted from Crassocephalum mannii flowers. There were no further attempts made to isolate and characterize each of these alkaloids.

The alkaloid solution which contained these two alkaloids decreased the tone and amplitude of the contractions of the isolated rabbit duodenum. As the solution contained two alkaloids it was not possible to associate their activity on the rabbit duodenum to either alkaloid. The effect of the alkaloid solution on the isolated rabbit duodenum was quite different from that of the freeze-dried alcoholic extract of C. mannii flowers. The alcoholic extract had increased the tone of the isolated rabbit duodenum. The different actions of the alkaloid solution and alcoholic extract on the rabbit duodenum were suggestive of

different compounds involved in eliciting these varying actions. The effect of the alkaloids on the isolated rabbit duodenum was possibly masked by other compounds in the alcoholic extract when the extract was tested on the rabbit duodenum.

Perhaps the testing of the alkaloids independently of each other on the isolated rabbit duodenum would give a clearer answer as to whether the effects of the alkaloids are quite different from those of the alcoholic extract. In this way it would be possible to give amore precise conclusion on the effects of the alkaloids and alcoholic extract on the isolated rabbit duodenum.

Also the gross and microscopic changes of the livers from the rats which had been fed on rations containing ground C. mannii flowers indicated the presence of a pyrrolizidine alkaloid in the flowers, perhaps isolation and characterization of the extracted alkaloids might have proved likewise.

The freeze-dried alcoholic extract of C. mannii flowers did not inhibit the growth of Staphylococcus aureus. As the freeze-dried alcoholic extract contains many compounds it would have been better to test each of these compounds separately on the growth of Staphylococcus aureus. However, the testing of the whole extract did not indicate bacteriocidal or bacteriostatic activity.

CONCLUSION

The plant Crassocephalum mannii was investigated for its possible pharmacological and toxicological effects.

The freeze-dried alcoholic extract from C. mannii flowers was found to possess the following biological activities:-

(a) Increase the tone of the intestine and enhance movement of the intestinal contents.

(b) Decrease the rate and force of contractions of the heart.

(c) Decrease the blood pressure.

The experiments on the isolated guinea pig ileum, isolated rabbit duodenum, isolated perfused rabbit heart and on the dog's blood pressure were suggestive of presence of compound(s) with parasympathomimetic or histaminic effects in the freeze-dried alcoholic extract of C. mannii flowers. The blockade of the expected increase in tone of the guinea pig ileum and the partial blockade of the fall in blood pressure in the dog by mepyramine maleate following the administration of the freeze-dried alcoholic extract to the test organ and animal preparation indicated either the presence of histamine releasing compound(s) or histamine like compound(s) in the alcoholic extract or that perhaps by virtue of the local anaesthetic properties pertaining to the antihistaminic drugs like mepyramine maleate, mepyramine maleate was able to desensitize the organ and animal preparations to the effects of the alcoholic extract.

Moreover the decrease in rate and force of contractions of the isolated perfused rabbit heart due to the alcoholic extract is contrary to the known effect of histamine on the heart. Histamine or histamine like or histamine releasing compound(s) would be expected to increase the heart rate. However the increase in tone of the guinea pig ileum and rabbit duodenum, the decrease in rate and force of contractions of the isolated rabbit heart and the fall in blood pressure in the dog following the administration of the freeze-dried alcoholic extract to these preparations let alone the specific blocking action of atropine sulphate (a parasympatholytic drug) to all these effects of the extract were highly suggestive of the presence of parasympathomimetic compound(s) in the extract.

The flowers of Crassocephalum mannii were found to contain a compound or compounds with hepatotoxic effects when toxic doses were administered. These effects included:-

(i) Centro-lobular necrosis, haemorrhages and injury to blood vessels of the liver.

(ii) In prolonged experiments there was connective tissue formation in the periportal areas, bile duct proliferation and megalocytosis.

(iii) In sub-lethal doses interfered with the ability of the liver to excrete the foreign compound sulfobromophthalein (BSP) at a rate comparable to the normal control livers.

