TOXICITY STUDIES OF AJUGA REMOTA BENTH IN RATS (*Rattus rattus*) AND GOATS (*Capra hircus*)

BADRU GALIWANGO, B.V.M.S.

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

Department of Veterinary Pathology and Microbiology
Faculty of Veterinary Medicine

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DECLARATION

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

25/07/1989

BADRU GALIWANGO

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

PROFESSOR G.M. MUGERA, D. Vet. Sc., M.Sc., Ph.D.

Dean: Faculty of Veterinary Medicine,
College of Agriculture and Veterinary Sciences,
University of Nairobi.

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ABSTRACT

Ajuga remota Benth. (Labiatae or Lamiacea) is a small shrub found in many areas of Kenya, especially where land has been disturbed by human activities. The plant is known to have therapeutic activities. It is used for treatment of abdominal illnesses and toothache in human and blackleg in cattle. This study was undertaken to investigate its aqueous extract effects on animals, particularly pathological effects. Establishment of the mode of toxicity of *Ajuga remota* Benth was attempted by relating the clinical manifestations to the histopathological lesions *in vivo*.

Weaner rats (*Rattus rattus*) of Sprague Dowley breed and goats (*Capra hircus*) were used to study the pathological effects of the toxic principles of *A. remota*. The whole plant excluding roots were extracted in distilled water by boiling for a few hours, filtered through cotton-wool, number 1 filter paper and then centrifuged at relative centrifugal force of 2.12×10^{4}g. Then the aqueous extract was dehydrated by freeze drying to obtain a powder. The powder was reconstituted in distilled water at required percentage. The aqueous extract was then sterilized at 121°C for 15 minutes. It was administered parenterally by subcutaneous route to experimental animals at various doses. Toxicity in rats was monitored by keen observation for symptoms, necropsy findings and histopathological study of tissues of

animals that succumbed and those killed at the end of the experiments. Toxicity in goats was studied by objective observation for clinical signs, serum biochemistry and haematological changes. macroscopic and microscopic lesions of goat which died of acute, prolonged toxicity and those sacrificed were recorded. Aqueous extract was found to be non toxic to rats at levels below 1.37 gm/kg body weight.

The lesions seen in rats depended largely on the dosage and duration of administration of extract to animals. Serially quantified doses were injected intraperitoneally into weaner rats to determine LD_{50}. Then rats were observed for toxic effects. The medium lethal dose (LD_{50}) for crude extract was found to be about 5.5 gm/kg of body weight. The rats that received doses much higher than the LD_{50} showed acute death with congestion, haemorrhages in visceral organs and central nervous tissues. 60% of rats which died of acute toxicity were found with intussusception in small intestine. Those that received doses below LD_{50} had poor growth, loss of condition relative to dosage and took longer time to succumb.

Histological sections from treated rats, were fixed in 10% formalin and stained with hematoxylin and eosin (H&E) revealed marked haemorrhages, cellular degeneration, and necrosis of uriniferous tubules, hepatocytes and neurons. Oedema and vacuolation were constant findings in brain and liver respectively. Organs concerned with detoxication and excretion of toxic substances from the body were mostly affected.

Intestines and brain were also to a certain extent involved. Perivascular and perineuronal vacuolation was marked in brains and spinal cords, while intracytoplasmic vacuolation, pyknosis and karyolysis were commonly found in hepatocytes. Epithelial cells of proximal tubules showed degeneration and necrosis with hyaline casts in lumina of tubules especially in the cortex. Lungs showed haemorrhages and congestion. Some portions could be seen with fibrin in the alveolar sacs. The lymphoid tissue follicles were depleted.

Goats administered with a dose of 900 mg/kg of body weight died of acute toxicity within 48 hours. Clinical manifestations included oedematous swelling along the ventral part of the abdomen for a few hours after administration, excitability, partial paralysis of limbs nearest to injection sites for a few hours, uneasiness, raised coats and loss of condition in prolonged treated cases.

Haematological studies indicated a decrease in packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell counts (RBC), total plasma protein (T.P.) by significant fractions, while mean corpuscular volume (MCV) remained unaffected.

Serum biochemical analysis of enzymes revealed slight decrease in levels of lactate dehydrogenase (LDH) blood urea nitrogen (BUN), serum creatinine and alkaline phosphotase. Alanine aminotransferase and aspartate aminotransferase levels in blood of treated animals elevated significantly.

Necropsy conducted on treated animals revealed petechial haemorrhages, congestion of parenchymal organs and central nervous tissues. Prolonged treatment was characterised by hydrothorax and ascites in goats which received 350 gm/kg body weight. Oedema of the brain was marked. Microscopically, there were marked cerebral haemorrhages, congestion, perivascular and perineuronal vacuolation. Lungs were congested and haemorrhagic. There was marked degeneration of uriniferous tubules, hepatocytes and neurons. Each of these degeneration invariably led to necrosis in their respective organs.

Ajuga remota daily doses of about 1.6 g/kg of body weight cause acute toxicity leading to death in rats. Death is likely to be due to extensive haemorrhages in various organs. A single dose of 900 mg/kg of body weight caused acute death in goats. Ajuga remota extract seems to have sympathomimetic effects which lead to increased peristaltic movement followed by intestinal invagination. Prolonged administration of low doses leads to degeneration and necrosis in liver, kidney and central nervous tissues. Extensive haemorrhages and perivascular vacuolation are likely to have been due to increased permeability and endothelial damage by active principles of Ajuga remota Benth..

1.0 INTRODUCTION

A variety of poisonous plants have caused extensive losses to the livestock industry in many parts of the world from time immemorial. They are still a significant economic problem in many areas, (Radeleff, 1964).

It is not possible to give the annual loss through mortality and reduced production caused by poisonous plants in East Africa but it is considerable, (Mugera, 1979). Culvenor (1986), reported that overall losses are estimated for the more important diseases of livestock in Australia due to poisoning of the grazing animals by naturally occurring toxic substances. The estimates based substantially on data from State Department of Agriculture or Primary industry, show that the total is of order of A$70-80 million per annum. Animal deaths are not often the major issue because of management intervention; a high proportion of the loss from many diseases is their effect in preventing realisation of the potential production..

Overgrazing coupled with periodic droughts as commonly experienced in Africa impairs or destroys many palatable and nutritious forage plants and permits the spread of less palatable and often poisonous ones to prevail. These plants are often well adopted to resist harsh environmental conditions.

Animals are often exposed to danger of poisoning both at range and zero-grazing conditions. Livestock

ingest the toxic plant material which may grow together with forage or when harvested with hay or their seeds mixed with grains. It is not as difficult, however, to ascribe a given cause of poisoning to particular poisonous plants (Verdcourt and Trump, 1969).

Ajuga remota Benth. (Labiatae) is widespread in Kenya. This plant is commonly used medicinally in many parts of Kenya and probably in other parts of Africa at large. Cattle keeping tribes of Kenya use it for treating various diseases in human and cattle. It is also claimed that livestock normally avoid grazing on A. remota. Due to its widespread occurrence in Kenya, it is being widely used as a medicinal herb and it being repugnant to grazing animals, it is not unlikely that it may be responsible for losses in livestock. It is claimed by Kikuyu elders that A. remota was the only plant which could always survive locusts invasion as such it was named Kerema ngigi (locust untouchable).

Since humans and animals alike are exposed to possible poisoning by A. remota Benth., and recognizing that nothing has been investigated on the mode of toxicity, this work represents an attempt to establish the effects of A. remota Benth. on animals. Clinical signs, changes in haematology and body fluids of live animals, gross and microscopic pathological lesions were used as parameters for monitoring the effects.

1.1 LITERATURE REVIEW

The common herb *Ajuga remota Benth.* belongs to a family known as Labiatae or Lamiaceae. It is a large family of mostly herbs and undershrubs consisting of many useful plants. Many are used as ingredient of spices due to their aromatic smell while others as medicinal herbs.

It also includes poisonous plants; a case in point is *Hoslundia opposite* which is an exceedingly abundant aromatic shrub in scrub and bushland. There have been several cases where material later authentically named has caused purging and vomiting followed by death in two days. The bark has been used to prepare a stomach medicine, (Verdicourt, 1969). Mettam, reported by Verdcourt (1969), found that 20 to 30 gm of this plant killed a sheep. The death was preceeded by poor condition, fairly profuse liquid and dark green diarrhoea. Postmortem finding showed lesions in abomasum and intestines. The mucosae were swollen, infiltrated and showed numerous petechiae. The kidneys showed congestion of the cortex.

Many texts have been published on traditional medicine and medicinal plants. It will not be possible to review all these texts but Watt and Breyer-Brandwijk's Medicine and Poisonous plants of Eastern and Southern Africa (1962) and Kokwaro's Medicinal Plants of Kenya (1976) are excellent expositions. However, these sources mention nothing about the toxicity of *A. remota* in ruminants.

1.1 LITERATURE REVIEW

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1.1.1 Distribution

Generally labiates are plants of open ground, only a few genera are found in tropical rain forests (Haywood, 1978). A large number of labiates are cultivated either as ornamental or kitchen herbs. Some are used in flavouring food but others are important sources of essential oil used in perfumery and pharmacy, (Heywood, 1978).

According to Nairobi Museum collection, it is clear that *A. remota* Benth. is widely distributed throughout Kenya. The herb has been sited at Muguga, Nairobi, Kericho, Nyeri, Narok district, Eastern Province and Masai land. Samples have been collected from Kabete too. *A. remota* Benth. is erect often rhizomatous, pubescent leaves and small subscessible axillary pale blue or purple flowers.

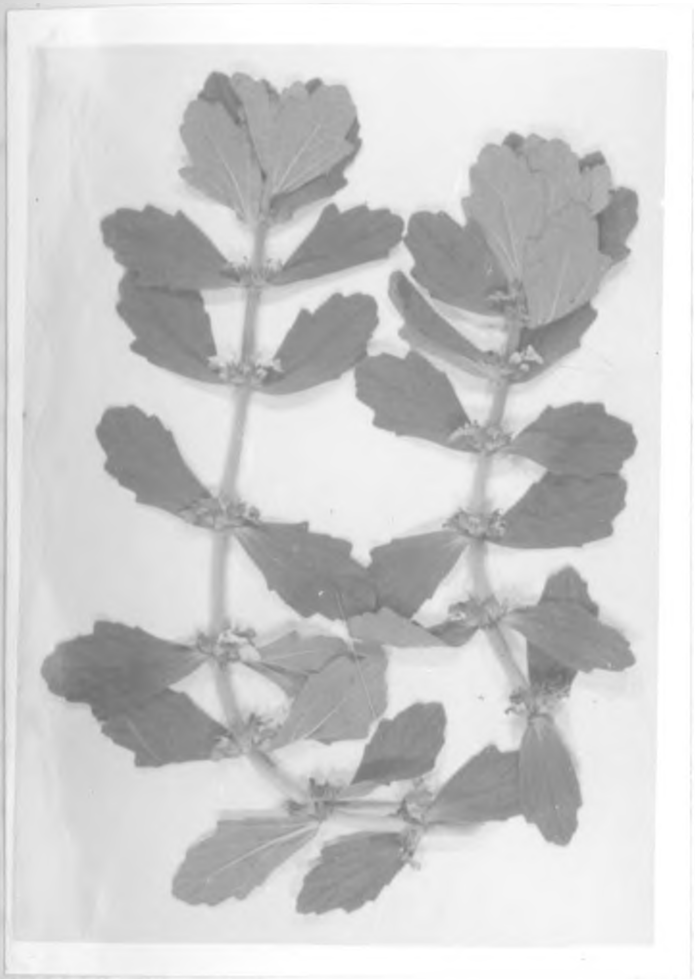


Plate 1: A. remota stem.

A. remota is commonly found in disturbed areas of grassland. The mountain form is more strictly rhizomatous with rosette of leaves at ground level and many merit specific status when it has been carefully studied (Agnew, 1967).

A. remota (Labiate) is known under various names by different people in Central Kenya. By some it is called Wanjiru wa Kieni, by others Wanjiru wa gana, Wanjiru wa rurii, Wanjiru wa Weru, nguri kuma and Kunini.

Table 1, various names are presented

Kunini	Kikamba
Osorara	Masai
Nguri Kuma	Kikuyu
Wanjiru wa Rurii	Kikuyu
Wanjiru wa Gana	Kikuyu
Wanjiru wa Weru	Kikuyu
Karema Ngigi (Locust untouchable)	Kikuyu
Malalina	Kipsigis
Cheborusiot	Kipsigis
Chebeiyat	Kipsigis
Osognoi (sogonoi means bitter)	Masai

1.1.2 Traditional Medicine Application of A. remota in relation to its therapeutic value

Wakori et al.,(1986), reported that preparation from a A. remota Benth. are claimed to treat toothache, headache, stomach-ache, joint pain, Asthma, Malaria, epilepsy, palpitation of the heart, epistaxis and post-delivery pains. Kokwaro (1976) reported that the leaves of A. remota which are bitter are pounded and steeped in cold water and the infusion drunk as a remedy for fever, toothache, dystentery and high blood pressure. From their investigations, Wakori et al., (1986), revealed that the herb A. remota contains a wide variety of chemical compounds. The traditional use of the plant for the many disease conditions may be due to these constituents. However, they concluded that A. remota shows some therapeutic activities that are not yet confirmed pharmacologically but are clinically effective.

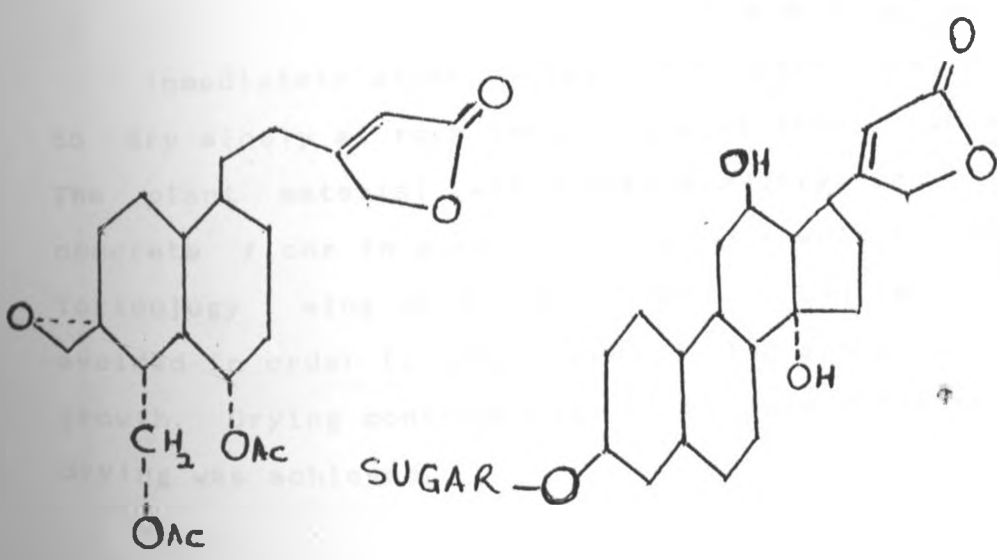
1.1.3 Active Principles of Ajuga remota Benth.

A cardioactive compound was isolated and characterized (Kuria 1976; Kuria and Muriuki, 1984) as well as a number of compounds having insect antifeedants were found to be neoclerodane derivatives, the ajugarins.

Njunge et al., (1986) while working on aqueous extract of A. remota leaves separated two fractions by column chromatography. They ran the fractions on thin-layer chromatography and visualized with three spray

reagents; Kedde reagent (alkaline 3,5 dinitrobenzoic acid), Carr-Price reagent (antimony trichloride), and ninhydrine reagent. The activities of two fractions were tested on isolated rabbit heart and cat blood pressure. Fraction A was found to cause arrhythmia, slowing of the heart rate and reduction in forces of contraction while fraction B was found to reduce the forces of contraction. On cat blood pressure, fraction A was found to cause a sustained fall in blood pressure which is followed by hypothermia, while fraction B causes a transitory fall in blood pressure. A. remota leaves contains principles with effects on the cardiovascular system. The compounds were found to be highly soluble in water but unstable.

Figure 1.
Structure of active principles of Ajuga remota



Ajugarin

Cardiac glycoside

2.0 EXPERIMENT ONE

PART I

MATERIALS AND METHODS

Ajuga remota Benth. herb was collected from the bush near Kabete Veterinary Laboratory Primary School along the road to Uthiru round-about from College of Agriculture and Veterinary Sciences at Kabete. Only stems together with their leaves were harvested. They were plucked carefully in order to exclude the roots. The plant material was then packed in plastic sacks, sealed and transported by vehicle to the Faculty of Veterinary Medicine, Department of Pathology and Microbiology.

Immediately after arrival, each batch was allowed to dry slowly at room temperature of about (18-24°C). The plant material was spread sparsely on a clean concrete floor in a well ventilated spacious room of Toxicology wing of the department. Overlapping was avoided in order to attain even drying and avoid fungal growth. Drying continued for 45-60 days until thorough drying was achieved.

2.0.1 Aqueous extract preparation

Soon after drying, aqueous extract from Ajuga remota was prepared. Because of the shrinkage of stems and strong attachment of leaves to stems it was found impracticable to detach the leaves from stems. So the whole plant (stems and leaves) was used.

Three kilograms of dry plant material were weighed out and steeped in distilled water for two hours in a boiler. Later the plant's active principles were extracted by boiling for 2-3 hours. Remains were filtered off, first through cotton-wool, then a clear dark-brown aqueous extract filtrate was collected. This filtrate was subjected to further filtration through five layers of filter paper with application of a vacuum pump to create a negative pressure. The filtrate was subsequently centrifuged at a relative centrifugal force (RCF) of 1.04×10^4 g for 10-15 minutes to remove any remaining solid fine particles.

A clear brown supernatant was collected into clean flat-bottomed flasks. Flasks were then connected to an electric freeze-drier to dehydrate the extract. Anhydrous brown powder was recovered, put into clean dry universal bottles and sealed immediately. Little by little quantities from this stock powder extract were weighed on an analytical electrical balance and reconstituted in distilled water to make a 25% concentration. This 25% concentration was used for parenteral administration.

2.0.2 Determination of the median lethal dose (LD_{50})

Forty eight Sprague-Dawley male and female rats at a ratio of twenty to fifteen respectively were obtained from the small animal unit at the International Laboratory for Research on Animal

Diseases (ILRAD) at Kabete. On arrival they were housed in the small animal unit of the Department of Veterinary Pathology. They were accommodated six per cage. Wood shaving bedding was provided and kept clean by regular replacement. Rats were provided with balanced commercial grain ration and drinking water ad libitum. The animals were allowed to acclimatize for five days before the inception of the experiment. The rats were weighed daily.

On day 5 the experimental animals were randomly divided into six groups of eight rats per experimental group. One group was set a side as a control group while the other five groups were used for intraperitoneal injection with aqueous extract. The animals were weighed and doses calculated as follows: Group 1, 2 and 3 received dosages of 9.8, 7 and 5.5 gm per kilogram body weight respectively, while 4 and 5 received 4.65 and 3.5 gm/kg body weight. The calculated dosages of extract were administered in a 30% concentration. The rats were then injected intraperitoneally using a five-millilitre syringe and a 20G needle. The sixth group of 8 rats received 2 millilitres of normal saline. The rats were then subjected to close observation for twenty four hours to determine their mortality. The results were used to compute the mortality percentage of the groups.

The mortality percentages in 24 hours were plotted on a graph against their respective dosage rates per kilogram of body weight and then LD_{50} was estimated. The lethal dose within 24 hours was found to be around 5.5 gm/kg body weight.

Table 2 The estimation of the LD_{50} response of weaner Sprague Dawley rats to an intraperitoneal injection of the extract of A. remota Benth.

Groups	1	2	3	4	5	6
Dosage rate (gm/ kg body weight)	9.8	7.0	5.5	4.6	3.5	-
Average wt. in gm	122.4	128.0	128.5	96.8	110.5	98.5
No. of dead rats per group (in 24 hours)	8/8	6/8	4/8	2/8	0/8	0/8
Percentages	100	75	50	25	0	0

2.0.3 Experimental animals for main experiment

Fifty six weanling 5-week-old male and female rats, Rattus rattus of Sprague-Dawley breed were obtained from the small animal Unit of International Laboratory for Research on Animal Diseases (ILRAD) situated at Kabete, Nairobi. They were put into large cages and transported to the Department of Veterinary Pathology by vehicle.

On arrival at the Faculty, rats were divided randomly into 7 groups (Table 3) each one consisting of 8 rats. This was done in order to have approximately equal mean body weights per experimental group. Rats were accommodated four per cage. German cages made of filtered polycarbonate with steel covers were used. A 5 cm thick wood-shaving bedding was provided. Bedding was regularly changed every after 4 days in order to maintain good standard of cleanliness. The animals were fed ad libitum on a commercial Unga mouse and rat grain ration and water supply in feeding bottles with metallic tubes was accessible to them throughout the experiment. All rats were marked by numbering with a special dye in order to identify them. Each morning rats were checked for any alteration in their demeanour and feeding habits. After every morning's general observation, all rats were weighed and weights recorded in grams.

Environmental temperature and humidity ranged between 18-24°C and 45-65% respectively. No deaths were recorded during acclimatization period of 11 days prior to initiation of the study. Experimental rats of 7-8 weeks of age weighing 107-197 gm were used and were injected with a water extract from A. remota using subcutaneous route.

Powder extract of Ajuga remota was weighed (on analytical electric balance) and reconstituted in distilled water to make a 25% concentration solution. This aqueous extract solution was subjected to sterilization for 10 minutes at 121°C under a pressure of 15 lbs. It was allowed to cool at room temperature.

Rats were examined and weighed as usual. Average weights for each experimental groups, i.e. 1, 2, 3, 4, 5 and 6 were calculated and dosages for each group were determined. Serial reduction of doses were from Group 1 to group 6. Group 7 (control) received 2 ml of physiological saline each.

2.0.4 Administration of the extract

Injections were administered subcutaneously. Disposable syringes of five millilitres and 20G x 1.5" hypodermic needles were used. Seventy per cent ethyl alcohol was applied as skin antiseptic agent. Group 1 of eight rats with average weight of 145.8 gm received the highest dose of 4280.8 mg per kilogram of body weight. Group 2 with average weight of 136.4 gm received 2291 mg/kg of body weight. The third group

with average weight of 140.4 gm received 1602.6 mg/kg of body weight. Group 4 with average weight of 159.0 gm received 1572.3 mg/kg of body weight. Group 5 with average weight of 137.0 g received 1368.6 mg/kg of body weight. Group 6 with average weight of 152.0 gm received the lowest dose of 1315.8 mg/kg of body weight. While group 7 with an average weight of 141.1 gm served as a control group received 2 ml of physiological saline solution. Repeat doseges were administered at an interval of 24 hours based on the day's average weight of the groups (Table 3).

Table 3: Total weights, average weight (gm) volume of extract, total doses, and dosage per kilogram of body weight in milligrams in various experimental groups of rats

Group	Total weight (gm)	Average weight (gm)	Vol. of extract solution (ml	Total dose (mg)	Dosage kg of B. wt. (mg)
1	1166	145.75	2.5	625	4280.8
2	1091	136.6	1.25	312.5	2291
3	1123	140.4	0.9	225	1602.6
4	1272	159.0	1.0	250.0	1572.2
5	1096	137.0	0.75	187.5	1368.6
6	1217	152.0	0.8	200	1315.8
7	1129	141.2	2.0	-	-

3.0 RESULTS

3.0.1 Response to the injections of the aqueous extract

The results observed in rats injected with the aqueous extract of Ajuga remota varied for each group and depended on the amount of the extract administered. Following the subcutaneous injections the rats were observed for 3 hours and inspected every 6-8 hours daily throughout the experiment to monitor clinical signs and immediate removal of any dead rats for necropsy.

Two to three hours after injections, rats in group 1-4 started showing some reactions. Among the main ones included raised coat appearance, an increased water intake accompanied by diuresis. These were more marked in rats that received more than 1.5 gm/Kg of extract. Reduced food intake was also noticed in extract recipients as compared to control group 7 which did not show any change. When at rest there was a tendency of each rat to separate and stay isolated from the rest. So there was even distribution in the cages while the control group rats collected themselves in one of the corners of the cages. 56.25% of rats in group 1 up to 4 had either stagnated or lost weight by the end of the first 24 hours. By the end of day two and day three of the experiment only 37.5% and 18.75%

respectively had gained weight while others were losing.

Only 2 rats from group 5 and 6 showed an initial weight loss then later regained. The rest of group 5 and 6 rats had a generalized reduction in weight gain though they did not lose. Group 7 (Control group) did not show any changes (Figure 1). On day 4 some rats had diarrhoea and this was accompanied by the soiling of hindquarters. Nasal discharge was a common finding and in some cases it was bloody in the 1st and 2nd groups. Arched back was common especially when the animals became weak and moribund. Some members of group 1 and 2 passed red coloured urine. Intoxicated rats were aggressive and nervous and could bite at any slightest mishandling. They showed marked nervous disturbances which included movements of heads from side to side and circular walking when put on a table. The first death occurred on day 4 of the experiment.

Deaths in experimental groups started occurring after the third day of the experiment. First death occurred in group 4 which received one of the lowest doses. On day 4 of the experiment 5, 5, 3, 2 and 1 rats of group 1, 2,3, 4 and 5 respectively were found dead and others in moribund state. On day 5 of experiment 3, 2, 1 and 3 rats from group 1, 2, 3 and 4 respectively died. On the sixth day two rats died one from group 1 and another from group 3.

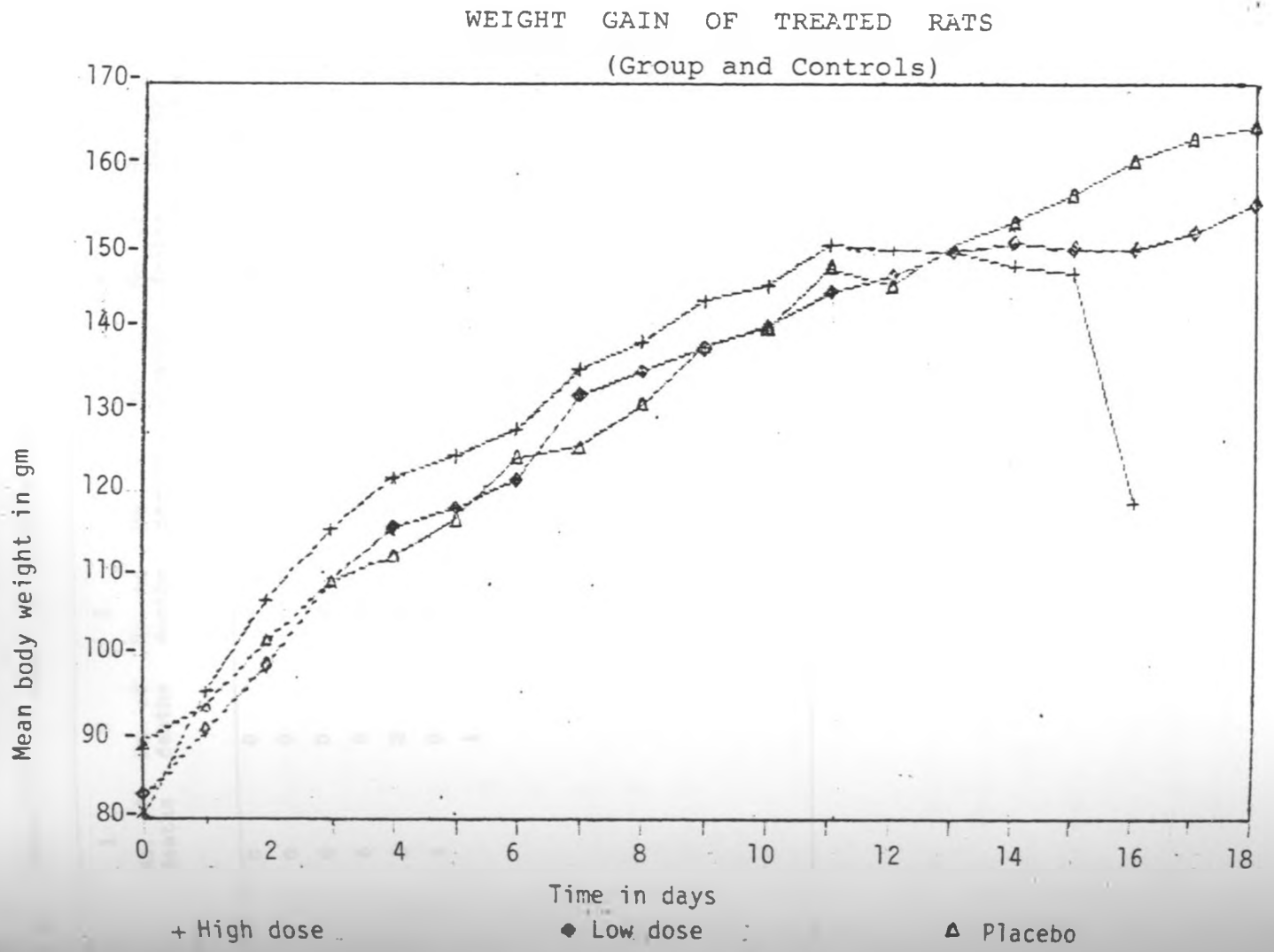


Figure 2: Dose-effect relationship to weight gain of experimental rats

Table 4: Mortality of rats in various groups in Experiment 2

Exp. Group Days of injection	1 No. of deaths	2 No. of deaths	3 No. of deaths	4 No. of deaths	5 No. of deaths	6 No. of deaths	7 No. of deaths (control)
1	0	0	0	0	0	Nil	Nil
2	0	0	0	0	0	"	"
3	0	0	0	1	0	"	"
4	5	5	3	2	1	"	"
5	3	2	1	3	0	"	"
6	1	0	1	0	0	"	"
7		1	1	1	0	"	"
8			2	0	0	"	"
9				0	0	"	"
10					0	"	"
11					0	"	"
12					0	"	"
13					0	"	"
14					0	"	"
15					0	"	"

Table 5. By day 8 mortality rate in various groups stood as follows:

Group	Percentage of mortality
1	100
2	100
3	100
4	87.5
5	0
6	0
7	0 (Control group)

No death so far had been recorded from groups 5, 6 and 7 (control). First deaths in group 5 and 6 occurred on days 19 and 22 of the experiment respectively.

3.0.2 Necropsy Procedure

All the rats were routinely inspected three times per day, every morning at 8.30 a.m., evening at 4.30 p.m. and before midnight at 11.00 p.m. Any rats which succumbed to the toxicity and those euthanized were subjected to thorough standard postmortem examinations immediately. All organs were scrutinized for macroscopic lesions, those found with some were noted. Organs and tissues were examined *in situ*. Then dissected from the carcasses and re-examined under ample light.

Samples were collected virtually from all organs. Specimens of brain, spinal cord, liver, myocardium, spleen, kidneys, lungs, pancreas, adrenal glands, mesenteric and ileocecal lymph nodes, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, urinary bladder, prostate gland, bones, and skeletal muscles were fixed in 10% neutral buffered formalin for at least 48 hours. After fixation in formalin, tissues were trimmed and processed further for histologic study. They were embedded in paraffin wax. Paraffin sections were prepared, cut at 5-6 microns thick, stained with hematoxylin and eosin (H & E) and examined by light microscopy.

3.0.3 Gross pathological lesions

At necropsy, gross lesions were mostly found in the contents of thoracic and abdominal cavities, and to some extent in the central nervous system.

Moderate dehydration was found in carcasses of rats that had been given high doses and had developed diarrhoea before death. There was brown stain, congestion and inflammatory reaction around recent sites of injections. Very few sites of some treated rats developed necrosis and healed quickly by scar formation. In abdominal cavities, congested mesenteric blood vessels stood out clearly. This was more marked in carcasses of group one which had small intestinal invagination (intussusception). Mesenteric lymph nodes were slightly enlarged. Six out of eight rats in group 1 which received about 4300 mg per kilogram of body weight and died of acute toxicity were found with invaginated small intestines (jejunum and ileum). Parts anterial to the intussusception were swollen and their ingesta stagnated. Livers and spleens of acute toxicity subjects were invariably congested. More than 50% of animals in group 1 and 2 had moderate ascites and hydrothorax while only two rats of group 3 had hydroperitoneum. Pulmonary congestion and petichiae were constant findings in most of extract recipients. However, the intensity varied according to the doses administered.

No lesions were seen in lungs of control group. Hydropericardium occurred especially in cases which

survived beyond the fifth day of the experiment. Those which died of acute toxicity showed slight increase in pericardial fluid. Rats which died later during the experiment and those killed in extremis had marked hydrothorax and hydroperitoneum respectively. Gelatinous materials were over their pericardium (necrotic pericarditis) and livers. The myocardia were flabby. Petechial haemorrhages in skeletal muscles especially those of hind quarter were common in rats which died of acute toxicity.

The central nervous tissues had gross lesions similar to those of lungs. The brains of most of the rats of group 1 - 3 showed severe congestion of blood vessels. The congestion was accompanied by subdural petechial haemorrhages in both the brain and the spinal cord. The petechoae were more marked on the cerebral hemispheres. The brain had a soft swollen appearance. The gyrae appeared flattened due to intracranial pressure against the cranium. A few cases of group 2 and 3 showed some tendency of herniation of the cerebella into the foramena magnum with consequential distortion of their caudal aspect.

3.0.4 Histopathological examination of the tissues

Histopathological sections from organs of necropsied rats were fixed, processed and stained with hematoxylin and eosin and examined under light microscopy for pathological changes.

Microscopically, the major pathological lesions observed included extensive haemorrhages in various organs mainly the liver, kidney, lungs and brain. Cellular degeneration and necrosis were also commonly noted in the neurons, hepatocytes, and tubular epithelial cells especially of subacute and chronic cases. Cellular necrosis and degeneration in some cases seemed to be preceeded by intracytoplasmic changes like vacuolations then the nuclei would undergo either karyolysis or pyknosis. The necrosis was very marked in the liver, kidney, spleen and central nervous tissues

The brains of high dose recipients showed marked petechial haemorrhages. Extravasated blood could be seen in some portion of the brains near blood vessels (Plate 4). Perivascular vacuolation was a common finding in both acute and chronic but more so in chronic cases. The vacuolation appeared as clear zones around congested blood vessels. The blood vessels in the brain and spinal cords were in most cases highly congested. Many neurons showed degenerative and necrotic changes including karyolysis and pyknosis of nuclei. The neurotoxic effect seems to have affected the purkinje cells of the celebella more than other neurons. Some neurons in brains and spinal cords had marked perineuronal vacuolation which appeared as clear zones around the affected neurons Plate 5.

The alimentary canal of rats which died of acute intoxication showed marked congestion of the blood vessels in the tunica submucosae. In some rats there

was necrotic enteritis where the mucosae were necrotic especially the apex of villi of small intestines. The invaginated portions of intestines showed marked necrosis of the mucosae especially of the outer layers. Blood vessels were highly congested. In other acutely intoxicated rats which did not have intussusception had little mucosal alternations, however, some showed haemorrhages into the lumina. The Peyer's patches were depleted in chronically affected rats which were killed at the end of the experiment.

Skeletal and cardiac muscles (striated m.) showed foci of haemorrhage. The haemorrhages were mostly clear in the thigh muscles. Patches of extravasated blood were seen in myofibres (Plate 30). Cross section of the muscles showed extrensive damage to blood vessels mainly capillaries (Plate 31). The endothelium appeared completely torn out. This led to escape of blood into myofibres. Myocytes also appeared to have undergone Zenker's degeneration and many had no nuclei.

Livers of acute cases showed marked congestion of blood vessels and haemorrhages. Hepatocytes were necrotic. Many of these cells had either pyknotic or karyolytic nuclei and intracytoplasmic vacuolation especially in the portal area. Cells appeared shrunk and looked smaller. Liver cells with intracytoplasmic vacuolation appeared with acentric nuclei. The nuclei were forced aside by the vacuoles in these cells. These intracytoplasmic vacuoles were negative to Sudan black B. stain for fat. This strongly suggests that

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The lymphoid tissues, in acute cases, were congested and haemorrhagic. Marked depletion of lymphatic nodules. The lymphocytic follicles depleted of the lymphoid cells especially in their lymphopoietic centres of chronic intoxication. Extensive lymphocytic cells degeneration and necrosis were mostly found in chronic cases. Hemosiderin depositions were also found in a few chronic cases.

