TOXICOLOGICAL INVESTIGATION OF GNIDIA LATIFOLIA (MEISN), A PLANT COMMONLY USED IN TRADITIONAL MEDICINE

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DECLARATION:

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

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

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ABSTRACT

TOXICOLOGICAL INVESTIGATIONS OF GNIDIA LATIFOLIA (MEISN), A PLANT COMMONLY USED IN TRADITIONAL MEDICINE

by

Japheth Chesire Kiptoon

Gnidia latifolia (Meisn) is an ubiquitous woody shrub found in the semi-arid areas of the Kenya highlands. Where it occurs, it is known to be both poisonous as well as therapeutic. It is used to treat open wounds and mange in goats. This study was undertaken to investigate its effects and those of its constituent extracts on animals. An attempt was made to establish the mode of toxicity of Gn. latifolia (Meisn) by relating the clinical signs to the tissue changes in the living animal.

Cattle and weaner rats were used to study the pathological effects of the toxic principle of Gn. latifolia (Meisn). The leaves and bark were pulverized and suspended in cold tap-water. The suspension was then used to drench bull calves, 4-9 months old at various doses. Toxicity in the calves was monitored by observing symptoms, serum biochemistry and haematological changes. Gross pathology and histopathology were recorded in all calves that succumbed.

Pulverized leaves, flower and bark were extracted using water, methanol, ether, chloroform and ethanol. Fractions obtained were tested for toxicity by injecting them intraperitoneally into weaner albino Wistar rats. Methanol, ether, chloroform and water extracts were non-toxic to rats at dose levels below 2 gm/kg. body weight. The ethanol extract was toxic to the rats and was used for subsequent toxicologic experiments. The length of time it took the various groups of animals to succumb to the toxic constituents were also recorded.

In calves on feeding trials for 2-3 months the most significant gross and microscopic lesions were observed in the liver, kidneys and the lymphatic system. There were marked tissue wasting accompanied by serous effusions into the peritoneal, pericardial and pleural cavities. The livers of cattle had extensive hepatic fibrosis especially around the portal triads. Varied degrees of degeneration of the hepatic cells and fibrous tissue proliferation were noted in all the calves fed pulverized leaves. Lymphopoietic follicles in the spleen and lymph nodes showed cell necrosis, haemorrhages, congestion and fibrosis. Chronic kidney lesions consisted of degenerative nephrosis and glomerulonephritis, accompanied by marked deposition of hyaline casts in the periglomerular spaces and proximal tubules. Haematological studies on calves showed slight anaemia and a marked lymphopaenia. Serum biochemistry showed slight increase in the aspartate amino transferase (AST), formerly called serum glutamic oxaloacetic transaminase (SGOT). There were also slight increases in lactate dehydrogenase (LDH) and blood urea nitrogen (BUN).

The pathological changes seen in the weaner albino Wistar rats depended on the dosage and the length of time the animals were injected with the toxic extract before succumbing. The ethanol extract was quantitated and measured amounts were injected intraperitoneally into the weaner rats. The rats were observed for toxic effects. The median lethal dose for the ethanol extract was 262 mg/kg. body weight. The rats that received doses much higher than the LD_{50} showed acute death with necrosis of tissues that have fast-multiplying cells. There were marked haemorrhages of the thymus, spleen, pancreas and the small intestines. Some of the rats injected with dosages below the LD_{50} showed poor body weight gains.

Histopathological sections from treated rats revealed a marked cellular degeneration in the parenchymatous organs with proliferation of the fibroblastic cell types replacing lymphoid and acinar cells. The organs that are involved in mononuclear cell production were most severely affected leaving either partially empty follicles or follicles filled with cell debris. The kidneys, liver and the adrenal body showed marked cellular degeneration with hyaline casts deposited and filling proximal tubules of the kidneys and sinusoidal spaces of the liver and adrenal body. In some of the histological sections, the albuminous casts were so extensive that they elicited inflammatory cell reactions. Petechial haemorrhages were seen in several organs such as the kidneys, the pancreas, the adrenal cortex and medulla, the thymus and the lymph nodes.

The ethanol extract from the pulverized leaves was precipitated in petroleum spirit yielding about 11 gm/100 gm of the dry matter. The extensive cellular damage seen in the vital body organs especially those with fast-multiplying cells suggests the need for additional research on the plant. It is important to establish whether the selective effect is constant for all dosage levels far below the LD_{50}. Further investigation should also be carried out on poisonous plants to determine the usefulness of their active principles.

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TOXICOLOGICAL INVESTIGATIONS OF GNIDIA LATIFOLIA (MEISN) IN CATTLE AND RATS

INTRODUCTION

The use of natural products of plant origin for the treatment of diseases and especially in the treatment of malignant disease conditions dates back to antiquity. Several types of herbs, shrubs, and trees have been claimed to be effective against specific types of ailments and others have been used to treat some particular clinical syndromes. Most of the medicinal plants are known to cause toxicity if proper regimen is not followed and some have caused other harmful side effects even when proper dosages were administered (Mugera, 1985). The problem of plant poisoning and the identification of the toxic substances involved has for a long time been frustrating because the amount of the toxic principle and even its presence or absence in the plant depend on the soil and climatic conditions. If one also considers the fact that the phytoconstituents can vary depending on other factors such as the time of harvesting and the part of the plant being tested, it is easy to appreciate the complexity of investigating plants for their constituents. It is further known that the plant species concentrate active chemical constituents in specific parts like the roots, stems, leaves or fruit (Perdue, Abbot and Hartwell, 1970).

In their investigations, Verdcourt and Trump (1969) dealt with

several hundred enquiries to determine the type of plants involved in accidental and malicious poisonings of man and animals. They discovered that some toxic plants were used in several parts of the world as herbal medicines and were claimed to have beneficial effects in the treatment of a variety of diseases.

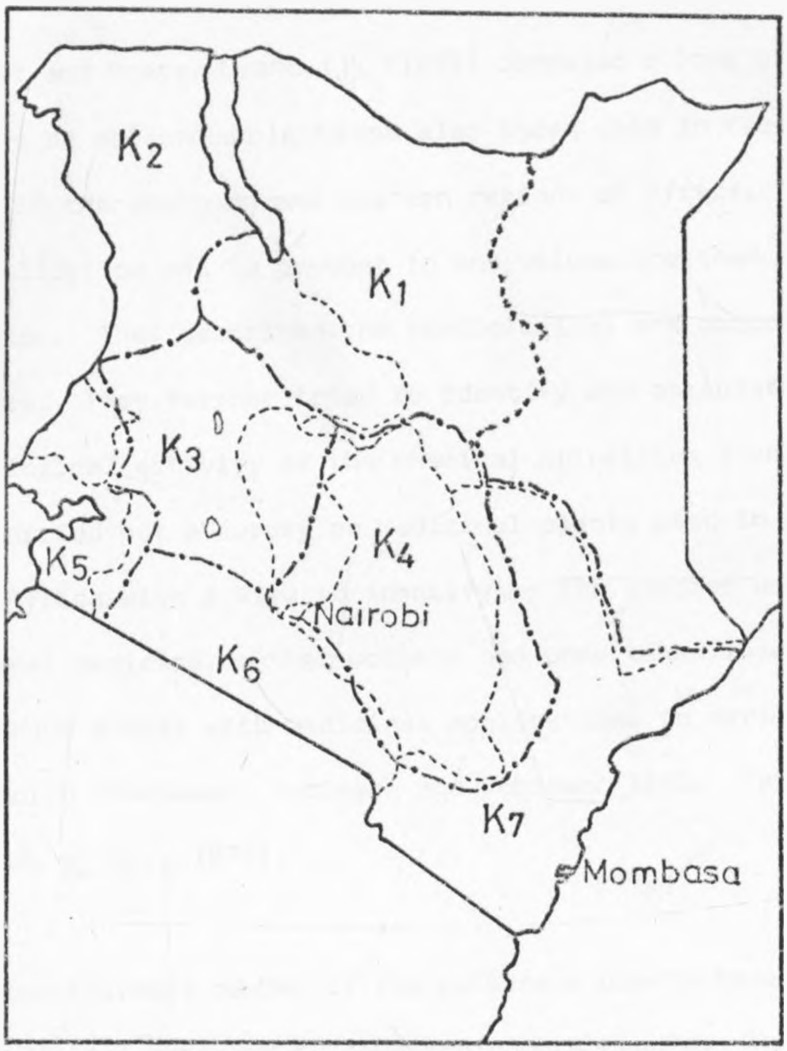
Species of the genus Gnidia (Meisn) or Lasiosiphon in the family Thymelaeaceae have been used for medicinal purposes in many parts of Africa. Plants in this genus have strong fibrous barks, simple leaves (alternate or opposite) and are usually small and exstipulate. Different parts of the plant have been used in the treatment of ailments ranging from mild skin disorders to severe internal sicknesses. Studies on the toxicity of some Gnidia species (Gn. burchelli, Gn. kraussiana, Gn. chrysantha, Gn. anthylloides) have confirmed the presence of toxic substances (Terblanche, Pieterse, Adelaar and Smit, 1966; Nwude and Parsons, 1978 a, b). Investigations conducted by Alexander (1928) showed that personnel involved in the preparation of Gnidia material suffered marked irritation in the mouth, nose and pharynx accompanied by persistent coughing, sneezing and headaches and nausea.