(iv) In higher but still sub-lethal doses stimulated bile production with the attendant enhanced excretion of sulfobromophthalein (BSP).

The alkaloid extract of the plant flowers were found to contain two alkaloids, the further identification of which was not attempted but the solution containing them decreased the tone and amplitude of the intestinal contractions.

The freeze-dried alcoholic extract of C. mannii flowers was found to have no bacteriostatic or bacteriocidal effects on growing Staphylococcus aureus.

REFERENCES

Agnew, A.D.Q. (1974) Upland Kenya wild flowers, pp. 472-474, Oxford University Press, Ely House, London

Benson, L. (1959) Plant classification, pp. 296-302, D.C. Heath and Company, Lexington and Massachusetts

Brodie, B.B. (1973) Enzyme activation of drugs and other foreign compounds to derivatives that produce tissue lesions. In: Pharmacology and the future of man, Vol. 2; Toxicological problems (Acheson, G.H. and Loomis, T.A., eds.), pp. 48-59, S. Karger A.G., Basel, Switzerland

Dale, I.R. and Greenway, P.J. (1961) Kenya trees and shrubs, p. 157, Nairobi Buchanan's Estates Ltd. in association with Hatchard's 187 Piccadilly, London

Goodale, R.H. (1964) Clinical interpretation of laboratory tests, 5th edit., pp. 318-319, Blackwell Scientific Publications, Oxford

Harbone, J.B. (1973) Phytochemical methods, Chapman and Hall Ltd., 11 New Fetter Lane, London

Hooper, P.J. (1978) Pyrrolizidine alkaloid poisoning, pathology with particular reference to differences in animal and plant species. In: Effects of poisonous plants on livestock (Keller, R.F., van Kampen, K.R. and James, L.F., eds.), pp. 161-176, Academic Press Inc., New York

Kavanagh, F. (1972) Analytical microbiology, Vol. II, p. 134, Academic Press, New York

Kokwaro, J.O. (1976) Medicinal plants of East Africa, pp. 62-63, East African Literature Bureau, Nairobi

Mehendale, H.M. (1976) Perfusion method, Drug Metab. Dispos. 4: 124

Mehendale, H.M. (1979) Modification of hepatobilliary function by toxic chemicals, Federation Proc. 38: 2240-2245

Miller, L.C. and Tainter, M.L. (1944) Estimation of the ED_{50} and its error by means of logarithmic probit graph paper, Proc. Soc. Exp. Biol. Med. 57: 261-264

Short, D.J. and Woodnott, D.P. (1969) The I.A.T. manual of laboratory animal practice and techniques, 2nd edit., p. 409, Granada Publishing Limited, London

Snedecor, G.W. and Cochran, W.G. (1967) Statistical Methods, 6th edit., The Iowa State University Press, Ames., Iowa, U.S.A.

Sognen, E. (1965) Effects of calcium binding substances on gastric emptying as well as intestinal transit and absorption in intact rats, Acta Pharmacol. et Toxicol. 22: 31-48

The Herbarium, P.O. Box 45166, Nairobi

The Staff of the Department of Pharmacology, University of Edinburgh (1970) Pharmacological experiments on isolated preparations, 2nd edit., Churchill Livingstone, Edinburgh

The Staff of the Department of Pharmacology, University of Edinburgh and MacLeod, L.D. (1970) Pharmacological experiments on intact preparations, 1st edit., Churchill Livingston, Edinburgh

Thirty First World Health Assembly, Geneva (1978) Drug policy and management, Official records of W.H.O. 248: 418-441

Turner, R.A. (1971) Beta adrenergic blocking agents. In: Screening methods in pharmacology, Vol. II (Turner, R.A. and Hebborn, P., eds.), pp. 21-40, Academic Press, Inc., New York

Verdcourt, B. and Trump, E.C. (1969) Common poisonous plants of East Africa, p. 50, Collins, St. James Place, London

Watt, J.M. and Breyer-Brandwijk, M.G. (1962) The medicinal and poisonous plants of Southern and Eastern Africa, 2nd edit., pp. 257-286, E. and S. Livingstone Ltd., Edinburgh and London