In acute poisoning lungs were haemorrhagic where extravasated blood could be seen in the alveoli. Congestion of blood vessels was constantly noted. In the lungs, changes were accompanied with fibrinous exudate. The alveolar septa were infiltrated with lymphocytic cells (Plate 11). Some of the subacute and chronic cases had fibrinous exudate in the alveoli. Some portions of lungs of rats sacrificed at end of the study showed emphysema. Alveolar sacs appeared ruptured while adjacent portions were atelectatic (Plate 13).

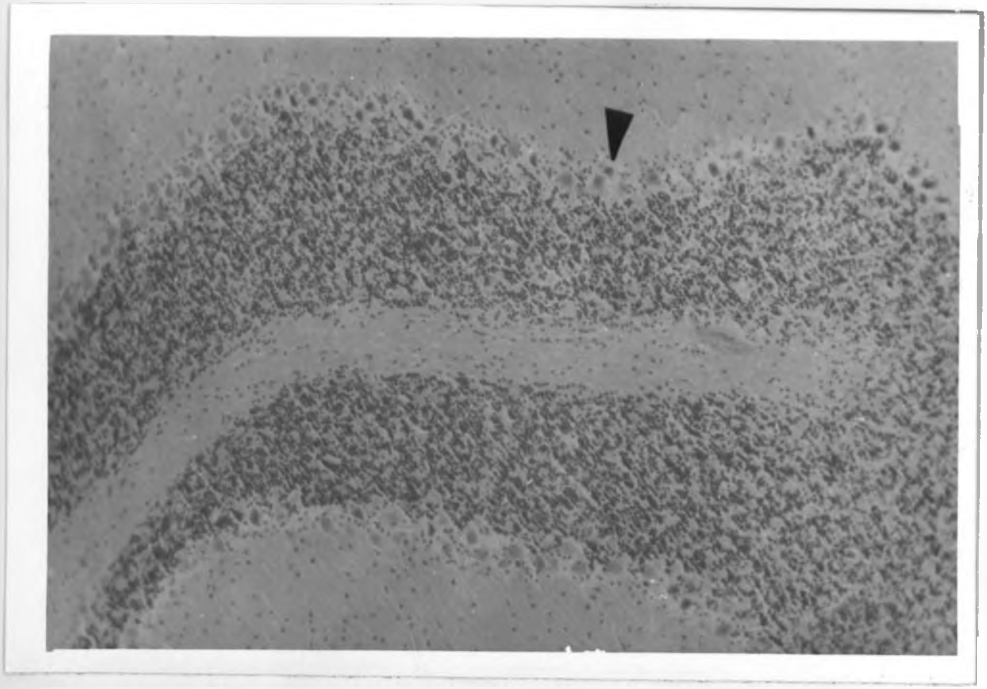

Plate 2: Rat Brain: Portion of cerebellum showing necrosis of purkinje cells in a folium. Many of these neurons are necrotic, their nuclei have undergone karyolysis (H&E x 400)

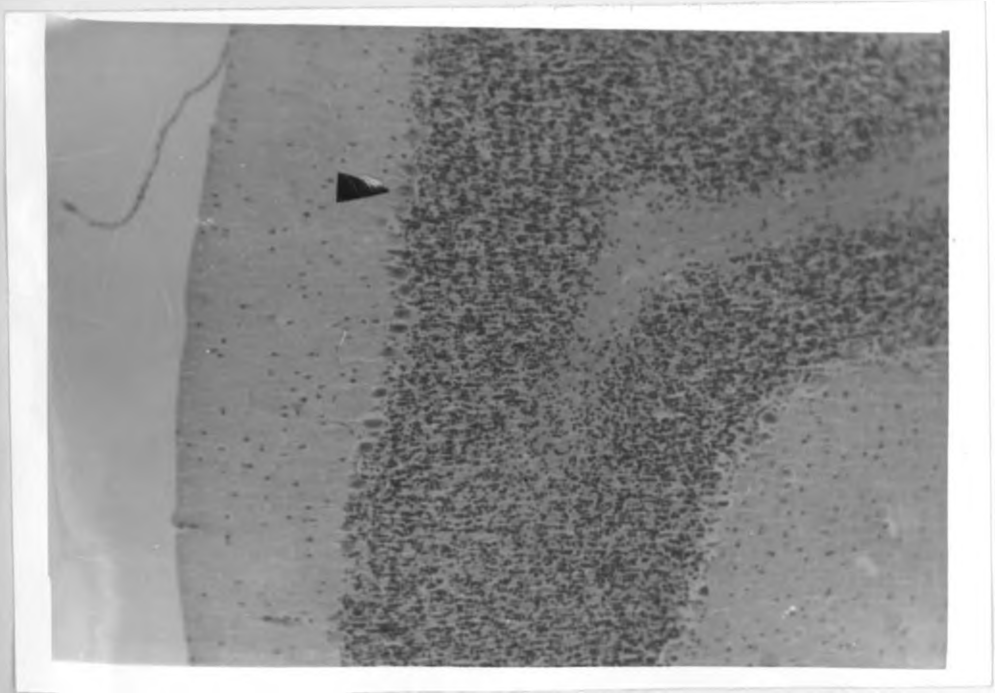

Plate 3: Rat Brain: Degeneration and necrosis of Purkinje cells in the brain. Many have lost their cellular integrity (H&E x 400).

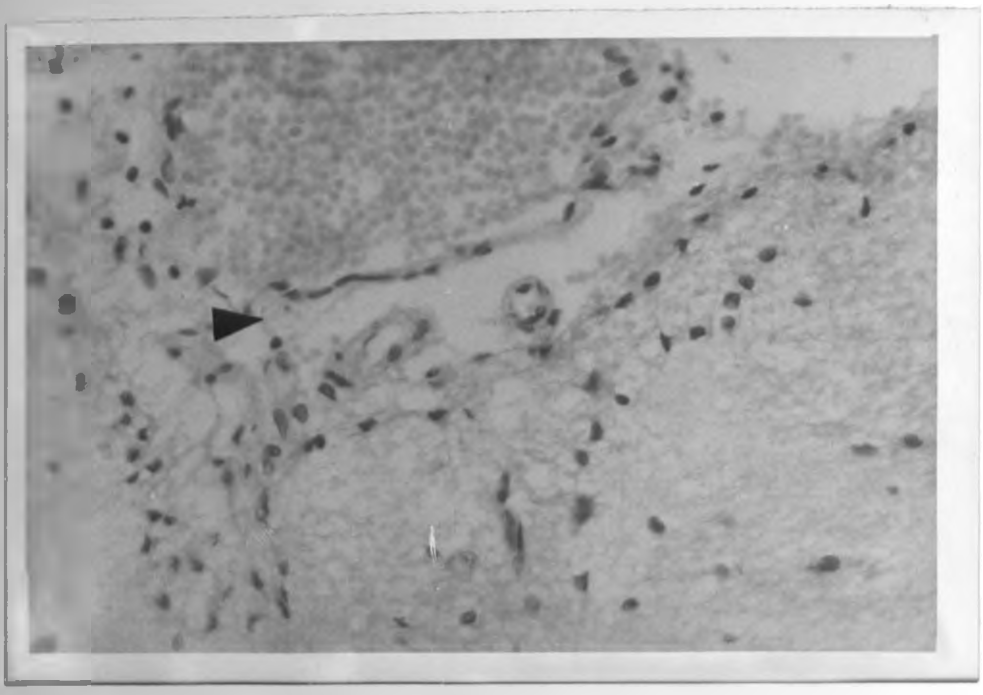

Plate 4: Rat Brain: Cerebral haemorrhage in the cerebrum of rat which died of acute toxicity after three repeat doses of 4.5 gm/kg. B.W. Damaged blood vessels and extravasated blood are seen (arrow). (H&E x 400)

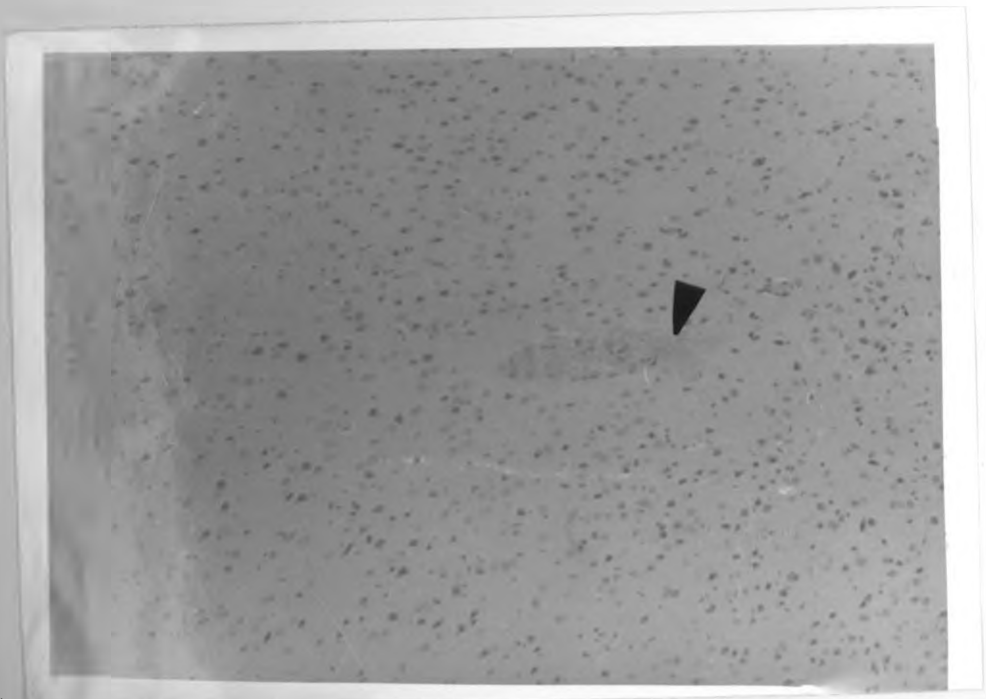

Plate 5: Rat Brain: Perivascular vacuolation and severe congestion of blood vessels. White zones around blood vessels and some neurons indicate vacuolation. One capillary has haemorrhagic zone (arrows). Such lesions were commonly found in rats which survived acute toxicity (H&E x 200).

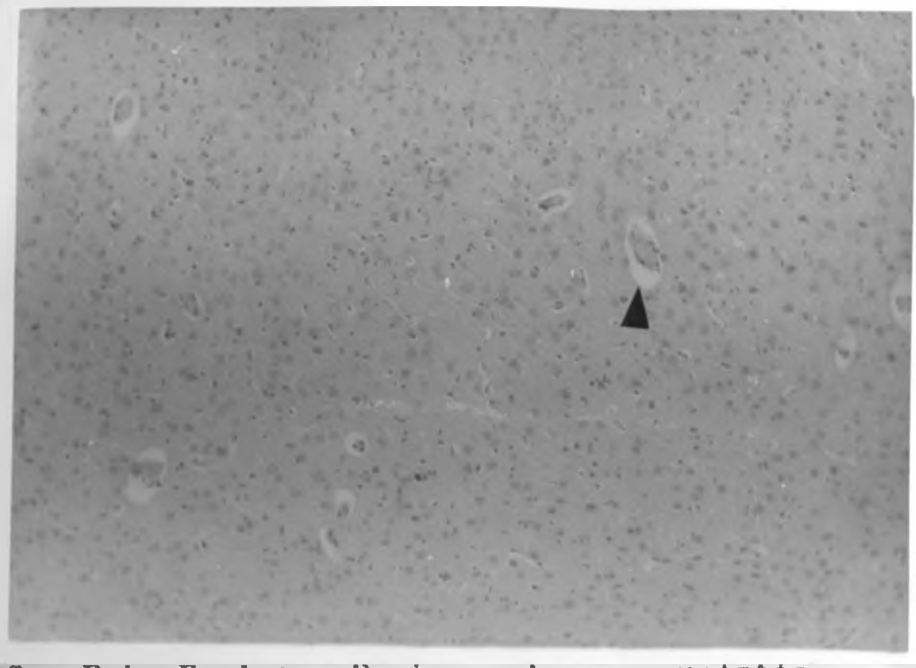

Plate 6: Rat Brain: Perivascular vacuolation and pyknosis of nerurons in the brain of rats. Nuclei of neurons appear contracted and roundish. This was mostly found in rats which received low dose and were killed in extremis (H&E x 100).

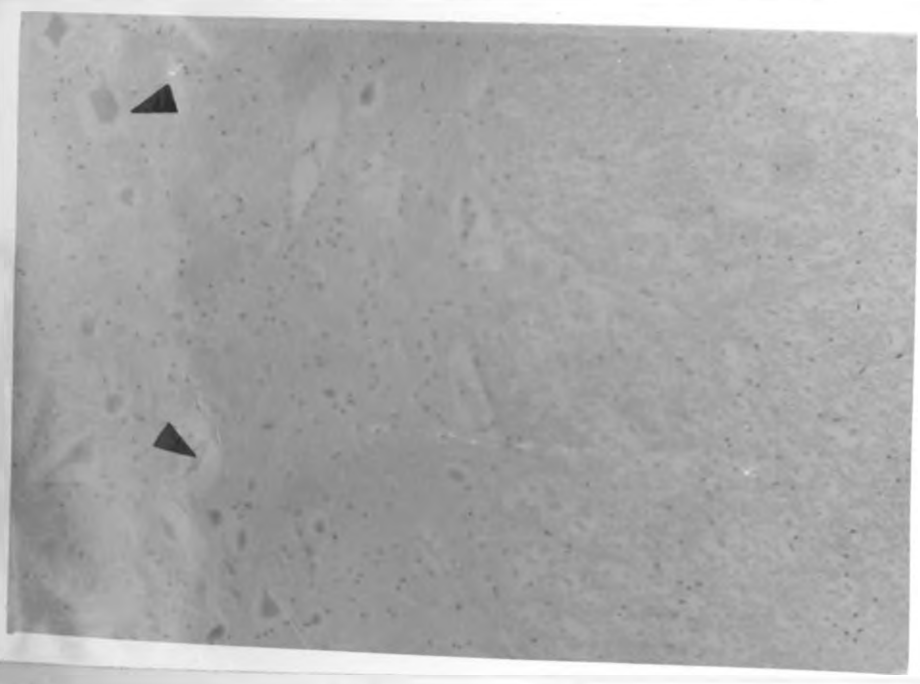

Plate 7: Rat Spinal Cord: Marked perineuronal vacuolation in the gray matter of the spinal cord. Nuclei of some neurons have already undergone Karyolysis (arrow). (H&E x 400).

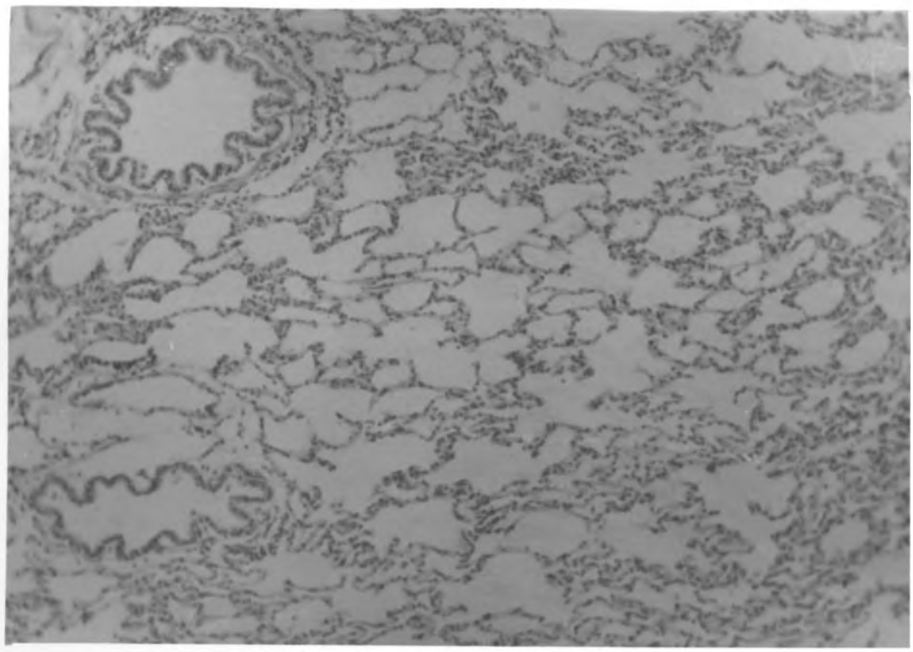

Plate 8: Rat lung: Normal lung from control rat which received innoxious saline (H&E x 100).

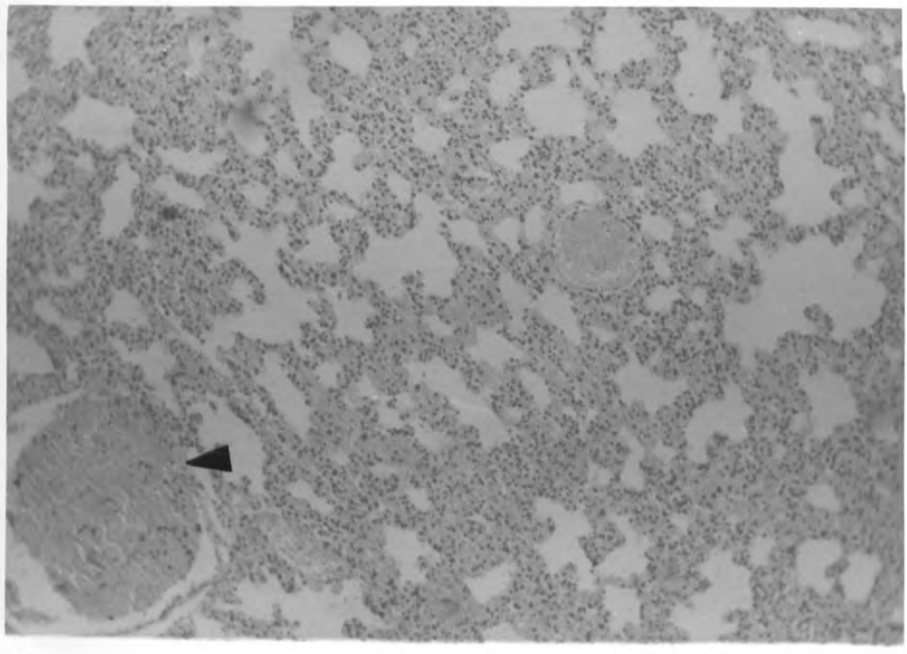

Plate 9: Rat lung: Severe congestion of blood vessels and all the capillary network of the alveolar sacs. The blood vessels walls appear to have been damaged and haemorrhage has occured (arrow). (H&E x 200).

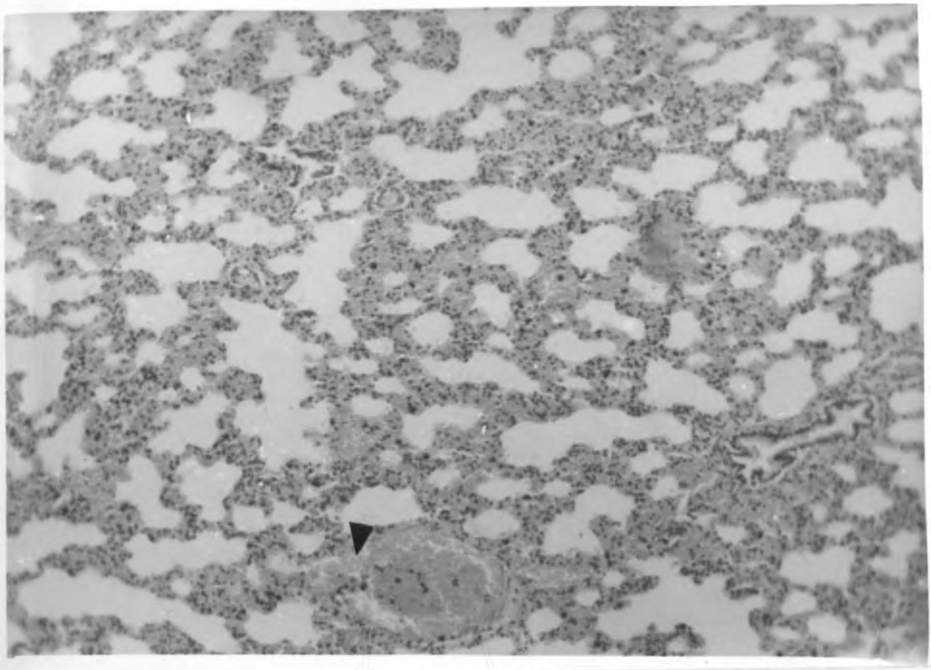

Plate 10: Rat lung: Severe congestion of blood vessels and all the capillary network of the alveolar sacs. The blood vessels walls appear to have been damaged and haemorrhage has occurred (arrow). (H&E x 200).

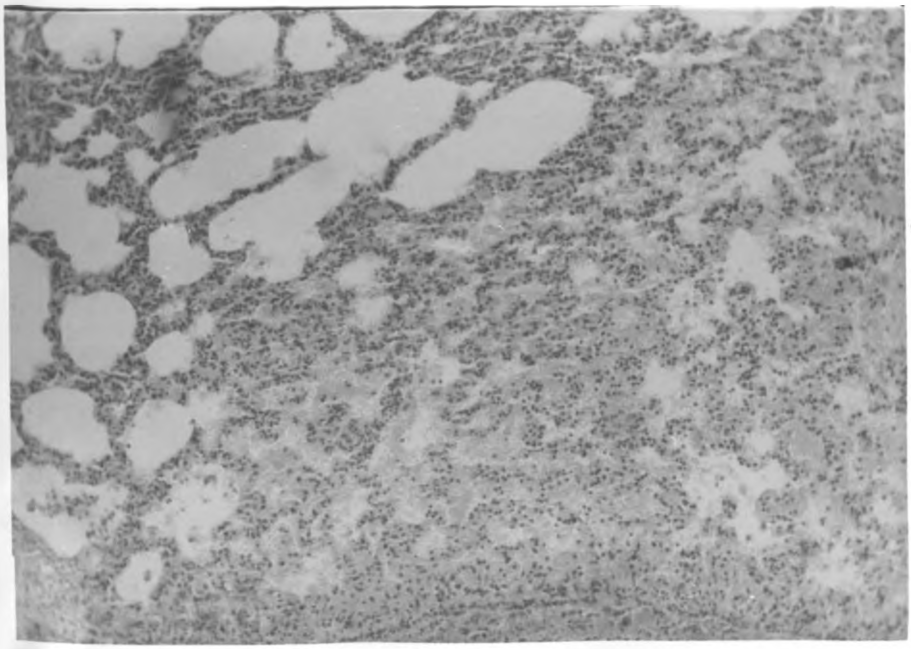

Plate 11: Rat lung: Extensive fibrinous exudate in the alveoli of which died of acute toxicity of A. remota. Emphysematous alveolar sacs are seen in the adjacent portion (H&E x 100).

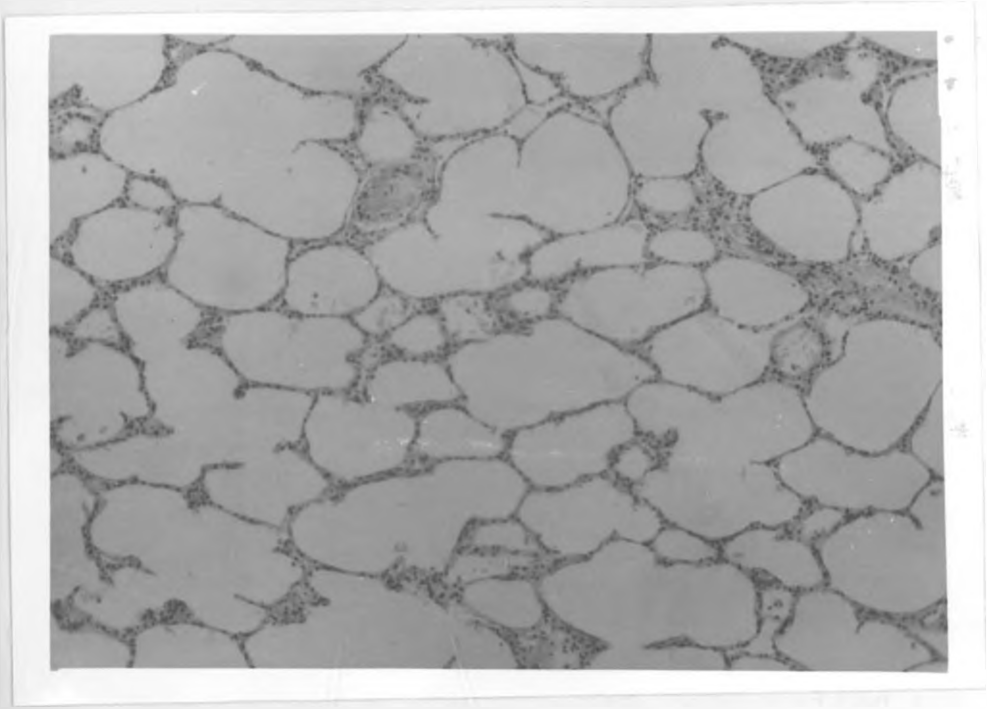

Plate 12: Rat lung: Emphysema in the lung. Alveolar sacs appear extended with gases. Some are ruptured. This was mostly seen in cases survived for more than two weeks of repeat doses. (H&E x 100).

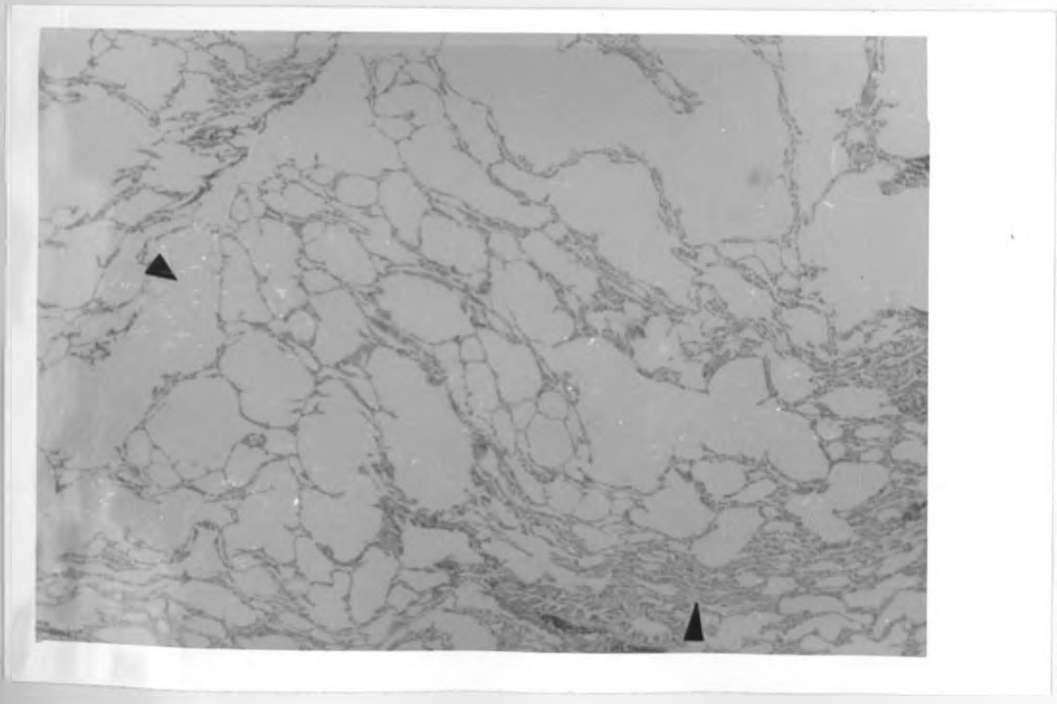

Plate 13: Rat lung: Emphysema accompanied with atelectasis. Marked alveolar sac rupture and collapse of alveoli in the adjacent portion. (H&E x 100).

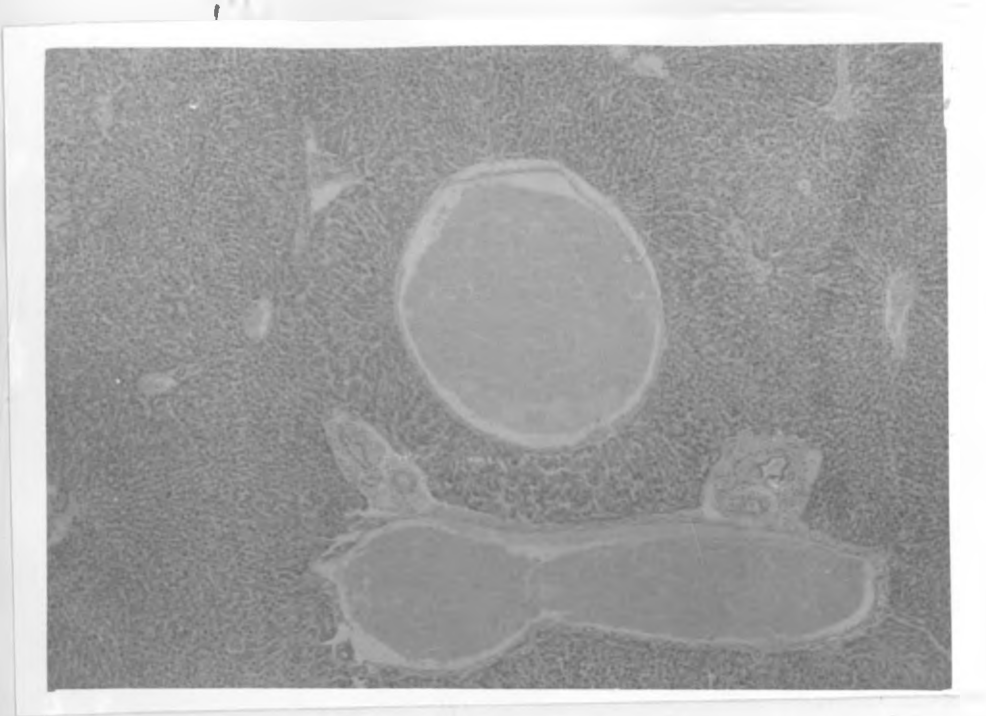

Plate 14: Rat liver: Severe congestion of blood vessels in the liver marked degeneration and necrosis hepatocytes. (H&E x 100).

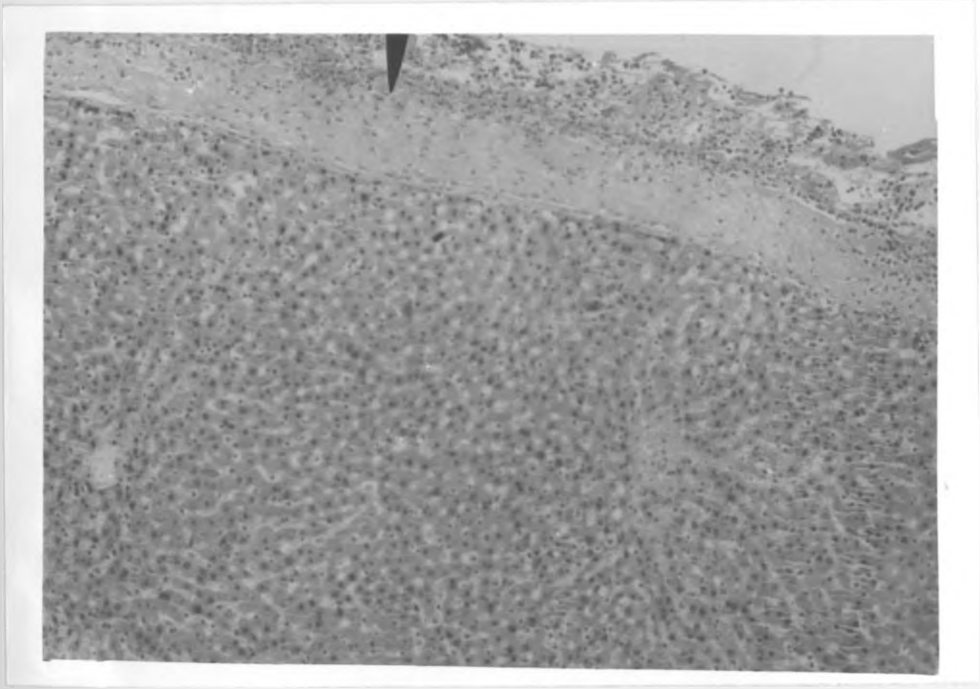

Plate 15a: Rat liver: Intracytoplasmic vacuolation in the hepatocytes. Many cells appear necrotic. Necrotic hepatitis is marked, the liver capsules is necrotic, with fibrin and inflammatory cells covering it. Similar lesions were seen where rats received low dose and survived long up to 20 days (H&E x 200).

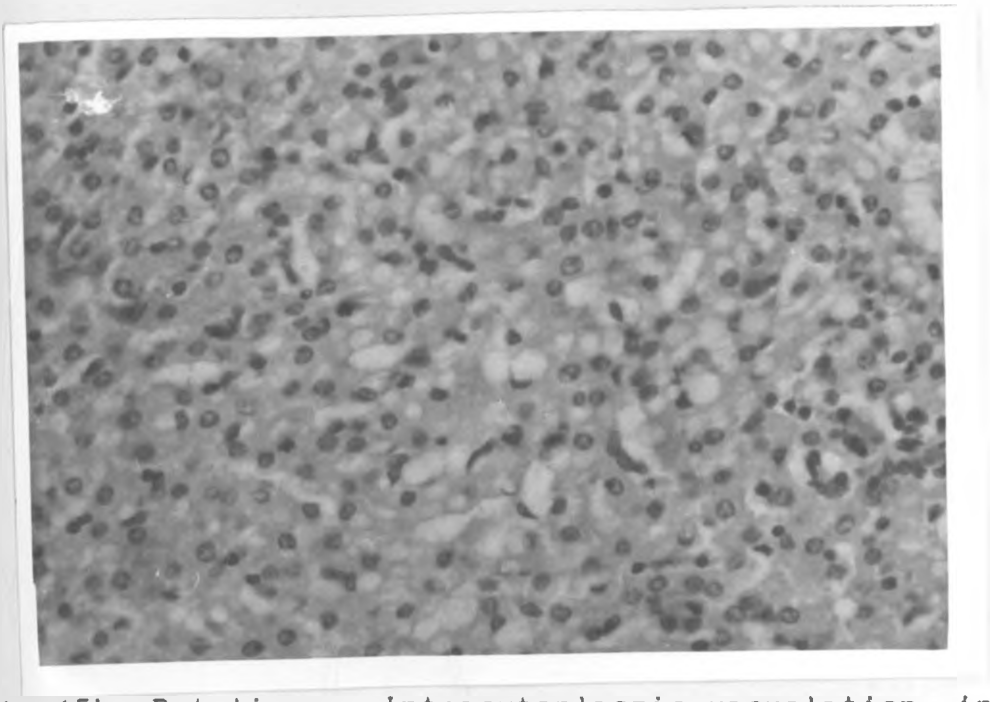

Plate 15b: Rat liver: Intracytoplasmic vacuolation in the hepatocytes, (H& E x 400)

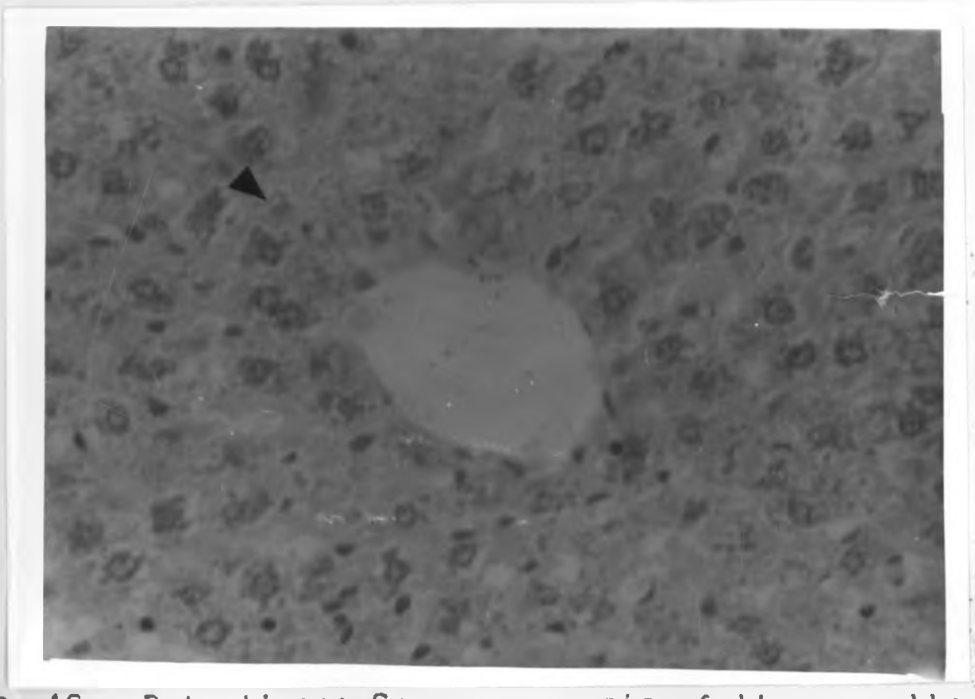

Plate 16: Rat liver: Severe necrosis of liver cells around the central vein. Many cells are swollen and their nuclei have undergone karyorrhexis. Nuclei are disintegrated. Such lesions were seen in rat which died of acute toxicity within 4-7 days of treatment (H&E x 400).