Gn. latifolia (Meisn) is a large many-stemmed shrub that grows to a height of 6 metres. Its leaves are narrowly oblanceolate or oblong and about 4-5 cm. long (Dale and Greenway, 1961). Small amounts of parts of the plant if eaten by an animal may cause slow death with clinical and postmortem signs of acute haemorrhagic gastroenteritis. Large doses of the plant cause acute abdominal pain and rapid death.

The plant was used extensively in traditional medicine in East Africa. It was used in Uganda and Western Kenya in the treatment of lymphoma and in particular, Burkitt's lymphoma. It was also used in several other conditions that showed clinical tenseness accompanied by swelling and pain. In the Baringo and Elgeyo/Marakwet districts, it was used to treat humans and animals suffering from skin infections and some internal sicknesses. A brew of the leaves was used to wash goats affected with sarcoptic mange and ringworm. Fresh leaves were crushed into a paste which was applied to swellings and abscesses, while the ground dried leaves were taken as snuff to relieve headache and blocked nose due to influenza and common colds. The leaves were also boiled and the brew used to wash children suffering from scabies and measles.

Roots sweetened with honey were chewed by people suffering from chronic pneumonia, sore throat and those with abdominal complaints. A dilute suspension of the root bark brew when mixed with goatmeat soup or honey was used to treat whooping cough in children.

A plant that causes toxicity to people and animals will often demonstrate some pharmacologic activity when proper dosages are administered. This project was therefore undertaken to investigate the toxicity of Gnidia latifolia (Meisn) in calves and rats using the leaves and bark of the plant. The calves were fed ground whole plant parts while the rats were inejcted intraperitoneally with crude and pur fied extracts of the plant.



—·—·— Botanical boundaries

------- Provincial boundaries

Fig. I Map of Kenya: Distribution of Cnidia latifolia (Meisn) in Kenya (K3, K4, K5)

K1 to K7 indicate Botanical zones.

REVIEW OF THE LITERATURE

Watt and Breyer-Brandwijk (1962) compiled a long and detailed literature on poisonous plants and also those used in traditional medicine in the southern and eastern regions of Africa. The aim of their publication was to present in one volume the then available information. They described the toxicological and medicinal uses of the plants. They further tried to identify and postulate on the pharmacolcgical activity of the chemical principles involved. Kokwaro (1976) carried out a survey on medicinal plants used in various parts of East Africa with a view to identifying the species used in traditional medicine. Other workers had undertaken investigations on poisonous plants with medicinal applications in various other parts of the world (Medewaar, Robinson and Robinson 1943; Farnsworth 1966; Dwuma-Badu et al., 1976).

A considerable number of the poisonous plants have been known for their medicinal purposes by some indigenous people. The poisonous or toxic plants are those plants whose chemical constituents have properties of biological activity on animal tissue and these relative observations of biological activity can be studied when the rate of their interaction with animal tissues is controlled (Farnsworth, 1966). If one considers plants which cause systemic toxicities, then it is possible that with proper dose administration, one can study the pharmacologic activity of the constituents. Documented evidence shows that plants used in traditional medicine have chemical compounds with

beneficial pharmacological activity (Medewaar et al., 1943). Studies on plant poisoning is sometimes a frustrating subject because many plants have not had their chemical constituents documented (Verdcourt and Trump, 1969). The amount of the active principle(s) is also influenced by the climate, soil and the growth of the plant (Farnsworth, 1966).

The genus Gnidia belongs to the family Thymelaeaceae which is a close evolutionary relative of the Euphorbiaceae and Tiliaceae. These families belong to the orders Euphorbiales and Malvales, respectively. The two orders and the order Thymelaeales arose from the ancestors of the Flacourtiales which were prevalent during the Tertiary Period (Takhtadzhian, 1966). The plants occur throughout the world especially in the tropical and sub-tropical regions and are commonly found in highland or mountainous zones of tropical Africa. A considerable number of plants in these families are poisonous to man and animals.

The genus Gnidia is also referred to in the older literature as Lasiosiphon. The genus is rather large and has several species about a dozen of which occur in eastern and central Africa where they are used extensively for medicinal purposes (Watt and Breyer-Brandwijk, 1962; Kokwaro, 1976). Since time immemorial, it has been known that Gnidia plants produce a bitter and persistent burning sensation in the mouth, tongue and throat. This property might have led to the belief that they would have therapeutic application for external as well as internal illnesses. The ability to cause a bitter and burning sensation in the

mouth is not altered by drying or other physical manipulation.

Toxicities of Gnidia species to humans and animals

Wherever Gnidia species occur, there have been several reports of poisoning in livestock and people either because the plant material was ingested accidentally or because its dosage was not determined before use. Traditionally, the plant was known to be exceedingly poisonous to livestock especially on sprouting after fire outbreak, armyworm and locust invasions (Verdcourt and Trump, 1969). The plants are highly poisonous when taken internally and are also intensely irritant when they come in contact with the mucous membranes of the mouth, eyes and the nose. Its latex is acrid, pungent and highly irritant. Those who collect and process the plant material usually suffer from marked irritation of the mouth, nose, pharynx and the eyes; symptoms which may be followed by coughing and headaches. Gn. anthylloides has for a long time been known to cause acute poisoning in livestock in southern Africa. Subsequent experiments confirmed that the flowering tip and leaves of the plant were very toxic. Death occurs rapidly after consuming the plant parts with acute abdominal pain as the only observable sign (Alexander, 1928; Watt and Breyer-Brandwijk, 1962). Gn. burchelli has also been shown to be very toxic to animals (Terblanche, Pieters, Adelaar and Smit, 1966). Both Gn. anthylloides and Gn. burchelli are reported to cause dyspnoea, nasal and lacrimal discharges followed by anorexia and profuse diarrhoea. Some other species of Gnidia (Gn. capitata, and Gn. chrysantha) have been reported to cause death to people who used them as abortifacients (Watt and

Breyer-Brandwijk, 1962). Gn. kraussiana is exceedingly poisonous and causes death rapidly (Shone and Drummond, 1965). Its fresh green leaves and the flowering tip have also been used as fish poison. The fish decompose quickly if not removed from the water. Clinical toxicity trials with Gn. kraussiana have proved that the plant is poisonous to a wide range of animals. Herdsmen in the subtropical savannah areas know it to be dangerous to livestock (Nwude and Parsons, 1978,a). Poisoned animals show loss of appetite, increased respiration, diarrhoea and intense thirst. Post-mortem examination shows several haemorrhagic lesions in the gastrointestinal tract, heart, kidneys and congestion of the liver. The plant has also been used for homicidal purposes (Verdcourt and Trump, 1969). When the present author was collecting Gn. latifolia from the Kenya highlands, he often inquired from the indigenous people about the uses of the plant. Invariably he was met with suspicion and most of the people would shun talking about the plant. The few who ventured to talk would ask for assurance that the author would not employ the plant for any malicious intent, suggesting that perhaps the plant was also used for homicidal purposes.

