STUDIES ON SOME PHARMACOLOGIC AND TOXIC PROPERTIES OF PEDDIAE VOLKENSII GILG AND SCUTIA MYRTINA (BURM. F.) KURZ.

by

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DECLARATION:

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

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

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ABSTRACT

Peddiae volkensii Gilg and *Scutia myrtina* (Burm.f.) Kurz have been used medicinally as laxatives, and have also been said to be toxic. One aim of the research reported in this thesis was to test whether methanol extracts of the dried leaves from the two plants had stimulatory effects on the smooth muscles of the rabbit duodenum and the effects of the various drugs that would block the stimulatory effect if present.

The second aim was to test the effects of the above named extracts on the blood pressure in an anaesthetised dog and the effects of drugs that block the observed effects.

The third aim was to observe the toxicity and clinicopathologic signs in rats fed rations containing ground dried leaves of the two plants for 90 days.

The last aim was to determine the median lethal dose (LD_{50}) of the freeze dried extracts of the leaves in mice injected intraperitoneally.

The clinical signs observed in 40 rate fed *P. volkensii* Gilg and 36 rats fed *S. myrtina* (Burm.f.) Kurz, included rough hair coats diarrhoea in all rats and weight loss in many.

For rats fed *P. volkensii* Gilg, the liver weight: body weight ratio was less compared to the control group. This was an indication of liver cell damage, a fact shown histologically.

The rats fed P. volkensii Gilg had marked hydroperitoneum hydrothorax and frothy trachea. There were no significant gross findings in rats fed S. myrtina (Burm.f.) Kurz. Microscopically, both plants produced pulmonary haemorrhages and severe alveolar thickening. P. volkensii Gilg also caused renal proximal convoluted cellular degeneration, hepatic and adrenal cortical haemorrhages, marked splenic hemosiderosis signifying increased red blood cell destruction.

Methanol extract of P. volkensii Gilg leaves caused a piece of rabbit duodenum to decrease in rate of rhythmic contractions, but increase in the tone and force of contractions. Pretreatment of the preparation with atropine had no effect on the activity of the extract. This suggests that the mechanism of stimulation was not cholinergic since atropine is a blocker of the muscarinic receptors of acetylcholine. Pretreatment of the preparation with mepyramine maleate blocked the stimulatory effects of the extract which suggests histamine-like properties since mepyramine maleate is a blocker of H_1 histamine receptors.

Methanol extract of S. myrtina (Burm.f.) Kurz produced increased force but decreased rate and tone of rabbit duodenum. Pretreatment with mepyramine maleate produced no change in the effects of the extract except that there was no increased force of contractions.

This was possibly due to the anticholinergic properties of mepyramine maleate. Pretreatment with 1 mg atropine sulphate completely abolished the stimulatory effect of the extract. This suggests that the stimulatory effect was cholinergic in nature.

P. volkensii Gilg methanol extract caused a decrease in the blood pressure in the dog. The systolic, diastolic and the pulse pressures were decreased. The heart rate was also increased possibly by baroreceptor mechanism. Pretreatment with propranalol, mepyramine maleate and atropine was inconclusive. In case of S. myrtina (Burm.f.) Kurz methanol extract, the effect on the blood pressure in the dog was as in P. volkensii Gilg, i.e. an overall decrease in blood pressure. However, pretreatment with mepyramine maleate blocked the decrease in blood pressure by about 75%, while pretreatment with atropine sulphate blocked the decrease in blood pressure by about 100%. This suggests that S. myrtina (Burm.f.) Kurz caused a decrease in blood pressure by both cholinergic and histamine-like mechanisms.

In conclusion, both P. volkensii Gilg and S. myrtina (Burm.f.) Kurz stimulate rabbit duodenum, decrease systolic, diastolic and pulse pressure in the dog which suggest they could be used as laxatives and antihypertensives. However, this is not advisable because both plants produce pulmonary haemorrhages and severe alveolar thickening. P. volkensii Gilg also cause renal proximal convoluted cellular degeneration, hepatic and adrenal cortical haemorrhages and marked splenic hemosiderosis signifying red blood cell destruction.

The acute (24 hr) LD_{50} for S. myrtina (Burm.f.) Kurz is (6.1) g/kg and that of P. volkensii Gilg is (6.3) g/kg of body weight in mice.

INTRODUCTION.

The study of toxic and medicinal plants indigenous to East Africa is a fairly recent development although certain plants have been known to contain poisonous principles which were used in arrow poisons as early as the beginning of the century. Indeed, some of the indigenous peoples of East Africa have a wealth of knowledge regarding the medicinal or toxic aspects of some of the plants within their locality which goes back to many centuries.

It was well known before 1927 that poisonous plants caused severe losses to livestock. The extent of the loss was and is still very difficult to assess for two main reasons. First some animals are found dead and it is difficult to ascertain whether they died of some infectious conditions, or due to poisonous plants. Secondly diagnosis of plant poisoning in animals is complicated by the fact that very few of these plants present pathognomonic signs or the so called typical lesions of a particular condition.

Nonetheless, the study of poisonous plants was relegated to a minor place in the field of Veterinary Medicine due to presence, at that time, of more devastating diseases of livestock such as rinderpest and East Coast Fever. In addition organized research in this field with respect to feeding and toxicity trials in animals was lacking.

At the present time, more emphasis has been given to the research on the pharmacologic and toxic effects of plants both in animals and humans.

Mettam (1929) carried out feeding trials in animals using seven toxic plants namely Gloriosa virescene; Cassia didymobotyra; Ornithogalum longibracteatum; Dichrocephala Chrysanthemifolia; Leonotis mollissima; Acokanthera Schimperiela et longiflora; Asclepias plalycalyx all of which are indingenous to Kenya. In this feeding trial, the clinical signs, and the post mortem lesions in poisoned animals were well described.

Hudson (1930) described tests in sheep using three indigenous plants namely Trifolium semipilosum; Ornithogalum longibracteum; and Hypoxis villosa. but no toxic effects were manifested by sheep fed these plants.

Mugera (1970) also did some work on some indigenous plants to determine whether they are toxic to livestock or not. Despite these studies many indigenous plants which have either been used medicinally by local tribes or are claimed to be toxic either to humans and or to animals have yet to be investigated to experimentally verify their toxic or medicinal properties. Such two plants are Peddiae volkensii Gilg and Scutia myrtina (Burm.f.) Kurz which have either been used medicinally or claimed to be toxic by the local people in the areas where they grow. The purpose of this investigation is to determine the pharmacologic and or toxic properties of the two plants.

Scutia myrtina (Burm.f.) Kurz

Peddiae volkensii Gilg

mainly found in North America, and the seeds, and the leaves being the most toxic (Sperry et al., 1965). Poisoned animals show weakness, trembling, incordination and respiratory distress. At post mortem, there is very severe pulmonary oedema. Microscopically, there is degeneration of some of the skeletal and cardiac muscles, toxic nephritis and hepatitis. (Sperry et al., 1965).

In Britain, two members of the Rhamnaceae family have been used as laxatives and are known to be toxic (Clarke and Clarke, 1975). The toxic plants are Rhamnus carthatica (common buckthorn) and Rhamnus flangula. The leaves and bark of both species have glycosides which yield the purgative emodin (trioxymethyl anthraquinone) on hydrolysis (Clarke and Clarke, 1975).. An allied species, Rhamnus purshiana, native to the American continent is the source of the purgative cascara sagrada, a stimulant carthatic. The signs of poisoning in cattle with R. frangula are diarrhoea, colic, moderate fever, and death occurs within hours of the onset of the clinical signs. Ventilago vininalis (Supple jack) is a useful fodder, but can cause poisoning in sheep owing to its high tannic acid content if it forms the major part of the diet for too long a period (Clarke and Clarke, 1975). Other members of Rhamnaceae family have been used as remedies for various ailments by different tribes in Africa (Mitchell and Breyer-Brandvijk, 1962).

Peddiae volkensii Gilg.

This plant belongs to the family Thymeleaceae. The plant is a shrub which can reach a height of 6 m and is found inside the forest. The leaves have entire margins, the fruit is pink and the flowers are greenish yellow (Dale and Greenway, 1961). P. volkensii Gilg is claimed to be toxic by the local tribe in the area where it grows, but no work has been done to verify this allegation. Some members of Thymeleaceae family have been used as purgatives; these are Gnidia latifolia and Synactolepsis alternifolia. Other members of the same family have been used as remedies for a variety of ailments (Kokwaro, 1976).

Several members of the family Thymeleaceae are toxic to animals. These include Gnidia species; Lasiosiphon spp. and Orthrosolen spp. (Mitchell and Breyer-Brandwijk, 1962). Gnidia and Orthrosolen spp. also cause abortion (Mitchell and Breyer-Brandwijk, 1962). Toxic members of the family mentioned above seem to be gastrointestinal irritants since they produce profuse diarrhoea (Mitchell and Breyer-Brandwijk, 1962).

In Britain, the family Thymeleaceae is represented by Daphane laureola (Spurge laurel) and Daphane mereum (Mezereon) (Clarke and Clarke, 1975). The bark and berries of the above plants contain mezerein. The latter is a resin and is the anhydride of mezereinic

acid. The bark also contains daphin, a harmless glycoside (Clarke and Clarke, 1975). Mezerein is not destroyed by drying and storage. The compound is a very strong irritant comparable to the cantharides in its action. The toxicity is high e.g. 30g of the bark of D. mezereum will kill a horse, and three berries are sufficient to kill a pig. The signs of poisoning are vomiting, intense colic, prostration and general collapse. Post mortem reveals severe gastritis with white patches (as of burns) in the upper part of the gastrointestinal tract (Clarke and Clarke, 1975).

In Australia and New Zealand, the plants involved in poisoning of livestock in this family are Pimelea spp. (Clarke and Clarke, 1975). P. prostrata (Strathmore weed) has caused death in horses. The plant has an irritating poison which causes ulceration of the mouth, tongue, and oesophagus.. The signs are colic, depression and loss of appetite plus watery diarrhoea. The species involved in causing poisoning in Australia are P. simplex (desert rice flower), P. decora and P. trichostachva (Clarke and Clarke, 1975).

In South Africa, Lasiosiphon burchelli (Harpius bos) has caused poisoning in sheep. The signs observed are diarrhoea, dysponea and stasis; 1.8g/kg body weight of the dried plant material is lethal orally. L. bipinnatum (ganskweek) causes abdominal pain and photosensitivity in calves. At post mortem, there is liver necrosis (Clarke and Clarke, 1975).

OBJECTIVES OF THE PROJECT.

Economic losses to livestock owners due to poisonous plants in Kenya are difficult to estimate, but are certainly a reality. At the present time, our knowledge of local poisonous plants is still limited. However, with increasing research being done in this area, more information is being gained every year with regards to which plants are poisonous to livestock in a particular area and which plants have antimicrobial properties.

It is well known that indigenous tribes of Kenya from time immemorial have used local plant population for its medicinal and or poisonous properties. In the later case for example, the Akamba people have used extracts of Acokanthera spp. which contain the cardiac glycoside Ouabain as an arrow poison. They have also used the root of the plant Adenia volkensii (Kiliambiti) which contain a cyanogenetic glycoside to poison enemies. While information from local tribes regarding the toxicity or the medicinal property of a particular plant to animals or humans is certainly valuable, this kind of information needs experimental verification to be recognized scientifically.

P. volkensii Gilg and S. myrtina (Burm.f.) Kurz have been used medicinally as laxatives, and have also been described as toxic. The work to be done will involve the following.

a) To test whether methanol extracts of the dried leaves from the two plants has stimulatory effects on rabbit duodenum, and the effects of various blocking drugs on the stimulatory effect if present.

b) To test the effect of these extracts on the blood pressure in the dog, and the effects of various drug blocking agents on the observed effect.

c) To observe the clinical signs, gross and microscopic lesions in the rats that have been fed rations containing ground dried leaves of the plants for 90 days.

d) To determine the median lethal dose LD_{50} of the freeze dried methanol extracts from the leaves. This will be tested in mice injected intraperitoneally at several dose levels and observing the number of deaths after 24 hours.

The results of the above work will show whether one or both plants have chemicals which can be used as a laxative. They will also show whether the plants contain chemicals which can be used as hypotensives. Whether the above is really practical will depend on the results of the toxicity studies in c and d. The results will also show whether the plants have chemicals which have harmful effects to body organs.

MATERIALS, METHODS, AND RESULTS.