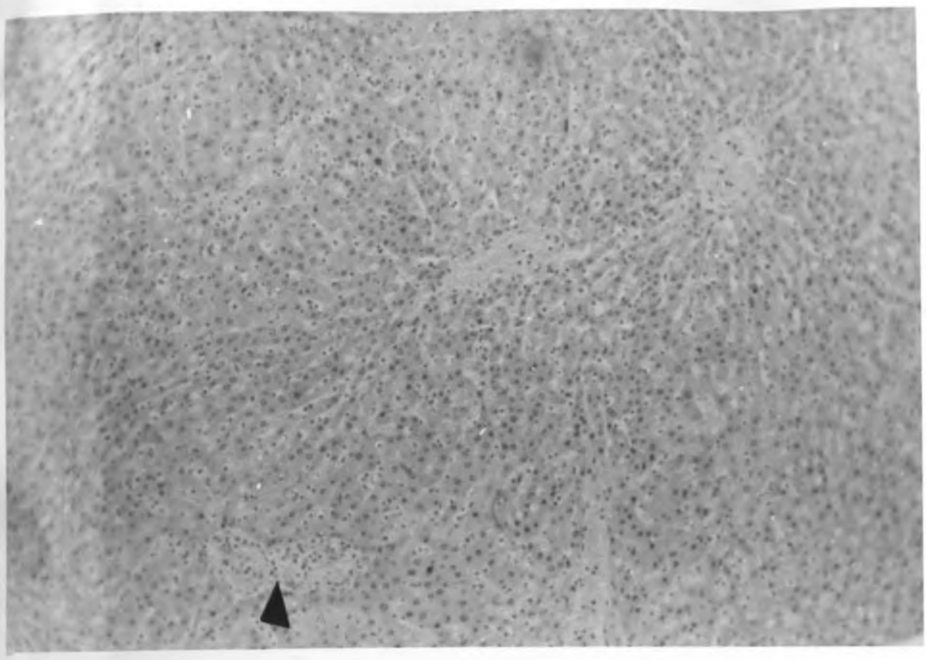

Plate 17: Rat liver: Necrosis of the liver cells. Many heptatocytes nuclei have undergone either pyknosis or keryorrhexis while fibrosis has started (arrow). (H&E x 200).

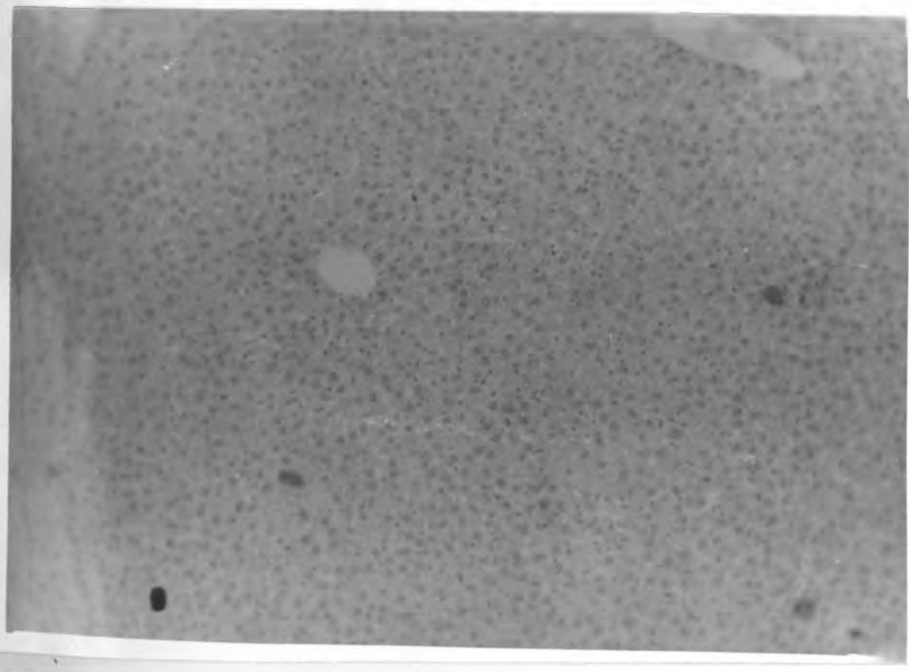

Plate 18: Rat liver: Necrosis of liver cells accompanied by marked pyknosis and karyolysis of nuclei. Many hepatocytes are necrotic. They have lost their cellular characteristics. (H&E x 200)

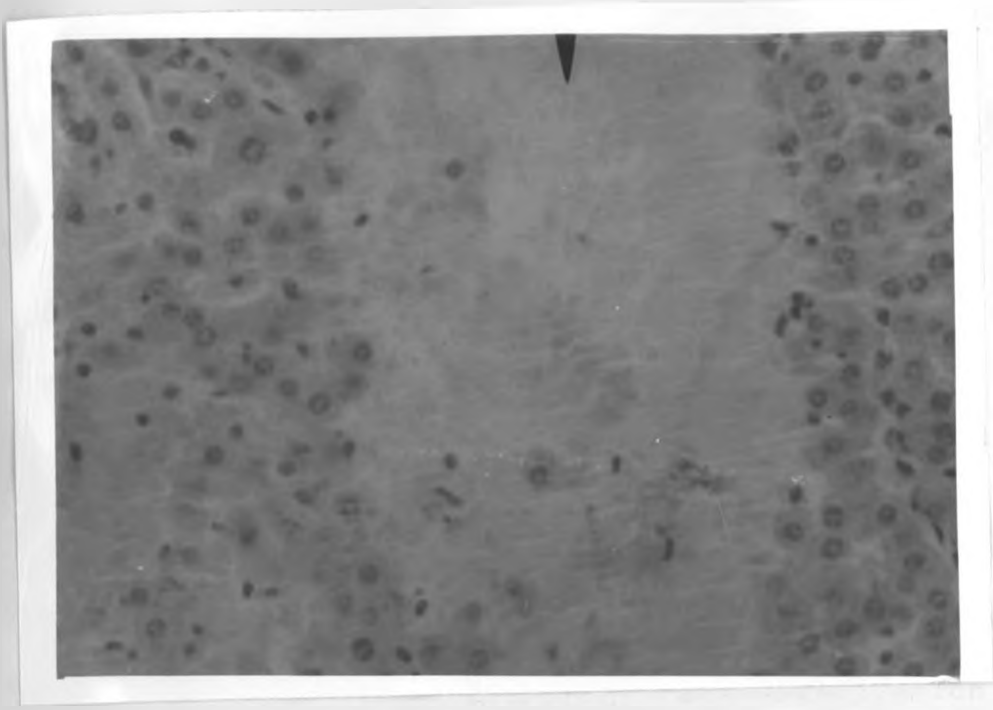

Plate 19: Rat liver: Focal coagulative necrosis of the liver. Entire field has necrotized while the neighbouring are undergoing degenerative changes (H&E x 400)

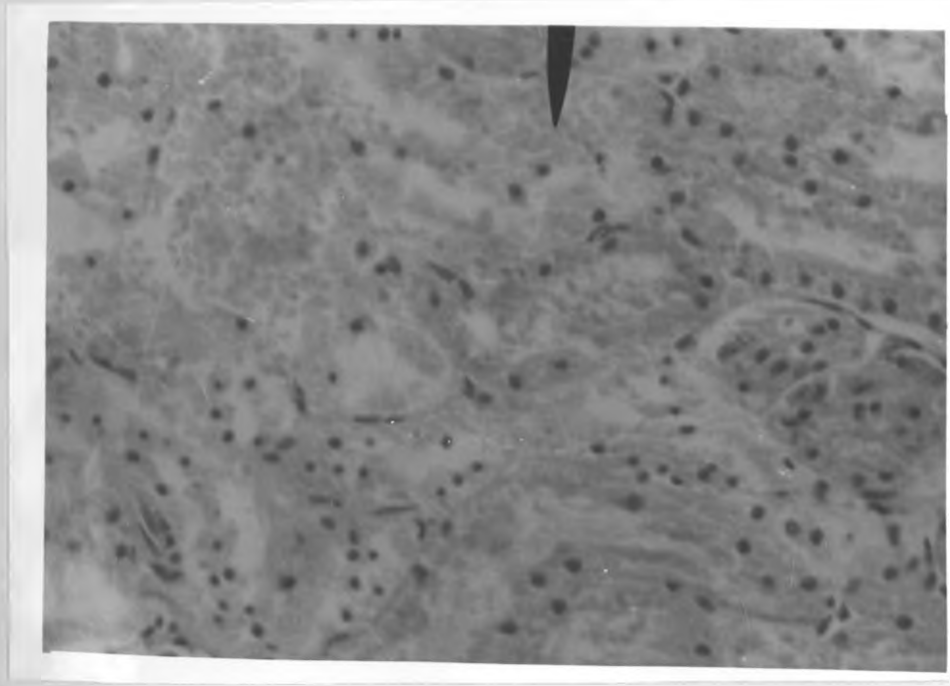

Plate 20: Rat Kidney: Extensive haemorrhage and diffuse coagulative necrosis of uriniferous tubules, in the cortex. Very few of the tubules remain with living cells. However, even those remaining are severely degenerated. The lumina appear filled with necrotic material. (H&E x 400).

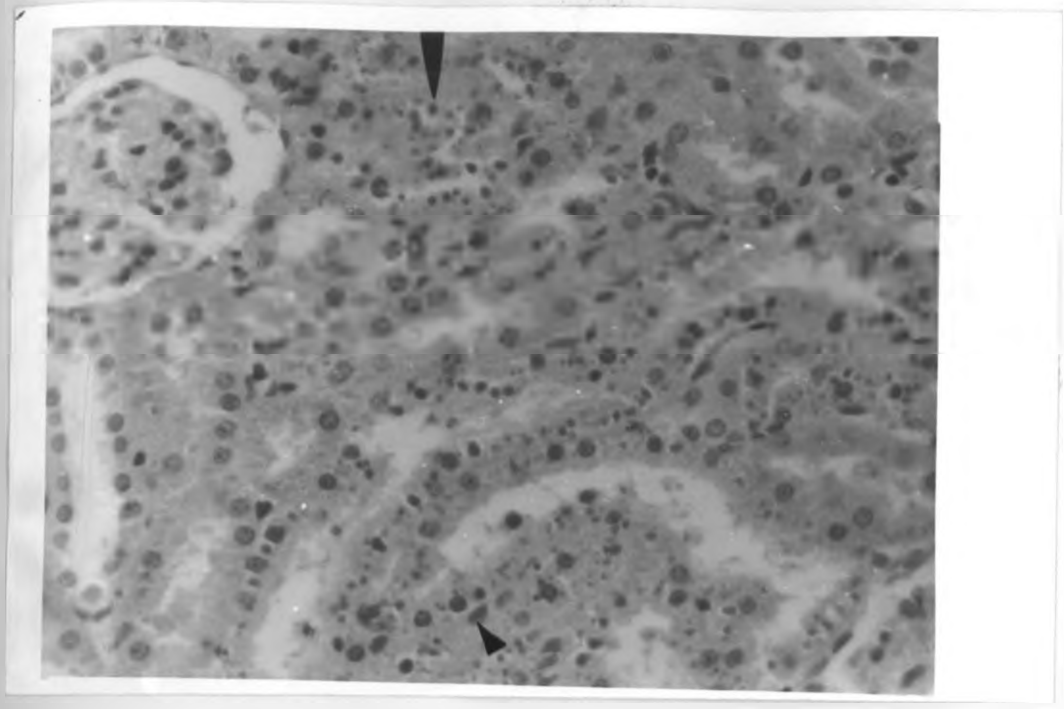

Plate 21: Rat Kidney: Hemosiderin granules which appear as dark roundish spots in the epithelial cells of tubules in the cortex. In the tissue stained with hematocylin and eosin, the deposits were reddish-brown polymorphic pigment particles in proximal uriniferous tubules (H&E x 400).

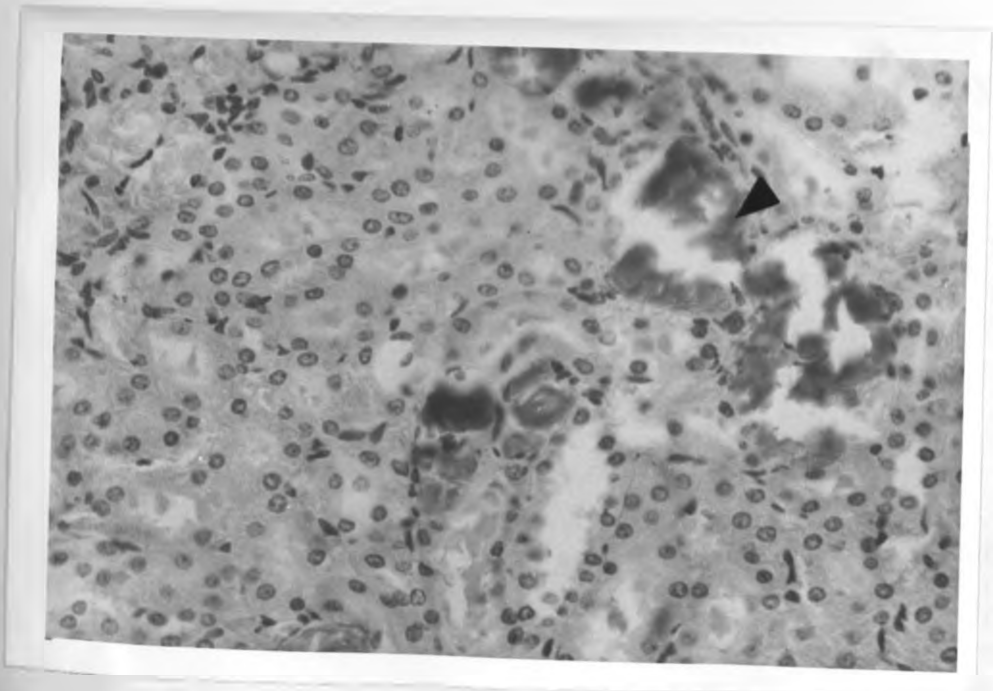

Plate 22: Rat Kidney: Focal liguidative necrosis of the kidney. A few fibrocytes have started surrounding the necrotized tissue (arrow). (H&E x 400).

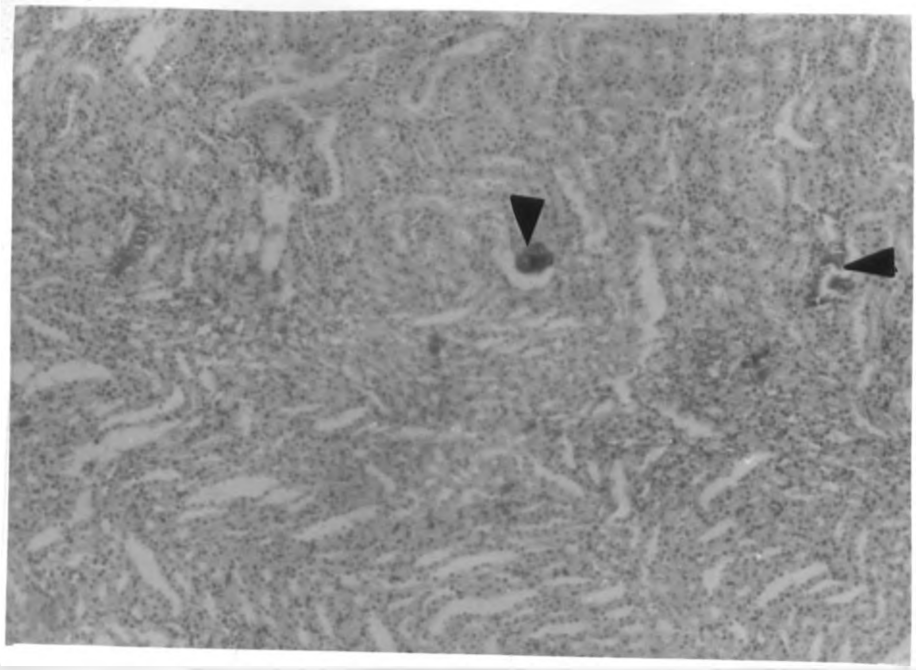

Plate 22a: Rat Kidney: Multiple focal coagulative necrosis. Foci of necrosis are seen (arrow) in the medulla. Such lesions were mostly seen in rats which died of acute toxicity. (H&E x 400).

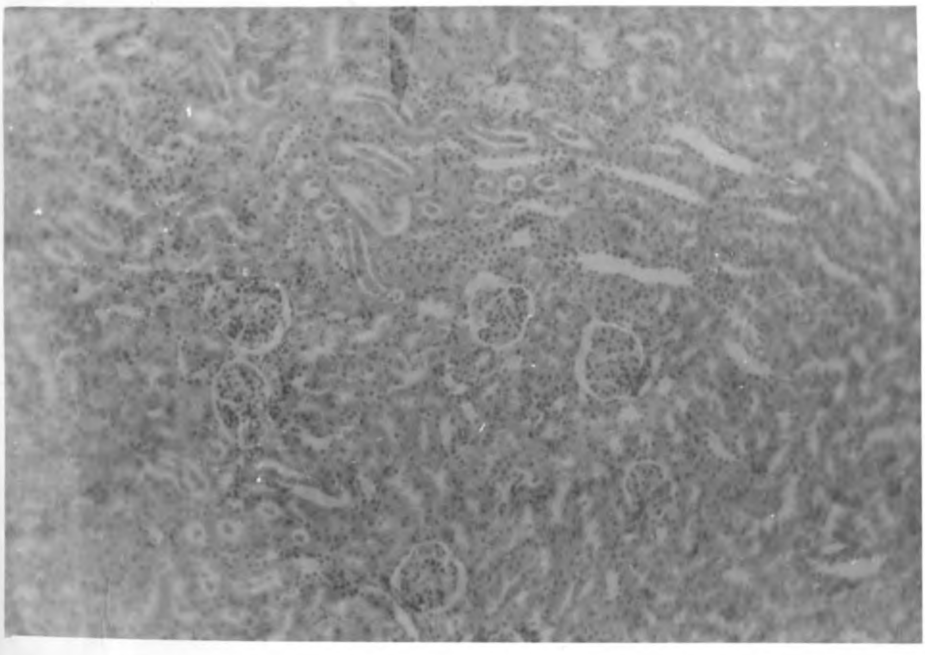

Plate 23: Rat Kidney: Diffuse degeneration of tubules in the cortex of the kidney of rat which received medium dosage and survival for about 20 days. Hyaline cast is seen in the lumina of the tubules. Many tubular epithelial cells demonstrate necrotic changes (H&E x 400).

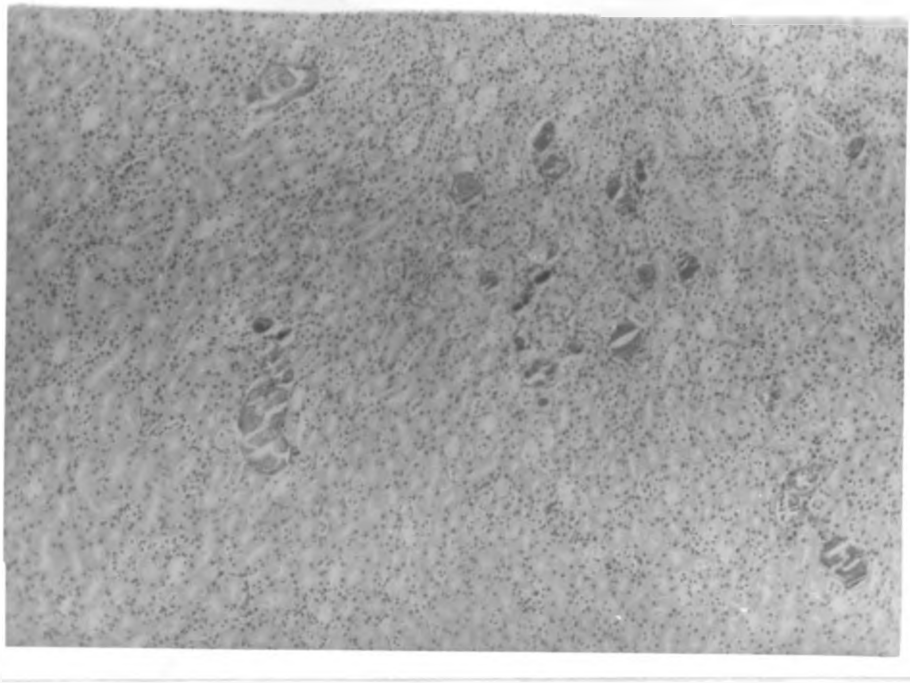

Plate 24: Rat Kidney: Multiple focal coagulative necrosis. Foci of necrosis are seen (arrows) in them medulla. Such lesions were mostly found in rats which died of acute toxicity (H&E x 100)

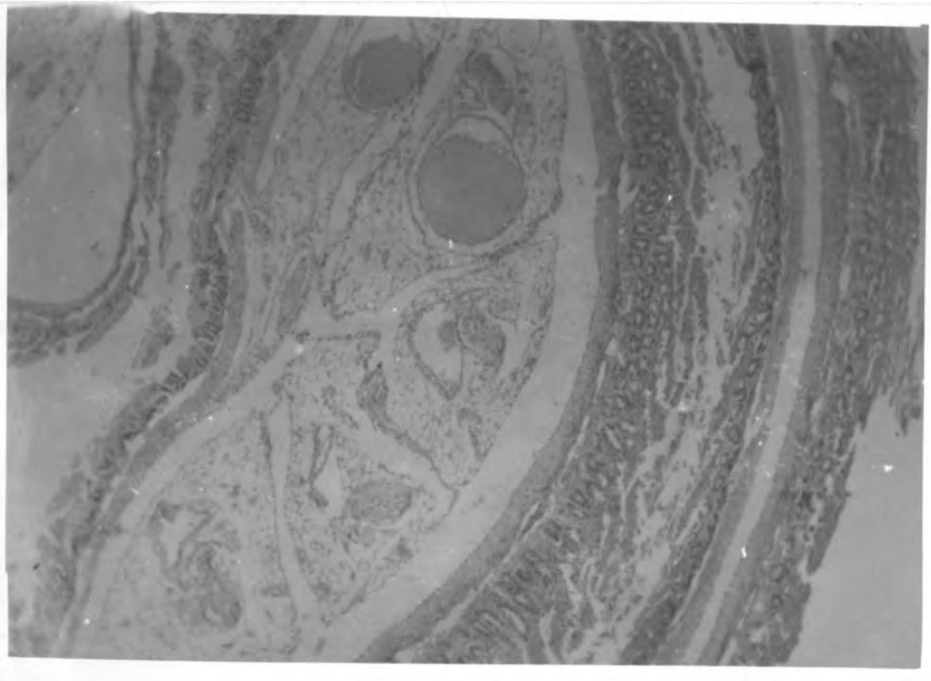

Plate 25: Rat intestine: Cross-section of small intestine (jejunum) it demonstrates cross-sectioned intussusceptum which is accompanied by severe congestion of adventitia connective tissue of the inner layer. The mucosae of all intestine wall appear necrotic. These cases were seen in in highest dose recipient (H&E x 100)

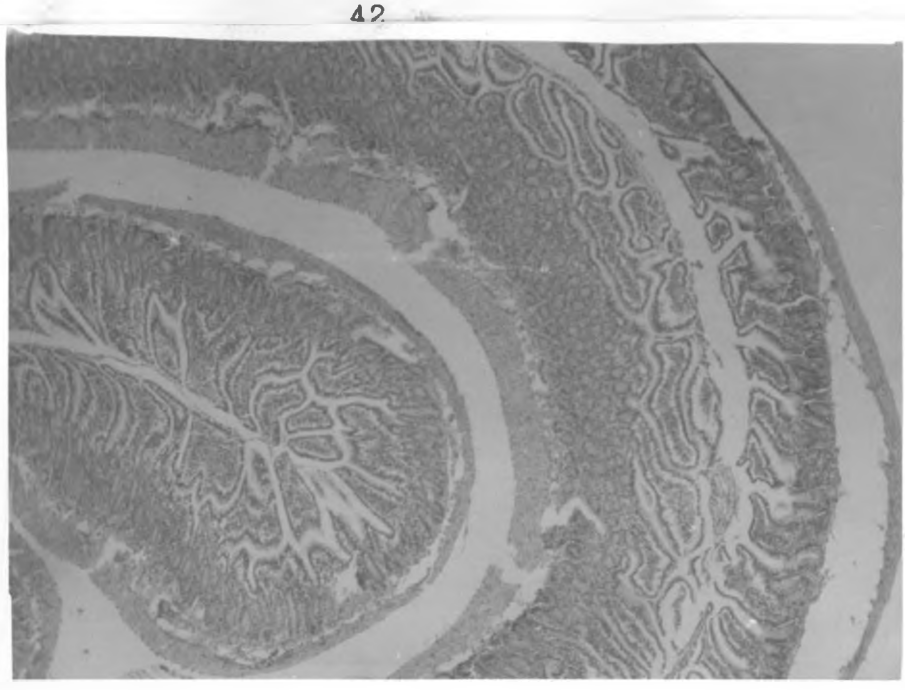

Plate 26: Rat intestine: Intussusceptum cross-section in the jejunum portion of small intestine (H&E x 50).

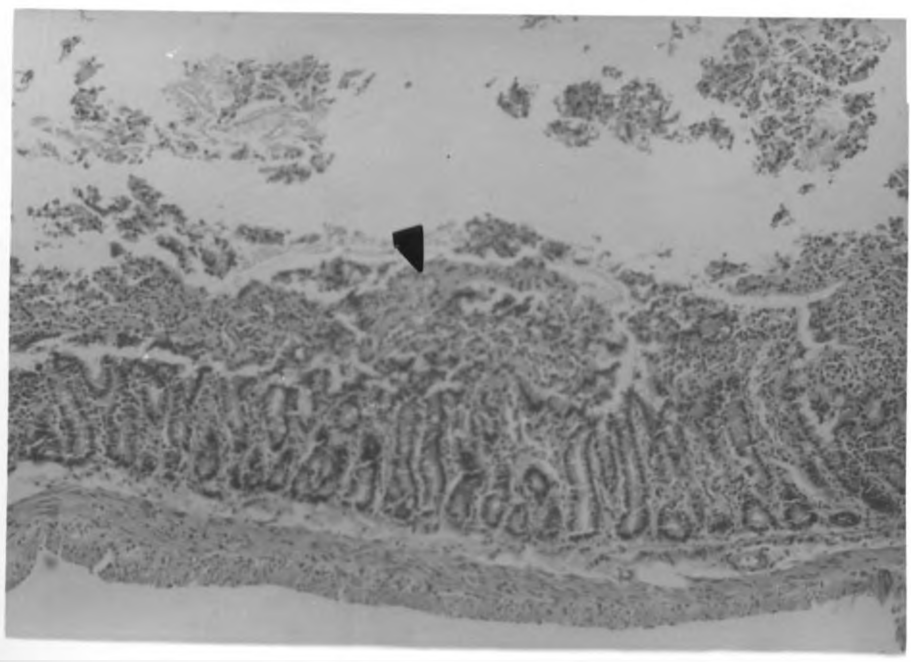

Plate 27: Rat intestine: Cross-section of intestine (duodenum) shows necrotic enteritis. A large portion of mucosa including the villi is necrotic. Lesions of this nature were mostly seen acute toxicity cases (H&E x100).

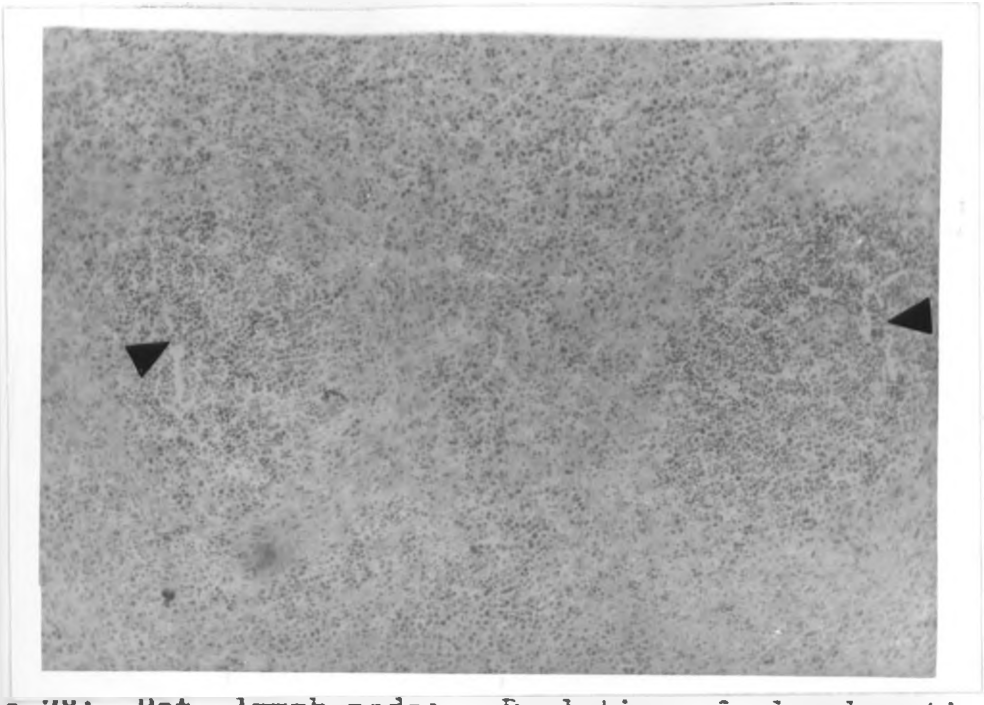

Plate 28: Rat lymph node: Depletion of lymphocytic nodules in the cortex of mesenteric lymphnode. Lymphoid cells area is greatly reduced and are being replaced with fibrocytes. (H&E x 100).

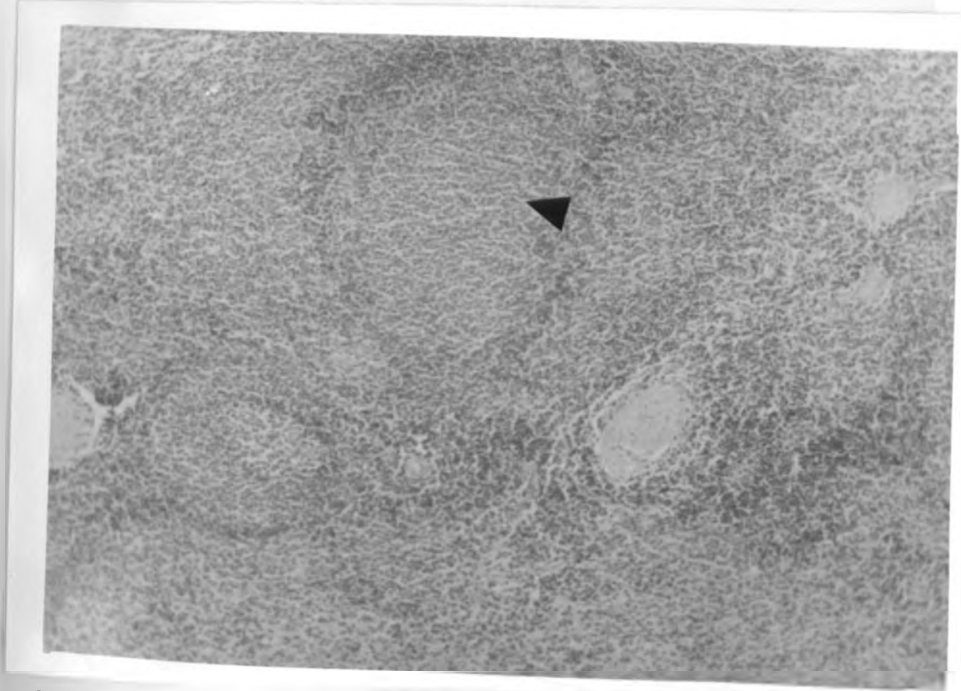

Plate 29: Rat spleen: Depletion of lymphocytic nodule in the spleen (H&E x 100).

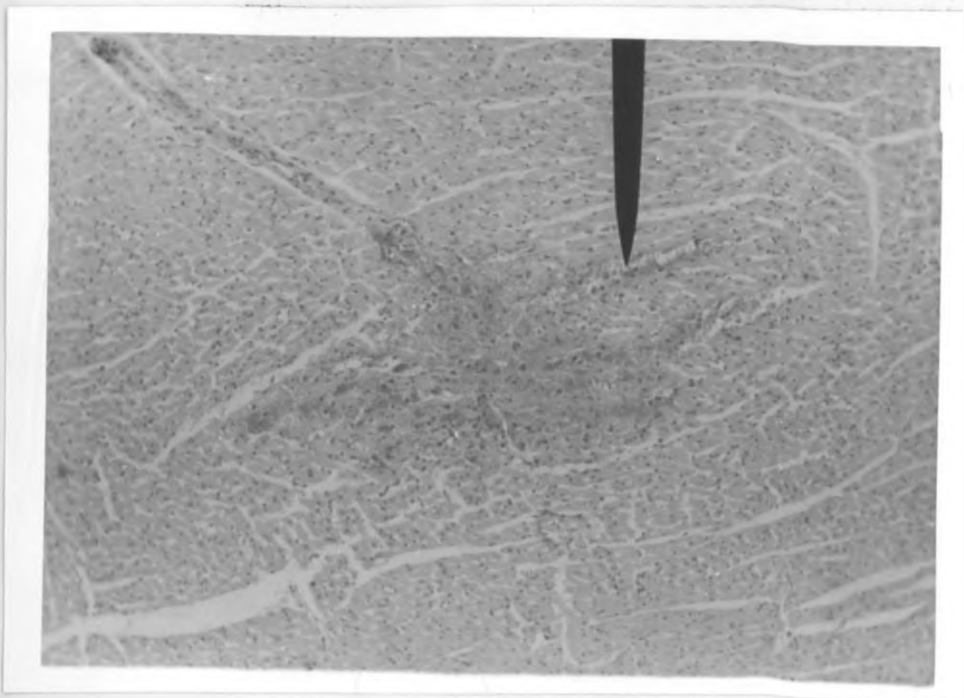

Plate 30: Skeletal muscle: Cross section thigh skeletal muscle showing extensive haemorrhage. Extravasated blood in myofibres (H&E x 100).