Medicinal Uses of Gnidia Species

Several plants of the genus Gnidia have been widely used in traditional medicine. Watt and Breyer-Brandwijk (1962) listed several species of the genus Gnidia that have been used for human teatment. They stated that Gn. chrysantha was used as a purgative. A little decoction of the root was also used as a "blood purifier". Several other Gnidia species were used as local applications to painful hollow

teeth, boils and abscesses. Gn enthylloides brew was drunk for coughs and influenza. Kokwaro (1976) recorded that Gnidia species were used extensively in East Africa as medicines by different tribes. He reported the use of Gn. buchanani root decoction in the treatment of bronchitis and abdominal pains. Gn. kraussiana and Gn. glauca roots were also reported to be used in a similar manner for internal complaints. A few species of genus Gnidia have been reported by the two groups of authors as being used as antidotes to poisons and especially as snake-bite remedy. Gn. latifolia decoction was taken internally as an antidote to poison or moderate amounts of the root bark brew were drunk as purgative and emetic. The leaves are made into a paste which is used to wash and dress indolent and callous ulcers. Gn. kraussiana leaves are also chewed and applied to burns, bruises and wounds.

In Kenya, Gn. latifolia (Meisn) occurs in the regions marked K3, K4 and K5 on Fig. 1. Many other species of the genus Gnidia occur in these areas especially on altitudes of 1200 to 2500 metres. The plant is mainly found on the mountainous slopes and plateau bordering arid zones such as Machakos, Kitui, Isiolo, Samburu, Baringo, Elgeyo/Marakwet, Western Nandi and on the Western Cheranganys and Elgon Mountains (Verdcourt and Trump, 1969; Dale and Greenway, 1961). Depending on the soil and climatic conditions the plant varies in height, but generally grows to a height of 5 to 6 metres. Most of the material used in this work was collected from the Kilungu and Kilome hills in the Machakos district.

In Kenya, Gn. latifolia is referred to by different names depending on the language of the region where the plant grows. Among the Kamba it is known as Mobila; among the Tugen, Keiyo and Nandi it is known as Songoro. It is further referred to as Mwata in Swahili, Mdaesun in Chagga and Jarambiri in Mshagalu (Kokwaro, 1976). Discussion with several local medicine men indicate that it is used for the treatment of a variety of sicknesses especially those affecting the respiratory and digestive systems. The leaves are usually used for treatment of superficial sicknesses whereas the roots are normally employed for internal sicknesses. The fresh green leaves are chewed or squashed with the hands and then pasted on open wounds, bruises and abscesses. They are also boiled and a dilute suspension of the brew used for washing children suffering from scabies, measles and chickenpox. A concoction of the plant mixed with other Gnidia species is made into an ointment with sheep fat and smeared on the head and body affected with ringworm. Dried leaves are also ground into powder and taken as snuff to relieve headaches and congested nasal passages. There are few reports of the leaves being mixed with charms to prevent ill effects when one is going on a journey or when anticipating contact with strangers (Kokwaro, 1976; Kipchenger, 1980). The roots are mixed with honey and chewed by people to treat sore throat, chronic headache and persistent pnuemonia, painful chest and abdomen. Where honey may not be available, the roots are either chewed with roast goatmeat or a brew of the leaves is mixed with goatmeat soup and drunk to treat similar sicknesses. Sometimes a teaspoonful of the brew is mixed with honey and administered (usual dose - 2 times a day) to children suffering

from whooping cough. Fresh leaves are boiled and the brew used to wash goats affected with sarcoptic mange. At the same time, each goat is drenched with approximately 40 - 50 ml. of the brew. This supposedly purifies the blood of the animal. A brew is also mixed with honey and rock-salts and used to drench a cow after abortion and before the animal is bred again. Some fresh leaves are burned to wilting and then crushed into a paste which is applied on abscesses and swellings. This treatment is supposed to quicken the ripening and subsequent healing of the abscess.

Experimental Toxicities of Gnidia species to Animals

(i) Farm Animals:

Nicholson (1947) reported the first experimental case of feeding farm animals with a plant of the family Thymelaeaceae. A case of suspected poisoning of a horse by the wood-laurel (Daphne laureola) had been reported to Nicholson in 1912 and samples were brought to his laboratory for toxicity trials. The plant was then commonly used as a deworming agent for horses in Britain. Several cases of unexplained colics had been reported following deworming. A horse was given 1 ounce of the ground leaves and developed inappetence, abdominal pain, dyspnoea and constipation, followed 48 hours later by profuse diarrhoea. The animal died and on post-mortem examination revealed extensive inflammation of the stomach and intestines, with watery foetid ingesta.

Alexander (1928) recorded the first systematic toxicological study

of a Gnidia species. Quoting a Dr. E.P. Phillips, he reported a history of heavy mortality in a flock of newly introduced sheep. The farmers had suspected the cause of death to have been due to the consumption of Gn. anthylloides. Many similar deaths had occurred in 1922 but Phillips' attempt to induce toxicity by feeding some undetermined doses of the plant to animals gave erratic results. Alexander (1928) then fed dried Gn. anthylloides leaves to sheep, cattle and horses (at various doses) and this led to dramatic observations. High doses (6 - 12 oz) produced death so quickly that no signs apart from acute abdominal pain were observed. Smaller doses (1 - 4 oz) caused anorexia, dyspnoea and profuse diarrhoea in all the animals. Post-mortem examination showed hyperemia, haemorrhages and fluid effusion into body cavities. Further investigation revealed that the plants were extensively used as medicines by indigenous people. Quoting another Dr. Andrew Smith, Alexander (1928) reported that Gn. anthylloides and Gn. meisneriana roots were used as snake-bite remedy, while the leaves and bark from Gn. kraussiana, Gn. linifolia and Arthrosolen gymnostachys were used extensively to bathe wounds and to relieve sore throat and headaches.

Steyn (1932) investigated Gn. burchelli for its toxicity in sheep. He drenched a sheep with 300 gm. of ground dried leaves and observed a similar clinical and post-mortem picture as shown by Alexander's experiment. He too had learned from farmers that the plant frequently caused death in stock and particularly in the newly introduced animals. He also observed severe inflammatory changes in the mouth and

respiratory systems of the people who helped prepare the material from Gn. burchelli.