Branches of both P. volkensii Gilg and S. myrtina (Burm.f.) Kurz were collected from Kangaita forest in Kerogoya, Kirinyaga District, S. myrtina (Burm.f.) Kurz was growing at the forest edge while P. volkensii Gilg was growing deep into the forest making it very cumbersome to transport to the forest edge where the vehicle was. It should be noted that for S. myrtina (Burm.f.) Kurz samples were in addition collected from Njoro (Ndothua) forest after exhausting those in Kangaita forest. After collection of the branches of the two plants, the material was transported to Kabete and dried in the open air separately. After drying, the leaves from each plant were separated from the branches and ground with an Arthur R.H. Thomas grinder Arthur H. Thomas Co. Phila. Pa. U.S.A., to a fine powder separately. Each of the ground material was then stored in a separate sack ready for feeding trials.

P. volkensii Gilg

Experiment I: LONG TERM FEEDING STUDY IN RATS.

Fifty white albino rats obtained from the Veterinary Research Laboratories, Kabete were divided into five groups, A, B, C, D, E. Each group consisted of ten rats (five females and five males). Each group was kept in separate metal cages with males and females being kept in separate compartments of the same cage. Initially, all rats were fed ground calf pellets ad libitum for seven days; during which time the animals

acclimatized to their new surroundings. Water was also supplied ad libitum. After seven days, all the rats were weighed individually and the weights (g) were recorded. The various groups of rats were then fed a ration consisting of a mixture of ground P. volkensii Gilg leaves and ground calf pellets. Control group was fed on ground calf pellets only. (Table 1 p. 11).

Table 1:

Designation of the groups of rats used in the experiment. and the corresponding concentration of P. volkensii Gilg fed to each group.

Rat Group	Plant material concentration (w/w%)[x]
A	2
B	4
C	8
D	16
E	0 (Control)

X % w/w is the number of grammes of ground plant material in 100 grammes of the mixture i.e. plant material + calf pellets. Thus 2% w/w contains 2 parts of plant material mixed with 98 parts of ground calf pellets.

Example: Suppose one wants 4 kg of the 2% mixture, the amount of plant material x kg in the mixture is

$$\frac{\text{Part } (x)}{\text{Whole}} \times 100 = 2$$

$$\frac{X}{4} \times 100 = 2$$

$$100 \quad x \qquad = 8 \qquad X = 0.08 \text{ Kg.}$$

Thus 0.08 kg of plant material mixed with 3.92 kg of ground calf pellets will make a 2% concentration.

The rats were fed with the appropriate rations ad libitum for twelve (12) weeks. Water, was also provided ad libitum. Each rat was weighed individually once every week and the weight (g) recorded. The animals were observed daily for any changes in their clinical appearance. Any such changes were recorded for each rat. Animals that died during the course of the experiment or were terminated at the end of the experiment were weighed and the weight (g) recorded. A complete post mortem examination was carried out and the gross pathological findings recorded. The liver was dissected, carefully weighed and the weight recorded. The ratio liver weight/body weight was calculated for each animal and recorded. In each case histological samples from all the body systems and organs were preserved in 10% buffered formalin for 1 - 2 days. The histology was done on thin sections (5 - 6 u) and stained with hematoxylin and eosin.

The control liver weight/body weight ratios for normal rats was established as follows: Ten (10) male rats and ten (10) female rats whose weight varied from 130g to 260g were selected from the test group at random. This range was choosen because the test group of rats fed on P. volkensii Gilg all weighed within the above range. Each rat was weighed and the weight recorded. The rat was then anaesthetised by pouring ether on to a cotton wool, the latter was then placed in a dessicator after which the rat was placed inside and the dessicator covered. The rat was removed from the dessicator when it became unconscious and then was decapitated. The abdomen was then opened and the liver was carefully dissected with scissors, blotted with Whatman No. 1 paper and weighed. The weight was then recorded in grammes.

RESULTS:

All the fifty rats used in this experiment had rough haircoat and a few showed evidence of diarrhoea. The diarrhoea was most pronounced in rats fed 8% and 16% levels of P. volkensii Gilg.

With the exception of rats fed 4% P. volkensii Gilg leaves (Table 11), all the other groups of rats lost weight (Table 10, 12 and 13) as compared to the control group (Table 14) which gained weight during the same period of time.

The liver weight: body weight changes were less than those of the control (Table 3 and 4). The ratio for the control was 3.82 ± 0.31 while that of the test group was 2.73 ± 0.36.

Overall, the percentage deaths were directly proportional to the concentration of P. volkensii Gilg leaves in the ration (Table 15).

At post mortem, all the cases examined had either yellow or blood tinged hydroperitoneum and hydrothorax. The liver was normal in size, but had petechiae. Some areas were pale or greyish. The lungs were voluminous, heavy, firm and pitted on pressure. There was white froth from the cut surface of the lung parenchyma as well as in the bronchi. The kidneys were enlarged and congested. The cortex was pale while the medulla was dark and congested. The spleen was dark. In some rats, there was softening of the brain, gastroenteritis, swollen stomach wall, and blood clots under the skull

between the cerebrum and the cerebellum.

Microscopically, the kidneys had glomerulonephrosis characterized by diffuse degenerative changes and erosions of the epithelium of the proximal convoluted tubules. The glomerular tufts were slightly swollen and degenerated. The severity of the degenerative changes (Fig. 1 p. 61) depended on the concentration of the plant material. Some cells had cloudy swelling, others had vacuoles in the cytoplasm and some were necrotic. In some cases, the swollen cells occluded the lumen of the proximal convoluted tubules.

In rats fed 16% P. volkensii Gilg there was loss of epithelium of the proximal convoluted tubules leaving a thin outline of the tubule without cells. In some cases, there were albuminous casts in the lumen of the tubule (Fig 2 p. 62). Such casts were usually accompanied by coagulative necrosis.

The lesions in the liver were degenerative in nature. The severity of the lesions was dependent on the level of feeding. At 2% level, some hepatocytes showed cloudy swelling. At 4% level of feeding, some cells had vacuoles in their cytoplasm primarily around the central veins. At 8% and 16% levels, there was necrosis of hepatocytes accompanied by massive diffuse haemorrhave (Fig. 3 p. 63).

In the adrenal gland, there was degeneration in the cells of the medulla at all levels of feeding. Some

cells showed cloudy swelling while others were necrotic. At 8% level of feeding, there was haemorrhage in the lower zone fasciculata and the whole of zona reticularis. The haemorrhage was more extensive at 16% level of feeding and extended into the medulla as shown (Fig. 4 p. 64).

In the spleen some lymphocytes had relatively large nuclei compared to the controls. There was golden yellow pigment in the red pulp. This pigment is hemosiderin like granules (Fig 5 p 65). The amount of the pigment increased as the plant material concentration in the feed increased.

In the lungs the two prominent lesions were thickened alveolar walls and haemorrhages (Fig 6 p. 66). The red blood cells were found in the lumen of the alveoli. The situation deteriorated as the concentration of the plant material increased, thus at higher concentrations, the lumen of the alveoli was completely obliterated, the haemorrhage being massive in nature. In addition to the haemorrhage, the alveoli contained pale pink staining homogeneous substance. This was oedema fluid in the alveoli (H&E).

Table 2a: The liver weight: Body weight ratios for control rats (females).

Rat No.	Body wt. (g)	Liver wt. (g)	Liver wt ratio body wt.	Ratio as %
1	165	6.00	0.0364	3.64
2	170	6.40	0.0376	3.76
3	175	5.80	0.0331	3.31
4	180	6.80	0.0378	3.78
5	183	7.00	0.0383	3.83
6	194	6.50	0.0335	3.35
7	198	7.40	0.0374	3.74
8	200	7.20	0.0360	3.60
9	210	7.80	0.0371	3.71
10	224	7.80	0.0348	3.48

$\bar{x} = 3.61 \pm 9.18$

Table 2b: The liver weight: Body weight ratios for control rats (males).

Rat No.	Body wt. (g)	Liver wt. (g)	Liver wt ratio body wt.	Ratio as %
1	130	5.16	0.0397	3.97
2	150	5.45	0.0363	3.63
3	204	8.00	0.0392	3.92
4	212	8.20	0.0387	3.87
5	218	9.50	0.0436	4.36
6	219	8.0	0.0365	3.65
7	222	9.50	0.0428	4.28
8	227	9.30	0.0410	4.10
9	236	10.50	0.0445	4.45
10	260	10.40	0.0400	4.00

$\bar{x} = 3.95 \pm 0.18$

NB: The combined mean ($\bar{x}$) for males and females

Table 3: The liver weight: Body weight ratios for rats fed P. volkensii Gilg at different concentrations (2, 4, 8, 16 %).

Rat No.	Body wt.* (g)	Liver wt (g)	Liver wt ratio Body wt.	Ratio as %
A I	142.0	4.0	0.0282	2.82
III	141.0	4.0	0.0284	2.84
IV	185.0	4.5	0.0243	2.43
VII	134.0	4.0	0.0299	2.99
VIII	145.0	4.7	0.0324	3.24
B I	173.0	4.0	0.0231	2.31
II	210.0	5.0	0.0238	2.38
V	160.0	3.5	0.0219	2.19
VIII	150.0	3.0	0.0200	2.00
IX	198.0	4.0	0.0202	2.02
X	140.0	5.0	0.0357	3.57
C I	140.0	4.0	0.0286	2.86
II	165.0	4.0	0.0242	2.42
III	180.0	4.5	0.0250	2.50
IV	175.0	4.8	0.0274	2.74
V	253.0	6.0	0.0237	2.37
VI	200.0	6.0	0.0300	3.00
VII	174.0	6.0	0.0345	3.45
IX	155.0	5.0	0.0323	3.23
X	124.0	4.0	0.0323	3.23
D IV	137.0	2.8	0.0204	2.04
V	171.0	5.0	0.0292	2.92
VI	137.0	3.8	0.0277	2.77
VII	138.0	4.5	0.0326	3.26
IX	194.0	5.0	0.0258	2.58

* Weights at death or on termination of the experiment.

Table 4: Mean and standard deviations (sd) of liver weight : body weight ratios for rats fed *P. volkensii* Gilg at different concentrations.

Concentration of *P. volkensii* Gilg	mean ± s.d. of the liver wt : body wt ratio
2	2.86 ± 0.30
4	2.41 ± 0.59
8	2.87 ± 0.39
16	2.71 ± 0.45

Table 5: Weekly body weights of 10 rats fed *P. volkensii* Gilg at a level of 2%.

	Body weight (g)									
	Rat number									
Day	1	2	3	4	5	6	7	8	9	10
0	240	184	177	227	250	167	179	161	154	175
7	197	191	196	242	291	166	214	201	184	207
14	234	237	246	289	310	177	219	208	199	216
21	221	221	223	270	292	169	209	202	187	208
28	202	209	215	251	272	165	213	193	195	206
35	210	203	207	253	266	159	203	196	190	199
42	211	206	215	252	261	161	200	186	183	187
49	199	197	195	240	251	153	190	180	177	179
56	191	194	189	224	235	151	191	184	177	176
63	183	188	180	225	230	153	190	179	177	174
70	178	182	175	225	227	151	183	171	170	172
77	178	176	174	215	223	150	181	165	167	171
84	171	171	168	209	218	148	178	161	164	169
91	164	166	162	203	210	141	170	156	162	166

Table 6: Weekly body weights of 10 rats fed *P. volkensii* Gilg at a level of 4%

	Body weight (g)									
	Rat number									
Day	1	2	3	4	5	6	7	8	9	10
0	217	187	139	191	150	160	158	164	172	152
7	266	249	-	234	196	205	197	202	223	185
14	298	270	-	273	210	209	208	199	226	200
21	272	243	-	262	198	199	195	196	199	190
28	259	221	-	255	196	199	196	190	226	187
35	268	213	-	255	220	201	170	190	225	185
42	280	207	-	273	227	203	177	187	229	189
49	265	204	-	257	224	195	170	183	217	175
56	266	190	-	256	223	200	175	170	214	184
63	250	196	-	245	200	188	162	177	205	172
70	234	179	-	242	210	187	165	175	216	172
77	234	178	-	239	198	183	160	170	207	169
84	226	174	-	234	191	179	-	168	200	164
91	214	168	-	228	180	173	-	162	195	160

Table 7: Weekly body weights of 10 rats fed *P. volkensii* Gilg at a level of 8%

	Body weight (g)									
	Rat number									
Day	1	2	3	4	5	6	7	8	9	10
0	148	171	195	191	227	181	178	135	187	158
7	162	165	220	202	280	223	218	171	194	192
14	174	-	206	174	298	226	221	169	199	191
21	194	-	215	-	298	230	214	174	198	195
28	193	-	219	-	296	215	206	170	188	195
35	186	-	210	-	276	206	206	162	174	192
42	179	-	207	-	268	204	197	152	171	175
49	172	-	201	-	268	204	194	150	170	165
56	172	-	188	-	276	214	209	155	165	170
63	150	-	168	-	250	187	184	142	155	172
70	160	-	174	-	255	190	180	132	-	153
77	140	-	170	-	245	185	175	130	-	148
84	-	-	165	-	235	180	171	116	-	124
91	-	-	162	-	227	176	170	-	-	-

Table 8: Weekly body weights of 10 rats fed *P. volkensii* Gilg at a level of 16%.