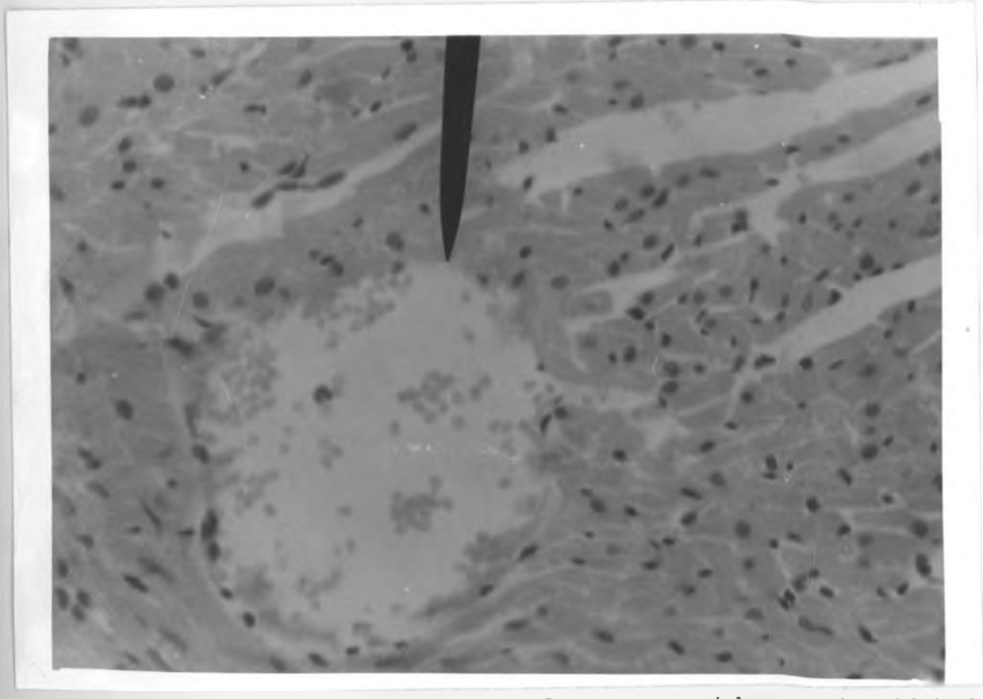

Plate 31: Skeletal muscle: Cross section of thigh muscle severely damaged capillary is shown with its endothelium completely torn out leading to escape of blood into the surrounding tissue (arrow). Myocytes also appear degenerated Zenker's degeneration (H&E x 400).

4.0 DISCUSSION

The nervous clinical signs induced by remota intoxication were in parallel to the finding of Kubo et al. (1976) that ajugarins did not exhibit neuromuscular blocking activity. Neuromuscular blocking signs were not observed in this study. Wakori et al., (1986) reported the use of A. remota in traditional medicine. The aqueous extract was used to treat stomach-ache, asthma, malaria, headache, nose bleeding, increased heart beat, bleeding in women, post delivery bleeding and pain. This study indicates that the aqueous extract has a strong stimulating activity on gastrointestinal musculature. The ability for the extract to cause intusussception in small intestine of rats, suggested that it has adrenergic effect on the sympathetic neuroeffector junction of the intestinal muscles. So it is assumed that by this mechanism the peristaltic movements are increased and as a result a purging effect is achieved. The lesion induced by the extract in the cardiovascular system show that the active principles act on these organs. It seems there is a decrease in cardiac output and endothelial damage leading to generalized congestion (congestive heart failure) and haemorrhages respectively. The flaccidity and Zenker's degeneration noted at necropsy and microscopic picture respectively, indicated the possibility of reduction of cardiac output due to decreased myocardial contraction. This may probably substantiate the traditional use of remota to treat bleeding. However, the potentiality which may be posed

by A. remota to cause hypotension in the subject should not be underestimated.

Hydroperitoneum and hydrothorax can be associated with necrotic and degenerative changes seen in the liver. These lesions indicate curtailed hepatic activity which might have led to low total plasma protein (T.P.) output.

The lesions induced by remota poisoning in the spleen, lymph nodes, adrenal glands were similar to those reported by Kiptoon (1981) in the rat exposed to an extract of Gn. latifolia. It appears lymphocytic cells are some of the target cells in remota intoxication. Hemosiderin deposition in these organ in addition to kidney of rats which underwent prolonged treatment indicates elevated destruction of red blood cells by histiocytes in lymphoid organs.

The pyknosis, karyorrhexis and karyolysis noted in the parenchymal organs and central nervous tissues are similar to results reported by Grisham (1979) when evaluating morphological irreversible changes in necrotic cells. Cytotoxic effects were exerted by the extract to cells of these organs. The perineuronal and perivascular vacuolations in the CNS together with macroscopic picture are indicative of cerebral oedema. Increased permeability and hyproteinemia are likely to have contributed.

5.0 EXPERIMENT ONE

PART II

This experiment was carried out in order to try to assess the development of lesions in various tissues following a single administration of a sub-lethal dose of aqueous extract *Ajuga remota* Benth.

5.0.1 Materials and Methods

Experimental Animals

Seven ten-week-old female rats of Sprague-Dowley breed weighing between 200 to 241 gm were obtained from small animal unit of International Laboratory for Research on Animal Diseases (ILRAD) at Kabete. They were accommodated in two German cages made of filtered polycarbonate with steel covers. Bedding of wood-shavings was provided. Good standards of cleanliness were strictly followed by regular changing of beddings and washing cages with detergents. An approved commercial Unga mouse and rat grain ration and water supply in feed bottles with metallic tubes were available *ad libitum*. An initial acclimatization period of 10 days was allowed prior to the inception of the experiment. Daily observation was done to monitor any changes in habits.

Generally they were treated as the previous colony in all aspects, except there was no daily weighing.

At the age of eleven and half weeks, after

acclimatization, rats were weighed. Quantities of doses were calculated and adjusted according to their respective body weights. It was decided that all rats receive a uniform dose of 3500 mg/kg of body weight subcutaneously.

An aqueous extract of a concentration of 30% was prepared from the stock powder extract. 30 mg of powder were weighed and dissolved into 100 ml of distilled water. It was put into universal bottles and sterilised under a pressure of 15 lbs at 121°C. It was allowed to cool at room temperature.

5.0.2 RESULTS

5.0.2.1 Clinical observations

Rats were closely observed for any Clinical signs arising from the toxicity. There was an appearance of raised coat in all rats 2-4 hours after receiving injections. A tendency of an increased water intake was noticed. Food consumption decreased during day one. On day 2 there was some tendency of arched back in most of the rats which remained. The arched back was not so pronounced as in previous experiment.

5.0.2.2 Necropsy procedure

Serial necropsies were started being carried out 24 hours after injecting aqueous extract. Rats were necropsied one per day until all the seven rats

were completed. Animals were picked from their cages at random. They were immediately euthanized with chloroform and post-mortem examination followed.

5.0.2.3 Gross lesions

Gross lesions were pronounced in the 1st and 2nd rats of first and second day respectively. Among the major macroscopic lesions seen included; slight inflammatory reactions around the site of injection, liver and cerebral congestion was marked in the first two cases. Some petechiae were seen in the liver of the first necropsy, but non in subsequent ones. Mesenteric blood vessels were also congested. All necrospies done from day 3 up to day 7 did not demonstrate any significant macroscopic lesions.

Specimens of tissues were collected from almost all organs as in experiment one. Fixation was done in 10% neutral buffered formalin for at least 48 hours.

Microscropic lesions

Tissues were trimmed and processed by an automatic processor. They were embedded in paraffin wax. Section of 5-6 microns thick were cut and stained with hematoxylin and eosin (H&E) then examined histopatholigically by light microscopy.

The brain of the rat killed on day one had congested blood vessels accompanied with haemorrhage in the cerebellum. Areas around blood capillaries showed

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The brain of the rat killed on day one had congested blood vessels accompanied with haemorrhage in the cerebellum. Areas around blood capillaries showed

some degree of development of perivascular vacuolation. Perineuronal vacuolation was not noted.

Lungs apparently appeared normal while the liver demonstrated endothelial damage to blood vessels (Plate 34). The blood vessels were congested. Haemorrhage was marked. Hepatocytes necrosis was noted. Some cells had undergone cloudy swelling with karyorrhexis of nuclei.

The kidney had foci of coagulative necrosis. This was seen in the subcortical zones of the kidneys of 1st and 2nd rats (Plate 36) Damage to endothelial lining accompanied by severe haemorrhage was marked mainly in the subcortical and medulla zones. Proximal renal convoluted tubule epithelial cells demonstrated degeneration. This was more severe in the rats killed on day one and two. However, subsequent cases showed some mild degeneration of tubules. Many tubular epithelial cells appeared to have lost their nuclei.

Intestinal lesions were mostly seen in the rat killed on day one. There was severe lymphocytic infiltration of the doudenal mucosa. The apex of the villi were necrotic and in some portions eroded and no cellular elements recognizable. The lymph node depletion was noted in the first case.

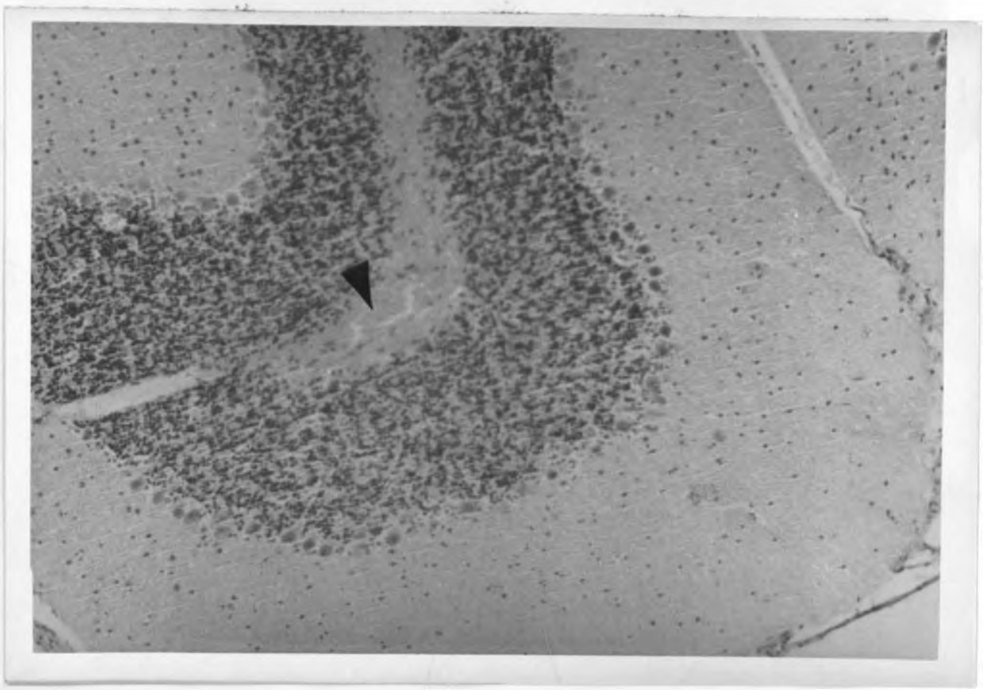

Plate 32: Rat Brain: Cerebral haemorrhage in the centre of a folium (arrow). This was noted in the rat killed on day one (H&E x 200).

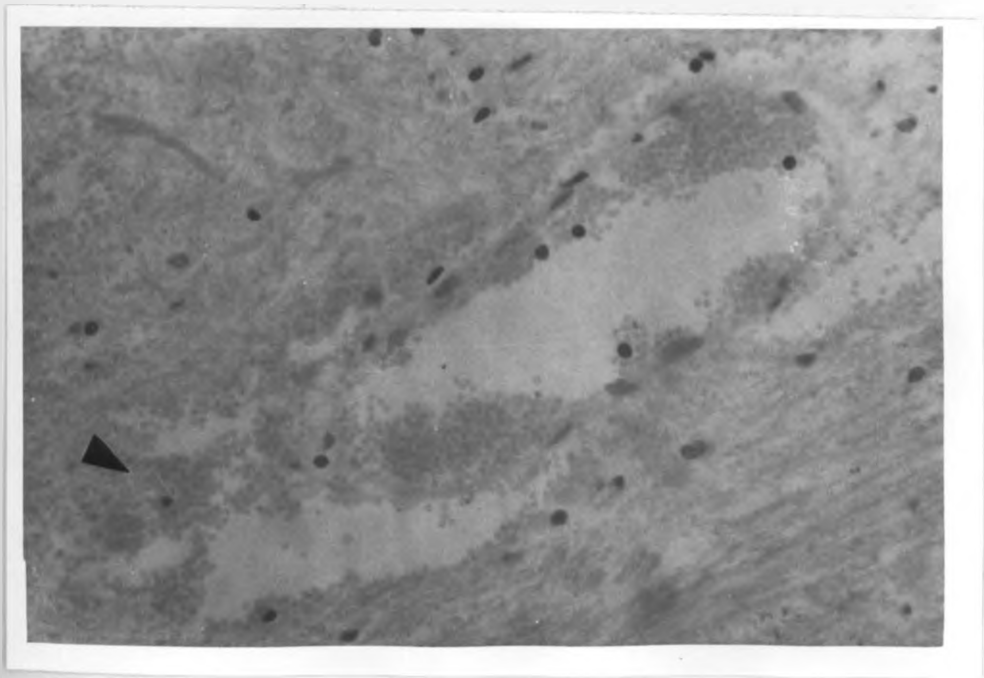

Plate 33: Rat Brain: Cerebral haemorrhage, the blood capillary is damaged and blood is seen to have been extravasated (arrow), (H&E x 400).

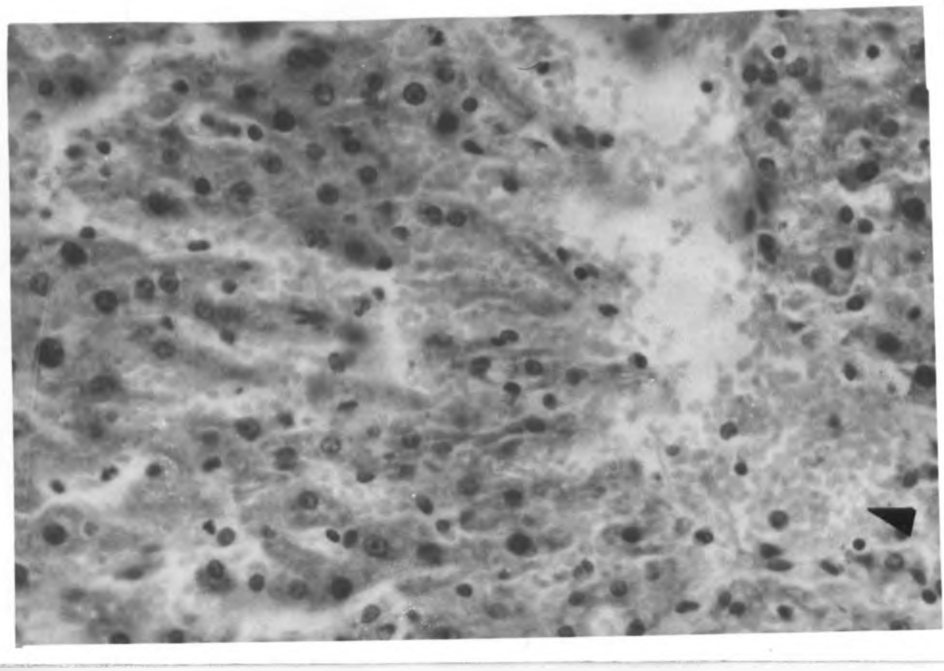

Plate 34: Rat liver: Extensive damage to endothelium accompanied with severe hepatic haemorrhage. Erythrocytes are seen spread in haemorrhagic zone (H&E x 400).

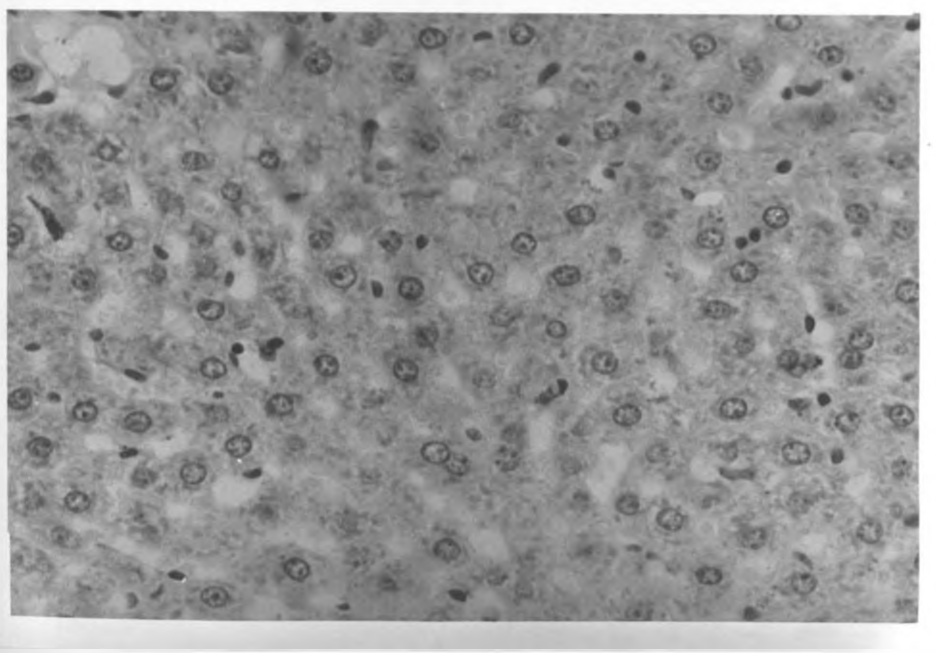

Plate 35: Rat Liver: Necrotic hepatocytes. Many liver cells have lost their cellular characteristics and their nuclei have undergone karyorrhexis, (H&E x 400).

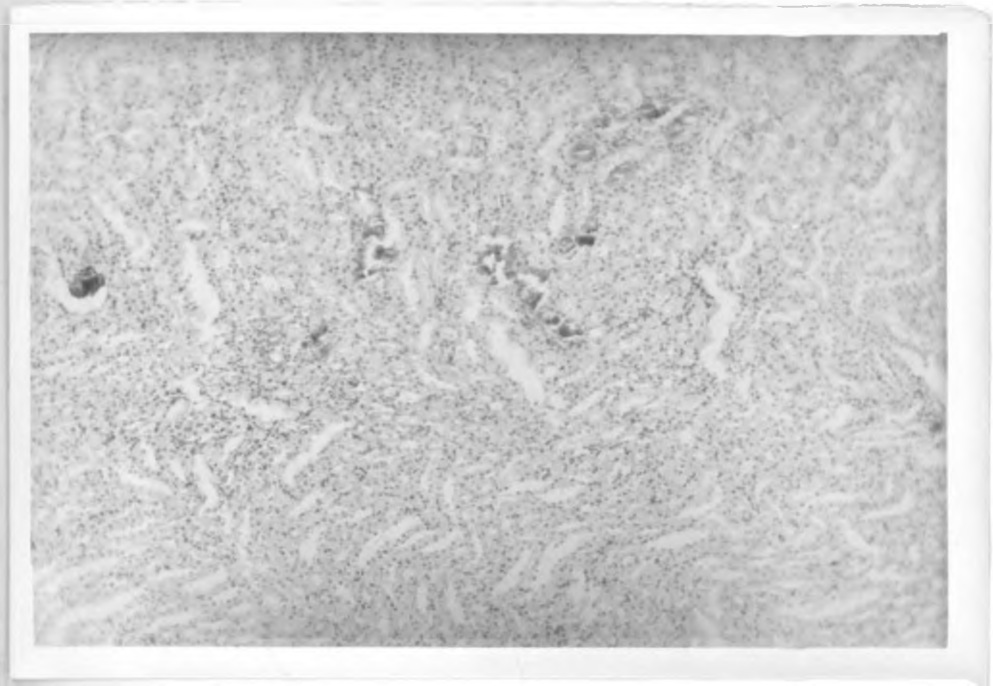

Plate 36: Rat kidney: Focal necrosis in the subcortical zone of the kidneys. Such lesions were found in five rats out of seven, (H&E x 100).

Table 6: Daily means of body weight of rats (in gm) which received high dose. Means of 4 experimental groups combined.

Days	Treatments 1, 2, 3, and 4 (High dose)	Treatments 5 and 6 (Low dose)	Control Group
	s.d.	s.d.	
1	80.5 ± 8.5	83.2 ± 9.4	89.5
2	95.6 ± 9.7	91.0 ± 10.4	94.1
3	107.0 ± 10.8	98.9 ± 11.2	102
4	115.7 ± 13.3	109.1 ± 12.7	109.1
5	121.8 ± 11.3	115.6 ± 11.4	112.3
6	124.5 ± 8.5	118.1 ± 11.6	116.8
7	127.9 ± 8.5	121.6 ± 12.4	124.4
8	135.2 ± 10.0	131.9 ± 13.5	125.6
9	138.5 ± 12.1	134.9 ± 13.8	130.8
10	143.6 ± 11.2	137.8 ± 11.0	138
11	145.4 ± 9.9	140.5 ± 9.8	141.1
12	150.5 ± 9.2	144.6 ± 10.7	147.6
13	149.8 ± 8.8	146.2 ± 13.2	145.4
14	149.4 ± 11.2	159.4 ± 9.3	150.3
15	147.9 ± 10.1	150.8 ± 8.1	153.3
16	148.9	149.8 ± 8.7	155.6
17	118.8	149.7 ± 8.8	160.6
18		151.9 ± 10.0	163.4
19		155.4 ± 9.5	165.0

s.d. = standard deviation

6.0 EXPERIMENT TWO

6.0.1 Acute and Prolonged toxicity study in goats, Capra hircus

Having studied acute and prolonged toxicity of Ajuga remota Benth. in rats (Rattus rattus) which represented toxicity in monogastric animals, attempts were made to investigate toxic effects in goats (Capra hircus) which represented ruminants. The study was intended to reveal clinical and pathological effects. Pathological lesions and targets organs were revealed.

6.0.2 Materials and Methods

6.0.2.1 Plant aqueous extract

Ajuga remota Benth. herb was collected from Uthiru near Kabete Veterinary Laboratory Primary School. It was packed in plastic bags and transported by a departmental van to the Department of Veterinary Pathology and Microbiology. It was dried in a spacious room of toxicology section of the Department of Veterinary Pathology and Microbiology, Drying period varied from 45 to 60 days as in experiment one.

Any remnants of roots were carefully removed from thoroughly dried plant material. Two kilograms were weighed and soaked in distilled water in a large boiler. Extraction was done by boiling for several hours. The extract was filtered through cotton wool

thrice. Then through five layers of filter paper (Whatman 1) with application of a negative pressure. The filtrate was then centrifuged at 1.04×10^4 g and supernatant freeze dried to recover a powder as described in experiment one. The extract was sealed in universal bottles to avoid rehydration. Aqueous extract was made from this powder by reconstituting it into distilled water at the time of administration.

6.0.2.2 Experimental Animals

A flock of twelve East African small goats Capra hircus weighing between 11 and 15.5 kilogram were used as experimental animals in the third attempt to study the toxicity of Ajuga remota Benth. Their ages ranged from 6 to 12 months. They were bought from one flock at Mogotia in Baringo District in Rift Valley Province. There were nine males and three females. General examinations were conducted to ensure that they were healthy and disease free and not carriers of any potential pathogens before being bought. Later in June 1987 goats were transported by road to the large animal pens of the Faculty of Veterinary Medicine at Kabete Campus.

On arrival the animals were divided into groups and housed in two thoroughly pre-cleaned pens. The pens had well designed drainage system. The floor was slightly elevated with rough concrete surface. Straw was always provided as bedding. A balanced ration of hay, wheat bran concentrate, mineral lick blocks and fresh drinking water were supplied ad libitum.

On the second day after arrival the animals were marked by fixation of ear-tags numbered 401 to 412. General examinations were conducted on the third day on all the goats for further scrutiny for any ailment precipitated by transport stress. Samples of blood were collected from each and every goat and sent to parasitology laboratory to be screened for blood parasites presence. Faecal samples also were taken directly from recta for examination in the laboratory for helminth eggs and coccidial oocysts.

Blood sample results were negative, while feacal sample examination revealed presence of eggs of gastro-intestinal parasites though physical examination had not indicated so. Though there was not much evidence of presence of ectoparasites, animals were subjected to general treatment by dipping in an approved potent acaricide to get rid of any ectoparasites. After tactical treatment for ectoparasites the animals were transferred to other fumigated stables to avoid any re-infestation. Goats that had positive results for gastro-intestinal parasites underwent deworming and those found negative just received prophylactic doses of active deworming agent, Valbazen (albendazole) orally. A week later other feacal samples were collected for screening again for any remnant eggs of gastro-intestinal parasites. Results were negative as such indicating total elimination of helminth. Then another general examination was conducted for all experimental goats to ensure their good health.

6.0.2.3 Haematological Basic Data Collection

A three weeks' acclimatization period was allowed to elapse before blood samples collection was started. Blood samples were collected once per week for four weeks prior to injection of the test extract. The following haematological parameters were studied:

i) The packed cell volume (P.C.V.)
ii) Haemoglobin concentration (H.b)
iii) White blood cells (W.B.C.) counts
iv) Red blood cell (R.B.C.) counts
v) Total plasma protein (T.P.)
vi) Mean corpuscular volume (M.C.V.)
vii) Mean corpuscular haemoglobin concentration (MCHC)

The methods employed in carrying out these parameters will be described together with blood sample collection in the main experiment later in this chapter.

6.0.2.4 Injection Experiment in goats

After five weeks of preliminary study, basic data taking and proper acclimatization the administration of extract was started.

All goats underwent physical examination of systems prior to administration of aqueous extract to ascertain their health status. All were physically healthy. Their weights varied from 11 to 15.5 kilograms. The goats which had been already numbered

by ear-tags number 401 to 412 were divided into four sub-experimental groups at random as follows:-

Group	1	consisted	of	two	goats	number 402 and 407
"	2	"	"	"	"	" 411 and 409
"	3	"	"	three	"	" 404, 410 and 405
"	4	"	"	five	"	" 401, 403, 406 408 and 412

Group one served as control group. It received a placebo of 10 millilitres of physiological saline i.e. 0.9% of sodium chloride solution in distilled water. The frequency of administration was similar to that of repeat dose treated animals i.e. 48 hourly. Group 2 of two goats received high doses, number 411 and 409 received single doses of 900 and 450 mg/kg body weight respectively. Group three consisted of goats number 404, 410, and 405. The first two of this group (404, 410) received 350 mg/kg body weight while 405 received 175 mg/kg b.w. per 48 hours. The fourth group of goats 401, 403, 406, 408 and 412 received repeat dose of 88.5 mg/kg body weight at an interval of 48 hours. Treatment continued for thirty days. The toxicity experiments on goats were carried out in those varying dosages of Ajuga remota Benth. extract so as to study the acute, subacute and prolonged responses, and also the clinicopathological changes that occurred due to each of the dosages. The aqueous extract was administered parenterally by subcutaneous route. Sterility and antiseptic measures were strictly

followed. Seventy percent ethyl alcohol was applied on skin as antiseptic agent before injecting.

Samples of blood for haematology and serum biochemistry were collected on alternating days to those of administration of extract. So, they were also collected at intervals of 48 hours. Jugular venipuncture was done on both the treated and control goats throughout the treatment period of thirty days.

Table 7: Dosages, subcutaneous administration of aqueous extract and survival data of goats

Goat Number	Body weight in kg.	Dosage rate mg/kg body wt.	Survival time in days
411	11.5	900	2.5
409	11.0	450 (once)	20
404	16.0	350	30
410	14.0	350	30
405	13.5	175	30
401	13.5	88.5	30
403	15.5	88.5	30
406	15.0	88.5	30
408	15.5	88.5	30
412	15.0	88.5	30
407*	17.5	10 ml of 0.9% NaCl	30
402*	14.5	10 " " " "	30

*Control

6.0.2.5 Collection of blood samples

All blood samples for haematological analysis were collected between 8.30 and 9.30 a.m. in the morning. Venepunctures were always done after overnight fasting prior to feeding and physical examinations. Also any form of disturbances, fear, mishandling and any other stress factors were strictly avoided to a minimum. Blood sampling were by jugular venepuncture using 19G x 1.5" disposable needles, and ten-millilitre plastic syringes. About one millilitre of whole blood was immediately transferred into clean bijou bottles containing about one milligram of dry disodium salt of ethylenediaminetetraacetic acid (EDTA) as anticoagulant and gently mixed to allow the crystals to dissolve in the sample blood. For serum biochemical determinations, about 5-6 millilitres of whole blood was collected at the same time into universal bottle without anticoagulant. The blood was allowed to stand on the bench at room temperature (20-23°C) for 1-2 hours to clot. The clots were always allowed to retract in order to get a maximum yields of serum. After retraction the clots were removed and the serum transferred into centrifugal test tubes and centrifuged at a relative centrifugal force (RCF) of 8.5 x 10^2 g for 10-15 minutes. The serum was pipetted off and used for enzyme activity determinations. For tests not carried out immediately, serum was deep frozen at <-20°C.

6.0.2.6. Clinical Examination

Daily clinical examination was conducted on the treated and control goats. Appearance of clinical signs due to toxicity were recorded. Rectal temperatures were read. The respiratory and pulse rates were counted on a per minute basis, digestive system and mucous membranes examined. Physical examinations were always done in the mid-morning after collection of blood samples and feeding the animals. Routine clinical observations were done in the afternoons to the treated goats.

6.0.2.7 Determination of Haematological Parameters

The estimation of blood values was done by carrying out routine haematology to determine the packed cell volume (P.C.V.), the haemoglobin concentration, total erythrocyte count (RBC), total white blood cell counts (W.B.C.), mean corpuscular volume (M.C.V.), mean corpusular haemoglobin concentration (MCHC), total plasma proteins (T.P.).

6.0.2.8 Packed cell volume (PCV)

The parameter was determined by the microhaematocrit method. Commercially available unheparinized microhaematocrit capillary tube of length

75 mm ± 1 mm inner diameter 1.1 - 1.2 mm were used. The tubes were filled with uncoagulated (EDTA) blood until between 2/3 and 3/4 full, then the dry ends sealed over a Bunsen burner flame. The sealed microhaematocrit capillary tubes were then placed into the grooves of the turn-table of a micro-haematocrit centrifuge (HAWKSLEY ENGLAND) with their open ends towards the centre. They were then span for five minutes at centrifugal force of 1.06×10^{4}* g.

$$*RCF \text{ (in g)} = 118 \times 10^{-7} \times r \times n^{2}$$

Where r = radius

n = rpm

They were removed and placed on the MSE (Patent U.S.A. 2948965) microhaematocrit reader and the PCV read and recorded in percentage.

6.0.2.9 The total plasma Protein Concentration (T.P.)

Determination of the Total Protein concentration was done using a refractometer (ATAGO, JAPAN). A drop of plasma from plasma layer of microhaematocrit tube used for packed cell volume (P.C.V.) was placed on the prism of the refractometer and viewed through the eyepiece by electrical illumination. The total protein value was read directly from the scales and recorded in grams per 100 millilitres of sample blood.

6.0.3 The Red Blood Cells Counts (RBC)

The red blood cell counts were done using the Coulter Electronic Counter (COULTER COUNTER ZM, Coulter electronics INC., HIALEAH FLORIDA U.S.A.) Model Zm with mercury manometer.

In order to count RBC, a blood dilution of 1:50,000 was used. To make the dilution, 40 lambdas of whole blood were mixed with 20 millitre of coulter ISOTON. Then one fifth of millilitre of the diluted blood was further mixed with a fresh 20 millilitres of ISOTON to yield the target dilution of 1:50,000. The diluted blood in auxilliary cuvette was placed on the platform of coulter counter and an electrode inserted into the diluted blood. The platform chamber door was closed and then switched on to count electronically. The readings displayed on the screen of the coulter counter machine were multiplied by a hundred to give the number of erythrocytes per cubic millimitre (mm^3) of the sample blood. The figures computed therefore were expressed as the number of erythrocytes in (millions) per microlitre.

6.0.3.1 The Haemoglobin Concentration

The haemoglobin concentration of blood was determined by cyanmethaemoglobin method using a Coulter Haemoglobinometer (Coulter Electronics, Inc., Hialeah France). The remaining amount of 20 millilitre after R.B.C. counts was used to determine haemoglobin. Six

drops of Zap-Oglobin (containing 300 mg Potassium Ferricyanide per 100 ml) were added and gently mixed with blood dilution. Immediate haemolysis of erythrocytes occurred. Then a few millilitres were poured into the tube of Coulter electronic haemoglobinometer. Haemoglobin concentration in grams per 100 millilitres of sample blood was displayed on read-out directly.

6.0.3.2 The White Blood Cell (W.B.C.) Counts

The total leukocytic counts were done using the Coulter Electronics Counter. The white cell counts were done using a blood dilution of 1:500 and this was prepared as a first step in the R.B.C. count. Six drops of Zap-Oglobin were dropped in the diluted blood then mixed. Haemolysis of erythrocytes occurred immediately within 10 seconds. The coulter counter machine was adjusted to WBC count level. Electrodes was inserted into the dilution then closed the door and switched on to count. The readings on the read-out of the counter were equal to the number of leukocytes per microlitre (mm^3) of sample blood.

6.0.3.3 The Mean Corpuscular Volume (MCV)

The Mean Corpuscular Volume (MCV) was automatically measured by the Coulter Electronics Counter machine simultenously as the red blood cells were being counted. The MCV was directly read from read-out screen of the machine.

6.0.3.4 The Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC was calculated by dividing the haemoglobin in grams per 10 litres of blood by the volume of PCV per 100 ml of blood. The result were expressed in grams of haemoglobin per 100 ml.

6.0.3.5 Determination of Serum Enzyme Activity

Studies on the serum were conducted to determine the activity of aspartate aminotransferase (Asap AT) formerly known as serum glutamicoxaloacetic transminase (SGOT), Alanine-aminotransferase formerly known as glutamic pyruvic transaminase (SPGT) lactate dehydrogenase (LDH), serum alkaline phosphatase (SAP), Creatine and blood urea nitrogen (BUN).

6.0.3.6 Serum Aspartate aminotransferase
[Serum glutamicoxalotransaminase (SGOT)]

The colorimetric determination of aspartate aminotransferase was done according to the method of Reitman and Frankel (1957) and using the bio Merieux reagents. Colorimetric determination of aspartate aminotransferase activity is according to the following reaction:

A.T: Aspartate + a-keto-glutarate $\rightleftharpoons$ oxalo-acetate + Glutamate

One millilitre of aspartate aminotransferase substrate consisting of (Phosphate buffer pH 7.5 and aspartate a-ketoglutarate) was dispensed into test-tubes and then incubated for five minutes at 37°C. Two hundred microlitres of sample serum was added, well mixed and incubated in waterbath at 37°C for exactly one hour. After incubation one ml. of colour reagent consisting of 2.4-dinitrophenylhydrazone was added, mixed well and let to stand for twenty minutes at room temperature. Then 10 ml of sodium hydroxide of 0.4N was added, mixed well and waited for five minutes at room tempeature before starting reading the optical densities. Optical densities and units per millilitre were read and printed by Coultronics France S.A. electro-spectrophotometer at a wavelength of 505 nm with zero adjustment of distilled water.

6.0.3.7 Serum Alanine-aminotransferase
[Glutamic pyruvic transaminase (GPT)]

The colorimetric determination of serum alanine-aminotransferase formerly known as glutamic pyruvic transaminase was done according to the method of Reitman and Frankel (1957) and the biochemical reagent Kit used was by bio Merieux. Colorimetric determination of alanine-aminotransferase activity is according to the following reaction.

Alanine + a-ketoglutarate $\underset{\text{AGT}}{\rightleftharpoons}$ pyruvate + glutamate

The pyruvate formed is measured in its derivative form, 2.4-dinitrophenylhydrazone.