Terblanche, Pieterse, Adelaar and Smit (1966) undertook some chemical extractions of Gn. burchelli and used the crude extracts obtained to drench both farm and laboratory animals. Initial trial were done on a merino ewe that was drenched with 3.3 gm/kg. body weight of the dried ground leaves and the animal died. They observed typical clinical signs as those already described by Alexander (1928) and Steyn (1932). In vacuo extraction of the dry ground leaves with acetone yielded a black tarry substance. Further purification with petroleum ether and cold water yielded an insoluble amorphous dark green substance which was toxic to animals. Nine sheep, two pigs and a dog were drenched with various doses of the dark green substance. The sheep developed the typical clinical picture of anorexia, dyspnoea, polypnoea, groaning, haemorrhagic nasal discharge and foetid diarrhoea. A few developed constant muscular tremors while one had a complete paralysis of the hind quarters. The pigs showed yellowish diarrhoea which later became haemorrhagic while the dog vomitted and then recovered.Haematological studies showed only a slight elevation of the blood urea nitrogen (BUN), but other parameters were not significantly affected. Pathological changes were however very marked in the lungs, liver, gastrointestinal tract, kidneys and heart. Hyperemic and haemorrhagic lesions were common. There was also a marked atrophy of the lymphoid tissues which on histological examination revealed karyorrhexis of the lymphocytes in the lymph follicles.

Nwude and Parsons (1978 a,b) fed Gn. kraussiana leaves to four sheep, four goats, three donkeys and six cattle. The sheep were fed 3.3. gm/kg. body weight per day for nine days while the goats were fed 4.0 gm/kg. body weight per day, again for the same period of time. The donkeys were fed dried ground leaves at 4.0 gm/kg. body weight per day while the cattle were fed fresh green leaves and tips through ruminal fistulae, at dosages of 8.0 gm/kg. body weight per day, again for a period of nine days. All the test animals showed depression, anorexia, nasal and ocular discharges and profuse diarrhoea. In cattle haematological studies revealed lymphopaenia, eosinopaenia and an increase in the BUN and LDH. However, there were no changes in the other serum enzymes (SGOT and alkaline phosphatase). All donkeys, cattle and two goats died but none of the sheep died. Gross pathological changes included haemorrhagic gastroenteritis, haemorrhagic myocarditis and brain congestion. Histopathological sections showed petechial haemorrhages in many body organs with mononuclear cell infiltration of the gastrointestinal tract and fibroblastic cell proliferation. There were no gross or histological changes in the kidneys and the liver apart from congestion and oedema. All the changes observed were attributed to an intense irritation by the active principle in Gn. kraussiana which the authors suggested was a corrosive poison. The same workers further postulated that the toxin either caused lysis of lymphocytes or the release of adrenocorticotropic substances which in turn caused lymphopaenia.

(ii) Laboratory Animals:

Chemical investigations of plants for toxic principles are usually

determined by the use of small laboratory animals but their susceptibilities vary greatly.

Christie (1958) had postulated that plant alkaloids were similar in chemical structure in that they were esters of basic alcohol possessing a pyrrolizidine ring. He injected a pyrrolizidine intraperitoneally into adult male Wistar rats at dosages of 320 mg/kg and the liver sections of the dying rats showed ovoid structureless hyaline vacuoles (1 to 10 μ in diameter) in the parenchymal cell cytoplasm. He stated that the cells swell prior to degeneration due to an increased cellular water load and partly due to the development of small fat droplets from damaged mitochondria.

Adamson, Dixon, Ben, Crews and Rall (1965) injected the alkaloid vincristine intraperitoneally into mice and rats. The animals developed muscular weakness and hind limb paralysis after 4 days. The clinical signs observed were depression, dyspncea, ataxia, sprawling of the limbs and an abdominal pain. On postmortem examination, the dying rats showed congestion of the lungs, kidneys and adrenals, accompanied by gastrointestinal inflammation and dilatation with gas in the duodenum and ileum. The pancreatic acinar cells were unevenly depleted of secretion granules. There were mild toxic changes in the liver and kidneys.

Peterson (1965) injected Wistar rats intraperitoneally with an aqueous solution of lasiocarpine at a dosage of 240 mg/kg. body weight and observed depression, inappetence, loss of weight, diarrhoea and death.

He studied the pathological changes caused to the liver parenchyma and observed suppression of nuclear and cell divisions. He further postulated that there was no interference in the synthesis of deoxyribonucleic acid (DNA) in the liver. He further stated that drug hepatotoxicity caused degenerative cellular changes leading to hepatocellular necrosis in the centriblobular zone, and necrosis of the parenchymal cells in the periportal zones within 24-48 hours of injection. Chronic alkaloid poisoning on the other hand was preceeded by the development of hepatic parenchymal cytomegally and karyomegally more particularly in the midzonal and periportal regions of the hepatic lobule. Acute toxicity showed haemorrhages and necrosis of hepatic parenchymal cells, while chronic exposure to plant toxins led to increased proliferation of fibrous tissue especially around the bile ducts.

Farnsworth (1966) also stated that biologically active plant principles should be investigated for their chemical constituents so as to derive methods of treatment of plant poisonings of both humans and animals and also to derive some new sources of economic materials useful as precursors of synthesizing other complex chemical substances. He stated that the initial selection of plants to be investigated should be made on the basis that certain chemicals are present in the plant and its relatives, and that these chemicals can demonstrate some biological activity.

O'Gara, Lee, Morton, Kapacha and Dunham (1971) tested some crude plant extracts for cytotoxicity by injecting the extracts subcutaneously

into rats and observing their reaction. The animals showed acute necrotizing nephrosis, pulmonary oedema, centrilobular hepatic necrosis and congestion. The chronically treated animals showed elevated BUN levels which were explained as resulting from damaged kidney tubules. Toxic plant constituents are absorbed from the stomach and intestines and when eliminated through the kidneys cause similar tubular damage leading to elevated BUN levels (Clark and Clark, 1978; Harborne, 1973).

Terblanche et al. (1966) extracted Gn. burchelli leaves with acetone. They drenched five rabbits, twenty two guinea pigs and seven rats with the extract. The guinea pigs were found to be highly susceptible. The LD_{50} of the extract was approximately 22.4 mg/kg body weight. In the rabbit and rat the LD_{50} values were 1-1.5 gm/kg and 200-300 mg/kg body weight, respectively. There were acute deaths in the guinea pigs with mortalities occurring within 5-20 hours without any other clinical signs being observed. The animals which survived for one to two days after the treatment showed profuse diarrhoea, listlessness and ruffled haircoat. The rats showed similar clinical picture while the rabbits died without any clinical signs. On postmortem examination the guinea pigs showed acute hyperaemic and haemorrhagic inflammations in the gastrointestinal tract. Grossly the liver was normal but histologically it showed cellular necrosis and hyaline degeneration. There was congestion in the heart, adrenals, kidneys, brain, pancreas and lymph nodes and haemorrhages in the heart and adrenals. The rabbits showed generalized congestion, hyperaemia and petechiae in the gastrointestinal canal. Their livers were congested and had neutrophil infiltrations;

with similar haemorrhagic zones in the spleen and lymph nodes. The rat on the other hand showed haemorrhagic enteritis accompanied by congestion of the liver and lungs. Terblanche et al (1966) then concluded that the toxic principle in Gn. burchelli affected primarily the heart and lungs and caused irritation of the gastrointestinal canal. It also caused liver, kidney and lymphoid tissue damage. No explanation was offered as to the cause of the nervous paralysis in the rat and guinea pig.

Kupchan, Sweeney, Baxter, Murae, Zimmerly and Sickles (1975) extracted the chemical constituents of Gn. lamprantha (Gilg) leaves using ethanol. They then demonstrated the effect of the extract on malignant cancer lymphocytes (P-388 leukemia cells) on mice. Their results indicated that the antileukemic activity of the ethanol extract was concentrated in a chloroform layer of a chloroform-water partition of the extract. The same extract was also found to be highly piscicidal when tested on goldfish.