	Body weight (g)									
	Rat number									
Day	1	2	3	4	5	6	7	8	9	10
0	192	172	144	185	162	175	175	166	186	163
7	179	-	-	190	178	172	176	150	200	157
14	144	-	-	159	164	150	185	-	178	-
21	-	-	-	137	173	137	202	-	202	-
28	-	-	-	-	211	-	220	-	223	-
35	-	-	-	-	240	-	237	-	234	-
42	-	-	-	-	226	-	215	-	222	-
49	-	-	-	-	205	-	209	-	207	-
56	-	-	-	-	201	-	211	-	215	-
63	-	-	-	-	199	-	200	-	215	-
70	-	-	-	-	187	-	182	-	197	-
77	-	-	-	-	185	-	170	-	190	-
84	-	-	-	-	182	-	154	-	183	-
91	-	-	-	-	178	-	138	-	177	-

Table 9: Weekly body weights of 10 rats fed on ground calf pellets (control diet).

	Body weight (g)									
	Rat number									
Day	1	2	3	4	5	6	7	8	9	10
0	188	212	172	246	158	176	185	151	168	173
7	247	212	201	301	213	224	227	194	211	208
14	259	290	245	318	230	240	235	206	221	212
21	239	272	227	286	207	227	223	188	203	207
28	234	260	231	280	207	222	223	181	196	207
35	242	255	230	281	209	238	233	190	218	210
42	230	246	221	268	204	237	226	190	211	210
49	220	242	209	256	198	228	220	180	198	204
56	249	264	235	272	214	236	227	192	209	205
63	225	240	210	257	198	227	220	185	198	201
70	231	241	215	254	197	229	218	181	203	200
77	230	240	215	254	200	230	223	180	200	201

Table 10: The body weights and weight changes in 10 rats fed 2% *P. volkensii* Gilg.

	Body weights (g)		Weight	Changes *	
Rat No.	Initial	Final	(g)	%	
1	240	178	- 62	- 26	
2	184	166	- 18	- 10	
3	177	174	- 3	- 2	
4	227	215	- 12	- 5	
5	250	223	- 27	- 11	
6	127	150	+ 23	+ 18	
7	179	181	+ 2	+ 1	
8	161	165	+ 4	+ 2	
9	154	167	+ 13	+ 8	
10	175	171	- 4	- 2	$\bar{x} = -3$

Table 11: The body weights and weight changes in 10 rats fed 4% *P. volkensii* Gilg

	Body weights (g)		Weight	Changes	
Rat No.	Initial	Final	(g)	%	
1	217	234	+ 17	+ 8	
2	187	178	- 9	- 5	
3	139	---	---	---	
4	191	239	+ 48	+ 25	
5	150	198	+ 48	+ 32	
6	160	183	+ 23	+ 14	
7	158	160	+ 2	+ 1	
8	164	170	+ 6	+ 4	
9	172	207	+ 35	+ 20	
10	152	169	+ 17	+ 11	$\bar{x} = +11$

* A negative (-) in front of a number indicates wt loss, + sign indicates wt gain. wt change = final wt - initial wt. % = $\frac{\text{wt change}}{\text{intial wt}} \times 100$

Table 12: The body weights and weight changes in 10 rats fed 8% P. volkensii Gilg leaves.

Rat No.	Body weights (g) Initial	Final	Weight Changes (g)	%
1	148	140	- 8	- 5
2	171	165	- 6	- 4
3	195	170	- 25	- 13
4	191	174	- 17	- 9
5	227	245	+ 18	+ 8
6	181	185	+ 4	+ 2
7	178	175	- 3	- 2
8	135	130	- 5	- 4
9	187	155	- 32	- 17
10	158	148	- 10	- 6

$\bar{x} = -5 \pm 5$

Table 13: The body weights and weight changes in 10 rats fed 16% P. volkensii Gilg leaves.

Rat No.	Body weights (g) Initial	Final	Weight Changes (g)	%
1	192	144	- 48	- 25
2	177	- *	-	-
3	144	- *	-	-
4	185	137	- 48	- 26
5	162	185	+ 23	+ 14
6	175	137	- 38	- 22
7	175	170	- 5	- 3
8	166	150	- 16	- 10
9	186	190	+ 4	+ 2
10	163	157	- 6	- 4

* Found eaten up by cage mates

$\bar{x} = - 9 \pm 10$

Table 14: The body weights and weight changes in 10 rats fed ground calf pellets (control group).

Rat No.	Body weights		Weight Changes	
	Initial	Final	(g)	%
1	188	230	+ 42	+ 22
2	212	240	+ 28	+ 13
3	172	215	+ 43	+ 25
4	246	254	+ 8	+ 3
5	158	200	+ 42	+ 27
6	176	230	+ 54	+ 31
7	185	223	+ 38	+ 21
8	151	180	+ 29	+ 19
9	168	200	+ 32	+ 19
10	173	201	+ 28	+ 16

$\bar{x}$ = + 19.6

S.D = 7.9

Table 15: Percentage deaths in rats fed P. volkensii Gilg at different concentrations.

% concentration of plant material w/w	Rats			
	Dead	Survived	Total	% Deaths
0 (control)	0	10	10	0
2	0	10	10	0
4	1	9	10	10
8	3	7	10	30
16	7	3	10	70

Experiment II: THE EFFECTS OF AQUEOUS EXTRACTS OF *P. volkensii Gilg* LEAVES ON ISOLATED RABBIT DUODENUM.

The aqueous extract was prepared as follows:

Dry, finely ground leaves of the plant were filled to one third of the volume of a round bottomed flask. Analytical grade methanol was then added to the flask so as to soak the material completely and finally leave a layer of methanol about the volume of the ground material on top of the flask's contents. The flask with its contents was placed in a constant temperature water bath maintained at 45°C. The flask was held in place using clamps.

After seventy two hours of extraction, the methanol was decanted into an empty flask. More methanol was added to the residue in the flask and the extraction continued for another forty eight hours after which the methanol was decanted into the flask again. The process was repeated until the plant material was completely decolourised. Depending on the initial amount of the material, the process took two to four weeks.

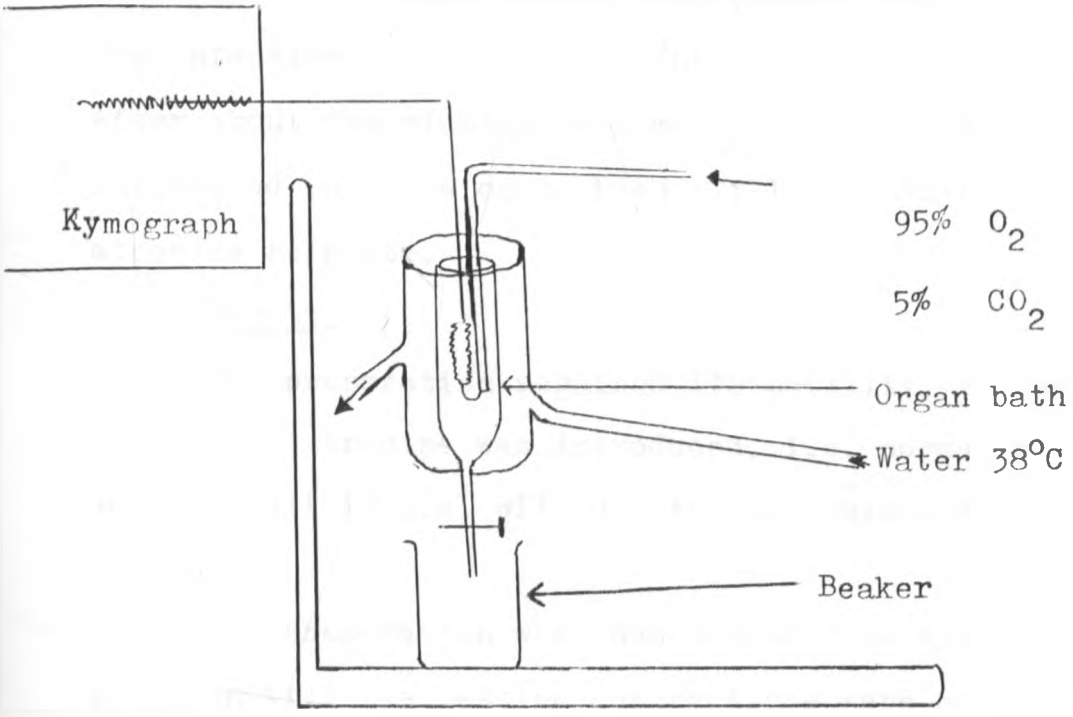
The combined extracts were then reduced in volume to about one quarter of the original volume. This process was done by evaporating off the methanol under vacuum using a rotavapor. The concentrated methanol extract was divided into two portions.

For experiment II, III, VI and VII methanol was evaporated to dryness and the residue reconstituted with distilled water to make an aqueous solution. For experiment IV and VIII the extract was cleaned up using activated charcoal and the methanol removed by freeze drying.

Procedure and results:

A piece of duodenum 4 - 5 cm long freshly removed from a rabbit was rinsed or flushed with saline blown through the lumen with a syringe.

The preparation was set up in an organ bath containing tyrode's solution kept under continuous oxygenation and a constant temperature about 37°C as indicated in the diagram below:



After the normal tone, amplitude and rate of contractions had been established, then the experiment was started. 0.2 ml of the extract was introduced into the bath.

i) Result.

There was a marked increase in the tone and also the amplitude. The contractions also became more

rhythmic.. The rate of contractions was however slowed.

The tyrode solution was then drained out and the preparation rinsed with tyrode solution until the resting contractions were regained. After this, 0.5 mg of atropine sulphate was introduced into the organ bath.

ii) Result.

There was a slight decrease in the tone of the intestine i.e. atropine sulphate caused relaxation of the intestine. The contractions were completely abolished. After about two minutes, 0.2 ml of the extract was introduced into the organ bath still containing atropine sulphate.

iii) Result.

The preparation regained its motility, to what it was before atropine was introduced, i.e. normal but not as in Result i) i.e. without atropine sulphate pre-treatment.

The preparation was then washed with tyrode solution till the resting contractions were established in part. 0.4 ml of 0.1% mepyramine maleate was introduced into the organ bath.

iv) Result.

There was a slight decrease in the tone of the intestine. The contractions were slightly bigger in amplitude. After two minutes, 0.2 ml of the extract was introduced into the organ bath containing mepyramine maleate.

v) Result.

The contractions were completely abolished and there was a decrease in tone as compared to the normal resting tone. After two minutes, the organ bath was washed three times with tyrode solution. 0.2 ml of the extract was then introduced into the organ bath.

vi) Result.

The duodenum preparation started contracting and the amplitude of the contractions was higher than the normal tracing. The tone was just about the same as the normal tracing, but the rate of contractions was slower than in the normal tracing.

Experiment III: EFFECTS OF P. VOLKENSII GILG AQUEOUS EXTRACT ON THE BLOOD PRESSURE IN THE DOG.

i) Anaesthesia.

The animal was weighed and then anaesthetised to a deep surgical plane with pentobarbitone sodium using a calculated dose of 30 mg/kg of body weight.

ii) Cannulation of the carotid artery.

A ventral longitudinal midline incision was made through the skin of the neck. By blunt dissection of the neck muscles, the trachea and one of the carotid arteries were exposed. The carotid artery was freed from the vagus for a distance of about 3 cm. The cephalad end of the exposed artery was occluded with a ligature and a clamp was placed approximately 2 cm below this ligature. A small incision was made between the clamp and the ligature, the tip of the cannula was then introduced toward the heart and tied securely in place. The other end of the cannula was then connected to a mercury manometer and the pressure readings were recorded on a smoked drum fixed to a kymograph.

iii) Cannulation of the femoral vein.

The pulsation of the femoral artery was palpated as it passes down the inner surface of the thigh at the so called femoral triangle. The overlying skin was rolled to one side and an incision approximately 4 cm long was made. The femoral vein (dark in colour)

was located alongside the femoral artery and cannulation done as in the carotid artery i.e. the distal end of the vein was ligated, the proximal end clamped, and the incision to fit the cannula was made in between.

iv) Procedure and results.

The injections were made utilizing the femoral cannula after which it was flushed with approximately 5 ml of heparinized saline after each injection. A short pre-injection recording was obtained prior to each injection. Injection of 0.1 ml of the extract showed no change in the blood pressure. When 0.2 ml of the extract was injected, there was an overall decrease in the blood pressure. The diastolic, systolic and the pulse pressures were reduced, the heart rate was increased. When 0.4 ml of the extract was injected after waiting for the blood pressure to return to normal, the blood pressure was reduced to such an extent that the dog died.