One millilitre of alanine-aminotransferase substrate consisting (phosphate buffer pH 7.5, alanine and a-ketoglutarate) was dispensed into test tubes and then incubated for five minutes at 37°C for exactly thirty minutes. One millilitre of colour reagents (2.4-dinitrophenylhydrazine) was added, mixed and let to stand for twenty minutes at room temperature. Ten millilitres of sodium hydroxide o.4N was added mixed well and waited for five minutes, then optical densities and international units were computed by Coultronics, France S.A. eletrospectrophotometer. Optical density and international units were read based on a wavelength of 505 nm with zero adjustment of distilled water.

6.0.3.8 Blood Urea Nitrogen (BUN) Levels

Serum Enzyme determination of Urea

Serum enzymatic determination of urea concentration was done following the method of Hallet, and Cook (Clinical Chemistry Acta, 1971). The biochemical reagents Kits used were by Coulter Urea Enzymatic, Luton, Beds, LU3, BRH England. The principle used to determine urea concentration in sample sera is measured by the following:-

$$\text{Urea} + H_2 \xrightarrow{\text{Urease}} 2NH^+_3 + CO_2$$

$$3NH^+ + \text{-Ketoglutarate} + NADH \xrightarrow{\text{Glutamate Dehydrogenase}} \text{Glutamate} + NAD^+ + H_2O$$

A working reagent was obtained by reconstituting a buffer reagent into lyophilized urease enzyme. One tablet was reconstituted with 8 ml of buffer and fifty microlitre of ∂-ketoglutarate solution then let stand for fifteen minutes. Test tubes were labelled as follows: reagent blank, standard, normal control, abnormal control and unknown. Ten microlitres of sample serum were pipetted into each tube. The reagent blank received the same amount of distilled water. Two millilitres of working reagent was pipetted into each tube, mixed, and then incubated the tubes for 10 minutes at 37°C. The Coultronics, France S.A. electrospectrophotometer was adjusted to zero absorbance with distilled water at 340 nm wavelength. The optical densities and mg/100 ml of blood were printed out automatically by the machine.

6.0.3.9 Serum Creatinine

The kinetic determination of creatinine was done according to Bartel H. et al (1972) method and using the reagents prepared by bioMeriuex company. The determination of creatinine was done without deproteinization. The complex formed by creatinine and picric acid in alkaline medium was measured for one minute. A working solution was prepared by mixing one to one (1:1) volumes of colour reagent (picric acid 8.8 mol/litre) and alkaline reagent (sodium hydroxide 0.4 mol/litre). To one millilitre of working solution at 25-30°C was added 100 microlitre of the sample and mixed. Then measured the increase in absorbance between 20 and 80 seconds. Digital readouts in milligrams per litre and optical densities were printed automatically by Coultronics, France S.A electrospectrophotometer.

6.0.4.0 Lactate Dehydrogenase (LDH opt.)

The measurement of the lactate dehydrogenase activity was done using an optimized standard method conforming to the recommendation of the Deutsche Gesellschaft fur Klinische Chemie, Anon (1970). The principle applied here was that LDH catalyses the oxidation of lactate to pyruvate with concurrent reduction of Nicotinamide Adenine dinucleotide (NAD) to the hydroxyl from NADH as indicated in the following reaction.

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightleftharpoons{\text{LDH}} \text{L-lactatate} + NAD^{+}$$

This is an "optimized standard method" conforming to the recommendations of the Deutsche Gesselschaft fur Klinische, Chemie. A working reagent was prepared by dissolving reagent tablet of NADH 0.18 micro mol/litre in buffer/substrate (phosphate buffer 50 micro mol/litre pH 7.5 and pyruvate 0.6 micro mol/l. The working solution was brought to assay temperature (25°C) before use. 0.1 millilitre of sample sera were pipetted into reagent solution in their original containers. The sample and solution were mixed well. Coultronic electrospetrophotometer was programmed to read initial absorbance, repeat reading after exactly 1, 2, and 3 minutes. Zero adjustment was made against the air at a wavelength of 340 nm. Reading were automatically printed out in units per litre.

6.0.4.1 Serum Alkaline Phosphatase (SAP)

Alkaline phosphatase measurements were done following the method of Kind and King (1954). Colorimetric determination of alkaline phosphatase activity according to the following reaction.

$$\text{Phenylphosphate} \xrightarrow[\text{pH 10}]{\text{Alkaline phosphatase}} \text{phenol} + \text{phosphate}$$

The phenol liberated is measured in the presence of amino-4-antipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction. Two millilitres of buffered substrate were pipetted into each of two test-tubes and incubated in the waterbath at 37°C for five minutes. Fifty microlitres of test serum was added to the first and further incubated at 37°C for exactly 15 minutes. A half millilitre of a reagent consisting of amino-4-antipyrine, sodium arsenate were added and mixed well. A half millilitre of a reagent which consisted of potassium ferricyanide mixed and let to stand for 10 minutes in the darkeness. Optic densities and Kind and King Units were automatically computed by the Coulter electronic spectrophotometer and printed out.

7.0 RESULTS

The weights of the goats, the dosage in grams of *Ajuga remota* extract they received and the survival time in days are given in Table 7.

7.0.1 Clinical observation

The clinical manifestations observed in goats treated with aqueous extract of *A. remota* subcutaneously, varied with dosage administered. Goats 411, 409, Group II; 404 and 410 Group III showed signs of intoxication between hours 3 and 8. They showed initial excitability by moving around their pens and knocked their penmates off with their heads. There was partial paralysis of the limbs nearest the sites of injections. These were followed by uneasiness, straddled stance of the affected limbs, flexion of the stifle or carpal joints. Oedema developed above these joints. They showed raised coats, slight elevation of respiratory, heart and pulse rates. However, heart and pulse rate reduced by day 2. Vigorous ruminal movements were noted. This could be seen from a distance at the left flank but the frequency did not seem to increase. Goat 411 which received high dose shed lumpy faeces instead of the usual pellets. By day 2 animals became dull with signs of leg weakness, unilateral swelling above carpal or stifle joints (Plate 52), inappetence and lacrimal discharges. After 24 hours another dose was administered to goat 411. Later on this goat started shedding softer loose

faeces, which was scanty, partially undigested and slightly pale brown in colour. During day 2 this goat developed oedema along the ventral part of the abdomen, brisket and in the submandibular space. Dyspnoea mainly of expiratory nature was noted. Cardiac auscultation revealed intervention of bradycardia whereby the heart rate had considerably fell from pre-administration rate of 84/min to about 60 per minute. There were arrhythmia and weak pulse which were accompanied by hypothermia of about 36.5°C. Sixty hours from the beginning of the study heart rate fell further to about 46 per minutes while the respiration rate had pronounced decrease with increased depth. These signs were followed by weak spasm all over limb muscles and recumbency. Muscle contraction occurred at intervals of 20 second. The animal fell unconscious and straightened its limbs. Frothy discharge came from buccal cavity. The animal succumbed soon after the 64th hour from the initial dose.

The animal which was treated with a dose of 450 mg/kg body weight just once showed a subacute form of toxicity and survived much longer up to twenty days. It developed diarrhoea on day 2 which continued up to day 10. Other signs were similar to that of 411 but not as intensive. Respiratory distress accompanied by nasal discharge was pronounced. There was a progressive loss of weight from initial weight of 11.5 to 9.0 kg by the time it died.

The third group of goats consisted goats 404, 410 and 405. This group received a dose of 350 mg/kg of

body weight. Group IV included goats 401, 403, 406, 408 and 412. This group received 88.5 mg/kg body weight. Both groups received injections at an interval of 48 hours for thirty days. On day 2 goats showed raised coats but more pronounced in goats 404 and 410. The intensity of raised coats declined as the experiment progressed. All treated goats showed partial paralysis of limbs as described in group II. Inflammatory oedema always developed 3-6 hours around the site of injection and subsided 30-40 hours later. They showed increased consumption of water. Two goats of Group VI had respiratory distress with nasal discharge. Regular auscultation of the heart revealed abnormal heart sounds in most of the goats. There was a general decrease of the heart rates. Comparison of the heart and pulse rates indicated pulse deficit in the majority of the goats especially high dose recipients (404 and 410). They showed worried look on their faces, but remained bright throughout the course of the experiment except those which succumbed. Softer faeces were shed by treated goats than those of control group. No abnormality in feeding behaviour was noted apart from increased water intake. The goats, however, showed a marked loss of body conditions. There was loss of weight in all treated goats while the controls gained steadily.

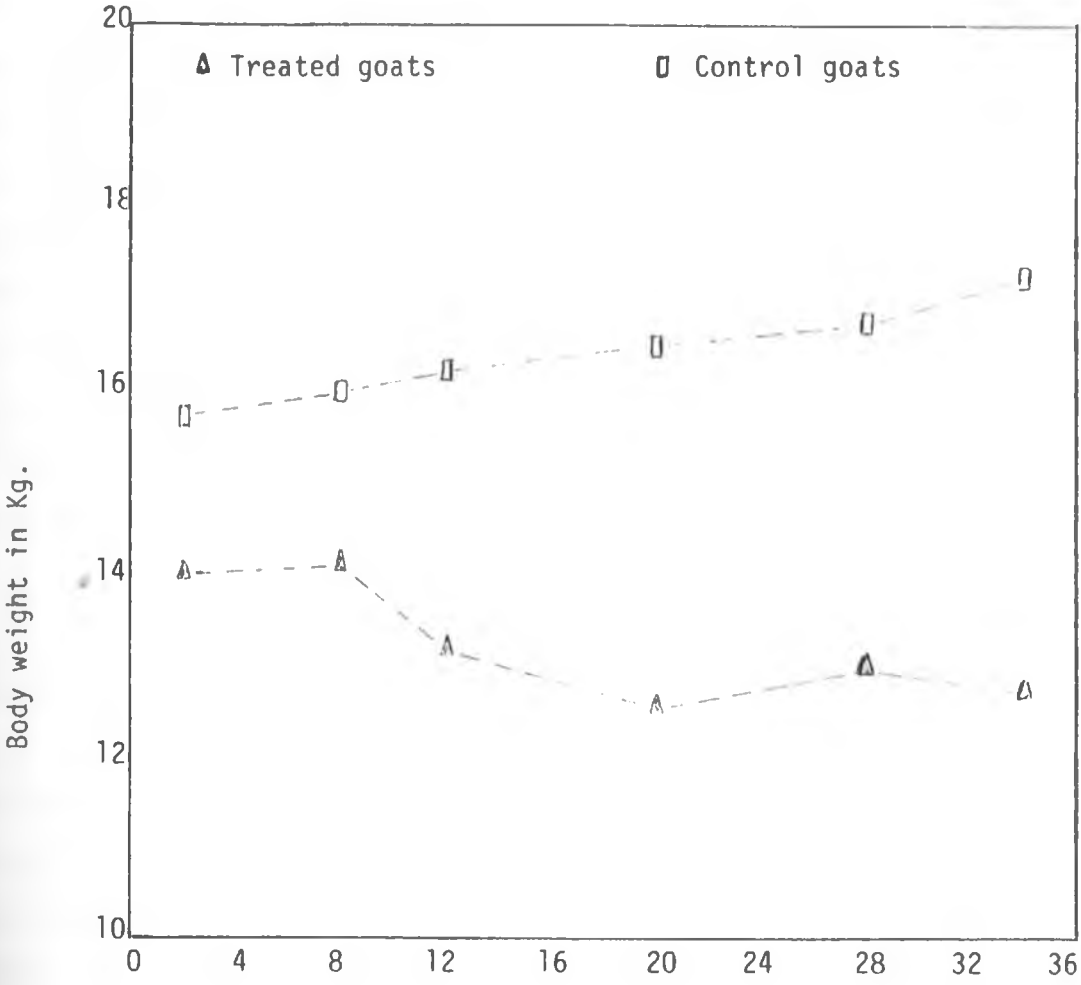

Figure 3: Effects of A. remota on weight gain of treated goats.

7.0.2 Haematological examination

The results of the haematological examinations are summarized in Figure 4 and Tables 8, 9 and 10. It is shown in Table 5, that there were some changes in red blood cell counts of the treated goats by the second day. By day 4 there was a sharp drop of erythrocytic counts. The means of red blood cells (RBC) counts of treated goats had dropped by two millions from pre-administration levels of 1.8×10^5 to 1.6×10^5 level. This was subsequently followed by a gradual drop in the levels of RBC counts throughout the course of the study in general.

Goat 411 which received one of the highest dose died of acute toxicity two days later without showing any marked changes in blood picture. There was a gradual decrease of RBC counts of 409 counts decreased from the initial level of 18.5 million cells per microlitre to 10.5 million by the time it succumbed. All other treated goats showed a general trend of decrease from their pre-administration levels to levels just below or close to normal count levels. The control goats showed insignificant decrease.

The packed cell volume (PCV) percentage showed a steady gradual drop in most of treated goats. The results of the packed cell volume are summarized in Figure 6 and Tables 8 and 14. There was a sharp drop in PCV corresponding to that of erythrocytic counts. This drop was followed by a gradual decrease of 0.7% per 48 hours. Goat 411 which died of acute poisoning

showed a marked drop from predosing level of 28% to 24%. However, the level did not go below the lower limit of the normal range of 20-38%. Goat 409 which died after three weeks, had a gradual decrease below the lower limit up to 14% at the time it succumbed. In general six out of ten treated animals demonstrated subnormal packed cell volume during the study either at the time of deaths or at the extreme on day 30. The active principles of *A. remota* significantly ($P<0.05$) affected the packed cell volume. The packed cell volume of control goats showed slight decrease, but remained in the normal limits Figure 6.

The total protein (T.P.) levels showed gradual decreases for all poisoned goats while the control group showed stable levels in normal limits throughout the course of the experiment. A half of the treated animals showed marked levels below normal. Goat 411 and 409 which died during the course of study, demonstrated subnormal levels as they approached death. Goats 404 and 401 also showed subnormal levels of T.P. These animals had marked serous fluid effusion in their body cavities and this was reflected before by the low levels of total plasma protein (hypoprotenamia). The effects of toxic agents of *A. remota* on total protein was significant ($P<0.05$).

Haemoglobin concentration took a bit longer to show any marked changes. The acute toxicity victim goat did not show any dramatic change as those which lived for longer time. The means of treated goats (Table 8) show that decline was marked by day 10. Goat

409 died after the third week. It had a well marked decrease of hemoglobin from predosing level of 11.7 gm to 5.6 gm per 100 millilitres. Fifty percent of treated animals showed levels below the lower limit or normal levels, while the other half indicated slight decline though remained in normal levels (Appendix V). The control goats showed mild decline and remained in the acceptable range throughout the course of experiment. Variance analysis for hemoglobin levels in treated goats was significant with ($P<0.05$).

Total leukocyte counts or white blood cell counts (WBC) was done using the Coulter electronic counter. The results are shown in Table 9 and Figure 7. A marked leukocytosis started developing on day 14. Acute toxicity did not seem to lead to leukocytosis and this is exemplified by goat 411. By the time it died after day 2, it showed decrease in leukocytic counts - leukopenia. WBC counts of 411 by the time it succumbed had dropped from predosing level of 1.3×10^3 to 0.88×10^3 per microlitre of blood. However, goat 409 and other treated goats showed leukocytosis changes progressively with time. Those which received higher doses showed higher counts. Goats 412, 408, 401 and 410 had elevation of leukocytic counts exceeding 2.0×10^4 per microlitre of blood. There were no significant changes observed in WBC counts of control goats 402 and 407.

The mean corpuscular volume (MCV) values were directly read off the coulter electronic counter readout screen during RBC counting. There was a slight

drop in MCV of treated goats. Acute toxicity did not demonstrate any effect on MCV. However, other goats showed little decline in volume. Goat 409 by the time it succumbed had reduced from pre-exposture level of 16.5 to 16.5. These decreases were not significant.

The average values for the MCHC in the treated and control goats were computed by dividing the haemoglobin in grams/10000 ml of blood by volume of PCV/100 ml (Table 10). The mean haemoglobin content of treated and control was normochromic. The values remained in the normal levels though they tended to be on upper limit. This indicated that toxic principles did not affect haemoglobin formation.

7.0.3 The serum biochemical examination

The serum biochemical parameters examined in the goats included the following: blood urea nitrogen (BUN) levels, the activities of aspartate aminotronsferase (Asp AT) formerly known as glutamate oxaloacetic transminase (GOT), alanine aminotransferase formerly known as glutamic pyruvic oxaloacetic transaminase (GPT), serum alkaline phosphatase (AP), lactate dehydrogenase (LDH), and serum creatinine levels.

Lactate dehydrogenase was examined once on samples collected before the treatment took place. Due to exorbitant costs of LDH test-kits, not many samples could be assayed. However, a few samples were ran and indicated that there was no marked alterations of lactic dehydrogenase levels in serum of both treated and control goats.

The blood urea nitrogen (BUN) levels in most of treated goats showed day to day variations, but the levels remained between 3-12 u mol/millilitres. Goat 411 showed elevated level in a sample collected a few hours before its succumbing. Generally, there was no significaht changes in levels of other treated and control goats. Kinetic determination of creatinine was done without deproteinization of the samples. The complex formed by creatinine and picric acid in an alkaline medium was measured for one minute. Predosing and control data (Table 11) showed a slight gradual drop of serum creatinine in treated goats. There was a sharp dramatic drop in treated goats in the last four days of the study. The levels in control goats were stable and did not vary very significantly (Table 15). Means of pretreatment levels of treated animals ranged between 70-80 micro mol/l. However, by day 15 levels had fallen to about 60 micro ml/l.

Colorimetric determinations of aspartate aminotransferase were carried out according to Reitman and Frankel method. Results are summarised in Tables 16 and 12 and Figure 8. The serum amounts of this enzyme markedly increased in treated goats. Goats 411 which died of acute toxicity did not show any elevation of the enzyme. However, goat 409 which died after administration of aqueous extract demonstrated high elevated level of aspartate aminotransferase as early as day 4. Levels exceeded 215 units per millilitre of blood on day 6, and this level remained high until death. The means of treated goats (Table 12) indicated

elevation of levels of AsAT, while the control goat showed slight increase of levels. Goats 409, 410, 405 which received 350 mg/kg body weight also showed slightly higher levels than those of low doses. Control goats 402, and 407 showed some variation of levels from one batch of samples to another these changes were not so marked. Their levels remained between 70 and 103 units. Significant increases ($P<0.05$) were observed in treated goats.

Alanine amino transferase levels were determined using a similar method to that of AspAT. Results of the former are summarized in Tables 12 and 16. Generally, studies conducted on the enzyme before dosing and those on control goats during treatment did not reveal any significant alterations. Treated and control goats seemed to have almost the same levels, though there were variations from batch to batch and sample to sample. Colorimetric determination of serum alkaline phosphatase activity was assayed once per week during the course of the study. Data collected before administration together with control levels showed no elevation of this enzyme in serum. There was some dropping in the levels from the original basic readings compared to those of postdosing levels.

Body weights of experimental goats were used to assess the impact of *A. remota* active principles on body weight gain. The means of body weight of treated goats showed that there was an initial stagnation of body weight in the first 7 days. Acute toxicity victim did not show any change in body weight. Goat 409 had

marked weight loss of 3.5 kg before it succumbed in the third week. Other treated animals did not gain any more weight after the starting of treatment, while the controls continued gaining steadily Figure 2.

7.0.4 Pathological Lesions

The animals which died of acute, prolonged and those which survived repeat dosing of *A. remota* solution were all subjected to thorough postmortem examinations. The survival periods of the individual animals are shown in Table 7. Acute case 411 had stretched limbs. There was a straw colour frothy discharge from respiratory tract which oozed out from the nostrils and the buccal cavity. The perianal area was soiled with faeces. Macroscopically, there was gelatinous appearance of the subcutaneous fascia especially the areas ventral to the abdomen. The abdominal and thoracic cavities contained moderate fluid effusion of serosanguinous nature, this indicated hydroperitoneum and hydrothorax respectively. Hydropericardium was pronounced. The pericardium contained straw-colour fluid. The myocardium betrayed flabbiness and petechial haemorrhages. Pulmonary hemorrhages and emphysema were eminent particularly in the cardiac lobe. There was frothy fluid along the trachea, bronchus and the bronchial tree in general. Petechiae on the trachea mucous membrane were noted. Froth could be seen when an incised piece of lung was subjected to pressure.

Gastrointestinal gross lesions in acute case, included severe congestion of mesenteric blood vessels. The rumen, reticulum omasum and abomasum had no any marked gross lesions. However, the blood vessels to these organs appeared severely congested. The liver appeared congested and slightly darker in colour. The gallbladder contained very little amount of yellowish bile instead of usual dark green bile. The spleen was slightly congested.

Goat 409 died of prolonged toxicity. The carcass was emaciated. The emaciation was associated with gelatinous atrophy of the body fat especially of the heart and in abdominal cavity. The lesions noted in the low dose intoxicated goats are given in details as follows:

Ascites and hydrothrax were marked in chronically treated cases. And it was more pronounced in goats which received 350 mg per kilogram body weight. The amount and colour varied acccording to cases. Acute cases had comparatively less fluid while chronically treated cases appeared to have more fluid of straw-colour nature in the abdomen and thorax. Only two goats which received low dose of 88 mg/kg body weight showed substantial amount of fluid in their cavities. Goat 409 which received a dose of 450 mg/kg body weight was found with the largest amount of fluid. The pericardia of four goats were found embedded with gelatinous atrophied fat. The pericardia contained moderate amount of fluids (hydropericardium). The myocardia were flabby and the left ventricles of three goats showed signs of weakness of the heart.

Lungs of these goats showed congestion and ecchymotic haemorrhages (Plate 47). Other portions of the lungs were emphysematous. Alveoli had been distended markedly. Microscopically, the acute cases showed severe congestion of blood vessels and pulmonary haemorrhages. Erythrocytes could be seen in the alveoli. In some cases there was lymphocytic infiltration of alveolar septa. Four goats out of 10 treated goats showed fibrin deposits in the lungs.

The Liver

The animal which succumbed to acute toxicity had fragile, congested liver with petechea on the surface. It was a bit enlarged. The second victim which died three weeks later and other goats killed in extremis had congested livers in some cases with petechea. Microscopically livers showed marked haemorrhages, congestion of central veins and capillaries, necrosis of hepatocytes in acute intoxicated goat. Whereas those of other goats which died later and those euthanized mostly showed marked degeneration of hepatocytes accompanied by necrosis of these cells. There was extensive pyknosis of nuclei. The nuclei of many hepatocytes were shrunk and roundish in shape (Plate 38). Diffuse intracytoplasmic vacuolation of liver cells was prominant. These vacuoles were not positive to Sudan black B stain for fat. In some cells vacuoles appeared to have pushed the nuclei aside. These lesions subsequently resulted in necrosis of cells (Plate 37, 38). Some cells were karyolytic where the nuclear material appeared completely dissolved and the chromatin was no longer differentiable.

The Kidney.

The kidneys in the acute intoxicated cases were soft and friable and had little colour alterations. The chronically treated cases looked pale and oedematous. A few cases showed congestion of the cortex grossly. Microscopically acute case showed severe congestion of blood vessels and extravasation of erythrocytes especially in the cortex. Cells of convuluted tubules showed some degree of necrotic degeneration. Prolonged treated goats showed marked degeneration of convuluted tubule epithelia. Many necrotic cells of uriniferous tubules appeared without nuclei (Plate 61). The tubules which are known to have cuboidal epithelial cells with brush border appeared having lost all these features. Karyorrhexis of the nuclei was common (Plate 61). Exudate could be seen in some of the glomerular space. Due to excessive exudate in the glomerular space large empty spaces were seen. Hyaline casts were seen in the lumina of the tubules in the cortex. The glomeruli themselves appeared rounded and shrunk due to excessive pressure exerted by exudate (Plate 62). These disorders invariably led to coagulative necrosis of the uriniferous tubular epithelial cells. The adrenal glands of treated goats did not show gross lesions. However, microscopic examinations showed haemorrhages in the zona reticularis of the cortex.

The Spleen and Lymph nodes

The spleen of both groups of acutely and chronically treated goats macroscopically appeared

slightly pale, but more so in chronically intoxicated cases. Those treated for one month, the spleens appared to be atrophied. Mesenteric lymph nodes were swollen . When sectioned the glands showed a marked gelatinuous eodema with slightly reduced cortical zone. Miscroscopic examination of histological section showed depletion of lymphatic nodules of the white pulp of the spleen. Necrosis of lymphoid cells was marked, especially lymphocytes of the germinal center. Lymphatic nodules were seen left with only a few lymphoid cells. Reticular fibres appeared devoid of their normal cellular elements like macrophages, lymphocytes monocytes and other leukocytes. Acute cases showed areas of haemorrhages and congestion accompanied by increased infiltration of neutrophilic leukocytes. There was intensive pigmentation in portions of red pulp of spleens of high dose recipients. Hemosiderin deposits were seen in the mesh networks of the germinal centre of lymph nodes and the red pulp of some spleen nodules. Hemosiderin presence was confirmed by modified Mallory's Method.

The Central nervous tissues.

The brain of goat number 411 which died of acute toxicity was highly congested with subdural petechiae on the cerebral cortex and the convolutions of the cerebellum. Congestion was noted along the spinal cords also. Goats treated with 350 mg/kg body weight showed marked congestion and cerebral oedema and haemorrhages. Convolution were flattened due to pressure against the cranial wall.

The brains appeared oedematous and felt softer than those of the control goats. Microscopic examination showed marked perivascular and perineuronal vacuolations in both the brains and the spinal cords of high dose treated animals. Some neurons had karyolytic nuclei. In the celebellum the purkinje cells in the folia and other neurons underwent various degenerative changes leading to necrosis, liquefaction necrosis of the these cells. Some of the necrotic nervous cells had pyknotic nuclei. Blood vessels were invariably congested in all treated animals. Extravasated blood was noted in the brain of acutely intoxicated goats and to a less degree in other high dose recipients (Plate 57). Lesions observed in the spinal cord mentioned earlier were mostly seen in white matter.

Muscles

The striated muscles (myocardium and skeletal) showed petechial haemorrhages grossly. Haemorrhage were also noticed in microscopic section (Plate 46). Zenker's degeneration was commonly found in treated goats especially high dose recipients. The sarcoplasm of the affected myocytes appeared pale and degenerated so that the striations were no longer visible.

The myocardia also had marked degenerative changes and demonstrated some degree of lymphocytic infiltration in some goats.

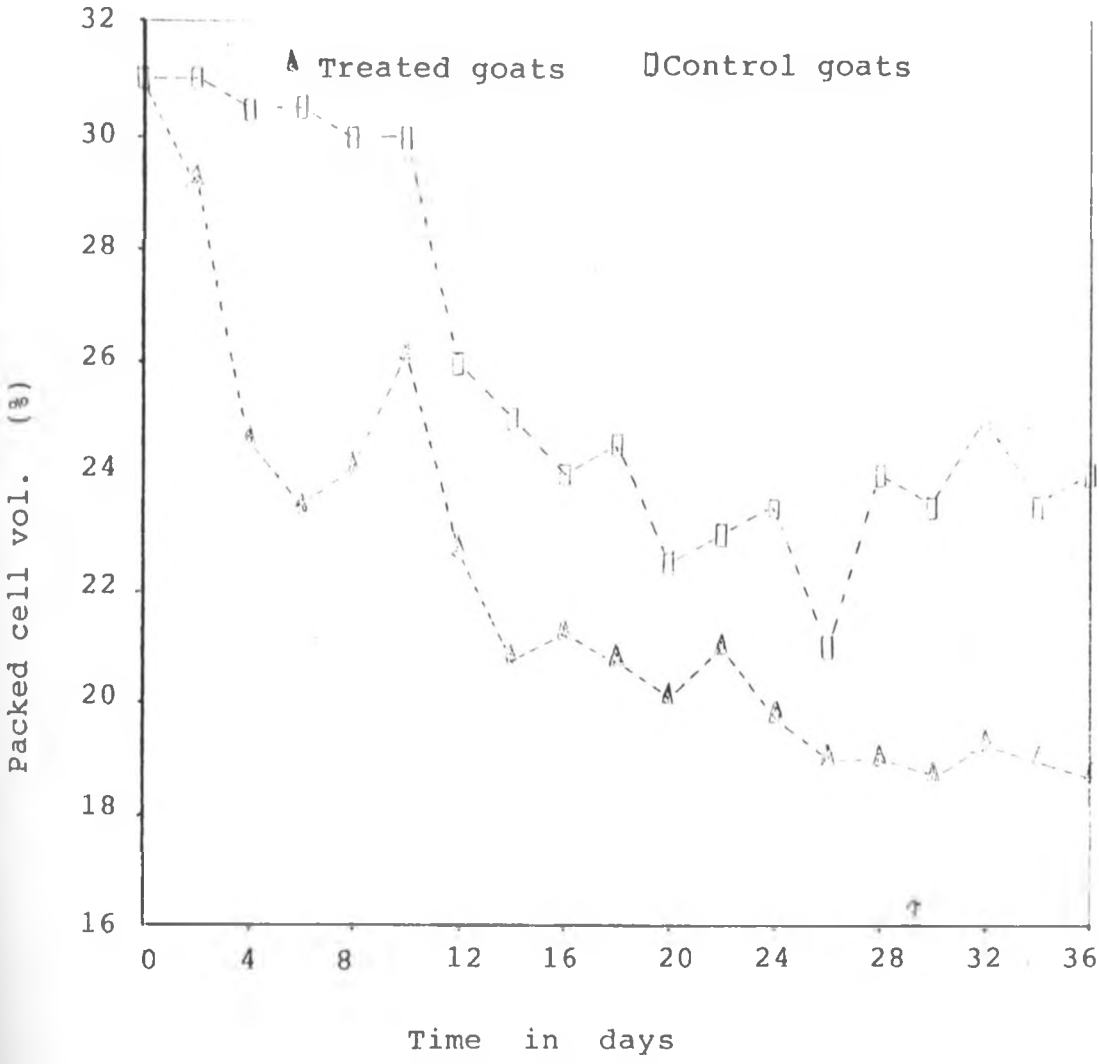

Figure 4: Changes in the Mean PCV of treated and control goats during experiment.

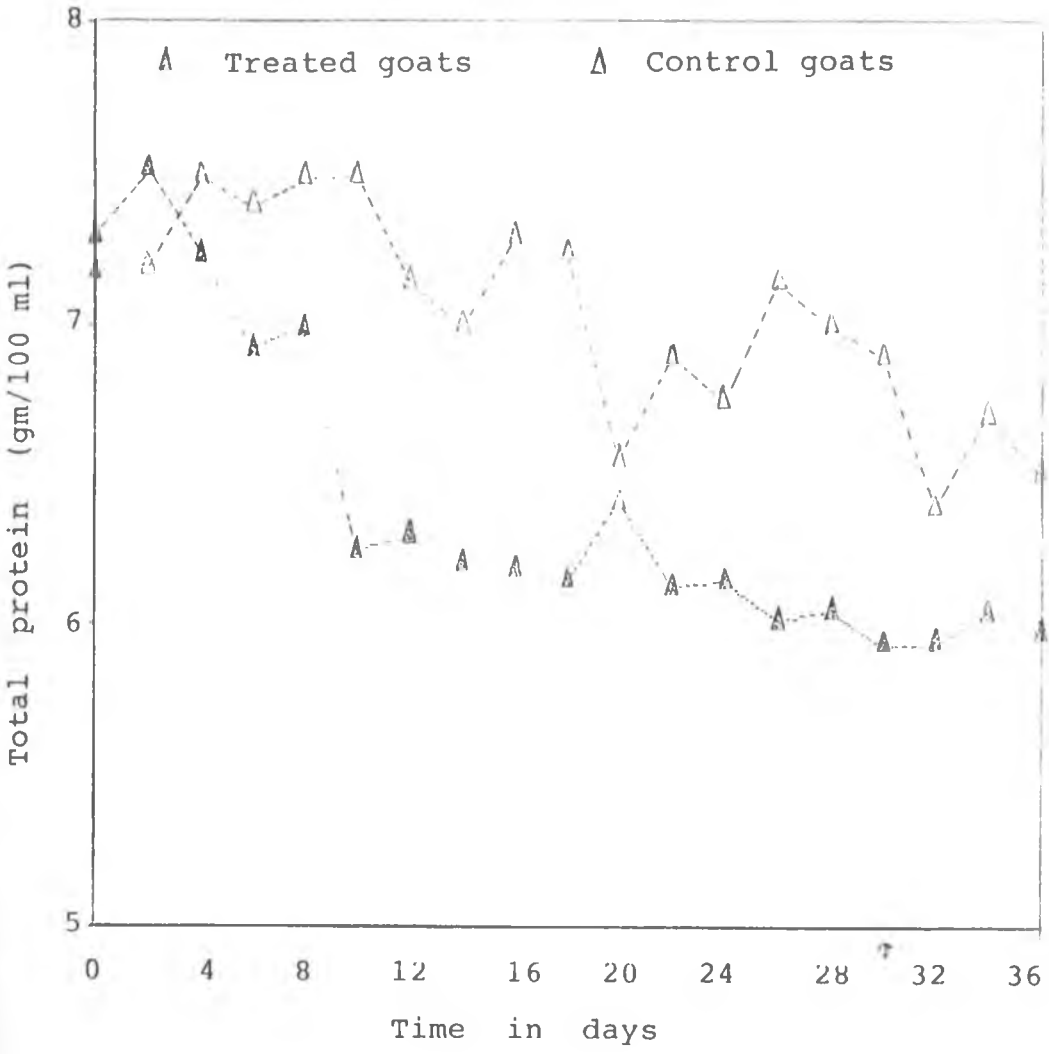

Figure 5: Total plasma protein picture of treated and control goats, based on their means.

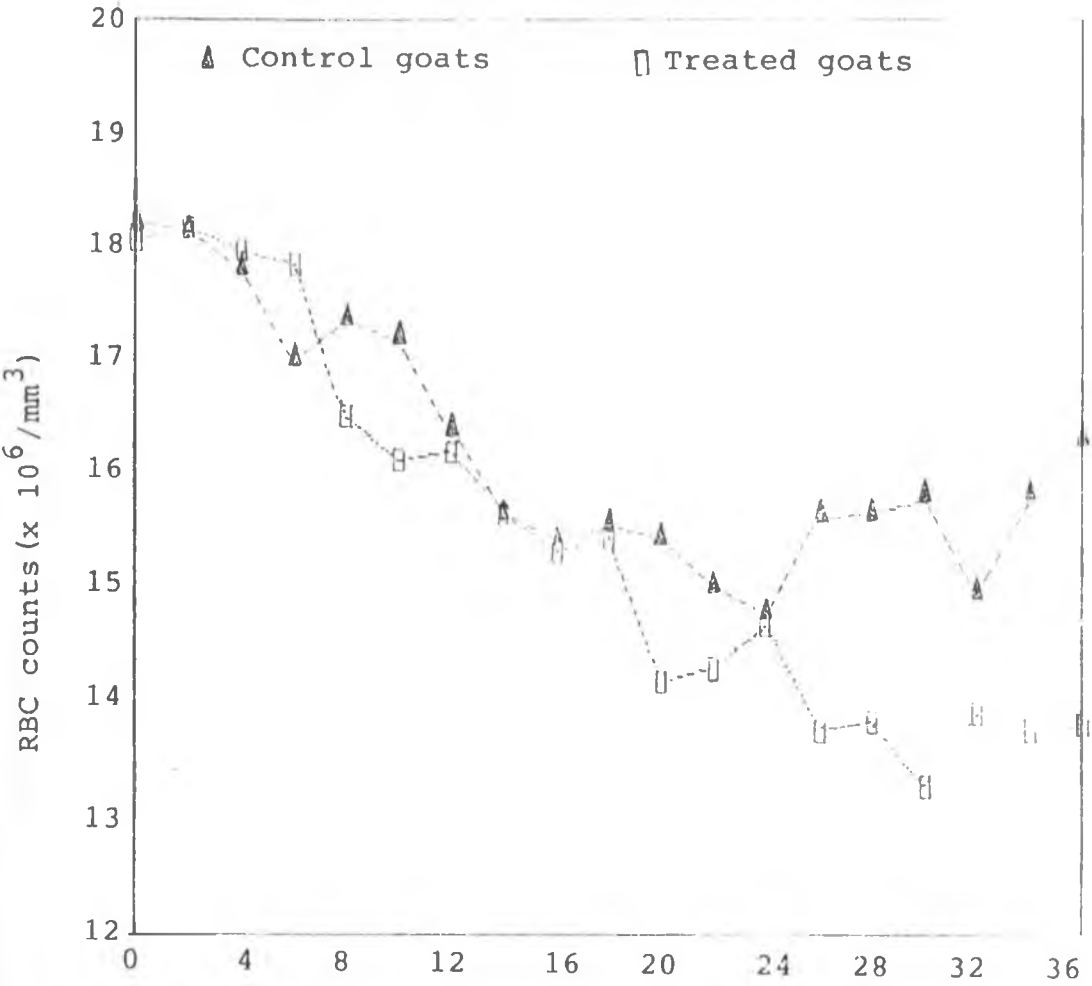

Figure 6: Variations of the means of the erythrocytic counts of treated and control groups of goats.