The Uses of Gnidia species in Traditional Medicine

Plants in the genus Gnidia have been used widely in traditional medicine. Watt and Breyer-Brandwijk (1962) listed several species of Gnidia which have been used for human treatment. Kokwaro (1976) reported that in East Africa, Gnidia species were used extensively as medicines by different tribes. Gnidia chrysantha was used by several people as a purgative and "blood purifier". Gnidia anthylloides was brewed and the decoction used to treat coughs and influenza. A root decoction from

Gn. buchanani was used in the treatment of bronchitis and abdominal pains. Root decoctions from Gn. kraussiana and Gn. glauca were also used in the treatment of internal complaints. A brew of Gn. latifolia was taken internally as an antidote for poison. A moderate amount of a root bark decoction, also prepared from Gn. latifolia, was taken as a purgative and emetic. The green leaves were also made into a paste and used to wash and dress indolent and callous ulcers. Gn. kraussiana leaves were also chewed and applied to burns, bruises and wounds.

Chemical Investigations of plants

Chemical investigations of several plants used in traditional medicine have been carried out (Crowley and Culvenor, 1959; Mattocks, 1967; Kupchan et al, 1972; Mitscher et al, 1972). Noble, Beer and Cutts (1958) stated that plant alkaloids have a basic chemical characteris containing a heterocyclic nitrogen and are synthesized in plants from amino acids or their immediate derivatives. Because of their chemical structure, these compounds react with animal tissues and induce pathological changes (Schoental, 1968). Much emphasis had originally been placed on the presumption that the toxic principles involved in the poisoning of grazing livestock were pyrrolizidine alkaloids. Plants however, synthesize and store an enormous variety of organic substances. These compounds vary in their chemical structures and molecular sizes. This variation influences their reaction with animal tissues. Plant alkaloids comprise the largest class of secondary plant chemical compounds and many of them are often toxic to man and animals. Those which show dramatic physiological activities have been

used as medicines. Plant alkaloids are also heterogenous in nature and many of them are terpenoid or best considered from their biosynthetic point of view as modified terpenoids. Gibb (1974) stated that terpenoid compounds are synthesized by condensation of acetic acid with acetoacetic acid through several stages to yield isopentenyl pyrophosphate ("active isoprene - a hemiterpene, the true structural unit of all terpenoids"). A considerable number of compounds are specific to one family or to a few related plants so that the pharmacological actions of these related plants are usually similar (Terblanche et al., 1966; Harborne, 1973). The function of the terpenoid compounds are largely unknown, but individual substances have been reported to be growth regulators while others act as insect repellents or attractants. Many plant compounds possess structures and chemical properties which suggest that they act by selective alkylation of growth macromolecules. This activity may interfere with the regulatory actions of nucleophilic groups of enzyme systems. The toxicity or biological activity of any plant substance depends on the stereochemistry of the basic as well as the acidic moiety. This is further influenced by the number of hydroxyl groups which by increasing water solubility affect the rate of elimination from the body (Schoental, 1968).

Work on Gnidia species has focused on the isolation and identification of their active constituents (Steyn, 1932; Marloth, 1932; Terblanche and Adelaar, 1965; Kupchan et al., 1972; Kupchan and Lacadie, 1972; Harborne, 1973). The investigations indicated the presence of an irritant resinous principle mezereine which blistered the skin, and

another toxic alkaloid daphnine from the family Thymelaeaceae (Marloth, 1932). The work of Gibb (1974) revealed that many members of Thymelaeaceae had several substances with chemical combinations of C_{14} and C_{20} mono-unsaturated acids in their leaves, stems and roots.

Recent investigations of Gnidia compounds have been directed at the isolation and identification of those compounds useful for disease treatment and especially against cancers of various types. Kupchan et al. (1975) successfully extracted the chemical constituents of Gn. lamprantha collected from Kenya. An ethanol extract when fractionated and assayed revealed that most of its antileukemic and piscicidal activity were concentrated in the chloroform layer of a chloroform-water partition. They separated the active layers by preparative thin layer chromatography on Chromar and identified the active principles. The fractions were further purified through successive column chromatography on silica-gel followed by partition chromatography on celite to yield three closely related compounds. Their structures were determined by a combination of spectral and chemical evidence and were recognized to be Gnididin (C_{37} H_{44} O_{10}; M.W. 27,750) and Gniditrin (C_{37} H_{42} O_{10}; M.W. 31,800). On methanolysis, both yielded 12-hydroxydaphnetoxin similar to an authentic mezereine sample. Preparative thin layer chromatography of the third active fraction on Chromar yielded Gnidicin (C_{36} H_{36} O_{10}; M.W.21,000). Kupchan et al. (1975) postulated that the ester affixed at C_{12} may act as a carrier moiety in the processes concerned with cell penetration or selective molecular complex formation. Christie (1958) reported that plant alkaloids were similar in chemical structure in that they were

esters of basic alcohol possessing a pyrrolizidine ring. The daphnetoxins of Gnidia species are macrolide diterpenoids (Kupchan et al., 1975). Macrolide compounds are plant chemicals distinguished from other compounds by their large lactone ring (Stahl, 1967).

The terpenoid plant compounds have a common biosynthetic origin based on the isoprene molecule $CH_2 = C(CH_3) - CH = CH_2$ and their carbon skeletons are built up from the union of two or more of these C_5 units. The resulting compounds of this union range from the essential oil components, the volatile mono-and sesquiterpenes (C_{10} and C_{15}) through the less volatile diterpenes (C_{20}) to the involatile triterpenoids and sterols (C_{30}) and carotenoid pigments (C_{40}), (Clarke and Clarke, 1975; Harborne, 1973).

Most natural terpenoids have cyclic structures with one or more functional groups (hydroxyl, carboxyl, amino, etc) so that the final steps in the synthesis of these compounds involves the cyclization and the oxidation or other structural modification. Terpenoids are lipid soluble chemicals and normally located in the cell cytoplasm of the plant and usually extracted from plant tissues with light petroleum, ethanol, ether and chloroform, and can further be seperated by chromatography on silica gel or alumina using the same solvents. Terpenes are readily absorbed from the skin, lungs and alimentary tract and are excreted primarily in the urine as a mixture of glucuronides which has an intense irritant action on the tissues especially the kidney tubules and also produces some narcotic effect (Clarke and Clarke, 1975). Isomerism is

common among these terpenoid compounds and they are mostly alicyclic in nature with the cyclohexane ring usually being twisted in the so called "chair" or "boat" forms and many other different geometric conformations are possible depending on the substitution around the ring.

The diterpenoids comprise a chemically heterogenous group of compounds all with a C_{20} carbon skeleton based on four isoprene units. Three classes of diterpenoids are common in plants.

(1) Resin diterpenoids which are rare and have a protective function in nature in that they are exuded from wood or the latex of herbacious plants.

(2) The toxic diterpenoids which are those occurring in the leaves of many species and are responsible for the poisonous nature of the foliage of these plants. One can quote the daphnetoxins of Thymelaeaceae as an example of these toxic diterpenoids which have similar chemical configurations as grayanotoxins and are oxidation and substitution complexes of agathic acid (Bonner and Varner, 1965).

(3) Gibberellins which are mainly hormones responsible for stimulating the growth of plants and their occurrence is known to be widespread in plants.

Experiment I: Experimental work on calves using whole plant parts of Gn. latifolia (Meisn)

MATERIALS AND METHODS

Introduction

Sixteen high grade (Guernsey, Ayrshire and Friesian) bull calves weighing between 50 and 150 kg were used as the experimental animals in the first studies on the toxicity of Gn. latifolia (Meisn). Their ages varied from four to nine months. The calves were divided into eight groups of two calves each and housed at the housing units at the Large Animal compound. Twelve of the sixteen calves were used as the experimental animals while four calves were kept separately in two groups as the controls. A balanced ration of hay, bran and calf boluses were fed and salt lick and fresh water continously supplied ad libitum. The calves were routinely examined both clinically and haematologically for any intercurrent diseases and parasites before they were used for the toxicity experiments. Faecal samples were examined in the laboratory for helminth eggs and coccidial oocysts. Those that were positive for the parasites were routinely treated with the appropriate drug. Faecal examination was repeated weekly to check for the parasites and any that were still positive were treated with higher dosages of the drug. Blood for haematology and serum biochemistry were obtained daily for five days prior to the feeding experiment to obtain baseline values. The calves were further sprayed once a week with an

organophosphate acaricide to prevent tick infestation.