Another dog was used and pre-treatment with propranolol 0.5 mg/kg) and atropine sulphate 0.2 mg/kg was done. The results of injecting 0.2 ml extract after the pre-treatments were inconclusive.

Experiment IV: DETERMINATION OF THE MEDIAN LETHAL DOSE (LD_{50}) OF FREEZE DRIED AQUEOUS EXTRACT OF *P. VOLKENSII* GILG IN MICE.

Twenty four white adult male mice were divided into 6 groups of 4 animals each corresponding to 6 dosage levels of freeze dried methanolic extract of *P. volkensii* Gilg leaves dissolved in distilled water. A control group consisted of four mice which were injected intraperitoneally (IP) with 0.3 ml of distilled water.* This volume was the highest used in the experimental groups.

The initial dose range finding experiment was conducted by trial and error using arbitrary dosages. In the first trial, a dose of 16000mg/kg was injected (IP) into four adult mice. All the animals died. Then a dose of 8000 mg/kg was tried on four mice. All the mice died. A dose of 4000 mg/kg was tried on four mice, none of the mice died. By such trials, the dose ranges which were used in the LD_{50} experiment were arrived at and were between 4000 and 8000 mg/kg. The dose ranges were in a geometric progression or had a logarithmic relationship.

Each member of the group was injected with the corresponding dose by the IP route. The number of animals dying within 24 hours after injection was recorded. The 24 hours LD_{50} was calculated using the method of Reed and Muench. The rationale of this method is based on two assumptions:

* Concentration of the solution was 1000 mg/ml.

1. Any animal surviving at a given dose level would have survived at a lower dose level.
2. Any animal dying at a given dose level would have died at a higher dose level.

Thus the results were expressed in a table of accumulated deaths and survivals (Table 16). The accumulated figures were obtained by having a column for surviving mice at the corresponding dosage levels. Thus, the "died" column was accumulated from low to high doses while the "survived" column was accumulated from high to low levels.

RESULTS:

Table 16: The death* - survival rates in mice injected with varying dosages P. volkensii Gilg freeze dried extract dissolved in distilled water.

Dose	log			Accumulated			Percent**
mg/kg	Dose	Died	Survived	Died	Survived	Total	mortality
4600	3.6628	1	3	1	9	10	10
6000	3.7782	2	2	3	6	9	33.3
6400	3.8062	2	2	5	4	9	55.6
6800	3.8325	3	1	8	2	10	80.0
7200	3.8573	3	1	11	1	12	91.7
7600	3.8808	4	0	15	0	15	100.0

* All the dying mice observed convulsed before death.

** Percent mortality % = $\frac{\text{Accumulated Died}}{\text{Total}} \times 100$

From table 16, the 24 hr LD_{50} lies between 6000 mg/kg and 6400 mg/kg. The calculation is as follows:

$$\frac{50\% - \text{Next lowest } \%}{\text{Next highest } \% - \text{Next lowest } \%}$$

Thus $$\frac{50 - 33.3}{55.6 - 33.3} = \frac{16.7}{22.3} = 0.7489$$

Log of dose increment i.e. from 6000 to 6400 is calculated as follows:

$$\begin{array}{rcl} \text{Log } 6400 & = & 3.8062 \\ \text{Log } 6000 & = & \underline{3.7782} \\ & & 0.0280 \end{array}$$

Then $0.7489 \times 0.0280 = 0.0210$

$$\begin{array}{rcll} \text{Log } LD_{50} & = & 3.7782 & \text{(log of 6400 - lower dose)} \\ & + & \underline{0.0210} & \\ & & 3.7992 & \end{array}$$

Antilog of 3.7992 = 6300 mg.

LD_{50} = 6.3 mg/kg.

S. myrtina (Burm.f.) Kurz.

Experiment V: LONG TERM FEEDING STUDY IN RATS.

Forty two white albino rats obtained from the Veterinary Research Laboratories, Kabete were divided into seven groups A, B, C, D, E, F, G. Each group consisted of six rats (three females and three males). Each group was kept in separate metal cages with males and females being kept in separate compartments of the same cage. Initially, all rats were fed ground calf pellets ad libitum for seven days; during which time the animals acclimatized to their new surroundings. Water was also supplied ad libitum. After seven days, all the rats were weighed individually and the weights (g) were recorded. The various groups of rats were then fed a ration consisting of a mixture of ground dried S. myrtina (Burm.f.) Kurz leaves and ground calf pellets as shown in (Table 17).

Table 17: Designation of the groups of rats used in the experiment and the corresponding concentration of S. myrtina (Burm.f.) Kurz fed to each group.

Rat group	Plant material concentration (w/w%).
A	4
B	8
C	16
D	32
E	50
F	100
G	0 (control)

The rats were fed with the appropriate rations ad libitum for twelve (12) weeks (control rats were fed on ground calf pellets only). Water was also provided ad libitum. Each rat was weighed individually once every week and the weight (g) recorded. The animals were observed daily for any changes in their clinical appearance and any such changes were recorded for each rat. Animals that died during the course of the experiment or were sacrificed at the end of the experiment were weighed and the weight recorded. A complete post mortem examination was carried out and the gross findings recorded. In each case, samples from all the body systems and organs were preserved in 10% buffered formalin for histology. The histology was done on thin sections (5-6 u) stained with hematoxylin and eosin.

RESULTS:

All the forty two rats used in this experiment had rough haircoat, sluggish movement and a few showed evidence of diarrhoea.

The weekly body weights of each rat as well as the weight changes are shown (Tables 18 - 31) with the exception of rats fed 4% S. myrtina (Burm.f.) Kurz (Table 25), all the other groups of animals lost weight (Tables 26 - 30). This is in contrast to the control group which gained weight during the same period of time (Table 31). For rats that died the number of deaths increased as the concentration of the plant material increased in the ration (Table 32). However, the percentage deaths was not directly proportional to the concentration of the plant material. This is in contrast with P. volkensii Gilg.

At post mortem, no significant gross lesions were observed. This is possibly because most of the rats died within a few weeks after the start of the experiment. Microscopically, characteristic lesions were found in the lungs. The alveolar walls were highly thickened with haemorrhage, oedema and fibrous connective tissue (Fig 7 p. 67). Apart from the red blood cells, the alveolar wall had been infiltrated by lymphocytes. The red blood cells were also found in the alveoli,

but the haemorrhage was not massive as compared to that in P. volkensii Gilg fed rats. The oedema fluid appeared as a light pink homogeneous mass in the alveoli when stained by H & E. The other pathological lesion was observed in the spleen. The latter had excessive deposition of hemosiderin in the red pulp (Fig. 8 p.68). This would indicate excessive red blood cell destruction probably related to the observed haemorrhage.

Table 18: Weekly body weights of 6 rats fed *S. myrtina* (Burm.f.) Kurz at a level of 4%

	Body weight (g)					
	Rat number					
Day	1	2	3	4	5	6
0	220	220	300	340	170	250
7	240	245	310	340	180	220
14	240	242	320	335	180	230
21	260	265	341	365	295	280
28	250	245	300	280	285	260
35	250	260	340	350	200	250
42	259	250	330	332	191	230
49	260	255	300	320	190	240
56	250	230	320	300	200	245
63	255	240	310	290	195	250
70	240	240	300	310	195	250
77	245	250	305	310	210	240
84	260	250	340	350	190	200

No of dead rats = 0

" " live " = 6

Total = 6

% death = 0

Table 19: Weekly body weights of 6 rats fed *S. myrtina* (Burm.f.) Kurz at a level of 8%.

	Body weight (g)					
	Rat number					
Day	1	2	3	4	5	6
0	250	195	250	260	180	222
7	242	205	270	300	210	260
14	260	225	290	300	230	245
21	280	230	300	290	210	250
28	260	200	270	265	200	240
35	260	255	280	250	200	210
42	228	174	248	260	205	228
49	220	175	250	260	210	200
56	200	-	230	250	210	240
63	150	-	180	255	215	245
70	-	-	-	245	220	245
77	-	-	-	245	200	200
84	-	-	-	210	230	265

No. of dead rats = 3

" " live " = 3

Total = 6

% death = 50%

Table 20: Weekly body weights of 6 rats fed *S. myrtina* (Burm.f.) Kurz at a level of 16%

	Body weight (g)					
	Rat number					
Day	1	2	3	4	5	6
0	290	205	255	170	205	160
7	270	230	290	195	220	170
14	265	240	290	210	220	190
21	290	260	315	200	230	190
28	245	200	310	195	210	180
35	220	230	210	195	200	180
42	210	192	225	200	220	194
49	200	200	220	200	200	190
56	-	130	170	-	-	-
63	-	-	-	-	-	-
70	-	-	-	-	-	-
77	-	-	-	-	-	-
84	-	-	-	-	-	-

No of dead rats = 6

" " live " = 0

Total = 6

% death = 100%

Table 21: Weekly body weights of 6 rats fed *S. myrtina* (Burm.f.) Kurz at a level of 32%

	Body weight (g)					
	Rat number					
Day	1	2	3	4	5	6
0	258	220	235	140	160	185
7	300	240	255	150	175	195
14	310	250	260	155	180	220
21	320	245	250	150	185	210
28	310	245	250	145	180	200
35	250	200	210	145	160	180
42	245	215	195	135	165	160
49	220	160	185	130	145	155
56	-	160	-	-	-	130
63	-	-	-	-	-	-
70	-	-	-	-	-	-
77	-	-	-	-	-	-
84	-	-	-	-	-	-

No of dead rats = 6

" " live " = 0

Total = 6

% death = 100%

Table 22: Weekly body weights of 6 rats fed S. myrtina (Burm.f.) Kurz at a level of 50%

	Body weights (g)					
	Rat number					
Day	1	2	3	4	5	6
0	210	250	175	175	205	235
7	210	240	160	158	180	225
14	210	240	160	155	175	160
21	220	260	180	209	205	205
28	210	210	160	220	195	180
35	195	200	145	170	185	210
42	190	200	140	165	160	200
49	-	-	-	-	-	-
56	-	-	-	-	-	-
63	-	-	-	-	-	-
70	-	-	-	-	-	-
77	-	-	-	-	-	-

No. of dead rats = 6

" " live " = 0

Total = 6

% death = 100%

Table 23: Weekly body weights of rats fed *S. myrtina* (Burm.f.) Kurz at a level of 100%

	Body weights (g)					
	Rat number					
Day	1	2	3	4	5	6
0	270	260	205	210	165	195
7	-	175	160	145	110	160
14	-	-	-	-	-	-
21	-	-	-	-	-	-
28	-	-	-	-	-	-
35	-	-	-	-	-	-
42	-	-	-	-	-	-
49	-	-	-	-	-	-
56	-	-	-	-	-	-
63	-	-	-	-	-	-
70	-	-	-	-	-	-
77	-	-	-	-	-	-
84	-	-	-	-	-	-

No. of dead rats = 6

" " live " = 0

Total = 6

% death = 100

Table 24: Weekly body weights of 6 rats fed on ground calf pellets (control group.)

	Body weight (g)					
	Rat number					
Day	1	2	3	4	5	6
0	240	240	220	150	160	185
7	250	240	220	142	155	175
14	245	230	210	145	150	185
21	260	230	210	160	150	190
28	250	200	195	160	145	165
35	220	195	200	160	150	185
42	235	210	190	170	150	180
49	245	210	210	170	155	185
56	230	200	200	180	160	180
63	240	210	230	185	145	190
70	240	195	240	200	160	185
77	260	195	235	195	150	185
84	280	240	210	210	185	185

No. of dead rats = 0

" " live " = 6

Total = 6

% death = 0

Table 25: The body weights and weight changes in 6 rats fed 4% S. myrtina (Burm.f.) Kurz leaves (ground).

	Body weight		Weight changes	
Rat No.	Initial	Final*	g	%
1	220	260	+ 40	+ 18
2	220	250	+ 30	+ 14
3	300	340	+ 40	+ 13
4	340	350	+ 10	+ 3
5	170	190	+ 20	+ 12
6	250	200	- 50	- 3

$\bar{x} = + 10 \pm$

Table 26: The body weights and weight changes in 6 rats fed 8% S. myrtina (Burm.f.) Kurz leaves.

	Body weight		Weight changes	
Rat No.	Initial	Final	g	%
1	250	150	- 100	- 40
2	195	175	- 20	- 10
3	250	180	- 70	- 28
4	260	210	- 50	- 19
5	180	230	+ 50	+ 28
6	222	265	+ 43	+ 19

* Last body weight recorded before death $\bar{x} = - 8 \pm 10$

Table 27: The body weights and weight changes in 6 rats fed 16% S. myrtina (Burm.f.) Kurz leaves.