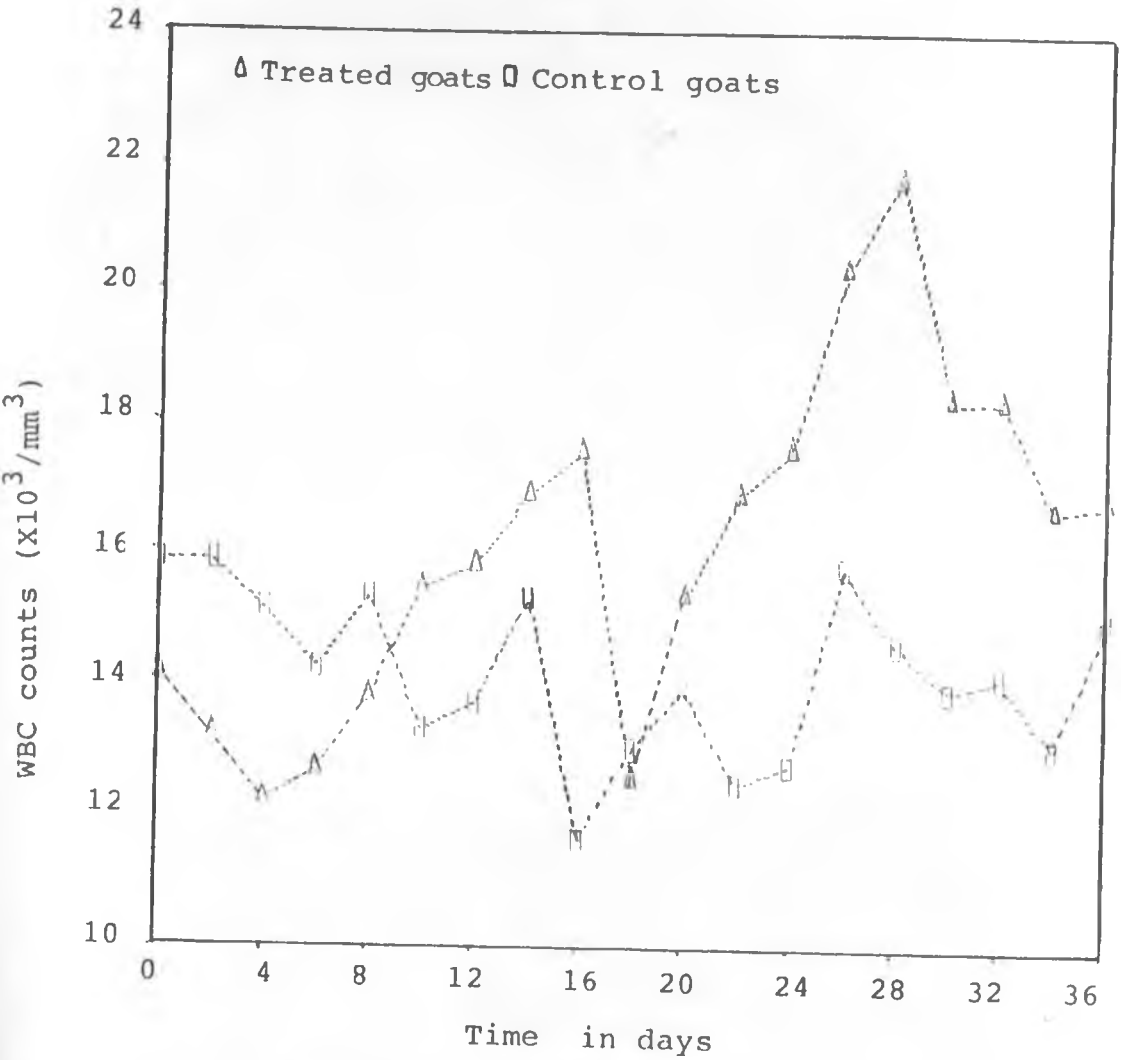

Figure 7: Changes in the means of total white blood cell counts of treated and control goats during the experiment.

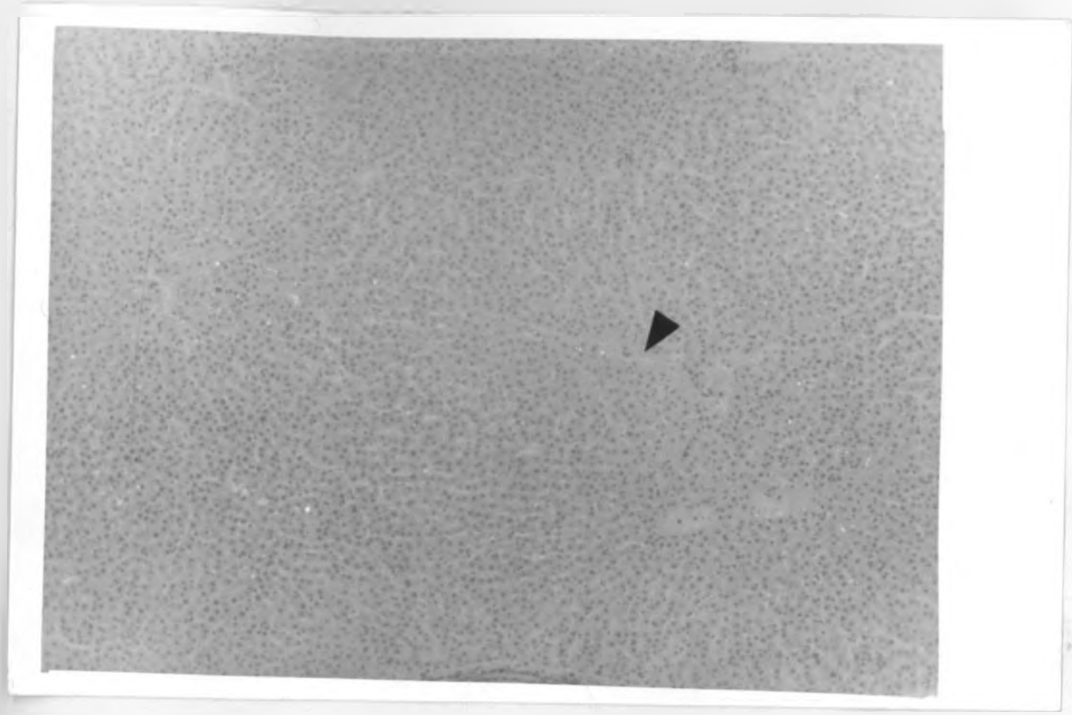

Plate 37: Goat liver: Haemorrhage in the central area near the central vein. Degeneration and necrosis of hepatocytes (H&E x 100).

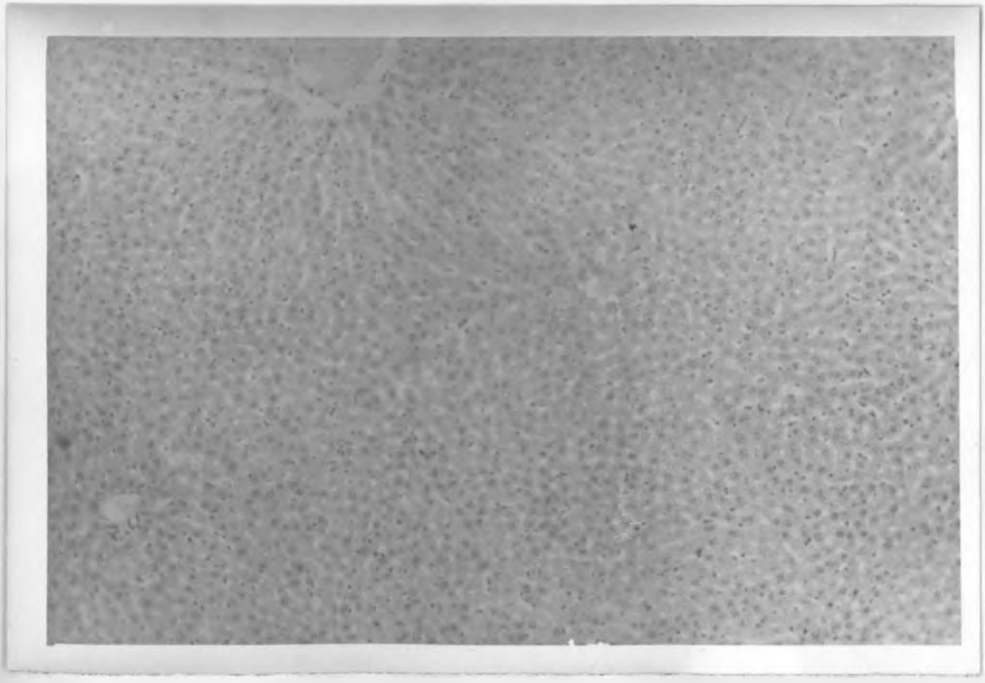

Plate 38: Goat liver: Congestion of the central vein necrosis of hepatocytes especially in and around the portal area. Intracytoplasmic vacuolation is seen in some of the cells. Many nuclei are Karyolytic (H&E x 100).

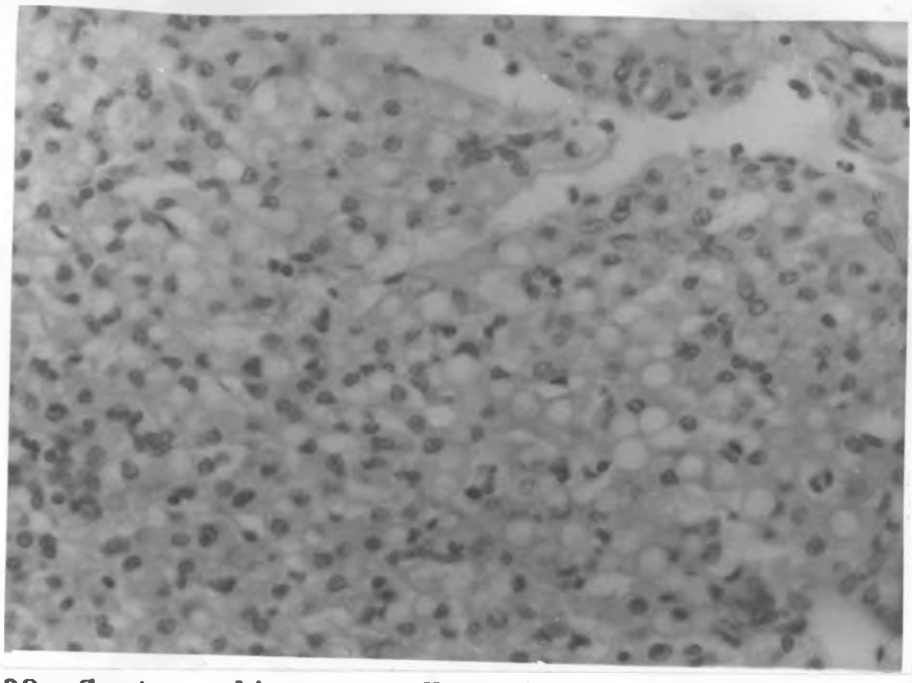

Plate 39: Goat liver: Hepatic intracytoplasmic vacuolation. Many nuclei are seen pushed aside by the vacuoles (H&E x 400).

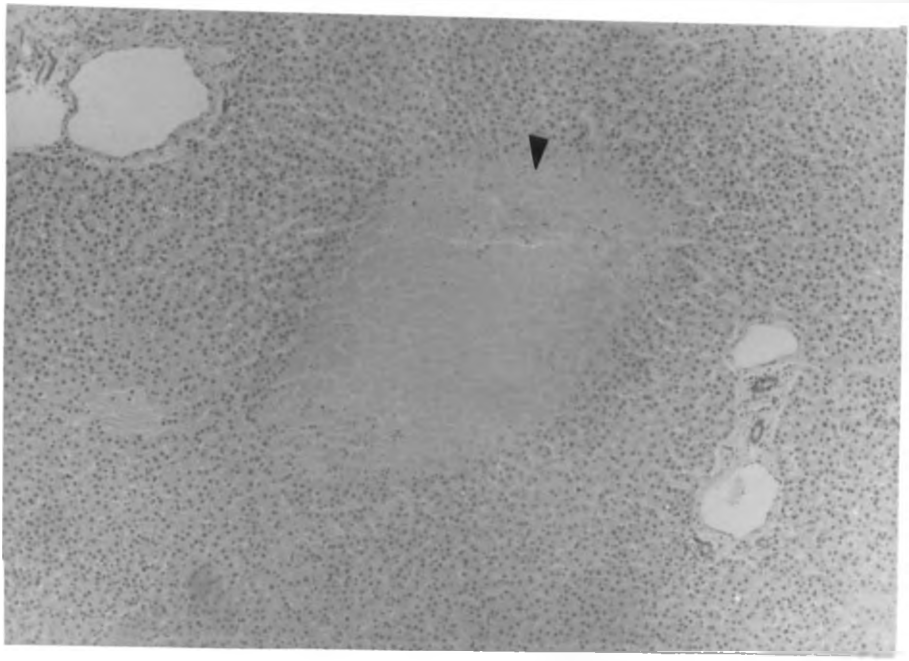

Plate 40: Goat liver: Focal necrosis in the liver with haemorrhages. Hepatocytes around the necrotic portion are undergoing degenerative changes (H&E x 100).

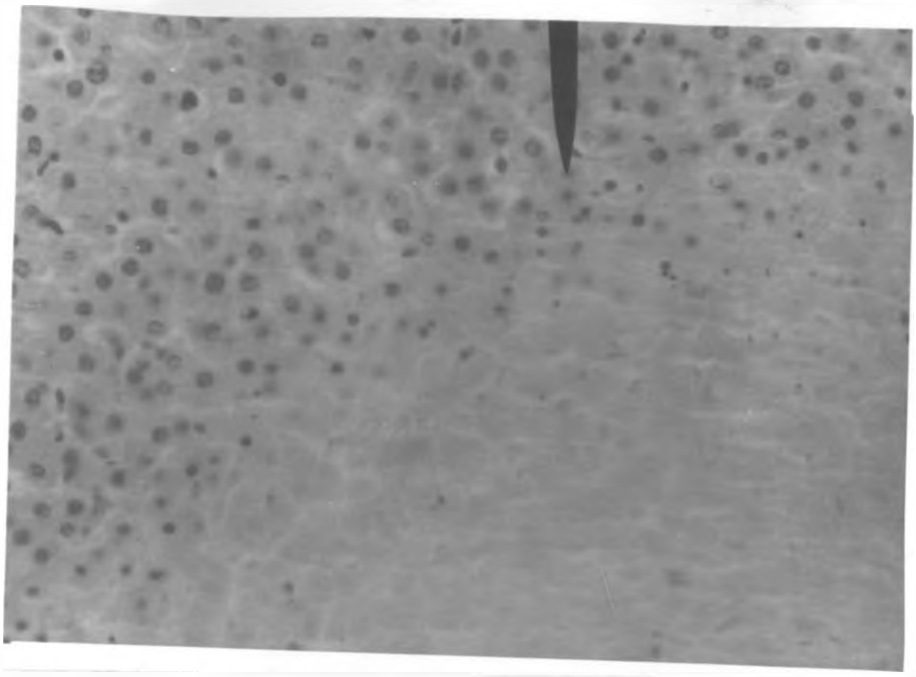

Plate 41: Goat liver: Focal necrosis and various degenerative changes in the hepatocytes. Some of cells nuclei are undergoing Karyorrhexis (H&E x 400).

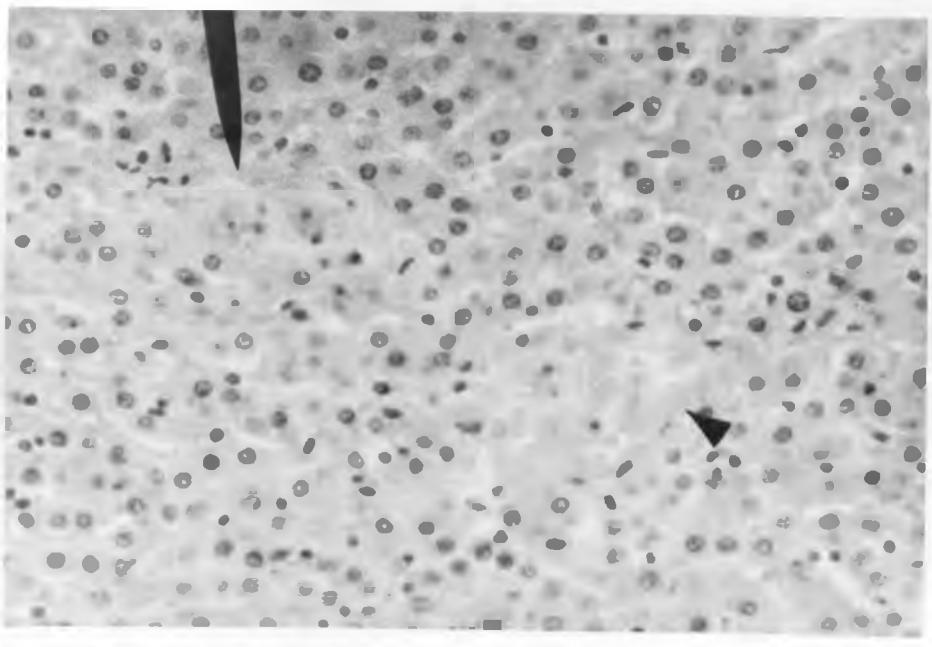

Plate 42: Goat liver: Haemorrhagic zone in the partal area of the liver. Some hep atocytes appear necrotic while others are seen with intracytoplasmic vacuoles (H&E x 400).

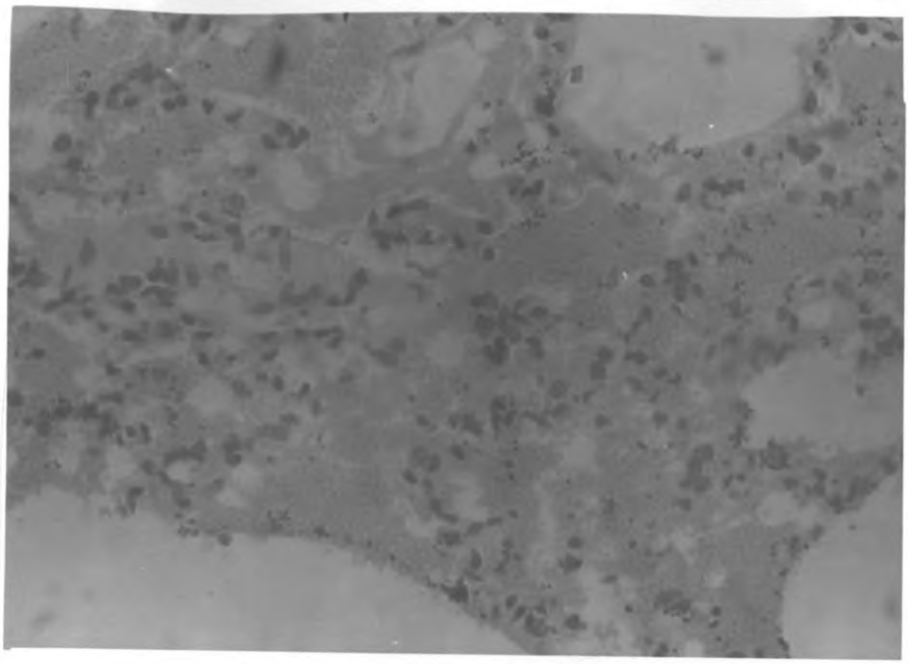

Plate 43: Goat lung: Severe pulmonary haemorrhage, alveolar sacs are seen filled with extravasated red blood cells. Fibrinous exudate is also seen in some alveoli. Lymphocytic cells infiltration of the alveolar septa is marked (H&E x 400).

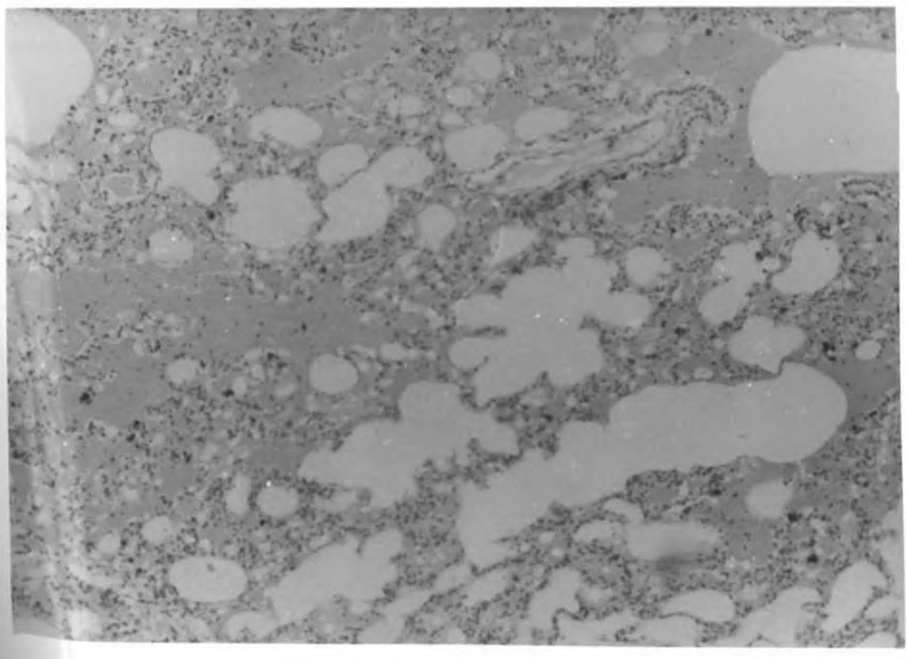

Plate 44: Goat lung: Fibrinous exudate in the lung. Some of the alveoli appear ruptured. This indicate oedema found in goats and treated with 350 mg/kg body weight (H&E x 200)

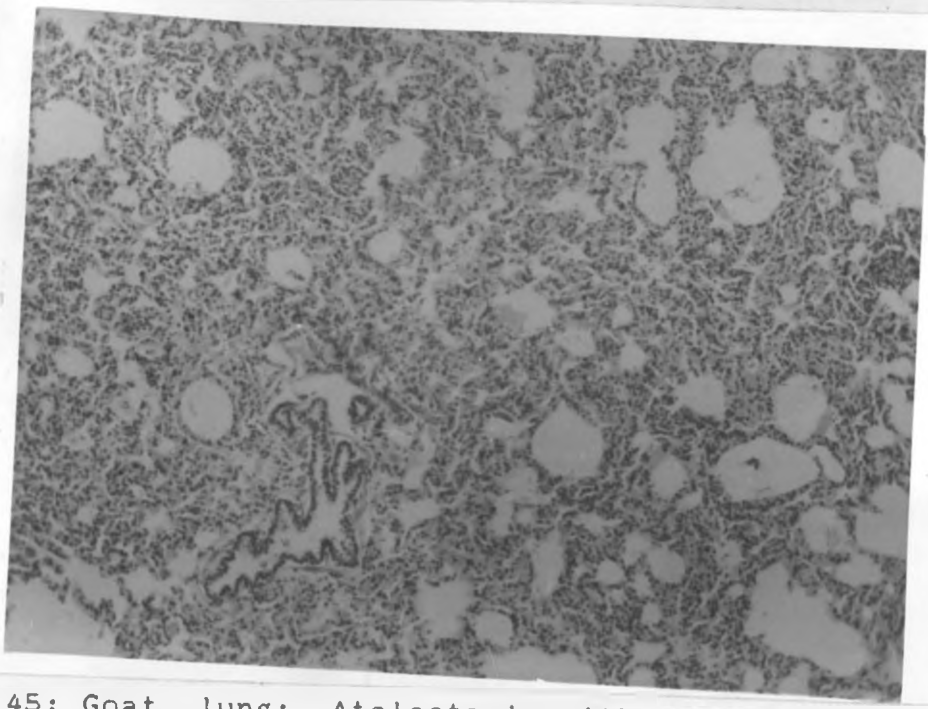

Plate 45: Goat lung: Atelectasis with some fibrious exudate in some of the alveoli (H&E x 100).

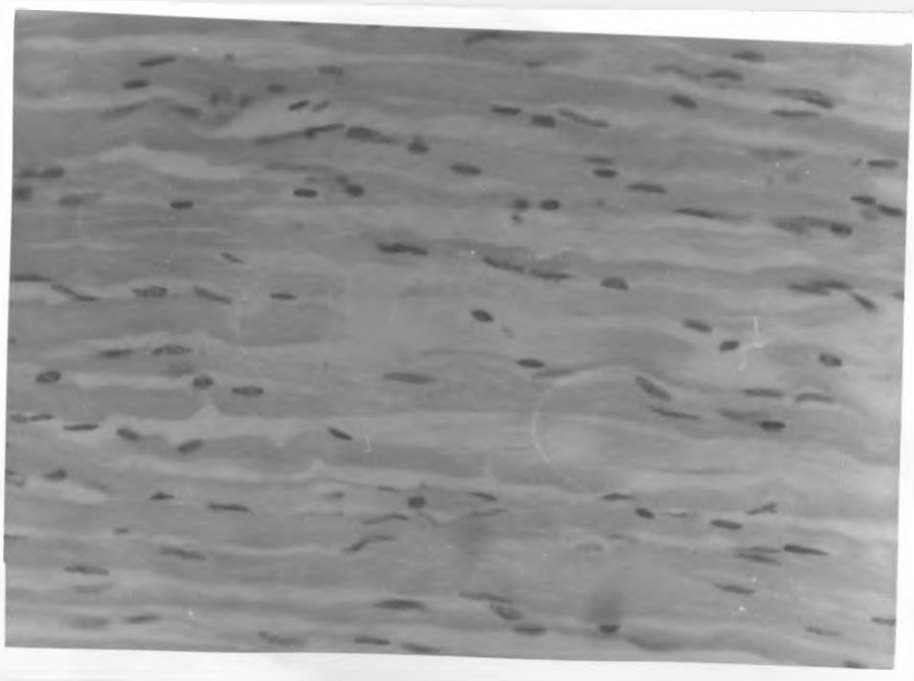

Plate 46: Goat myocardium: Zenker's degeneration. Some myocytes have lost their nuclei they cytoplasms appear coagulated exudate is seen in inter myofibre spaces. (H&E x 400).

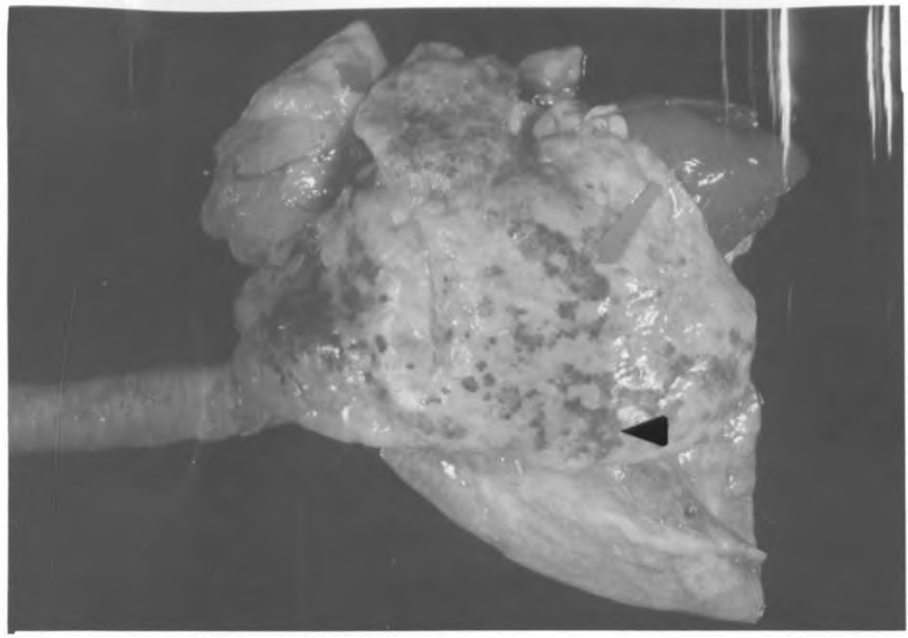

Plate 47: Goat lung: Severe ecchymotic haemorrhages in the lung of goat which died of acute toxicity of A remota extract.

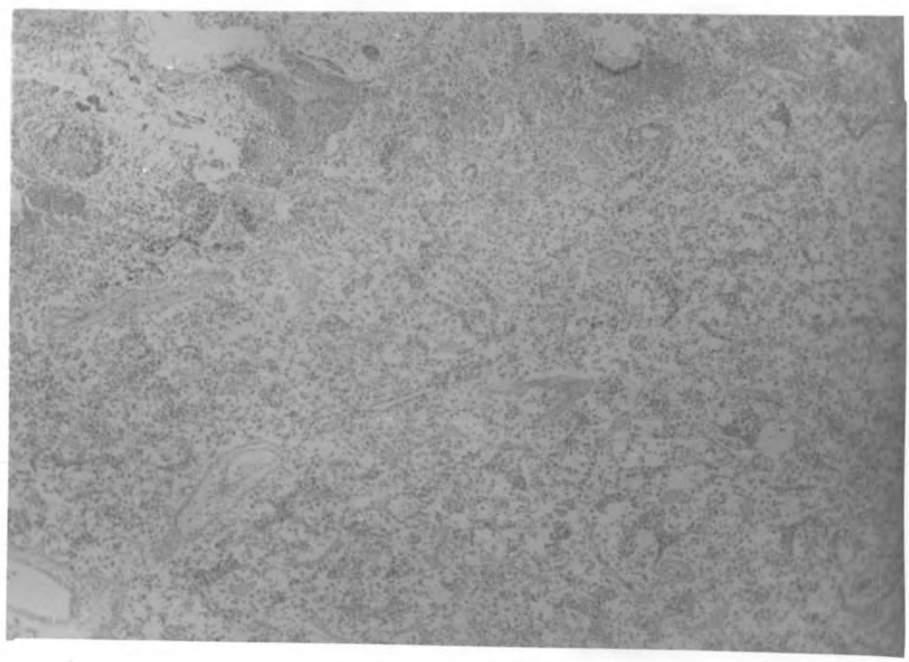

Plate 48: Goat spleen: Severe depletion of white pulp of lymphoid foci. Necrosis of lymphocytic elements of the spleen. The red pulp is also depleted. Hemosiderin granules deposits are also seen near a depleted lymphocytic nodule (H&E x 100).

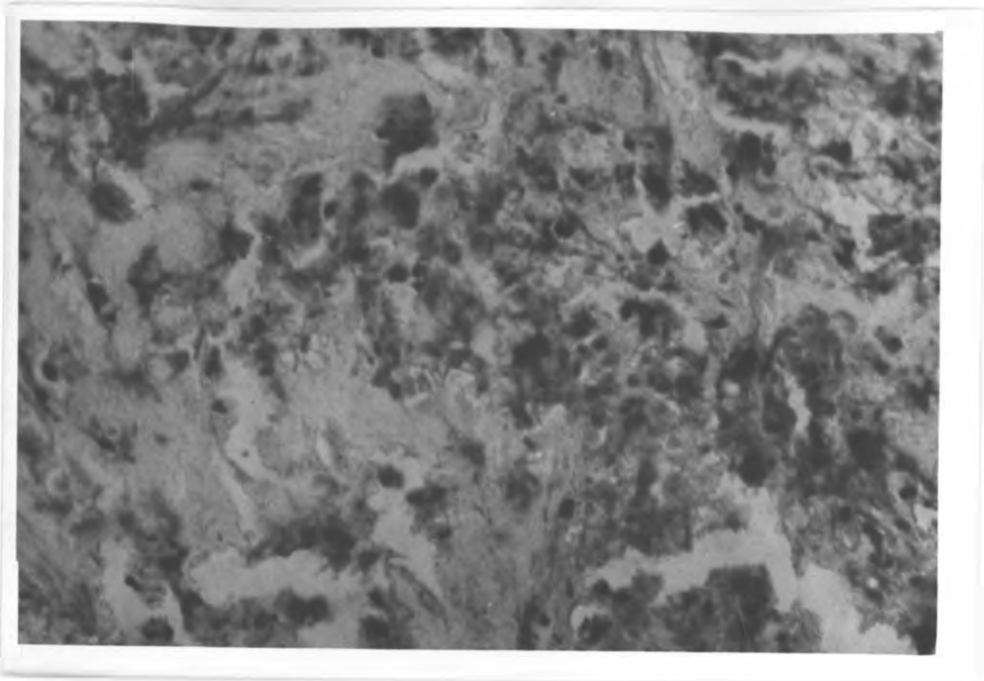

Plate 49: Goat Spleen: Hemosiderin granules deposits in the red pulp of the spleen of one of the goats which received high doses (Mallory's method x 400).

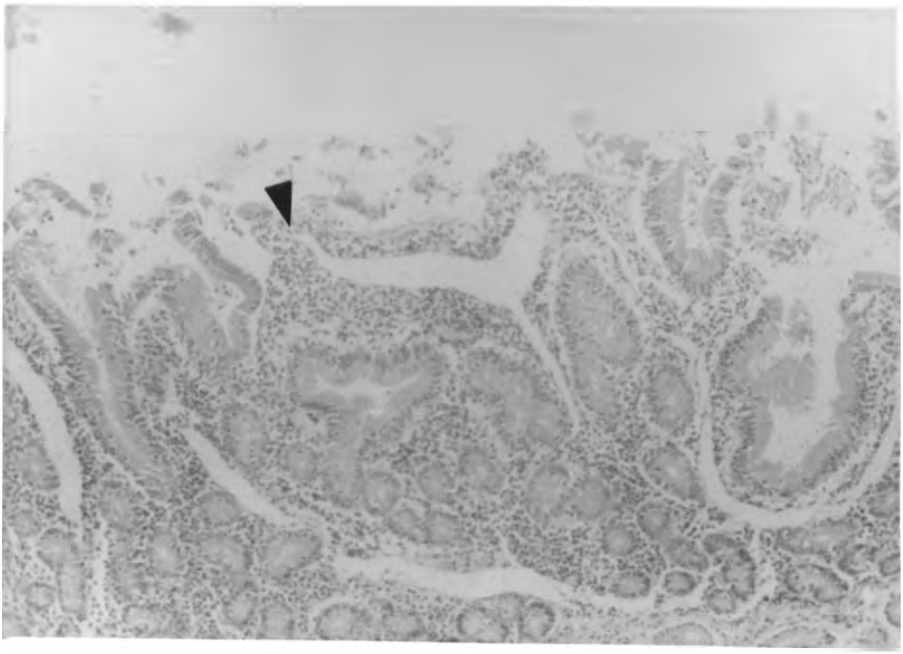

Plate 50: Small intestine: Necrosis of the mucosa of duodenum. The crests of the villi are eroded off (H&E x 400).