Two bull calves weighing 82 kg and 54 kg were drenched with leaf material at dosage rates of 2 gm/kg. per day and 1 gm/kg. per day, respectively. Two other animals weighing 81 kg and 58 kg were drenched with Gnidia bark material at dosage rates of 2 gm/kg. per day and 1 gm/kg. per day, respectively. The third group which weighed 98 kg and 112 kg respectively, were given dosages of 500 mg/kg of ground Gn. latifolia leaves while the fourth group of two bull calves weighing 81 kg and 96 kg were drenched with ground leaves at 200 mg/kg per day. The fifth and sixth groups consisted of four bull calves which weighed 74, 115, 154 and 85 kg and the two groups were drenched at a dosage rate of 100 mg/kg. per day with leave material. The control calves were similar in age, breed and body weights to the test animals and were kept under similar conditions as the test calves, but they were not drenched with any Gnidia material.

The Gnidia material used in this experiment and all later experiments was collected from Kilungu and Kilome hills of Machakos district and prepared as follows: The young branches and leaves of Gn. latifolia (Meisn) were collected and transported to the Faculty of Veterinary Medicine, Kabete. The branches were dried at room temperature ($22^{\circ}C$) for two to three days and the withered leaves separated. It was then possible to carry out separate experiments using the leaves and bark. The flowers were picked separately before drying. The separated leaves were further spread out and dried for another two days on gunny

bags and packing paper sheets at room temperature (22°C). Once dry, the leaves were ground separately into a fine powder using an electric grinding machine[a]. The thinner young branches with the bark were then ground separately using the same machine. After grinding, the leaves and the barks were stored in powder form in polythene bags at room temperature until needed.

The calf toxicity studies were done separately for each group of animals so as to observe daily clinical parameters in the treated groups. The calf experiments were carried out for a period of fifteen months. Each calf was weighed at the commencement of the dosing experiment and the calculated daily intake of the pulverized Gnidia material was administered by drenching orally. The calculated amount of material was suspended in clean tap water and then the calf was drenched with the suspension using either a long-necked bottle or a stomach tube. Blood samples for haematology and serum biochemistry were collected daily from the treated and control calves throughout the treatment period. The control animals were not given any test material but clinical examination was always done on them. The toxicity experiments were carried out with varying dosages of the Gnidia material so as to examine the acute, subacute and chronic response, and also the clinico-pathological changes that occurred with each of the dosage rates.

Clinical Examination:

Daily clinical examination was carried out on the treated and the

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a - Standard Model No. 3, Arthur H. Thomas Co., Philadelphia, U.S.A.

control calves. The appearance of the haircoat, and the animals' demeanor were recorded. Rectal temperatures were taken. The respiratory and pulse rates were counted on a per minute basis and the digestive system and mucous membranes examined. Special emphasis was laid on the consistency of the faeces, frequency and strength of the ruminal contractions and intestinal borborygmi. Clinical examinations were always done in the mid-morning before the daily drenching and sampling were carried out. Routine clinical observations were also done in the mid-afternoon on the treated calves.

Collection of Blood samples:

All samples for haematological analysis were collected about ten minutes after the clinical examination in the morning. Blood samples were collected from the jugular vein using a 1½" x 18G disposable needle and a plastic (10 ml) syringe. About four millilitres of whole blood was transferred into a bijou bottle containing approximately 4-5 mg. of dried sodium ethylenediamine tetraacetate (E.D.T.A.) as anticoagulant and shaken gently to allow the blood and the anticoagulant to mix. For serum biochemical determinations, about 20 millilitres (ml) of whole blood was collected at the same time into a universal bottle with no anticoagulant, and then allowed to stand on the bench at room temperature (22^{o}C) for about 45 minutes to clot. The serum was pipetted off and used for serum enzyme activity determinations.

Estimation of Blood Values:

Routine haematology was done to determine the packed cell volume

(PCV), the haemoglobin concentration (Hb), the red blood cell counts (RBC), the white blood cell counts (both total and differential, WBC) and the total serum protein (TP).

(i) The Packed cell volume

This parameter was determined by the microhaematocrit method. Commercially available unheparinized Microhaematocrit capillary tubes (75 x 1.3 mm; Arthur H. Thomas Co., Philadelphia, U.S.A) were used. The tubes were filled with the uncoagulated (E.D.T.A.) blood until about $\frac{3}{4}$ full, then the dry ends sealed over a Bunsen flame. The sealed tubes were then placed in a microhaematocrit centrifuge (Measuring and Scientific Equipment Ltd., Sussex, England; MSE) with their sealed ends towards the periphery and spun for 15 minutes at 12000 rpm. They were removed and placed on an MSE microhaematocrit reader and the PCV read.

(ii) The Haemoglobin Concentration:

The haemoglobin concentration of blood was determined by the cyanmethaemoglobin method using a coulter Haemoglobinometer (Coulter Electronics Inc., Hialeah, Florida, U.S.A). A volume of forty lambdas of whole blood was diluted 1:500 with ISOTON[a]. Then six drops of Zap Oglobin[b] (containing 300 mg Potassium Ferricyanide per 100 ml) was added and gently shaken. The mixture was left to stand at 22°C for about 10 minutes and then the solution placed in the cuvette of the Coulter Haemoglobinometer. The readings were directly recorded as grams per 100 ml of blood (with an error margin of ± 0.1 gm).

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[a] ISOTON - Coulter Electronics, Hialeah, Fla. U.S.A

[b] Zap-Oglobin - Couleter Electronics, Hialeah, Fla. U.S.A

(iii) The Red Blood Cell Counts (RBC)

The red blood cell counts were done using the Coulter Electronic Counter (Coulter Electronics Inc., Hialeah, Florida, U.S.A.): Model Z_B with a mercury manometer. For the Rbc counts, a blood dilution of 1:50,000 was used. To make the dilution, 40 lambdas of whole blood were mixed with 20 ml of ISOTON. Then, one fifth millilitre of the diluted blood was further mixed with a fresh 20 ml of ISOTON to yield the desired dilution of 1:50,000. The diluted blood in an auxilliary cuvette was placed on the platform and electrodes inserted into the fluid. The readings on the readout of the coulter counter multiplied by a hundred gave the number of red blood cells per cubic millimetre (cu.mm) of the sample blood. The figure computed thus was expressed as the number of red blood cells (in millions, 10^6) per cu.mm.

(iv) The White Blood Cell (WBC) Count:

Both the total and differential White blood cell counts were done. The total white blood cell counts were done using the Coulter Electronic Counter. The white cell counts were done using a blood dilution of 1:500 and this was prepared as the first step in the Rbc count. The diluted blood was then placed in an auxilliary cuvette and electrodes were inserted into the mixture. The readings on the readout of the coulter counter were equal to the number of the white blood cells per cubic millimetre of sample blood.

For the differential white cell counts, blood smears were made, air dried and fixed with methanol then stained with Wright's stain.

The differential white cell count was done under the microscope using the Battlement Method as described by Schalm (1965). Two hundred white blood cells were counted, recording lymphocytes, neutrophils, basophils, eosinophils and monocytes with a Marbel Blood Cell percentage calculator (Marbel Blood Calculator Co., Illinois, U.S.A). The total count of each blood cell type was then expressed as a percentage of the total number of the cells counted. The absolute number of the lymphocytes was further computed to correlate the lymphocytic response to the toxicity.