	Body weight		Weight changes	
Rat No.	Initial	Final	g	%
1	290	200	- 90	- 31
2	205	130	- 75	- 37
3	255	170	- 85	- 33
4	170	200	+ 30	+ 18
5	205	200	- 5	- 2
6	160	190	+ 30	+ 19

$\bar{x}$ - 11±13

Table 28: The body weights and weight changes in 6 rats fed 32% S. myrtina (Burm.f.) Kurz leaves.

	Body weights		Weight changes	
Rat No.	Initial	Final	g	%
1	258	220	- 38	- 15
2	220	160	- 60	- 27
3	235	185	- 50	- 21
4	140	130	- 10	- 7
5	160	145	- 15	- 9
6	185	130	- 55	- 30

$\bar{x}$ = - 18±9

Table 29: The body weights and weight changes in 6 rats fed 50% S. myrtina (Burm.f.) Kurz leaves.

	Body weights		Weight changes		
Rat No.	Initial	Final	g	%	
1	210	190	- 20	- 10	
2	250	200	- 50	- 20	
3	175	140	- 35	- 20	
4	175	165	- 10	- 6	
5	205	160	- 45	- 22	
6	235	200	- 35	- 15	$\bar{x} = -16^{\pm}$

Table 30: The body weights and weight changes in 6 rats fed 100% S. myrtina (Burm.f.) Kurz leaves.

	Body weights		Weight changes	
Rat No.	Initial	Final	g	%
1	270	-	-	-
2	260	175	- 85	- 33
3	205	160	- 45	- 22
4	210	145	- 65	- 31
5	165	110	- 55	- 33
6	195	160	- 35	- 18

$\bar{x} = -27^{\pm}7$

Table 31: The body weights and weight changes in 6 rats fed ground calf pellets (control).

Rat No.	Body weights		Weight changes		
	Initial	Final	g	%	
1	240	280	+ 40	+ 17	
2	240	240	0	0	
3	220	210	- 10	- 5	
4	150	210	+ 60	+ 40	
5	160	185	+ 25	+ 16	
6	185	185	0	0	$\bar{x}$ = + 1

Table 32: Percentage deaths in rats fed *S. myrtina* (Burm.f.) Kurz at different concentrations.

% concentration of plant material w/w	RATS			
	Dead	Survived	Total	% Deaths
0 (control)	0	6	6	0
4	1	5	6	16.7
8	3	3	6	50
16	3	3	6	50
32	6	0	6	100
50	6	0	6	100
100	6	0	6	100

Experiment VI: THE EFFECTS OF AQUEOUS EXTRACTS OF *S. myrtina* (Burm.f.) Kurz ON ISOLATED RABBIT DUODENUM.

The preparation of the methanol extract was done in the same way as was in *P. volkensii* Gilg.

A. Effects of the extract on isolated rabbit duodenum.

Procedure and results.

The set up was the same as in the case of *P. volkensii* Gilg.

After the normal tone, amplitude, and rate of contractions had been established, 0.2 ml of pure methanol was introduced into the organ bath.

i) Result.

There was no change in the tone, amplitude or rate of contractions.

The tyrode solution was then drained several times to rinse the preparation till the normal or original condition were obtained. 0.4 ml. of the extract was then added into the organ bath.

ii) Result.

There was a decrease in tone (relaxation), a decrease in the rate of contractions and an increase in the force of contractions i.e. amplitude. After rinsing the preparation as usual 0.4 ml of 0.1% mepyramine maleate was introduced into the organ bath.

iii) Result.

There was no effect on either the tone, the rate or the amplitude of contractions. 0.4 ml of the extract

was then introduced into the organ bath before mepyramine maleate was rinsed.

iv) Result.

There was a marked decrease in the tone and rate of contraction however, the force of contraction was the same as the control tracing. After rinsing the preparation as usual, 0.5 mg of atropine sulphate was introduced into the organ bath.

v) Result.

There was a decrease in tone and also the force of contraction (amplitude). The rate of contractions was also decreased slightly. 0.2 ml of the extract was then introduced into the organ bath before rinsing the preparation.

vi) Result.

There was an immediate increase in the tone to slightly above the control tracing. The force of contraction was bigger than the control tracing, but the rate of contractions remained the same as control. The preparation was then rinsed several times till the normal tracing was regained. A mixture of 1 mg atropine sulphate and 0.2 ml of the extract was then introduced into the organ bath.

vii) Result.

There was a very slight increase in the tone and slight increase in the force of contraction. The rate of contraction remained the same as the control tracing.

Experiment VII: EFFECTS OF S. myrtina (Burm.f.) Kurz AQUEOUS EXTRACT ON THE BLOOD PRESSURE IN THE DOG.

The anaesthesia, cannulation of the carotid artery as well as of the femoral vein and the method of injection and recording was done as in the case of P. volkensii Gilg.

Results.

After the control recording of the blood pressure was obtained, 0.2 ml. of pure methanol was injected. There was no change in the pattern of the recording i.e. there was no effect on the blood pressure parameters.

After the blood pressure returned to the control level, 0.2 ml of the extract was injected. This resulted in a marked decrease in the blood pressure. The diastolic, systolic and pulse pressures were greatly reduced. There was also an increase in the heart rate.

After the blood pressure returned to the control reading, mepyramine maleate was injected at a dosage of 1 mg/kg, followed by 0.2 ml extract 10 minutes later. The effects of the extract alone were blocked by about 75% i.e. there was less decrease in blood pressure parameters by the above percentage.

After the blood pressure returned to normal, propranol was injected at a dose of 0.5 gm/kg

followed by 0.2 ml extract 10 minutes later. The propranol did not block or potentiate the effects of the extract alone.

After the blood pressure returned to normal, atropine sulphate was injected at a dose rate of 0.2 mg/kg followed by 0.2 ml of the extract 10 minutes later. The result is that atropine sulphate blocked the effects of the extract almost completely.

Experiment VIII: DETERMINATION OF THE MEDIAN LETHAL DOSE (LD_{50}) OF FREEZE DRIED AQUEOUS EXTRACT OF S. Myrtina (Burm.f.) KURZ IN MICE.

Twenty four white adult male mice were devided into six groups of four animals each. These groups corresponded to six dosage levels of the freeze dried extract of S. myrtina (Burm.f.) Kurz leaves dissolved in distilled water. A control group consisted of four mice which were injected with 0.8 ml. distilled water intraperitoneally. This volume was the highest used in the experimental groups.

2.5 g of the freeze dried extract was dissolved in 10 ml of distilled water. The concentration was

$$\frac{2.5 \times 1000}{10} \text{ mg/ml} = 250 \text{ mg/ml.}$$

Calculation of the volume of extract to be injected:

Supposing the dose to be injected is 2000 mg/kg; and the mouse weighs 20 g ; 2000 mg/kg = 2 mg/g.

∴ Total dose of extract to be injected = 2 x 20 = 40 mg

1 ml of the solution was 250 mg.

X ml " " " " 40 mg

$$\frac{1}{X} = \frac{250}{40}$$

$$250\,X = 40$$

$$X = \frac{40}{250} = 0.16 \text{ ml.}$$

As in the previous plant, the dose range to be used was arrived at by trial and error using arbitrary - dosages at two extremes i.e. very high and very low dosages. A dose of 8000 mg/kg killed all the four mice. A dose of 3000 mg/kg killed none of the four mice. A dose of 6000 mg/kg killed 1 out of 4 mice while a dose of 5000 mg/kg did not kill any mouse. A dose of 7000 mg/kg killed all the mice. So it was decided to use dose range of 5000 - 7000 mg/kg.

Each member of a dosage group was injected with the corresponding dose by intraperitoneal route. The number of mice dying within 24 hrs after injection were recorded (Table 33). The acute or 24 hr LD_{50} was calculated using the method of Reed and Muench as before.

Table 33: The death* - Survival rates in mice injected with varying dosages of S. myrtina (Burm.f.) Kurz freeze dried extract dissolved in distilled water.

Dose mg/kg	Log Dose			Accumulated			Percentage Mortality
		Died	Survived	Died	Survived	Total	
5000	3.69897	0	4	0	9	9	0.0
6000	3.77815	1	3	1	5	6	16.7
6200	3.79239	3	1	4	2	6	66.7
6400	3.80618	3	1	7	1	8	87.5
6500	3.81291	4	0	11	0	11	100.0

* All the animals observed dying initially had little activity, laboured breathing followed by erratic movements, convulsions and death.

From the above results, the 24 hr LD_{50} lies between 6000 and 6200 mg/kg and is calculated as follows:

$$\frac{50\% - \text{Next lowest \%}}{\text{Next highest \%} - \text{Next lowest \%}} \quad \text{is}$$

$$\frac{50 - 16.7}{66.7 - 16.7} = \frac{33.3}{50.0} = 0.6667$$

The log dose increment i.e. from 6000 to 6200 mg/kg is calculated as follows.

$$\begin{aligned} \log 6200 &= 3.79239 \\ \log 6000 &= 3.77815 \\ &\hline 0.01424 \end{aligned}$$

Then $0.6667 \times 0.01424 = 0.00949$

$$\begin{aligned} \text{Then } \log LD_{50} &= 3.77815 \quad \text{(log of lower dose)} \\ &+ \underline{0.00949} \\ &\quad 3.78764 \end{aligned}$$

Anti log of 3.78764 = 6132.6 mg

$$LD_{50} = 6.1 \text{ g/kg.}$$

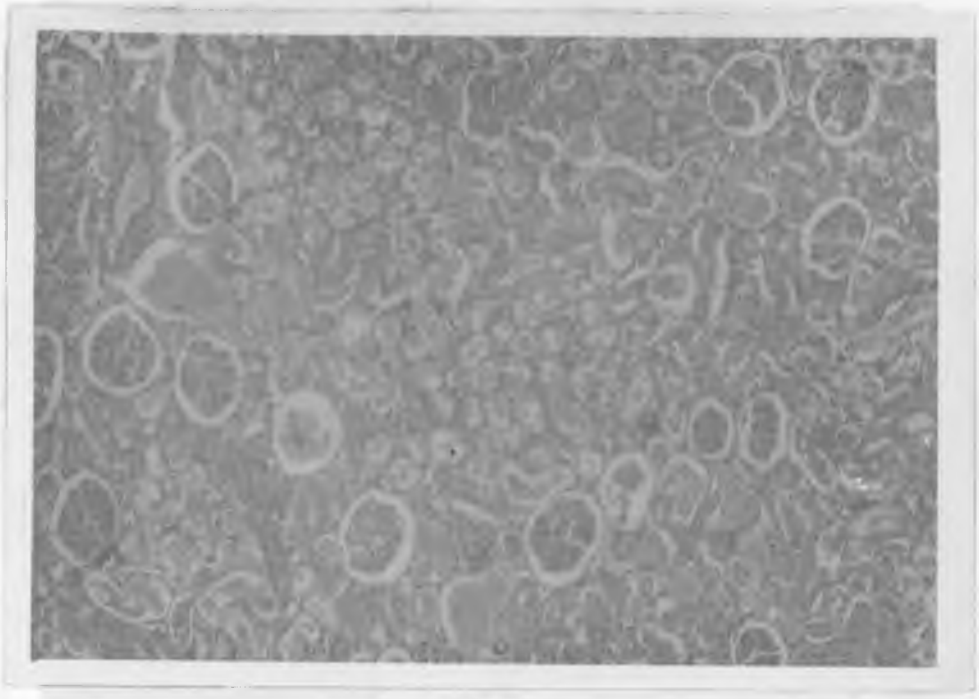


Figure 1: Degenerative changes in the cells of the proximal convoluted tubules of the kidney in a rat fed 8% P. volkensii Gilg leaves. (x 10 objective).

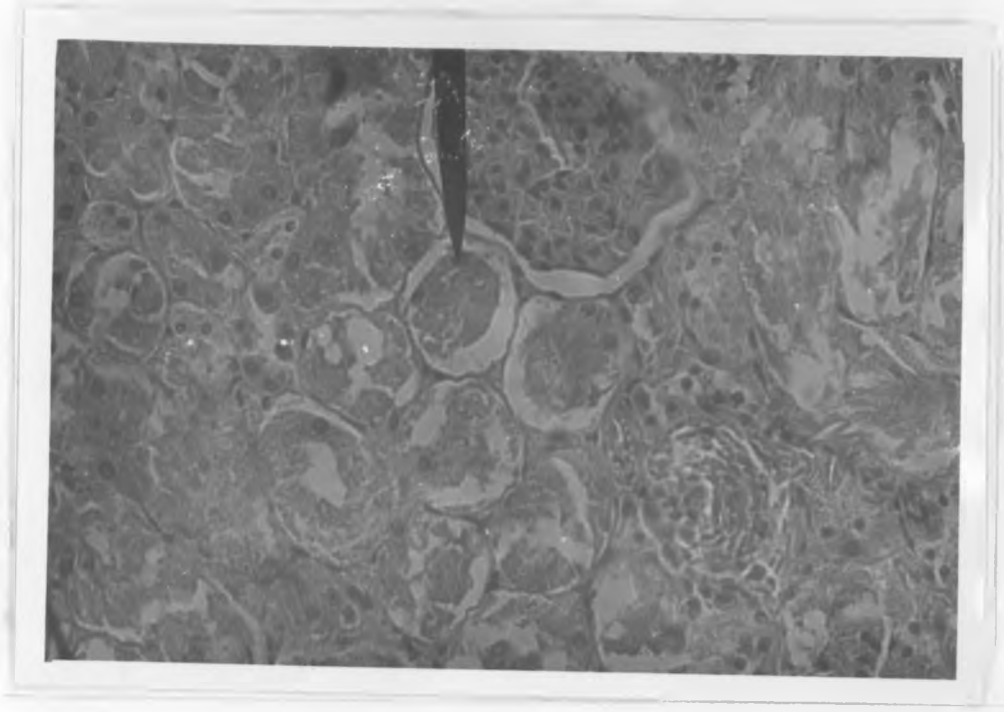


Figure 2: Hyaline cast in the proximal convoluted tubule (arrow); swollen and degenerated glomerular tuft in the kidney of a rat fed 16% P. volkensii Gilg leaves.
(x 40 objective).