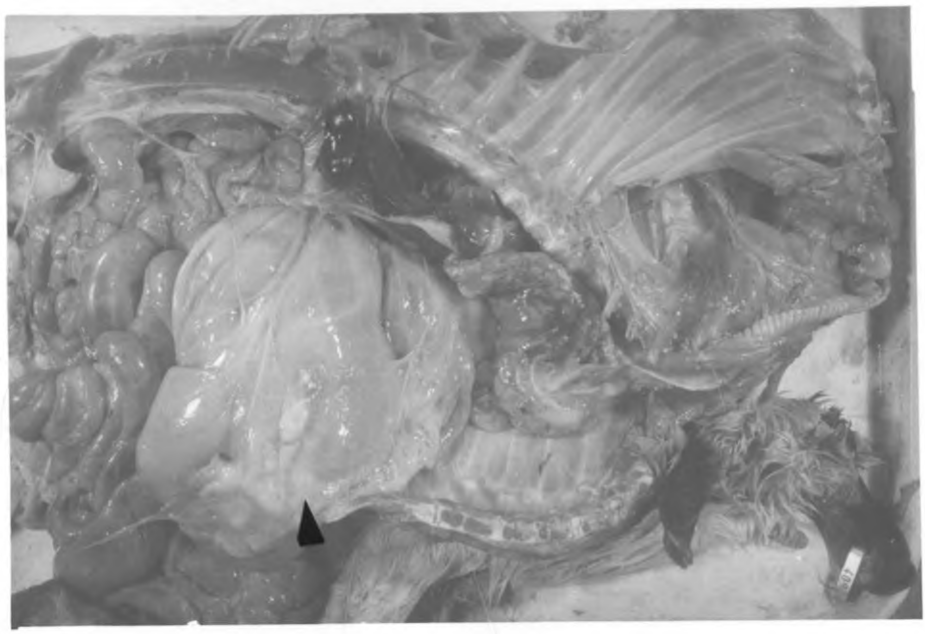

Plate 51: Goat thoracic cavity: Open thoracic cavity, gelatinous material covering the pericardium found in a goat which died after 20 days.

Plate 52: Goats developed oedema in limbs and along the ventral part of the abdomen around the sites of injection which used to subside 20-30 hours after administration.

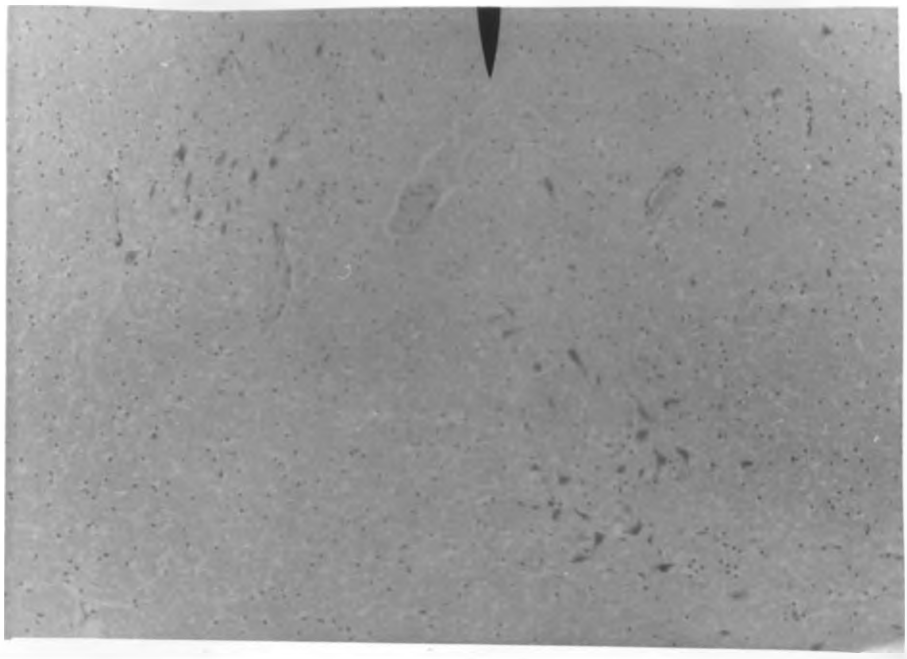

Plate 53: Goat brain: Cerebral haemorrhage and degeneration of neurons. Many neurons appear to have lost their cellular details (H&E x 100).

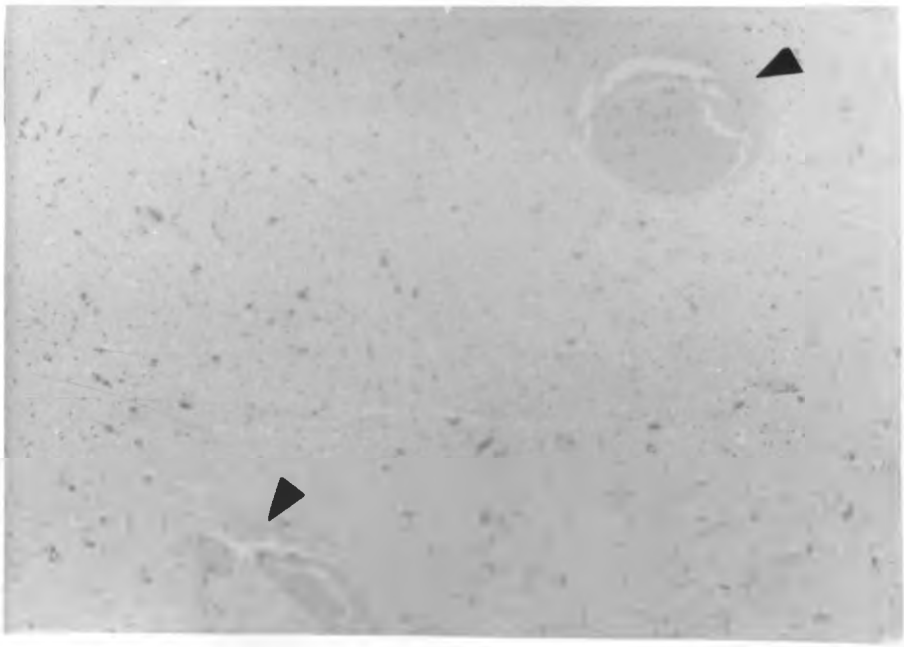

Plate 54: Goat brain: Severe cerebral congestion of blood vessels accompanied with extravasation of blood into brain tissue, (H&E x 200).

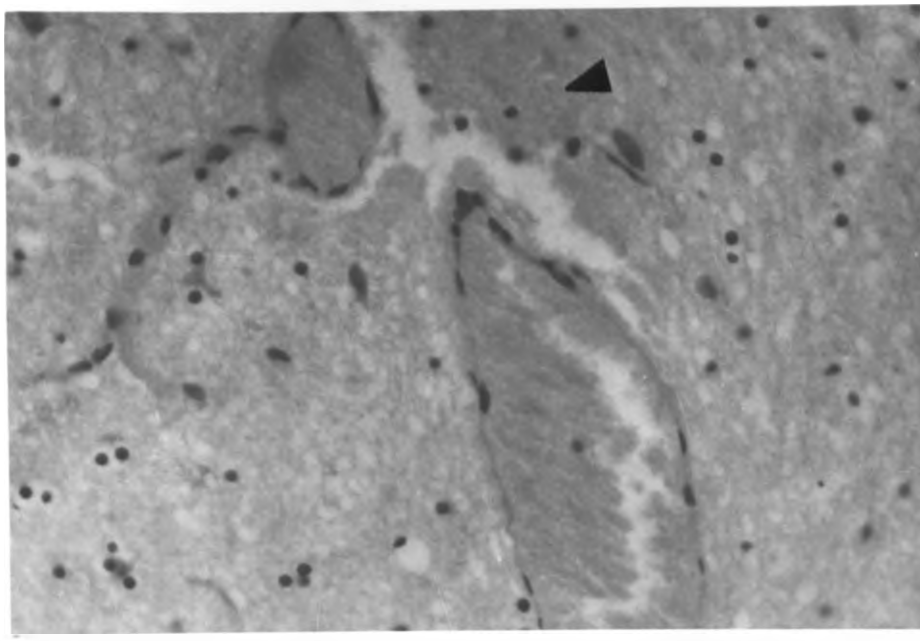

Plate 55: Goat brain: Cerebral haemorrhage, the capillaries appear damaged and a haemorrhagic zone (arrow) is seen (H&E x 400).

Plate 56: Goat brain: Severe cerebral congestion of blood vessels accompanied with marked perivascular vacuolation (H&E x 200).

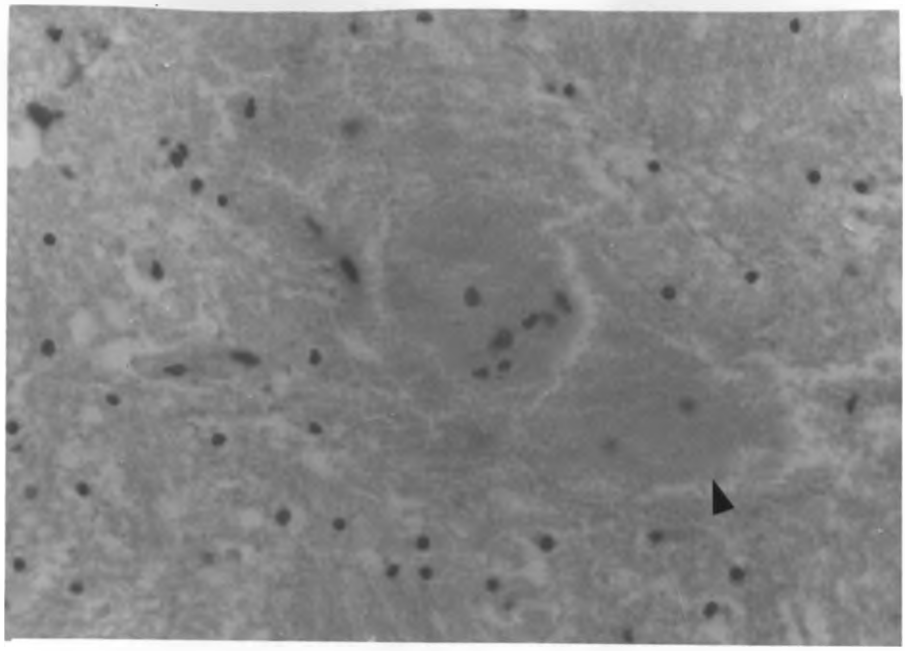

Plate 57: Goat brain: Severe cerebral haemorrhage. Extravasated blood is seen intermingled with the nervous tissue (H&E x 400).

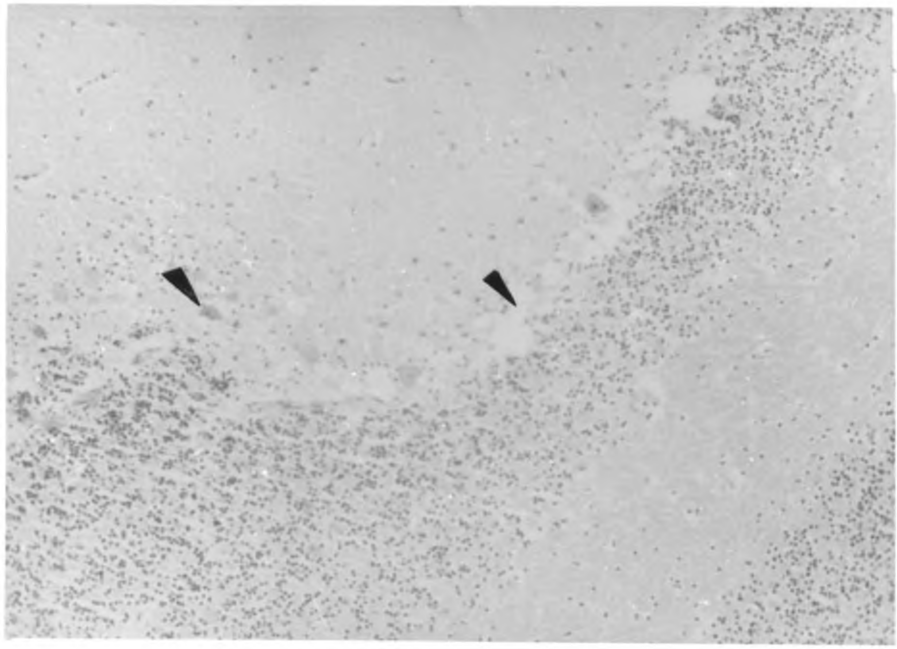

Plate 58: Goat brain: Cerebral oedema indicated by vacuolation in a portion of cerebellar folium along a layer of Purkinje cells. Necrotic cells are also seen (H&E x 400).

Plate 59: Goat brain: Focal liquifactive necrosis of the brain. Marked degeneration of neurons and necrosis. The nuclei of most neurons are Karyolytic (H&E x 200)

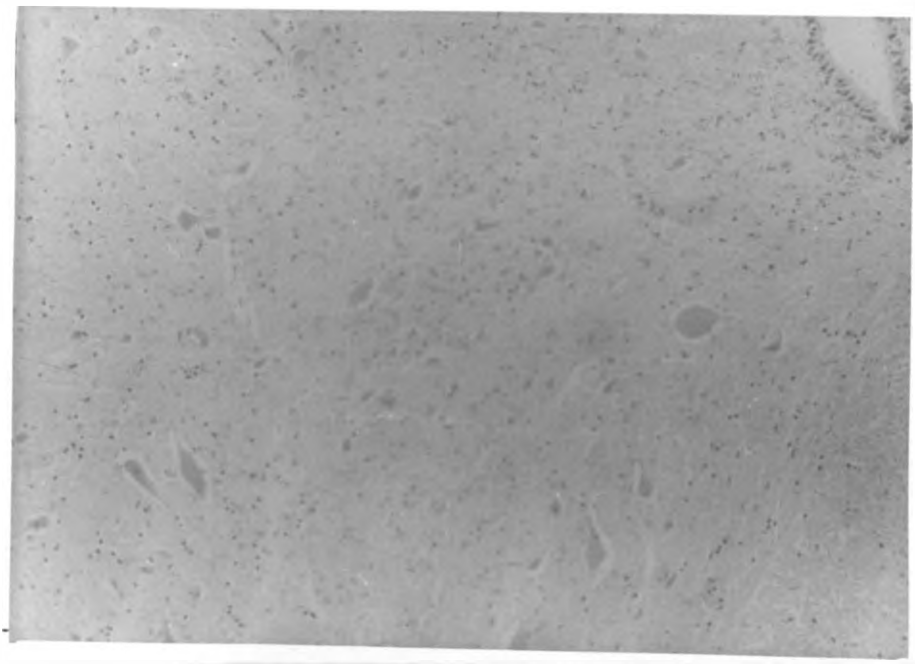

Plate 60: Goat Spinal Cord: Grey matter congestion of blood vessels. Perivascular and perineuronal vacuolations. Neurons show degenerative changes (H&E 200).

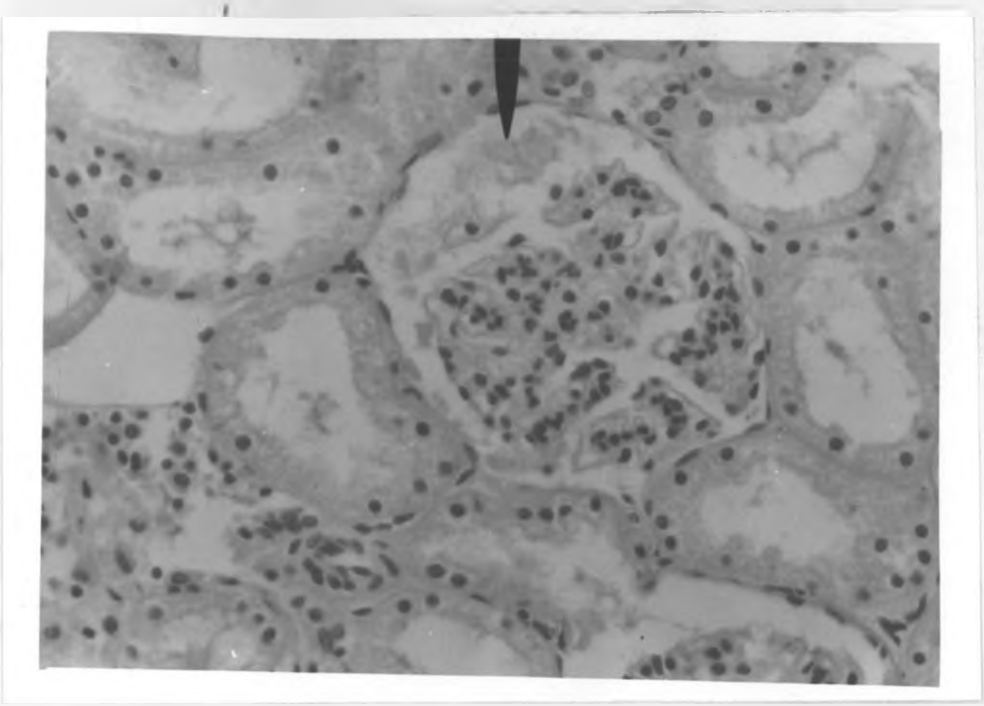

Plate 61: Goat kidney: Exudate in the periglomerular spaces (arrow). Coagulative necrosis of the uriniferous tubular epithelia. Most of the tubules are remaining with very few living cells along the lumina. Other cells are karyorrhectic and necrotic material is seen in the lumina (H&E x 400).

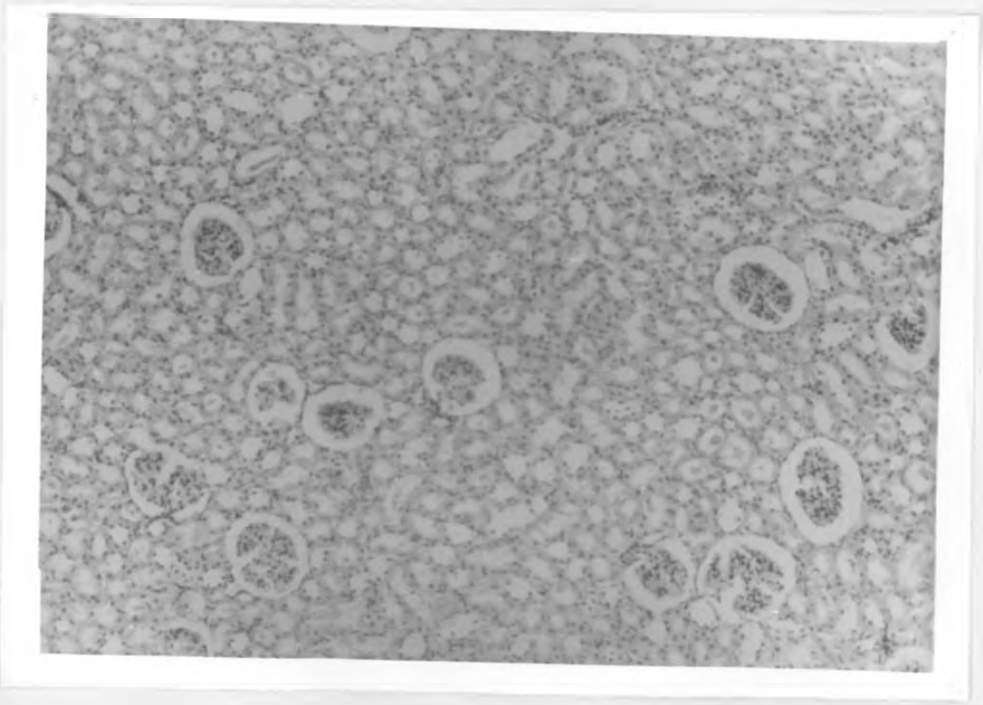

Plate 62: Goat kidney: Kidney cortex excessive increase of periglomerular space. The glomeruli themselves appear compressed most likely due to pressure exerted by accumulated exudate (H&E x 100).

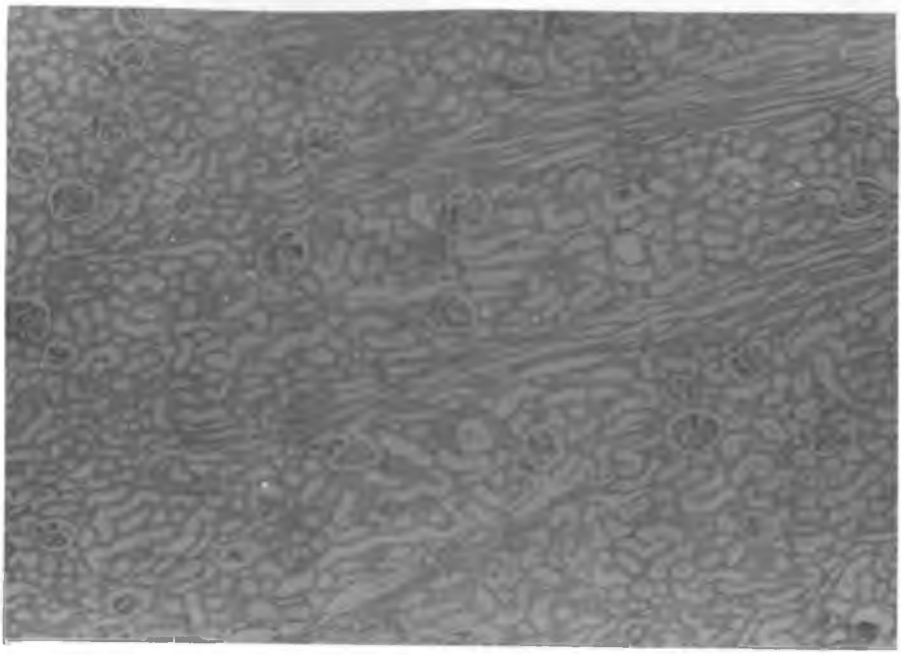

Plate 63: Goat kidney: Kidney medulla, degenerative changes in the straight collecting tubules (H&E x 100).

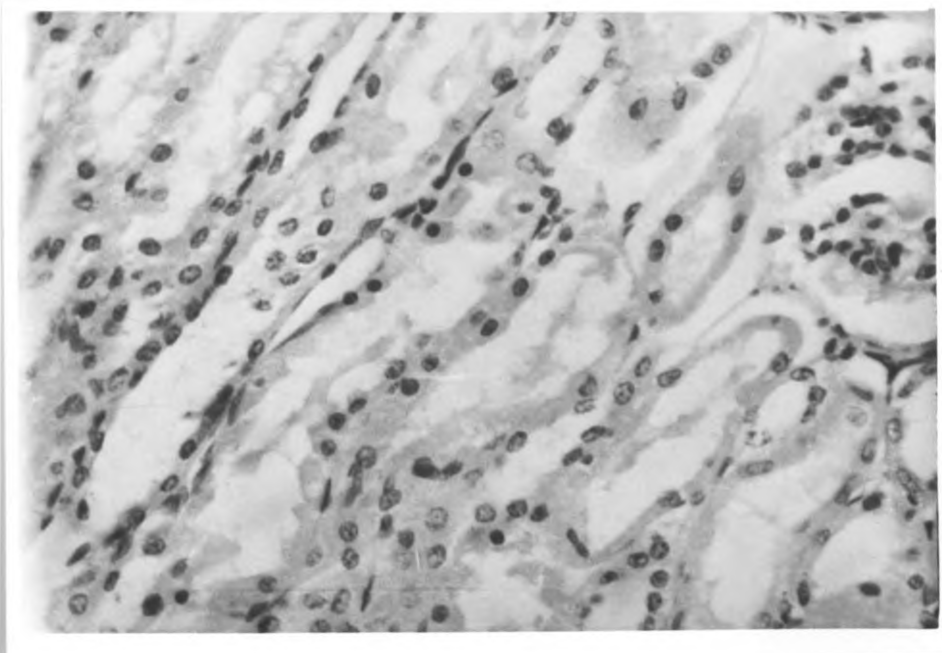

Plate 64: Goat kidney: Renal medulla, necrotic changes in the straight collecting tubules (H&E x 400).

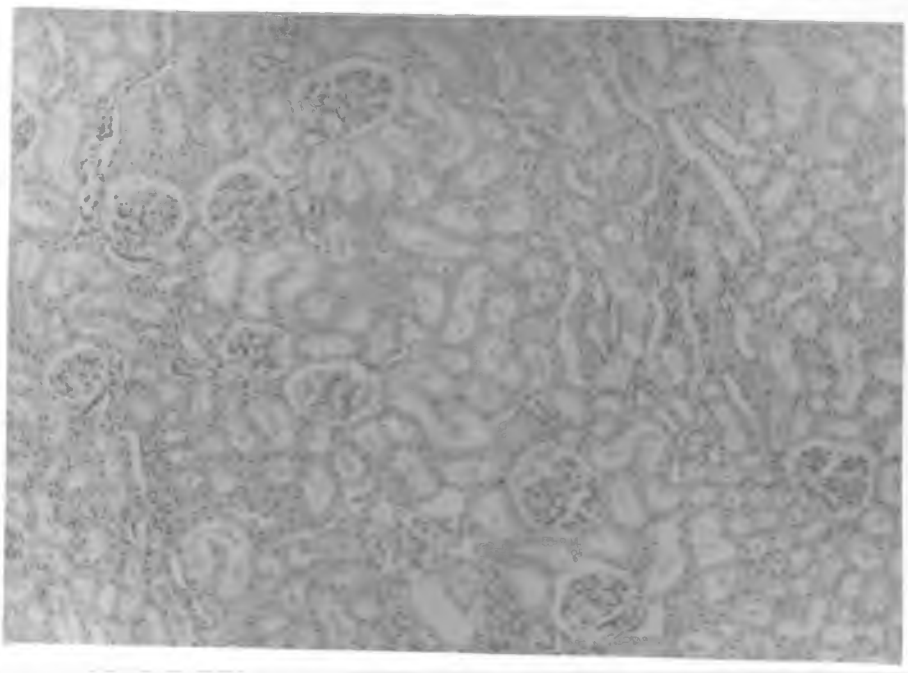

Plate 65: Goat kidney: Marked coagulative necrosis of proximal convoluted tubules in the cortical zone of the kidney. Hyaline casts are seen in the lumina of the tubules (H&E x 100).

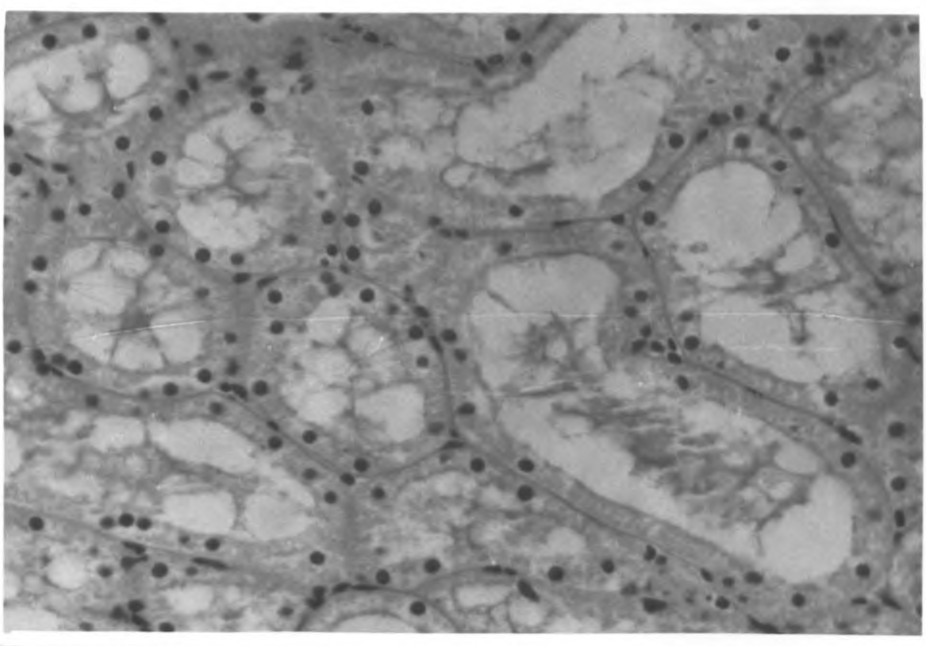

Plate 66: Goat kidney: Severe coagulative necrosis of proximal convoluted tubular epithelial cells. Most of the tubules have lost many of their cellular elements. Hyaline casts are seen in the lumina (H&E x 400).

8.0 DISCUSSION

The results indicated that when treated at rates from 90-900 mg per kilogram per fourty eight hours, the dried leaves and stems of *A. remota* extracted in water are toxic in goat and cause deaths at higher doses. The major lesions occur in the liver, kidneys, central nervous tissues and cardiovascular system.

A cardioactive compound was isolated and characterized (Kuria 1976, Kuria and Muriuki, 1984) also a number of compounds which have insect antifeedant activity (Kubo *et al*., 1976; Kubo *et al*., 1980, Kubo *et al*., 1982). They found that the insect antifeedants were neoclerodane derivatives, the ajugarins, but the cardioactive compound was not identified. Pharmacological activities of cardioactive compounds of fresh leaves extract in water were studied by Njunge et al., (1986). The extract was divided into two fractions A and B by column chromatography. Fraction A gave a positive reaction with Kedde's reagent for ajugarins. Fraction B gave 2 spots positive with Carr-Price reagent indicating triterpenoids four sports positive with ninhydrin reagent (one of these was Carr-Price positive) indicating sugar or amino acids. A nitrogen fusion test was negative so no amino acids were present. Fraction A was found to act on rabbit heart in vitro to cause arrhythmia slowing of the heart rate and reduction of force of contraction while fraction B reduced the force of contraction of myocardium.

When these fractions were tested in the cat, fraction A caused a sustained fall in blood pressure, was accompanied by fall of body temperature. Fraction B was found to cause a transitory fall in blood pressure.

Arrhythmia was noted in acutely and some of chronically treated goats in this study. The slowing and abnormal sounds of the heart were detected when aqueous crude extract was used in carprine. The effect was more marked in goats which received doses of 170 mg and above per kilogram body weight. Cardioactive compounds appear to have affected the cardiac muscles both physiologically and histologically. Zenker's degeneration was noted in histological sections of the myocardia of the treated goats. This also might have contributed the flabbiness of the heart muscle seen at postmortem.

Haemorrhages were a constant feature of the pathological finding in most of the acute and chronic cases. It is likely that the cardioactive compounds isolated and characterized (Kuria, 1976; Kuria and Muriuki, 1984), do not limit their activity on the heart only but extend to the vascular portion especially small vessels causing elevation in permeability and endothelial damage, thus leading to extensive extravasation of blood in various organs. These compounds seem to be responsible for congestion of blood vessels. Reduction of blood pressure might have affected the venous return leading to generalized congestion.

Venous stasis is likely to intensify the active principle activities on blood vessels leading to their subsequential damage. It is also assumed that the reduction of blood pressure decreased the kidney clearance as such leading to toxic substances accumulation in the body. However, more investigations are required on the activity of cardioactive compounds on vascular network and blood itself because no thrombosis was noted.

Another constant feature of the pathological picture in *A. remota* intoxication, are the nervous, hepatic and renal lesions. The presence of haemorrhages, congestion, perivascular vacuolation, perineuronal vacuolation in the brain and grey matter of the spinal cord are in accord with clinical evidence of circulatory, respiratory and nervous disturbances. Perivascular and perineuronal vacuolation in the central nervous tissues coupled with flattening of the gyrae of brain by intracranial pressure noted at necropsy strongly suggested oedema of the brain. The severity of the nervous signs during the course of *A. remota* toxicity might have been aggravated by the damage done on the liver cells. The lesions included congestion, haemorrhage, intracytoplasmic vacuolation, degeneration and necrosis. The elevation in aspartate amino transferase activity serum and a fall in the total plasma protein reflect disruption of hepatic cells as a result of necrosis or altered membrane permeability. However, this does not exclude the possibility of destruction of other tissues, since there is a wide distribution of

AspAT in other tissues such as muscles (skeletal and cardiac) as such it is of value in diagnosis of muscular degeneration.

Therefore extra-hepatic lesions like those in the kidneys and myocardia may have also contributed to leakage of Aspartate amino transferase into blood plasma. The release of AspAT into serum of ruminants (sited by Ahmed and Adam, 1950), to have been previously observed for example, in seneciosis in cattle (Ford, Richie and Thorpe, 1968) and in *Acanthospermum* poisoning in goat Ali and Adam 1978).

The lack of change in alanine amino transferase activity in serum confirmed previous experience with this enzyme in the ruminant by Cornelius (1960).

Elevation of alkaline phosphatase activity in serum indicated hepatic insufficiency and malfunction in the treated goats. This was in accord with hepatic lesions observed microscopically. The decreases in the means of levels of serum urea and creatinine despite the kidney damage observed, microscopically in the treated goats, is likely to have been caused by a direct mild diuretic effect of the toxic principles of *A. remota*.

The results obtained from haematological examinations indicate reduction in packed cell volume (PCV) together with red blood cells (RBC) counts from 31 to 18.7% and 18 to 13.8 millions respectively. Observation of plasma layer of haematocrit tube did not

show any presence of free haemoglobin (i.e. haemolysis). The mean corpuscular volume (MCV) of the treated goats did not show any significant alteration.The red blood cells may have become the target of antibodies which are likely to have coated their surfaces due to membrane deformities, and caused them to be removed from the circulation by the reticuloendothelial (RE) system. In the R.E. system macrophages in the spleen, Kupffer cells of the liver removes these abnormal red blood cells leading to immune mediated anemia. The normocytic anaemia was in accord with the finding of endogenous hemosiderin deposits in the spleen and the livers of some goats. Iron deposited in hemosiderin following uptake of affected red cells by reticuloendoethelial system could be considered either endogenous in origin if ferritin formation is an intermediate step or exogenous in origin if the iron aggregates directly after being dissociated from the haemoglobin of the red cell (Wixom *et al.*, 1980).

9.0 CONCLUSION

Injecting aqueous extract of A. remota Benth. to goats produced lesions in their livers, kidneys and central nervous system. The lesions mainly consisted of portal and centrilobular degeneration and necrosis of the liver cells and kidney uriniferous tubular damage in the cortex. Extensive haemorrhages also occurred in various organs including brains, lungs, kidneys and livers. The kidneys and livers were affected most followed by the central nervous tissues. The uriniferous tubular epithelial cells showed marked nephrosis. The livers had degenerative changes, intracytoplasmic vacuolation and necrosis of hepatocytes. The brain showed degeneration of neurons, perivascular and perinuerenal vacuolation and haemorrhages.

Haematological examinations revealed a marked erythrocytopenia in remota toxicity. This finding was in accord with the microscopical lesions observed in lymph nodes. Haemosiderin deposition was a common finding in spleen, and lymph nodes. Blood biochemical examinations revealed marked increase in aspartate amino transferase (GOT) and slight increase of alanine amino transferase (GPT) levels which were more marked in the terminal stages of the intoxication. It appears thus, the active compound(s) of A. remota Benth. are highly toxic to parenchymatous tissues and especially the liver and kidney. The striated muscles

also might have been involved. This is an important finding as regard to biochemical significance in *remota* toxicity.

Histopathological lesions were also noted in weaner Sprague Dowley rats injected with the aqueous extract of *A. remota* Benth. The lesions were similar to those noted in the carprine and were much more severe in the rats that received high dosages. Rats administered with sub-lethal doses of the extract for a long time, had curtailed growth rates, loss of body weights and general loss of conditions. There were marked haemorrhages in various organs mainly the viscera and central nervous tissues. Seventy percent of the highest dose croup showed intestinal invagination of small intestines. Degenerative and necrotic changes were noted in the livers, renal tubules and the brain. These lesions were in accord with clinical signs observed at antimortem.

This study suggests the need for more investigation on the *A. remota* , materials in purer forms and its implications in traditional medicine in relation to lesions caused in the body of man and animals.