(v) The Total Serum Protein Concentration (T.P)

Determination of the Total Protein content was done using a refractometer (ATAGO, JAPAN). A single drop of serum from the Wintrobe Microhaematocrit tube was placed on the prism of the refractometer and viewed through the eyepiece by electrical illumination. The total protein value was obtained directly from the scales and recorded as grams per 100 ml of sample blood.

Determination of Serum Enzyme Activity

Studies on the serum were done to determine the activity of aspartate amino transferase (AST), formerly called serum glutamate oxalacetate transaminase (SGOT), serum alkaline phosphatase (AP), lactate dehy'rogenase (LDH), the total protein (TP), creatine phosphokinase (CP') and the blood urea nitrogen (BUN).

1) Serum Glutamate Oxalacetate Transaminase (SGOT) (Aspartate amino transferase)

The measurements of SGOT were done following the method of Reitman and Frankel (1957) and using the DADE SGOT reagents[c]. One fifth millilitre of the test serum was mixed with 1.0 ml of the buffered substrate (Aspartate Ketuglutarate) and incubated in a waterbath at 37°C for 60 minutes. A volume of 0.1 ml dinitrophenylhydrazine colour developer was added and allowed to stand at room temperature (22°C) for twenty minutes. Ten millilitres of 0.4 N sodium hydroxide was further added to develop the brownish colour whose optical density was read with an Eppendorff photometer using a mercury 546 filter. The activity of the SGOT was expressed in Reitman-Frankel units per millilitre.

2) Serum Alkaline Phosphatase (AP)

Alkaline phosphatase measurements were done following the method of Kind and King (1954). Two millilitres of buffered substrate were measured into each of two test tubes and incubated in the waterbath at 37°C for five minutes. Then, a tenth of a millilitre of test serum was added to the first tube and further incubated for exactly 15 minutes. A volume of 0.8 ml of 0.5 N sodium hydroxide were added to each tube and mixed. Some 1.2 ml of 0.5 N sodium bicarbonate were added to each tube and mixed. One tenth millilitre of double distilled water was added to the blank and 1:0 ml of amino-antipyrine reagent were added to each tube and mixed followed by the addition of one millilitre potassium ferricyanide to each tube. The optical density of the solutions was

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c = DADE SGOT Reagents - DADE Diagnostics Inc., Miami, U.S.A.

then read from the Eppendorff photometer using mercury 546 filter, and the results obtained expressed in international units (I.U.)

3) Lactate dehydrogenase (LDH):

The measurement of the lactate dehydrogenase activity was done using the C-Zyme LDH reagent[d]. 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 form NADH as indicated in the following reaction:

$$\text{Lactate} + \text{NAD} \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH}$$

$$\text{NADH} + \text{INT} \longrightarrow \text{INTH} + \text{NAD}$$

The INT is a tetrazolium salt which on acquisition of the electron transfer into INTH forms a highly coloured reaction product whose optical absorbance is proportional to the LDH activity in the test serum.

The lyophilised LDH substrate was reconstituted with double distilled water and then a volume of 1.0 ml. of the reconstituted LDH was put in each of the 3 tubes labelled test, control and blank. The three tubes were then incubated in a waterbath at 37°C for five minutes. One tenth millilitre double distilled water was pipetted into the blank while a volume of 0.1 ml. of the reconstituted control was pipetted into the control. One tenth millilitre of test serum was pipetted into the test tube and the reaction allowed to proceed in the tubes for exactly ten minutes in the waterbath at 37°C. The reaction was then stopped with 5.0 ml. of 0.1 N hydrochloric acid. The optical

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d: C-Zyme LDH reagent - Coulter Electronics Inc., Hialeah Fla, U.S.A.

absorbances of each tube were read from the Eppendorff photometer using mercury 546 filter and the activity of the LDH was expressed in Wacker Units (w.u.) per 100 ml. serum.

4) Creatine Phosphokinase (CPK)

The measurements of the CPK were done using the C-Zyme CPK reagent (C-Zyme CPK Reagent[a], and following the method of Oliver (1955) as modified by Rosalki (1967). The principle of the test provides an enzymatic CPK measurement in the solution by the following reaction:

1. Creatine Phosphate + ADP $\xrightarrow{\text{CPK}}$ Creatine + ATP

2. ATP + Glucose $\xrightarrow{\text{Hexokinase}}$ ADP + Glucose - 6 - phosphate

3. Glucose - 6 - phosphate + NADP $\xrightarrow[\text{Dehycrogenase}]{\text{Glucose - 6 - phosphate}}$ phospho-glutarate + NADPH

4. NADPH + INT $\xrightarrow{\text{Diaphrose}}$ NADP + INTH

The lyophilised CPK substrate was reconstituted with double distilled water in vials and labelled Test, Control and Blank. The three vials were incubated in a waterbath at 37°C for five minutes. One tenth millilitre of double distilled water was pipetted into the blank while 0.1 ml reconstituted control was pipetted into the control. Similarly, 0.1 ml of the test serum was pipetted into the test tube and the reaction was allowed to proceed in the tubes for exactly ten minutes in the water-bath at 37°C. The reaction was stopped by the addition of five millilitres of 0.1 N hydrochloric acid to each bottle. The optical absorbances of the solutions were then read from an Eppendorff

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a - Coulter Electronics, Hialeah, Florida, U.S.A

spectro-photometer using a mercury 546 filter, and the CPK activity was expressed in international units per litre of the serum.

5. The Total Serum Protein Levels(TP)

The total protein concentrations were measured using the improved Biuret method of Reinhold (1953). A volume of 9.5 ml of a 23% solution of sodium sulphate was pipetted into a test tube and 0.5 ml. of the test serum was added and mixed gently. Three millilitres of the mixture was then withdrawn and put in a tube. To the remaining seven millilitres of the solution were added 0.2 ml of "Tween 80"[a] and five millilitres of analytical ether were added before the bottle was stoppered with a cork. After a thorough mixing by tipping the tube twenty times, the mixture was incubated in a waterbath at 37°C until the globulins had separated out and formed a pellet. The suspension was then centrifuged at 2,000 r.p.m. for five minutes. Three millilitres of the lower solution was removed and put into a test tube marked "Albumin" and then mixed thoroughly before being allowed to stand at room temperature (22°C) for thirty minutes. The optical density of the two solutions were then read in the Eppendorff spectrophotometer using mercury 546 filter. The readings were compared to a standard serum (Versatol-A)[b] and calculated according to the formula:

$$\frac{\text{Test sample}}{\text{standard sample}} \times \text{Conc. of standard sample} = X$$

Where X = protein gm/100 ml serum.

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a - "Tween 80" = Sorbitan monooleate, a synthetic "wetting agent"

b - Versatol - A, General Diagnostic Div. Warner & Lambert Co., New Jersey, U.S.A.

6. Blood Urea Nitrogen (BUN) Levels

Concentration of BUN were estimated using urea nitrogen test strips (URASTRAT)[a]. The principle used is a modified method of Conway (1958) microdiffusion technique. The serum travels through the URASTRAT chromatography paper by capillary action encountering first urease which produces ammonia from the urea nitrogen. The ammonia is liberated as gas in the next band by potassium bicarbonate. The ammonia gas diffuses upwards to reach the indicator band containing bromocresol green and tartaric acid. The concentration of the BUN was indicated by the height of the colour of the indicator band after 30 minutes.

A volume of 0.2 ml serum was placed in a 10 x 75 mm. test tube and the URASTRAT strip was placed into the tube and left to stand for thirty minutes. The blue band on the strip was then read against the chart and the BUN values were expressed in milligrams per 100 ml. of blood serum.