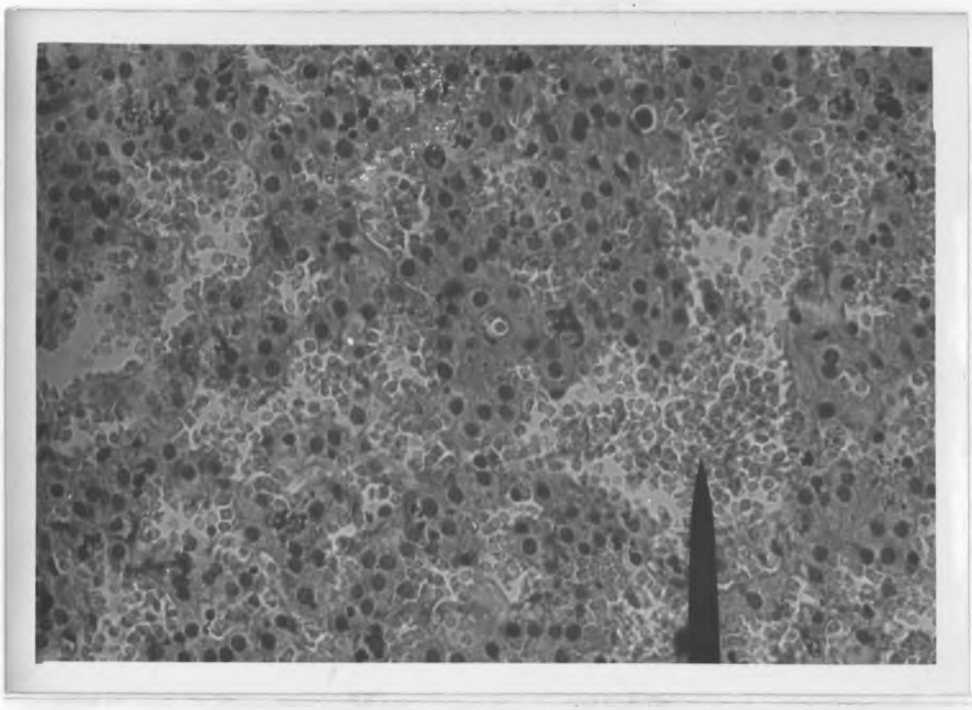


Figure 3: Extensive haemorrhage in the liver (arrow) and degenerative changes in the hepatocytes of a rat fed 8% P. volkensii Gilg leaves. (x 40 objective).

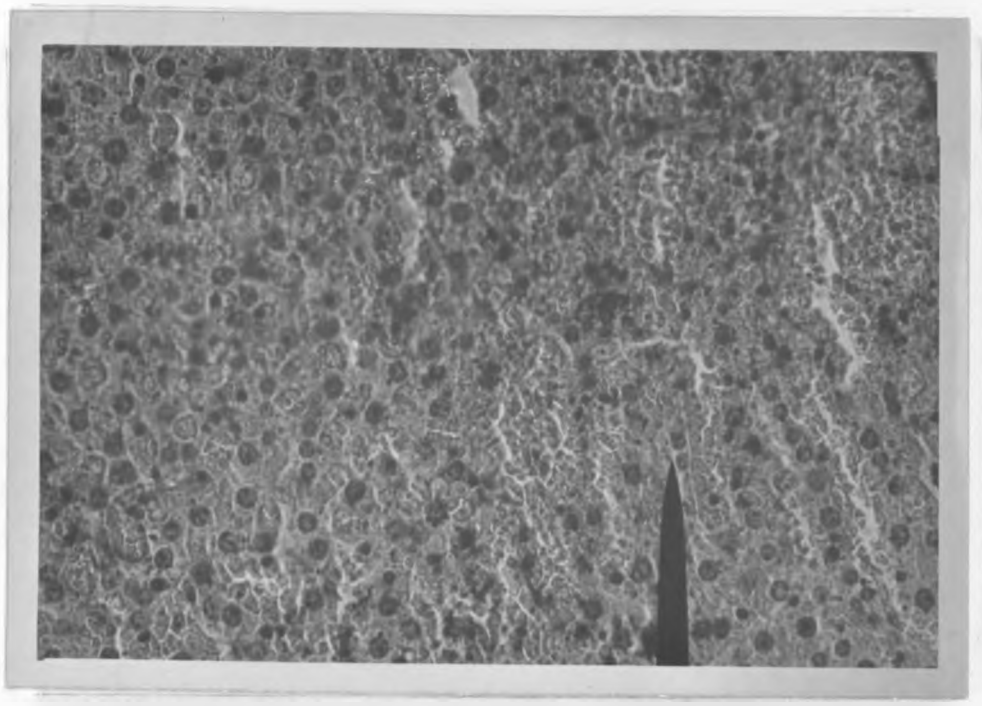


Figure 4: Haemorrhages and necrosis in the adrenal cortex of a rat fed 16% P. volkensii Gilg leaves. (x 40 objective).

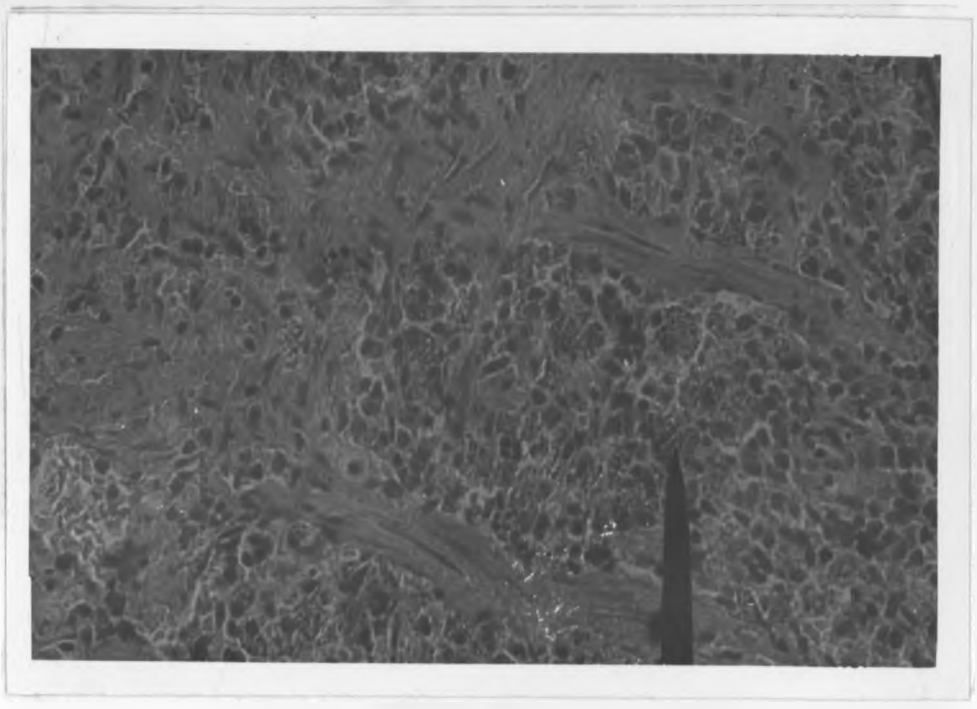


Figure 5: Extensive pigmentation in the spleen from a rat fed 8% P. volkensii Gilg leaves. Arrow indicates hemosiderin-like granules.

(x 40 objective).

Figure 6: Extensive haemorrhage (arrow), thickened alveolar walls and oedema of the alveoli and septa in a lung from a rat fed 8% P. volkensii Gilg leaves.
(x 40 objective).

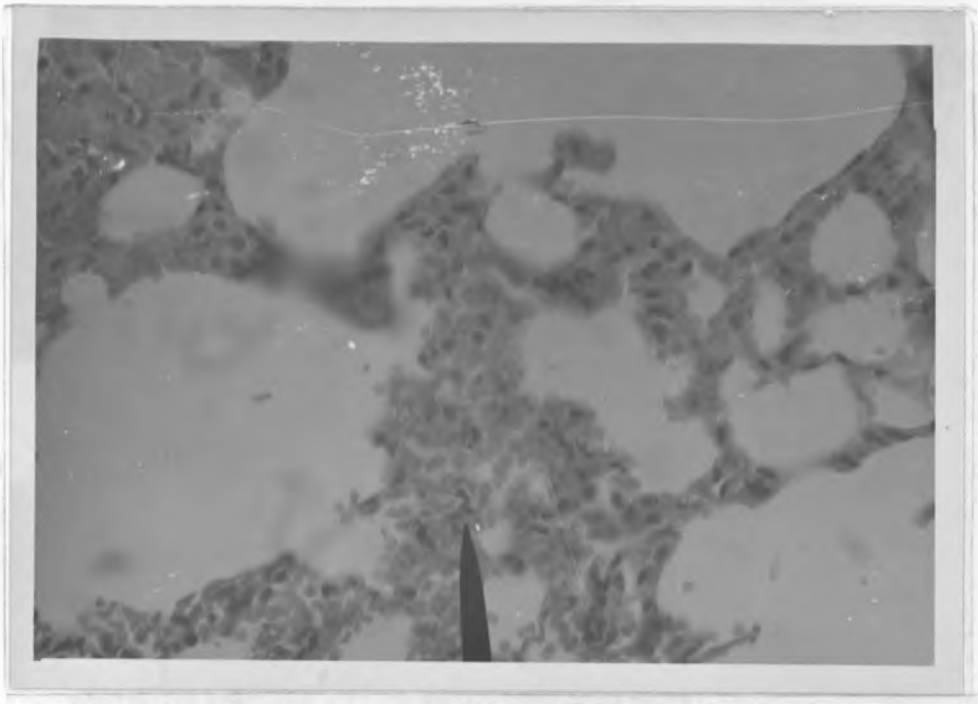


Figure 7: Haemorrhage and septal fibrosis (arrow) and oedema in the lungs of a rat fed 16% *S. myrtina* (Burm.f.) Kurz. (x 40 objective).

Figure 8: Extensive pigmentation in the spleen from a rat fed 16% *S. myrtina* (Burm.f.) Kurz. (Arrow indicates hemosiderin-like granules). (x 40 objective).

DISCUSSION AND CONCLUSIONS:

The results of the present study indicate that when 40 rats in 4 groups of 10 rats each were fed the dried ground leaves of P. volkensii Gilg at concentrations of 2%, 4%, 8% and 16%, all the rats had a rough hair coat while a few had evidence of diarrhoea. All the rats lost weight during the course of the experiment as compared to the control group over the same period of time. The only exception was in rats fed 4% P. volkensii Gilg. The latter group gained weight.

The same results were obtained when 36 rats in 6 groups of 6 rats each were fed the dried, ground leaves of S. myrtina (Burm.f.) Kurz at concentrations of 4%, 8%, 16%, 32%, 50% and 100%. The only difference was that the rats fed S. myrtina (Burm.f.) Kurz had a sluggish movement in addition to the other signs observed in rats fed P. volkensii Gilg.

The rough hair coat was an indication that the rats were not healthy any more after feeding on the leaves of the two plants. As far as the diarrhoea is concerned, this could be due to direct irritation of the gastrointestinal mucosa causing degenerative changes. Experiments conducted later (expt. II and VI) would also suggest direct stimulation in a manner similar to that of acetyl choline and histamine.

The loss in weight observed in most rats may be interpreted to mean reduced feed intake due to

decreased appetite. However, one can only be conclusive if feed intake was measured at the same time (Paget, 1970). The loss in weight could also be due to interference with carbohydrate and protein metabolism due to necrosis of the adrenal gland.