Table 8: The mean ± S.D. of haematological values of goats treated with Ajuga remota Benth. extract

Days	PCV (%)	Hb (gm/100 ml)	RBC (x 10^6/mm^3
0	31.0±8.4	10.66±0.96	18.04±3.13
2	29.2±6.61	10.68±0.88	18.17±2.30
4	24.6±2.0	10.93±0.89	17.96±2.16
6	23.44±8.5	10.54±0.72	17.84±2.10
8	24.1±1.51	10.25	16.5±1.55
10	26.11±3.52	8.94	16.12±0.92
12	22.66±0.07	8.77	16.18±1.91
14	20.77±1.82	8.26	15.67±1.85
16	21.22±1.37	7.99	15.35±1.78
18	20.77±1.82	8.03	15.46±1.55
20	20.11±2.48	8.13	14.21±1.07
22	21.0±1.59	7.41	14.32±1.56
24	19.75±2.84	7.29	14.70±2.21
26	19.0±3.59	7.12±1.04	13.80±2.39
28	19.0±3.59	7.53±1.19	13.3±1.92
30	18.75±3.84	7.53±1.19	13.3±1.92
32	19.3±2.92	6.93±0.9	13.93±1.48
34	19.00±2.93	6.48±0.85	13.8±1.39
36	18.74±2.43	8.0±1.13	13.84±1.39

Table 9: The mean ± S.D. of haematological values of goats treated with *Ajuga remota* Benth. extract

Days	MCV (fl*) (sd**)	WBC
0	18.42±1.46	14158±3400
2	18.44±1.83	13238±3380
4	17.85±1.23	12209±4830
6	18.05±1.28	12680±2760
8	17.75±146	13820±3430
10	17.56±0.92	15510±3600
12	16.90±100	15850±4650
14	17.11±1.19	16880±6670
16	17.00±0.97	17550±4500
18	17.39±1.67	12560±4730
20	17.00±0.83	35380±6170
22	16.94±1.13	16880±3600
24	17.22±1.87	17590±4790
26	1672±1.12	20370±5870
28	16.75±0.89	21780±5870
30	16.56±1.02	18351±5610
32	16.5±0.85	18350±5610
34	16.5±1.04	16670±4650
36	16.69±0.84	16830±4530

fl* = Femtolitre

s.d.** = Standard deviation

Table 10: The mean ± S.D. of haematological values of goats treated with *Ajuga remota* Benth. extract

Days	P.* (gm/100 ml)	MCHC gm/100
0	7.3 ± 0.47	34.84 ± 1.44
2	7.52 ± 0.42	34.98 ± 3.09
4	7.24 ± 0.51	34.92 ± 2.44
6	6.97 ± 0.66	37.94 ± 5.17
8	6.99 ± 0.77	41.86 ± 5.22
10	6.26 ± 0.66	38.84 ± 2.94
12	6.31 ± 0.62	36.56 ± 2.11
14	6.22 ± 0.62	35.27 ± 2.47
16	6.2 ± 0.51	36.1 ± 6.63
18	6.16 ± 0.38	38.44 ± 2.47
20	6.42 ± 0.53	39.84 ± 4.39
22	6.14 ± 0.38	36.21 ± 5.58
24	6.16 ± 0.55	35.74 ± 3.77
26	6.02 ± 0.32	35.25 ± 4.44
28	6.06 ± 0.63	38.05 ± 5.15
30	5.94 ± 0.56	36.59 ± 3.81
32	5.94 ± 0.49	34.21 ± 4.66
34	6.05 ± 0.49	36.48 ± 5.35
36	5.99 ± 0.54	

*Total plasma protein measured in gm/100 ml of blood

Table 11: The mean ± S.D. of serum biochemical values of goats treated with Ajuga remota Benth. extract

Time in days	LDH[1]	BUN[2]	Serum Creatinine U mol/litre
0			84.87±21.3
2			
4			84.87±21.3
6	252±92	7.16±2.25	71.42±20.8
8			89.48±17.29
10		8.26±2.51	
12			
14		6.60±1.84	
16			
18	198±87	4.02±1.46	71.14±19.45
20		5.57±2.29	63.46±12.36
22			60.28±13.15
24		4.56±1.01	68.14±29.1
26	198±6.5		80.5±16.63
28			87.74±22.62
30			67.6±28.34
32			73.2±25.79
34			59.15±7.31
36			48.11±8.23

[1]Lactate dehydrogenase measured in mg/100 ml of blood.
[2]Blood urea nitrogen measured in U mol/100 ml of blood.

Table 12 The mean ± S.D. of serum biochemical values of goats treated with *Ajuga remota* Benth. extract

Time in days	Serum alkaline Phosphatase K&K u/100 ml	Alanine Aminotransferase micro mol/ml	Aspartate Aminotransferase micro mol\ml
0			
2		76.75±3.7	99.17532.7±
4		20.66±5.6	91.0±24.94
6	68.0±50.5	21.88±5,5	40.85±18,12
8		22.58±6.3	99.0±51
10		32.8±4.9	109.2±22
12		36.6±7.1	115
14		21.1±5.3	123.22±37
16		23.6±5.2	116.78±41
18		26±5.9	112.44±46
20	45.33±39.4	28.9±3.1	123.0±36.6
22		23.7±3.5	140.4±36.7
24		22.2±3.6	124.9±39.2
26	45.00±51	29.9±3.1	99.0±47.2
28		28.9±3.4	93.8±27.7
30			85.0±28
32	49.78±51.8		122.75±18
34		28.6±8.5	129.8±41
36		30.6±15.9	120.4±44.8

Table 13: The mean ± S.D. of haematological values of goats used as controls in experiment with *Ajuga remota* Benth. extract

Days	WBC	RBC (x 10^6/mm^3)	MCV
0	15950±2616		
2	15950±2620	18.15±0.6	18.8±1
4	15200±3959	17.8±0	18.5±1.4
6	14285±3372	17.0±0.4	18.0±0
8	15400±1838	17.33±0.95	18±0.7
10	13330±3068	17.17±0.06	18.3±0.4
12	13730±1513	16.38±0.32	17.5±0
14	15350±3323	15.68±0.25	17.5±0
16	11665±2877	1543±0.53	17.8±0.4
18	13040±1357	15.58±0.11	18±0.7
20	13950±1767	15.45±0.78	17.8±.4
22	12515±3230	15.03±0.46	17.5±0
24	12795±1140	14.8±0.14	17.5±0
26	15850±70	15.65±0.14	17.8±.4
28	14660±1800	15.68±.46	17.8±0.4
30	13970±3090	15.8± .28	17.5±0
32	14150±2470	15±0	17.3±6
34	13100±140	15.8±1.3	17.5±0
36	15050±2190	16.3±.28	17.5±0

Table 14: The mean ± S.D. of haematological values of goats used as controls.

Time in days	PCV (%)	T.P. (in gm/ 100 ml)	Hb Conc. (gm/100 ml)	MCHC (gm/100 ml)
0	31±1.4	-	-	34.9±0.21
2	31±1.4	7.2±.28	10.8±.42	34.95±1.06
4	30.5±0.7	7.5±0	10.65±.07	36.5±1.70
6	30.5±0.7	7.4±.28	11.15±.78	36.5±2.83
8	30±0	7.5±.14	10.7±.57	33.25±2.76
10	30±1.4	7.5±0	9.95±.35	36.4±3.25
12	26±4.2	7.15±.21	9.4±.7	35.0±0.85
14	25±0	7.0±.28	8.75±.21	35.05±1.48
16	24±1.4	7.3±.28	8.4±.14	34.3±0.14
18	24.5±0.7	7.25±0.07	8.4±.28	36.6±2.83
20	22.5±2	6.55±.49	8.2±.14	34.8±1.27
22	23±0	6.9±0.14	8.0±.28	34.85±0.78
24	23.5±0.7	6.75±.35	8.2±.42	38.25±6.72
26	21.5±2.1	7.15±0.21	8.15±.6	34.35±1.48
28	24±0	7.0±0	8.25±.35	34.6±3.11
30	23.5±2.12	6.9±.42	8.1±0	33.2±0.56
32	25±0	6.4±.28	8.3±.14	34.3±0.71
34	23.5±.7	6.7±.42	8.05±0.07	33.85±2.62
36	24±1.4	7.2±28	8.1±0.14	

Table 15: The means ± S.D. of serum biochemical values of the goats used as controls (saline recipients)

Time in days	LDH	BUN (m mol/100 ml)	Serum Creatine (m mol/100 ml)	Alkaline Phosphatase (m mol/100 ml)
0				
2				
4			86.35±11.67	
6			71.6±0.99	
8	199.5±22	6.08±1.39	105.5±15.5	58.5
10		8.96±2.66	85.0±4.7	
112				
14		4.01±1.03		
16		5.28±0.62		
18			85.0±4.7	
20	234.5±14.8	4.70±0.4	61.6±7.57	33.5±9.2
22			71.4±1.9	
24		5.48±.23	62.9±4.4	
26	186±0		85.0±20	24±5.65
28			74.3±16.7	
30			83.7±8.6	
32			57.8±2.19	28.5±2.12
34			53.1±5.09	
36			72.95±6.6	

Table 16: The means ± S.D. of blood biochemical of goats used as control in the experiment with *A. remota Benth.* extract.

Time in days	Aspartate amino-transferase (GOT)	Alanine amino-transferase in IU
0	87.5	
2	87.5	13±2.8
4	75.5	17.3±2.5
6	32±16.9	16.25±1
8	39.6±8.1	21±2.8
10	70.5±4.9	33.5±6.4
12	102.5±21.9	31.5±3.5
14	116.5±26.2	21±7
16	138±67	23±0
18	136.5±26.2	24±7
20	146.5±55	37.5±6.4
22	76.5±48.7	20±4.2
24	128±9	27±1.4
26	105±45/3	34.5±0.7
28	89.5±13.4	30.5±2
30	61±5.7	30.5±2
32	123.5±3.5	17.5±2.12
34	103±57.9	30.5±2.12
36	103±25.5	24±2.8

Table 16: The means ± S.D. of blood biochemical of goats used as control in the experiment with A. remota Benth. extract.

Time in days	Aspartate amino-transferase (GOT)	Alanine amino-transferase in IU
0	87.5	
2	87.5	13±2.8
4	75.5	17.3±2.5
6	32±16.9	16.25±1
8	39.6±8.1	21±2.8
10	70.5±4.9	33.5±6.4
12	102.5±21.9	31.5±3.5
14	116.5±26.2	21±7
16	138±67	23±0
18	136.5±26.2	24±7
20	146.5±55	37.5±6.4
22	76.5±48.7	20±4.2
24	128±9	27±1.4
26	105±45/3	34.5±0.7
28	89.5±13.4	30.5±2
30	61±5.7	30.5±2
32	123.5±3.5	17.5±2.12
34	103±57.9	30.5±2.12
36	103±25.5	24±2.8

Table 17: Means of body, weight of experimental goats in Kilograms

Time in days	Treated goats	Control
2	14.5	15.75
8	14.15	16.00
12	13.0	16.5
20	12.55	16.25
28	13.0	16.75
34	12.75	17.25

Table 18: Means of experimental groups of rats on daily (body weight) basis

Day	T1	T2	T3	T4	T5	T6	T7
1	81.3	69.6	80.8	90.3	89.8	76.5	89.5
2	94.9	83.3	97.0	107.0	98.3	83.6	94.1
3	106.0	92.6	111.4	118.1	106.8	91.0	102.0
4	114.3	100.4	121.6	125.0	118.1	100.1	109.1
5	119.8	107.1	127.0	133.4	123.6	107.5	112.3
6	124.1	113.1	127.3	135.5	126.3	109.9	116.8
7	126.6	118.0	128.0	138.6	130.3	112.8	124.5
8	132.4	124.3	135.6	148.3	140.5	121.4	125.6
9	136.8	123.0	142.1	152.1	144.6	125.1	130.8
10	143.5	132.4	139.6	158.9	145.5	130.0	134.8
11	145.8	136.4	140.4	159.0	147.5	133.5	138.0
12*	152.3	140.8	146.4	162.4	152.1	137.0	141.1
13	153.4	138.8	147.4	159.4	155.5	136.9	147.0
14	149.0	136.8	147.6	164.0	156.0	142.8	145.4
15	147.9	135.9	147.0	160.6	156.5	145.0	150.5
16				146.9	155.9	143.6	153.0
17				118.8	155.9	143.4	156.3
18					159.0	144.8	160.6
19					162.1	148.6	163.4
20					163.0	152.9	165.0

*Day of administration

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11.0 APPENDICES

Appendix I: Daily total weight gm of rat groups treated with Ajuga remota Benth. extract.

Days of treatment	Rat groups 1	2	3	4	5	6	7 (Control)
0	1106	1091	1123	1272	1180	1068	1114
1	1218	1125	1171	1299	1217	1096	1129
2	1227	1110	1179	1275	1244	1095	1176
3	1192	1092	1181	1321	1248	1025	1163
4	1183	1089	1176	1285	1252	1160	1204
5	756	549	528	1026	1247	1149	1224
6	291	290	440	713	1242	1147	1250
7			416	279	1272	1158	1285
8			156	262	1297	1189	1307
9					1304	1223	1320
10					1329	1251	1362
11					1342	1248	1352
12					1356	1280	1363
13					1382	1302	1422
14					1392	1324	1420
15					1416	1371	1439
16					1436	1371	1471
17					1418	1373	1463
18					1422	1374	1479
19					1439	1424	1510
20					1120	1422	1520
21					1138	1420	1511
22					1157	1435	1555
23					1169	1444	1569
24					1084	1330	1594
25					1217	1355	1628
26					1224	1337	1643
27					1241	1366	1646
28					1233	1361	1669
29					1251	1397	1697

Appendix II: Packed cell volume PCV (in percentage)

	Control goats			Treated goats		
Days	402	407	411	409	405	404
2	30	32	30	30	32	33
4	31	30	28	30	31	34
6	30	31	27	34	31	33
8	30	30	27	28	24	34
10	31	29	24	30	20	29
12	23	29		19	22	30
14	25	25		21	22	33
16	23	25		20	23	31
18	24	25		23	20	29
20	21	29		17	22	27
22	23	23		17	22	29
24	23	24		15	22	29
26	23	20		14	24	24
28	24	24			23	26
30	25	22			23	24
32	25	25			21	22
34	24	23			21	23
36	25	23			20	24

	410	401	403	406	408	412
2	33	34	27	26	30	31
4	30	37	29	25	33	31
6	29	35	28	25	34	30
8	29	33	30	26	32	29
10	29	23	22	24	23	24
12	25	22	22	25	24	22
14	27	23	22	24	24	21
16	29	21	22	23	23	20
18	20	21	19	23	24	25
20	22	18	20	22	20	19
22	22	18	20	24	21	18
24	23	18	19	24	20	17
26	20	18	21	24	18	18
28	23	17	18	23	18	20
30	19	16	18	21	20	17
32	18	15	20	23	17	16
34	20	15	18	22	17	16
36	19	16	18	18	17	18

Appendix III: Total erythrocyte counts (10^6/mm^3)

Days	402	407	411	409	405	404
2	17.8	18.5	17.5	11.35	20.5	18.0
4	17.8	17.8	17.0	18.5	19.0	17.35
6	17.25	16.75	16.0	18.5	18.5	19.85
8	16.65	18.0	15.75	17.5	20.5	21.2
10	17.2	17.1		14.85	15.6	19.9
12	16.6	16.15		13.4	16.2	19.0
14	15.85	15.5		12.6	16.7	19.45
16	15.8	15.05		13.4	15.7	19.45
18	15.65	15.3		12.6	15.85	19.1
20	14.9	16.0		13.9	16.7	18.3
22	14.7	15.35		13.4	15.2	15.05
24	14.7	14.9		11.85	15.6	17.15
26	15.55	15.73		11.15	18.0	17.1
28	16.0	15.35		9.85	16.25	16.75
30	16.0	15.6		10.5	17.35	17.35
32	15.0	15.0			15.6	16.5
34	16.75	14.85			16.5	14.3
36	16.5	16.1			16.3	14.7

	Treated				
	410	401	403	406	408	412
2	18.0	22.8	19.0	13.0	20.0	20.0
4	18.4	21.8	17.15	12.5	21.0	19.7
6	16.6	21.0	17.5	13.5	18.53	19.55
8	17.0	19.75	18.0	14.5	17.4	16.75
10	17.45	16.95	16.1	14.8	16.55	16.4
12	15.8	19.33	16.0	14.7	15.3	15.35
14	17.4	17.2	15.85	14.75	15.4	16.25
16	17.65	14.3	15.3	15.85	14.85	14.55
18	16.4	14.5	15.35	15.3	14.5	14.45
20	15.1	14.1	17.15	14.4	14.6	14.85
22	14.9	12.9	14.44	14.2	12.9	13.85
24	15.3	13.07	13.95	14.35	14.3	13.30
26	16.0	13.0	15.05	15.5	13.5	13.00
28	14.9	10.15	14.6	14.0	13.85	13.85
30	13.25	11.85	13.1	14.8	13.4	13.2
32	12.65	10.35	13.1	12.5	12.55	13.15
34	14.2	11.6	14.8	14.0	13.5	12.50
36	13.5	11.4	14.2	14.0	13.4	13.2

Appendix IV: Total plasma protein (gm/100 ml)

Days	402	407	411	409	405	404
2	7.0	7.4	7.2	7.2	6.2	7.2
4	7.5	7.5	7.3	7.3	6.5	7.8
6	7.2	7.6	7.0	8.0	6.4	6.8
8	7.4	7.6	6.2	6.9	6.2	7.3
10	7.5	7.5	5.3	6.7	6.5	7.1
12	7.0	7.3	5.2	5.2	5.9	7.0
14	6.8	7.2		5.9	6.2	6.0
16	7.5	7.1		5.2	6.5	6.0
18	7.3	7.2		5.5	6.9	6.0
20	6.9	6.2		5.8	6.0	5.7
22	7.0	6.8		6.5	7.0	6.0
24	7.0	6.5		6.6	6.5	5.8
26	7.3	7.0		5.9	6.7	5.6
28	7.0	7.0		5.8	6.6	5.7
30	7.2	6.6			6.2	5.0
32	6.6	6.2			6.2	5.2
34	7.0	6.4			6.0	5.3
36	7.4	7.0			6.5	5.5
38	7.4	7.3			6.3	5.2

Days	Treated goats					
	410	401	403	406	408	412
2	8.0	7.6	8.0	7.2	7.4	7.2
4	8.0	7.5	8.0	7.3	8.0	7.5
6	7.6	7.1	7.6	6.8	8.0	6.8
8	7.4	6.5	7.9	6.8	7.5	7.0
10	7.8	6.7	7.9	6.9	7.6	7.4
12	6.9	6.2	6.5	6.3	6.9	6.5
14	6.6	6.0	6.9	6.1	5.7	6.4
16	6.8	5.7	6.9	5.6	6.8	6.5
18	6.5	5.6	6.9	6.0	6.0	6.4
20	6.7	6.0	6.3	6.0	6.1	6.8
22	6.2	5.5	6.8	7.0	6.8	6.0
24	6.4	5.4	6.4	6.0	6.2	6.0
26	6.8	5.4	7.0	6.0	6.0	6.0
28	6.0	5.7	6.5	6.0	5.9	6.0
30	6.6	5.2	6.4	6.0	6.0	6.5
32	6.5	5.0	6.2	5.8	6.2	6.4
34	6.5	5.2	6.3	5.8	5.9	6.5
36	6.4	5.2	6.3	5.8	6.2	6.5
38	6.3	5.1	6.2	6.0	6.3	6.5

Appendix V: Hemoglobin concentration (GM/DL i.e. gm/100 ml)

Days	402	407	411	409	405	404
2	10.5	11.1	10.3	11.2	11.4	11.2
4	10.6	10.7	11.2	11.7	11.1	11.4
6	10.6	11.7	9.9	11.3	11.6	11.0
8	10.3	11.1	10.1	10.9	10.6	11.5
10	9.7	10.2	9.9	10.6	10.0	11.2
12	8.9	9.9		7.7	8.6	10.6
14	8.9	8.6		8.1	7.5	11.3
16	8.3	8.3		6.8	7.5	10.3
18	8.2	8.6		6.8	6.9	10.4
20	8.1	8.3		6.8	7.9	11.0
22	7.8	8.2		6.9	8.7	10.0
24	7.9	8.3		6.5	7.9	7.9
26	7.7	8.6		5.8	7.4	8.9
28	8.0	8.5		5.6	7.9	9.2
30	8.1	8.1			8.5	9.1
32	8.2	8.4			7.9	8.4
34	8.1	8.0			7.5	7.4
36	8.0	8.2			8.2	7.5

Treated goats

Days	410	401	403	406	408	412
2	10.7	12.3	9.6	9.9	10.0	10.9
4	10.4	12.0	9.9	9.1	10.7	9.4
6	10.9	12.4	10.6	8.9	11.1	11.1
8	10.5	11.5	10.8	9.1	10.0	10.4
10	9.8	10.6	10.6	9.1	10.2	10.1
12	9.6	8.5	9.5	9.2	8.2	8.2
14	10.1	8.5	8.6	8.4	8.2	8.2
16	9.5	8.0	8.0	8.4	7.9	7.9
18	8.9	8.0	8.1	8.3	7.2	7.3
20	9.1	7.4	7.8	8.0	7.5	6.8
22	10.1	7.2	8.1	7.5	8.1	6.6
24	9.0	6.5	8.2	7.9	6.8	6.5
26	8.3	6.2	7.2	7.6	6.5	6.2
28	8.3	6.2	7.0	8.3	6.8	6.3
30	8.0	6.1	7.6	8.4	6.5	6.0
32	6.7	5.8	6.6	7.8	6.5	5.7
34	6.7	5.1	6.3	7.1	5.9	5.8
36	6.1	5.1	7.2	8.1	6.6	5.7

Appendix VI: Mean corpuscular volume MCV
(In Femtolitre fl.)

Days	402	407	411	409	405	404
0	18.0	19.5	17.5	18.5	16.0	19.5
2	17.5	19.5	18.5	21.0	15.5	20.0
4	17.5	18.0	18.0	18.0	15.1	19.0
6	18.0	18.0	18.5	18.5	16.0	19.5
8	17.5	18.5	17.5	18.5	15.5	18.5
10	18.0	18.0		17.5	18.0	18.0
12	18.0	19.0		16.5	15.1	18.0
14	18.0	19.5		17.5	15.5	18.0
16	17.0	17.5		16.5	19.0	18.0
18	18.5	18.0		16.5	20.5	18.0
20	17.5	17.5		16.5	18.5	16.5
22	17.5	17.5		16.5	16.0	19.0
24	18.0	17.5		16.0	15.5	17.5
26	17.5	18.5		16.0	15.0	17.5
28	17.5	18.0			16.0	17.5
30	17.5	17.5			16.0	17.5
32	17.5	17.0			16.0	17.5
34	17.5	17.5			16.5	17.5
36	17.5	17.5			16.0	18.0

Treated goats

Day	410	401	403	406	408	412
0	19.0	17.0	19.0	21.5	18.5	17.0
2	17.5	17.0	19.3	21.5	17.0	17.0
4	18.5	17.0	17.5	20.1	18.5	17.0
6	18.0	17.5	17.5	20.5	17.5	17.0
8	18.0	16.5	17.5	21.0	17.5	17.0
10	17.5	16.5	17.0	19.5	17.5	16.5
12	17.5	16.5	16.5	18.5	17.0	16.5
14	17.5	16.0	16.5	19.5	17.0	16.5
16	17.5	16.0	16.5	16.5	16.5	16.5
18	17.5	16.5	15.5	19.5	16.5	16.0
20	18.0	17.0	16.5	17.5	16.5	16.0
22	17.5	16.0	16.5	18.5	16.5	16.0
24	17.5	16.0	16.0	18.5	16.5	21.5
26	17.0	18.5	16.5	18.0	16.0	16.0
28	17.0	16.0	16.5	18.5	16.5	16.0
30	17.5	16.0	16.5	18.0	16.0	15.0
32	17.5	16.0	16.5	18.0	16.0	15.0
34	16.5	16.0	16.5	18.0	16.5	14.4
36	16.5	16.0	16.5	18.0	16.5	16.0

Appendix VII: Mean corpuscular haemoglobin concentration (MCHC)

Days	402	407	411	409	405	404
0	35.0	34.7	34.3	37.3	35.6	33.9
2	34.2	35.7	40.0	39.9	37.0	33.5
4	35.3	37.7	36.7	33.3	37.4	33.3
6	34.3	38.3	37.4	38.9	44.2	33.8
8	31.3	35.2	41.3	35.3	50.0	38.6
10	38.7	34.1		40.5	39.1	35.3
12	35.6	34.4		38.6	34.5	34.2
14	36.1	34.0		34.0	32.6	33.2
16	34.2	34.4		29.6	30.0	38.5
18	38.6	34.6		40.0	35.9	40.7
20	33.9	35.7		40.6	39.5	45.5
22	34.3	35.4		43.3	33.6	25.5
24	33.5	43.0		41.4	30.8	37.1
26	33.3	35.4			34.3	35.4
28	32.4	36.8			36.9	37.9
30	32.8	33.6			37.6	38.2
32	33.8	34.8			35.7	32.2
34	32.0	35.7			41.0	31.3

	Treated goats					
Days	410	401	403	406	408	412
0	32.4	36.2	35.6	34.6	33.3	35.0
2	34.7	32.4	34.1	36.4	32.4	30.3
4	37.6	35.4	30.3	35.6	32.6	37.0
6	36.2	34.8	49.0	37.9	31.3	35.9
8	36.3	46.1	48.2	36.4	44.3	42.1
10	38.4	43.2	43.2	36.8	35.8	37.3
12	37.4	36.6	39.1	35.0	34.2	39.1
14	32.8	38.1	36.4	36.5	34.3	39.5
16	44.5	44.4	42.6	36.1	30.0	29.2
18	41.4	41.1	39.0	36.4	35.7	35.8
20	45.9	40.0	40.5	31.3	38.6	36.7
22	39.1	36.1	43.2	32.9	34.0	38.2
24	41.5	34.4	34.3	31.7	36.1	34.4
26	27.0	41.2	38.8	36.1	37.8	31.5
28	32.1	47.5	42.2	40.0	32.5	35.3
30	32.2	44.0	33.0	33.9	38.2	35.6
32	25.5	42.0	35.0	32.3	34.7	36.3
34	32.1	31.9	40.0	45.0	38.8	31.7

Appendix VIII: Total leukocyte counts (WBC/mm^3)

Days	402	407	411	409	405	404
2	1400	17800	13000	12100	15100	11000
4	12400	18000	12200	8260	14400	12700
6	11900	16670	17900	10600	11090	12240
8	14100	16700	8880	14100	8880	12500
10	11160	15500	8880	15070	13190	13290
12	12660	14800		14280	13190	9570
14	13000	17700		15300	11900	10450
16	9630	13700		13980	13100	10600
18	12080	14000		15400	11160	12970
20	12700	15200		14900	10300	10800
22	10230	14800		16900	13200	9660
24	11990	13600		17500	12650	18240
26	15900	15800		17790	12980	18300
28	11990	14960		16790	12890	19000
30	11780	16150			13360	20470
32	12400	15900			11740	13400
34	13000	13200			12600	11860
36	13500	16600			12300	12700
38					11400	14900

Treated goats

Days	410	401	403	406	408	412
2	16000	13100	12100	12200	17500	21900
4	11700	14300	8800	11500	14500	20100
6	11390	10400	9340	10970	15240	19770
8	15700	15500	11790	10100	13200	16200
10	11170	12850	14400	12340	15100	21990
12	15340	18680	14730	16000	15000	22700
14	12700	17750	12000	21700	17000	23850
16	12200	25900	11870	18900	15770	29600
18	15980	21100	15390	20700	20900	25136
20	14270	17100	11400	17160	15160	19650
22	15350	12120	12350	19300	19608	19900
24	24000	19790	12450	12980	15480	17070
26	21900	24500	15180	10740	13920	23000
28	29000	26800	14400	16780	21000	26700
30	25000	29780	14600	19200	24900	26900
32	21700	16300	16400	17700	19800	29770
34	13000	12900	11608	18700	18600	21300
36	19600	24500	13800	14800	14380	21800
38	21800	19800	14600	105600	20100	22000

Appendix IX: Serum Lactate Dehydrogenase LDH levels(mg/100 ml)

Days	402	407	411	409	405	404
6	215	184	280	225	447	216
20	245	224		425	181	195
30	189	186		358	199	203

Treated goats

Days	410	401	403	406	408	412
6	208	382	311	226	95	260
20	151	173	149	138	425	183
30	187	128	173	191	358	147

Appendix X: Level of serum urea (micro mol/litre)

Days	402	407	411	409	405	404
2	5.10	7.06	-	8.87	6.04	5.33
6	10.84	7.08	-	8.26	12.99	11.60
14	3.28	4.75	-	7.62	9.85	7.67
18	5.71	4.84	-	4.31	6.66	2.83
22	4.41	4.98	-	-	10.19	4.96
24	5.51	5.64	-	-	5.96	3.18

Days	410	401	403	406	408	412
2	4.43	6.73	6.53	11.52	8.07	4.99
6	6.99	8.18	6.82	8.55	6.36	4.57
14	4.19	5.17	6.64	4.77	7.99	5.52
18	4.18	2.24	2.83	3.34	3.93	5.95
22	5.80	4.66	4.02	7.36	3.20	6.09
24	4.39	6.23	4.04	4.10	3.84	4.18

Appendix XI: Serum Alkaline phosphatase (Kind and King units/100 ml)

Days	402	407	411	409	405	404
4	60	57	45	56	50	24
16	40	27	-	23	33	30
24	28	20	-	26	26	18
28	27	30	-	-	41	46

	Treated goats					
Days	410	401	403	406	408	412
4	205	40	58	397	113	40
16	100	25	23	126	35	14
24	89	19	19	168	26	14
27	96	15	19	170	26	17

Appendix XII: Serum, Aspartate Aminotransferase (Reitman - Frankel units/ml)

Days	402	407	411	409	405	404
2	102	73	48	116	102	155
4	85	66	52	94	131	73
6	44	20	29	49	38	23
8	36	37	32	105	32	57
10	67	74		215	47	102
12	118	87		155	108	80
14	135	98		210	121	136
16	186	90		210	111	112
18	155	118		215	121	68
20	186	107		215	116	132
22	111	42		215	101	131
24	155	101		215	156	115
26	137	73		215	87	83
28	99	80			155	67
30	65	57			138	47
32	126	121			111	143
34	144	62			215	102
36	121	85			215	91

	410	401	403	406	408	412
2	67	79	116	80	110	131
4	80	108	151	76	84	112
6	29	30	87	38	49	54
8	29	54	35	36	40	38
10	130	122	67	67	74	67
12	117	122	95	102	87	117
14	102	137	87	95	95	126
16	143	119	76	110	72	98
18	80	116	62	122	135	88
20	106	177	127	108	95	106
22	132	83	132	138	95	143
24	107	103	127	101	108	112
26	62	99	117	87	57	80
28	80	98	73	94	80	102
30	94	143	98	73	57	95
32	111	143	143	115	94	122
34	80	143	111	143	102	143
36	102	152	132	99	83	97

Appendix XIII: Serum Alanine Aminotransferase (Reitman - Frankel units/ml)

Days	402	407	411	409	405	404
0	11	15	20	16	22	15
2	15.5	19	23	21	22	17
4	15.5	17	35	22	23	17
6	23	19	37	22	35	20
8	38	29	-	29	38	35
10	34	29	-	38	46	51
12	16	26	-	28	29	34
14	23	23	-	17	31	23
16	19	29	-	29	32	23
18	33	42	-	28	29	29
20	17	23	-	29	28	23
22	26	28	-	-	26	19
24	34	35	-	-	31	28
26	29	32	-	-	33	24
28	-	19	-	-	26	17
30	28	29	-	-	46	19
32	16	29	-	-	66	20
34	32	26	-	-	57	19

	410	401	403	406	408	412
0	19	12	15	20	20	22
2	12	21	21	32	15	24
4	24	21	25	27	17	19
6	16	16	24	35	19	21
8	35	32	35	22	38	29
10	29	35	36	29	33	32
12	29	12	18	29	21	22
14	21	20	17	29	23	23
16	29	35	23	26	29	16
18	26	29	33	29	32	22
20	24	17	23	23	26	20
22	29	16	26	26	19	22
24	29	32	34	32	35	29
26	22	26	29	35	29	26
28	32	17	19	23	15	17
30	29	17	20	29	30	32
32	20	22	26	35	26	31
34	22	16	16	29	24	22

Appendix XIV: Serum Creatine (micro mol/litre)

Days	402	407	411	409	405	404
4	78.1	94.6	59.8	64.7	128.7	99.5
6	70.9	72.3	70.0	62.5	71.8	35.7
8	116.5	94.6	91.5	114.2	79.0	93.5
18	88.3	81.7	-	62.5	75.4	69.6
20	56.2	66.9		60.0	49.5	74.1
22	70.0	72.7		58.9	74.5	55.8
24	66.0	59.8		88.2	103.1	126.7
26	70.8	99.1		90.1	106.7	74.5
28	62.5	86.1			91.9	94.2
30	77.6	89.7			95.0	32.1
32	59.3	56.2			90.6	39.7
34	49.5	56.7			59.3	49.5
36	68.3	77.6			56.7	42.8

Treated goats

Days	410	401	403	406	408	412
4	65.6	92.4	107.6	85.7	84.3	58.0
6	49.5	69.6	124.1	70.9	77.2	82.5
8	69.6	78.1	85.7	111.1	74.1	66.0
18	56.5	12.9	83.0	74.4	65.6	42.4
20	50.4	62.9	60.2	66.5	57.5	86.6
22	53.1	48.6	81.2	74.5	51.7	44.2
24	52.2	44.6	57.5	58.0	47.3	47.7
26	63.8	72.7	97.7	66.0	86.1	95.9
28	77.6	53.5	96.4	75.4	136.6	75.8
30	80.3	38.8	108.9	75.8	45.6	42.4
32	45.5	91.0	85.7	56.7	113.4	68.1
34	61.6	44.6	58.9	60.2	55.8	43.3
36	47.7	40.1	52.2	60.2	36.1	49.1

Appendix XV: : Total body weight of rats in experimental groups (wt. in gm)

DAY	T1	T2	T3	T4	T5	T6
1	650	557	646	722	718	612
2	759	666	776	856	786	670
3	849	741	891	845	854	728
4	914	803	973	1000	945	801
5	958	857	1016	1067	989	860
6	993	905	1018	1084	1010	897
7	1013	944	1024	1109	1042	902
8	1059	994	1085	1186	1124	971
9	1093	984	1157	1217	1157	1001
10	1148	1059	1117	1271	1164	1040
11	1166	1091	1127	1272	1180	1068
12	1218	1126	1171	1299	1217	1096
13	1227	1110	1179	1275	1244	1095
14	1193	1094	1181	1312	1248	1142
15	1183	1087	1176	1285	1252	1160
16	756	549	582	1028	1247	1149
17	291	290	440	594	1247	1147
18	292	148	416	276	1272	1158
19	151	290	156	262	1297	1189
20	140	140	159		1304	1223

Appendix XVI: The mean body weight gain in grams per rat group

Days of treatment	1	2	3	4	5	6	7
0	145.75	136.38	140.38	150.00	147.50	133.50	139.25
1	6.50	4.25	6.00	3.38	4.63	3.50	1.88
2	7.63	2.38	7.00	0.38	8.00	3.38	7.50
3	3.25	0.38	7.25	5.00	8.50	- 5.38	4.25
4	2.13	-0.88	6.63	1.63	9.00	11.50	11.25
5					8.38	10.13	13.75
6					7.75	9.88	17.00
7					11.50	11.25	21.38
8					14.63	15.15	24.13
9					15.50	19.38	25.75
10					18.63	22.88	31.00
11					20.25	22.50	29.75
12					22.00	26.50	31.13
13					25.25	29.25	38.50
14					26.50	32.00	38.25
15					29.50	37.88	40.63
16					32.00	37.88	44.63
17					29.75	38.13	43.63
18					30.25	38.25	45.63
19					32.38	44.50	49.50
20					-10.00	44.25	49.50
21					- 5.25	44.00	49.63
22					- 3.63	45.88	55.13
23					- 1.38	47.00	56.88
24					-16.00	32.75	60.00
25					6.17	35.88	64.25
26					7.33	38.43	66.13
27					10.17	42.57	66.88
28					8.83	44.71	69.38
29					11.83	47.00	72.88