In general, although the liver weight : body weight ratio is a little bit higher in males compared to the females (Tables 2a and 2b) over the same body weight range, the differences in the two ratios are not that great. Absolute liver weights will of course differ between each individual animal and also between the various animal species.

The weight of the liver relative to the body weight of the same animal has been found to be a very useful indication of the general involvement of the liver in the pathological process and has been recommended as a routine determination (Ernest and Webster et al., 1941; Liljergren and Webster, 1952; Jackson and Capiello, 1963; Francis, 1964). In experiment I, the ratio liver weight : body weight for the experimental groups was less than that of the control group (Table 4). (The ratio liver weight : body weight for the control group is shown in Tables 2a and 2b). This would suggest that there was loss of liver cells or necrosis caused by the leaves of P. volkensii Gilg. This phenomena was confirmed histologically (Fig. 3 p. 63).

In a living animal, it would be highly advantageous to determine whether the liver has been injured by some foreign compound being administered to the experimental animals without killing them. This has been done by measuring the concentrations or activities of certain enzymes in the plasma as a function of time. The enzyme to be chosen for the assay should be in its highest concentration in the liver. Thus, liver damage will result in the enzyme being released into the plasma, and the levels of the enzyme in the plasma should be a good indicator of the extent of the hepatocyte damage.

Zinkl et al. (1971) did a study to compare the plasma activities of several enzymes in a dog poisoned with carbon tetrachloride, a liver toxicant. In this study, he came to the conclusion that Sorbitol dehydrogenase (SD) and glutamic pyruvic transaminase (GPT) were the best indicators of liver damage in the dog.

Separate studies done by Ford,(1967);Irving et al., (1965); Makato et al. (1963) and Shaw (1974) concluded that (SD) was the best indicator for liver damage in both humans and the domestic animals. Freedland et al. (1965) in a comparative study to that of Zinkl et al. (1971) came to the conclusion that (GPT) was the best indicator of liver damage not only in the dog, but also in the horse.

Zein et al. (1970) and Lum et al. (1972) found out that the enzyme serum gamma-glutamyl transpeptidase

was a good diagnostic aid in cases of liver disease though not specific since it is found in high concentrations in the bone and pancreas.

Serum glutamic oxalacetic transaminase (SGOT) is widely distributed in many tissues but its activity is easy to measure in the laboratory. In the present study, one would expect high levels of SGOT, SD and gamma-glutamyl transpeptidase because of the degenerative changes in the liver of rats fed P. volkensii Gilg. If the levels of blood urea nitrogen (BUN), creatinine and inorganic phosphorus had been measured in the blood, one would expect elevation of the levels above normal. This would be compatible with the kidney damage observed histologically.

The gross pathological lesions were found in rats fed P. volkensii Gilg, but not in those fed S. myrtina (Burm.f.) Kurz. In the former case, the presence of excess fluid in the cavities as well as froth in the trachea and the lungs suggests capillary damage resulting in increased permeability and oozing of fluids and possibly some other constituents of blood out of the vessel forming oedema fluid. The froth in the trachea is formed when the oedema fluid mixes with air.

Microscopically the lungs of rats fed P. volkensii Gilg had highly thickened alveolar walls, there was haemorrhage in the alveoli and alveolar wall, and

there was light pink homogeneous mass (H&E) stain within the alveoli. The homogeneous mass was oedema fluid. The thickening of alveolar walls was mainly due to oedema fluid and the red blood cells. At 16% level of feeding, the haemorrhage was massive in nature (Fig. 6 p. 66). The presence of oedema fluid and haemorrhage indicates capillary damage resulting in increased permeability.

In the rats fed P. volkensii Gilg, the spleen had excess golden yellow pigment (hemosiderin) in the red pulp (Fig. 5 p. 65). The amount of pigment increased as the concentration of the plant material in the ration increased. The lesion produced by P. volkensii Gilg in the spleen is identical with that produced by S. myrtina (Burm.f.) Kurz (Fig. 8 p.68).

The spleen is an important organ in the body due to the fact that it performs some important functions which include the following i) the production of lymphocytes in the white pulp. The lymphocytes are important in the immune response of an animal ii) Production of the red blood cells, this is more so in young animals iii) the spleen also stores blood which is useful in emergency situations e.g. severe haemorrhage and hypoxia. iv) the reticuloendothelial system mainly the macrophages destroy red blood cells and v) the spleen stores the iron from the hemoglobin

of destroyed red blood cells as a golden yellow pigment known as hemosiderin.

Normally, there is some hemosiderin in the spleen (Bloom and Fawcett, 1962; Runnels et al., 1965). Massive amounts of hemosiderin, however indicate excessive destruction of red blood cells by the macrophages.

The gross pathological lesions and the microscopic lesions in the kidney, liver and the adrenals are comparable with those obtained by Kiptoon (1981). The lesions were observed when calves were drenched with Gnidia latifolia (Meisn) for 15 months, or when rats were injected (IP) with ethanol extracts of Gnidia latifolia (Meisn).

Certain plants when grazed by animals in sufficient quantities will produce lesions in one or several of the organs mentioned in P. volkensii Gilg poisoning. One of these is Lantana camara L. This plant is an ornamental which was introduced to Kenya early in the century. However, this plant had been

the cause of hepatogenous photosensitivity to ruminants (Gopinath and Ford , 1969). On microscopic examination of the kidney, the most prominent lesion was sporadic coagulative necrosis of the cells of the proximal convoluted tubular epithelium and hyaline casts were present in the lumen of the proximal convoluted tubules (Seawright and Allen, 1972). Louw (1948) isolated two chemicals i.e. Lantadene A and lantadene B which are the active principles in Lantana camara L.

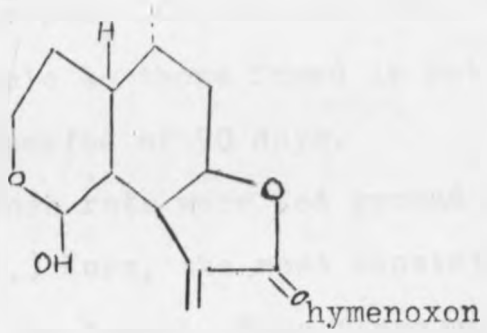
Among other lesions, calves fed Elaeodendron buchananii and Acokanthera longiflora have excess fluid in the thoracic cavities and lung oedema (Mugera, 1970). In Kenya, Senecio moorei is abundant in some areas and can cause liver lesions similar to those caused by P. volkensii Gilg (Mugera, 1970).

In the South Western United States of America, there is a plant called Hymenoxys odorata (Western bitterweed) which has caused considerable losses in sheep mainly, but occassionally goats and cattle are affected. Witzel et al. (1977) fed sheep with the ground material of Hymenoxys odorata at a dose rate of 1g/kg/day for 15 days resulting in subacute poisoning. Out of 7 sheep, 5 died and 2 were moribund within 15 days. On post mortem and microscopic examination, the consistent lesions were in the kidneys and the liver. The kidneys had severe glomerulonephrosis characterised by vacuolar degeneration and dilatation

of proximal and distal convoluted tubes, proteinaceous casts in the proximal convoluted tubules, swollen and degerated glomerular tufts, degeneration and necrosis of the inner renal cortex and outer medulla. There was mild to moderate toxic hepatosis characterized by vacuolar degeneration of hepatocytes around the central veins. The lesions are consistent with the findings in rats fed P. volkensii Gilg leaves.

Aanes (1960) administered lethal doses of Hymenoxys richardsonii (pangue) to sheep. On post mortem, there were haemorrhages in the epicardium, endocardium and the abomasum. There were marked hydroperitoneum, hydrothorax and pulmonary oedema. The hydroperitoneum and hydrothorax were consistent with the findings in rats fed P. volkensii Gilg.

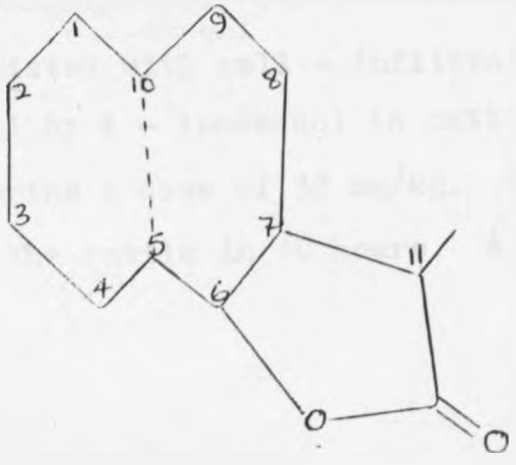
Ivie et al. (1975a) isolated a sesquiterpene lactone called hymenovin (hymenoxon) from Hymenoxys odorata



hymenoxon

The chemical hymenovin is lethal to sheep and its action is probably by alkylating sulphydral (-SH) groups in the cells. Denis et al. (1977) later isolated and identified hymenoxon from Baileya multiradiata and Helenium hoopsii.

Other sesquiterpenes that have been isolated from plants include tenulin; the major sesquiterpene lactone in Helenium amarum (Ivie et al., 1975b). All the sesquiterpene lactones isolated from the various different plants have their structure derived from germacranolides



germacranolide

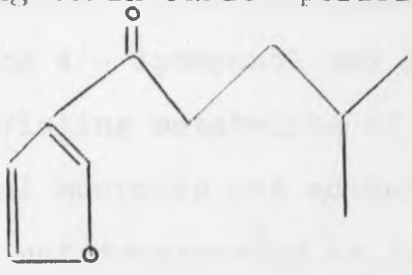
The sesquiterpene lactone isolated from Baileya multiradiata and Helenium hoopsii is the same as that from Hymenoxys odorata (Denis, 1977). The lesions produced by the sesquiterpene lactone from the above three plants are comparable to those found in rats fed P. volkensii Gilg over a period of 90 days.

When rats were fed ground dried leaves of S. myrtina (Burm.f.) Kurz, the most consistent and specific lesion was in the lungs. Thus there were oedema fluid in the alveoli and alveolar walls, haemorrhage in the alveolar walls and into the alveoli and septal fibrosis. The latter plus haemorrhage in walls resulted in thickened alveolar walls.

Doster et al. (1978) did some studies in cattle using the chemical 4 - ipomeanol. The latter is a product from mold damaged sweet potato Ipomoea batatas and is a furan compound. The mold involved originally was Fusarium solani. Intraluminal administration of non lethal doses (7.5 - 9 mg/Kg) of 4 - ipomeanol produced lung oedema and emphysema within 2 days. Later, there were haemorrhage into the alveoli and septal fibrosis associated with cell - infiltration. Acute toxicosis caused by 4 - ipomeanol in cattle was produced by administering a dose of 32 mg/kg. This resulted in death of the cattle in 10 hours. At

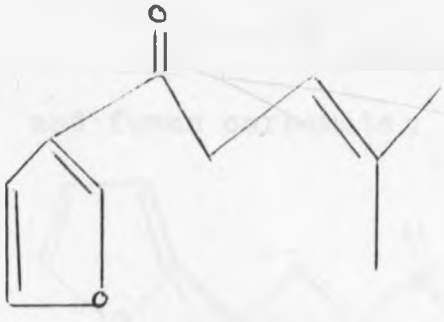
postmortem, the only lesion was massive pulmonary oedema. Wilson et al. (1971) isolated 4 - ipomeanol from contaminated sweet potatoes and showed that 1 - 6 mg of 4 - ipomeanol when injected into mice by intraperitoneal route, produced acute intoxication. Histologically, this was characterised by excess pleural fluid, lung congestion and oedema.

The plant Perilla frutescens Britton contains a lung toxin called perilla ketone.

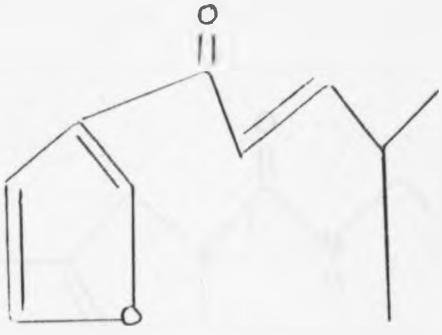


perilla ketone

This furan compound causes pulmonary oedema and effusions in mice and pulmonary oedema and congestion in sheep. These lesions are comparable with those produced by 4 - ipomeanol. Perilla frutescens has two other lung toxins namely, egomaketone and isoegamaketone



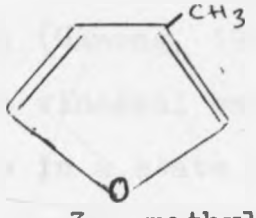
egomaketone



isoegamaketone

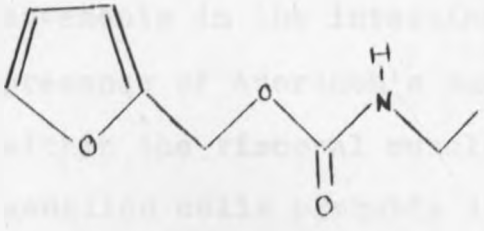
Boyd et al. (1975) did a study to find out the distribution of the lung toxin 4 - ipomeanol in the rat after intraperitoneal injection of the toxin labelled with C - 14. The greatest concentration of radioactivity, and therefore of the toxin was in the lungs. Thus binding of 4 - ipomeanol occurs maximally in the lungs. Other areas with significant concentrations of the toxin were the liver, the kidneys and the gastrointestinal tract. The kidney effects have been demonstrated in the rat. Boyd and Dutcher (1978) did experiments using 4 - ipomeanol and showed that the reactive alkylating metabolite of 4 - ipomeanol that produce renal necrosis was actually formed in the target tissue and not transported by the blood stream.

The pathological results in rats administered 4 - ipomeanol and other substituted furans like perilla ketones, 3 - methylfuran,

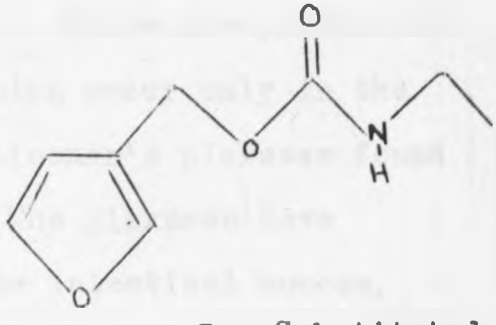


3 - methylfuran

and furan carbamates



2 substituted furan carbamate.



3 - Substituted furan carbamate (more toxic)

are mainly in the lungs, the lesions being comparable with those seen in rats fed S. myrtina (Burm.f.) Kurz.

In this study, aqueous extracts of both P. volkensii Gilg and S. myrtina (Burm.f.) Kurz were found to have some effects both on the isolated rabbit duodenum, and the blood pressure in an anaesthetized dog.

The intestines and the uterus have visceral smooth muscles in their walls. These muscles consist of sheets of cells which are closely adherent to each other through low resistance bridges. Therefore, each fibre does not necessarily receive a nerve ending (Ganong, 1975).

The visceral smooth muscles of the intestines have inherent rythmic contractions originating from the muscle itself (Ganong, 1975). This is because visceral smooth muscle has an unstable membrane potential (Ganong, 1975).

The visceral smooth muscle maintain the intestine in a state of partial contraction. Thus the predominent tone in the intestine is parasympathetic (Jones et al., 1977). There are peristaltic movements in the intestine which occur only in the presence of Auerbach's and Meissner's plexuses found within the visceral muscles. The plexuses have ganglion cells probably in the intestinal mucosa.

Serotonin and substance P, a polypeptide also found in the brain could be the transmitter substances (Horrobin, 1968 and Granong, 1975)..

Histamine and acetyl choline are two naturally occurring compounds within the animal body that can affect the motility of the intestine (Goodman and Gilman, 1975). The effect of histamine on the intestine is stimulatory so is that of acetyl choline i.e. one expects increased force of contraction in both.

Histamine acts on two types of receptors i.e. H_1 and H_2 (Ash and Schild, 1966). H_1 receptors are found in the intestine and bronchi, while H_2 receptors are responsible for gastric secretions. Mepyramine (pyrilamine) blocks H_1 receptors only (Goodman and Gilman, 1975) and has some anticholinergic activity as well (Beckman, 1961).

Acetyl choline has two receptors i.e. muscarinic and nicotinic receptors. The muscarinic receptors are located at postganglionic parasympathetic neuroeffector junctions e.g. parasympathetic innervation to the heart muscle, smooth muscles, and exocrine glands. The nicotinic receptors are in the autonomic ganglia, the neural muscular junction and the adrenal chromaffin cells (Jones et al., 1977).

Atropine blocks muscarinic receptors and also smooth muscles with no cholinergic innervation e.g. the intestine (Jones et al., 1977). Apart from being muscarinic blocker, atropine has slight antihistaminic effects (Beckman, 1961).

The results of the present study indicate that prior treatment of the duodenum with mepyramine maleate completely blocked the stimulatory effect of P. volkensii Gilg extract while only slightly or partially blocking those of S. myrtina (Burm.f.) Kurz extract. On the other hand pretreatment of the duodenum with atropine partially blocked the stimulatory effect of P. volkensii Gilg while completely blocking the stimulatory effects of S. myrtina (Burm.f.) Kurz. This would suggest that P. volkensii Gilg methanol extract had more of a histamine - like substance while that of S. myrtina (Burm.f.) Kurz was more of the cholinergic type compound.

The heart is innervated by both the sympathetic and parasympathetic nervous systems. In the heart, the vagus nerve represents the parasympathetic system. Normally, both of these systems have a continuous discharge The mechanisms involved in the maintenance of the normal blood pressure are both central and peripheral (Horrobin, 1968). The latter component is represented by the baroreceptors in the carotid sinus and the aortic arch.

The former component is the vasomotor centre in the medulla oblongata. The baroreceptors are stretch receptors and increase in the arterial blood pressure increases the rate of their discharge to the vasomotor centre. The latter then initiates effects that lower the blood pressure. These are the slowing of the heart rate (negative chronotropic effect) and a decrease in the force of contraction of the heart (negative inotropic effect). This is due to increased vagal discharge. In the blood vessels, there is inhibition of the sympathetic tone to the vessels, thus the cholinergic fibres to these vessels cause vasodilation, decrease in peripheral resistance and resultant decrease in blood pressure associated with the two phenomena. This is because blood pressure is equal to the peripheral resistance x cardiac output. The cardiac output is equal to the heart rate x stroke volume. The latter being the volume of blood pumped by the ventricles per beat.

When the blood pressure is lower than normal, the baroreceptors' discharge decreases and the blood pressure is brought to normal by increasing the heart rate (positive chronotropic effect) and an increase in the form of contraction of the heart (positive inotropic effect) through increased activity of the sympathetic system to the heart. In the blood vessels, there is increased vasomotor tone and therefore an increase in the peripheral resistance (Ganong, 1975).

In the present study, administration of 0.2 ml of P. volkensii Gilg aqueous extract to a dog IV resulted in a decrease in the blood pressure. The heart rate remained the same as the control reading. No further tests were done using this extract because the results were erratic and unpredictable.

Intravenous administration of 0.2 ml of S. myrtina aqueous extract (Burm.f.) Kurz into the anaesthetised dog resulted in a decrease in the blood pressue and an increase in the heart rate. Injection of mepyramine maleate at a dose rate of 1 mg/kg resulted in very transient fall in the blood pressure returning to normal in a few seconds. Administraction of S. myrtina (Burm. f.) Kurz methanol extract after pretreatment of the dog with mepyramine maleate resulted in decrease in blood pressure, but not as when the animal had not been pretreated with mepyramine maleate. Thus there was partial blockage. There was no alteration in the heart rate. Administration of atropine sulphate at 0.2 mg./kg. resulted in a slight increase in the blood pressure and the heart rate. Administration of the extract 10 minutes later resulted in a very slight and transient decrease in blood pressure, the heart rate being the same as control. Pretreatment of the dog with propranol had no effects i.e. the results were as if there was no pretreatment drug.

The blood pressure can be lowered by either affecting the central or the peripheral control system. In this study the increase in heart rate after administrat of S. myrtina (Burm. f.) Kurz can be attributed to the baroreceptor stimulation and the resultant mechanism to raise the blood pressure (Ganong, 1975). The transient fall in the blood pressure is common when there is rapid intravenous injection of H_1 antagonist (Goodman and Gilman 1975). The partial block in the effect of the extract by mepyramine maleate could be due to the anticholinergic properties of this compound (Beckman, 1961; Goodman and Gilman, 1972). The slight increase in the heart rate and the blood pressure after administration of atropine is attributed to the blockage of the vagus by atropine. This leaves the sympathetic system under control resulting in the observed results.

The fact that atropine sulphate almost completely blocks the effect of the extract suggest that the lowering of the blood pressure is by a cholinergic type of mechanism since as has been mentioned earlier, atropine blocks muscarinic receptors of acetyl choline (Jones et al 1977). The fact that propranol did not block the effect of the extract on the blood pressure indicate that the extract does not lower blood pressure through stimulation of B_2 receptors which are dilators of blood vessels. Propranolol is a B_1 and B_2 blocker (Jones et al., 1977).

It causes hypotension by blocking B_1 receptors in the heart and B_2 receptors in the blood vessels. This results in depression of heart rate and peripheral resistance resulting in decreased cardiac out put. This is most pronounced in a heart which was under the dominance of the sympathetic tone.

Certain other chemicals are also antihypertensive. One of these is histamine which has already been mentioned in connection with the rabbit duodenum. In the dog, histamine has no direct action on the heart muscle, rather, it causes dilatation of capillaries and terminal arterioles resulting in decreased blood pressure. There is also increased capillary permeability resulting in oozing of fluids into the tissues (Goodman and Gilman, 1975).

In humans, there is a condition called angina pectoris. This condition is manifested by poststernal pain probably due to cardiac hypoxia. Some of the drugs that have been used to manage this condition are the so called antihypertensive drugs and most of them are vasodilators acting directly on the smooth muscles of the arterioles causing a lowering in peripheral resistance and a decrease in blood pressure. These include amyl nitrite which causes vasodilation and increased coronary blood flow. (Goodman and Gilman, 1975). Nitroglycerin also improves the perfusion of diseased myocardium in patients with coronary artery disease (Lawrence et al., 1971). Certain diuretics also act on the arteriole

smooth muscles causing dilatation and lowering the blood pressure. Among the diuretics are the thiazides (Keneth and Horward, 1972). Unlike the above named chemicals, methyldopa and clonidine produce hypotension by acting on the hypothalamus (Laurence, 1961; Smith and Varma, 1972; Keneth and Howard, 1973; Girdwood, 1974).

In Pharmacology, there are two types of responses that are observed when a drug is administered to a biologica system. These are the graded dose effect relationship, and the quantal or the all-or-none response (Levine, 1973). In the graded dose effect relationship, it is assumed that the individual units of the biological system increase in their response as the dose of the drug is increased up to a maximum when the drug has occupied all the receptors. In the quantal type of response, the assumption is that the individual units of the system respond to their maximum capability or they do not (Beckman, 1961). Thus in toxicity studies, one criterion could be whether the animal dies or lives. The dose of a drug or chemical required to kill 50% of a population of animals is called the median lethal dose (LD_{50}), while the dose required to produce therapeutic effect in 50% of a population of animals is the median effective dose (ED_{50}) and the ratio $\frac{LD_{50}}{ED_{50}}$ is the margin of safety (Loomis, 1974).

The method used for determining dose range in the LD_{50} experiment has been recommended by several researchers (Buck et al., 1976; Loomis, 1974; Paget, 1970 and Reed and 1938). From the results, S. myrtina (Burm. f.) Kurz with LD_{50} of 6.1 g /kg is more toxic than P. volkensii Gilg extract with an LD_{50} of 6.3 g/kg. Since the extracts were injected via the intraperitoneal route, there was a possibility of the active chemical(s) being destroyed in the liver or there could be enterohepatic circulation. If any or both of these two conditions occurred, then the figure for LD_{50} would be smaller i.e. the extract would be more toxic than the calculation shows.

In determination of LD_{50}, there are two groups of methods used. These are the graphic and the calculation methods. Graphic methods include those of Miller and Tainter (1944) and Litchfield and Wilcoxon (1949). The calculation ones include those of Reed and Muench (1938) and Weil (1952).

According to Loomis (1974) the following is a practical classification of toxicities which can serve a useful purpose.

	Classification	LD_{50}.
1.	Extremely toxic	1 mg/kg or less.
2.	Highly toxic	1 to 50 mg/kg.
3.	Moderately toxic	50 - 500 mg/kg.
4.	Slightly toxic	0.5 to 5 g/kg.
5.	Practically non toxic	5 to 15 g/kg.
6.	Relatively harmless	more than 15 g/kg.

When a chemical is to be used for therapeutic purposes, it is very important not just to know the LD_{50}, but also to know the margin of safety (Wilson et al., 1975). A good therapeutic agent should have a wide margin of safety and few undesirable side effects.

In conclusion, both P. volkensii Gilg and S. myrtina (Burm.f.) Kurz stimulate rabbit duodenum, decrease systolic, diastalic and pulse pressure in the dog which suggest they could be used as laxatives and antihypertensives. However, this is not advisable because both plants produce pulmonary haemorrhages and severe alveolar thickening. P. volkensii Gilg also cause renal proximal convoluted cellular degeneration, hepatic and adrenal cortical haemorrhages and marked splenic hemosiderosis signifying red blood cell destruction.

The acute (24 hr) LD_{50} for S. myrtina (Burm.f.) Kurz is (6.1) g/kg and that of P. volkensii Gilg is (6.3) g/kg of body weight in mice.

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