NECROPSY PROCEDURE

All the calves which succumbed to the toxicities were subjected to a thorough standard postmortem examination. Gross changes in the organs were noted and prepared for histopathological studies. Routinely, the sections were selected from all macroscopic lesions and especially from the liver, heart, spleen, kidneys, lungs, lymph nodes, pancreas, duodenum, jejunum and the adrenals. The tissues were preserved in

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a Urastrat - General Diagnostic Div., Warner & Lambert Co., New Jersey, U.S.A.

buffered 10% formalin solution for at least 48 hours before sectioning. Then, sections were prepared for histopathological examination. All routine sections were prepared and stained with haematoxylin and eosin stain. The histological preparations and staining procedures were done following standard methods given in the Manual of Histological and Staining Methods (3rd Edition, 1968).

R E S U L T S

Clinical Observations:

The clinical signs observed in calves drenched with Gnidia material varied with dosage used. The animals which received high doses (2 gm/kg. and 1 gm/kg.) developed clinical intoxication within 24 hours. Those which received 2 gm/kg. per day showed acute abdominal pain within a few hours, followed by lying down, uneasiness and a slight increase in body temperature, pulse and respiratory rates. They also showed a worried look on the faces with panting, straining and some chewing movements of the mouth and tongue. The animals became dull on the second day with signs of hind leg weakness, inappetence, very dry muzzle and some stringy nasal and lacrimal discharges. Signs of abdominal pain included grunting, difficult breathing, continuous teeth grinding and occasional groaning on lying down or when waking up. There were few weak ruminal movements and intestinal borborygmi. Later on these conditions were followed by diarrhoea or loose faeces which were scanty, partially undigested and yellowish brown in colour. The first

calf that received 2 gm/kg leaf material died on the fourth day while the one on the bark material survived for about three weeks. The dosages and the duration of the drenching experiments of the calves are summarized on Table I.

The animals that were drenched with 1 gm/kg per day of Gnidia material showed a subacute form of toxicity and survived much longer. The calves, invariably, showed signs of weak hind legs on the day following the first drenching. These were accompanied by muscle tremors, slight frothing at the mouth, grinding of the teeth, grunting, depression and a tendency to lie down most of the time. These signs were then followed by diarrhoea (brownish faeces), difficult breathing, inappetance and a dry muzzle. The ruminal movements and intestinal borborygmi were weak and the haircoat was stary. The calf drenched with leaf material at dosage of 1 gm/kg/day, recovered uneventfully when feeding was suspended after 60 days. The calf, however, showed a marked loss of body condition and loss of hair on several areas of the skin. The calf that was drenched with bark material progressed to death with a very marked loss of body condition, loss of hair over the face and shoulders and a slight oedema of the submandibular area .

The two groups of calves that received 200 mg/kg/day and 500 mg/kg/day took much longer to develop similar clinical picture. They were depressed, showed marked loss of body condition, had diarrhoea and submandibular oedema. It usually took about 6-8 weeks for them to succumb to the toxicity and die.

The four calves which were drenched with the leaf material at 100 mg/kg per day developed chronic enteritis with intermittent diarrhoea and loose faeces. This was followed by loss of the body weight before the calves died.

Haematological examination

The results of the haematological examinations are summarized in Tables II and III. As shown in Table II, there were no changes in the packed cell volumes of the treated calves for the first two weeks, from the beginning of the drenching experiments. The calves then developed a gradual drop in the levels of the P.C.V. till they died. There were a few day to day variations for each set of animals but the gradual drop in the P.C.V. was observed in most (8/12) of the animals. The drop in the P.C.V. was more marked on the last two days or so before death.

There was only a slight drop in the levels of the red blood cell counts (Rbc). The calves showed some day to day variation in their erythrocytic counts but the range for each animal remained within a constant level. It was therefore assumed that the toxic principles of Gn. latifolia (Meisn) did not affect the red blood cell counts of the bovine to any significant extent.

The total protein levels did not show any alteration for all the experimental animals. Although several animals showed a generalized oedema of the fatty tissue and serous fluid effusion into the body cavities, these changes did not affect the serum protein levels, which remained constant at around 5 to 7 gm/100 ml serum. The haemoglobin

concentrations showed a slight drop in three of the calves that were drenched for more than a month with Gn. latifolia (Meisn) material. The levels of haemoglobin concentration dropped from about ten milligrams per 100 ml serum to about six milligrams per 100 ml. These changes were more marked on the last three to four days before the death of the animal. They were noted in calves No. 5 fed leaf material at 500 mg/kg per day for 28 days, and calves 10, 11 and 12 fed at 100 mg/kg/day each for 51, 36 and 38 days, respectively.

Table III shows the most striking change in the haematological parameters of the bovine in the case of Gn. latifolia toxicity. There was a marked drop in the total white cell counts in all the treated animals. The toxicity affected the mononuclear series more than the granulocytes of the white blood cells. The lymphocytic counts dropped by more than 50 per cent of their original values. Calf No. 1 which was fed Gnidia leaf material at 2 gm/kg/day died on the fourth day of feeding. Even at this early mortality the haematology had already shown a white cell count drop from 9,600 to 5,600 per cubic millimetre before the calf died. These haematological changes were progressively noticed depending on the dosage rates and the survival period of the animal. In calves No. 9 and 10 it was noticed that total white cell counts returned to their original levels after 72 hours following suspension of the feeding. The drop in the white cell counts was further noticed in the same animals when the feeding was resumed. These haematological changes in the white cell counts were shown to be due to a marked drop in the lymphocyte counts. This observation tallies well with the

histological observation of a lymphocytic necrosis in spleen and lymph node tissue sections. The absolute lymphocyte count dropped from an average of 6.7×10^3 cells per cubic millimetre at the start of the feeding experiments to an average cell count of 2.9×10^3 cells per cubic millimetre just prior to the deaths of the calves. Many of the calves which survived for longer than three weeks had their lymphocyte counts drop to levels below 2×10^3 cells per cubic millimetre towards the time of death. There were no marked haematological changes in the absolute neutrophil counts in both the acutely and the chronically poisoned calves.

The Serum Biochemical Examination

The serum biochemical parameters examined in the calves included the serum albumin levels, the blood urea nitrogen levels, and the activities of glutamate oxalacetate transaminase (SGOT or AST), alkaline phosphatase (AP), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) enzymes. There were no changes in the levels of serum albumin in treated calves. There were few recognizable day to day variations but the albumin levels remained around three to five milligrams per 100 ml. of blood in poisoned calves (Table V.) The levels of blood urea nitrogen remained low till just a few days prior to death and then showed a slight increase. Levels of 5 to 15 mg/100 ml of serum were initially recorded in the treated animals, but these levels rose to between 20 and 50 mg/100 ml serum in the last three to five days preceeding the death of the animals. One animal (No. 5) which received 500 mg/kg/day had markedly high levels of blood urea nitrogen for the last six days preceeding

death (up to 102 mg/100 ml serum), (Fig. 3.)

There were no changes in the activity of the creatine phosphokinase, and serum alkaline phosphatase in both the treated and control calves. The creatine phosphokinase levels remained between 25 and 60 international units per litre of serum while the alkaline phosphatase levels remained between 5 and 30 King-Armstrong units per millilitre of serum. The serum glutamate oxalacetate transaminase (SGOT) levels were slightly elevated compared to the control animals. The levels remained in the range of 30 to 100 Reitzman-Frankel units per millilitre of serum. Two calves, Nos. 2 and 4, that were fed at a dosage of 1 gm/kg/day had slightly elevated SGOT levels after two weeks of daily dosing. The levels of S.G.O.T., however, returned to their normal original values two or three days before the calves died. The S.G.O.T. changes are summarized on Table IV.

Table V is a summary of the three biochemical parameters that were measured in the calves fed with Gn. latifolia (Meisn). There were dramatically marked elevations in the levels of the lactate dehydrogenase (LDH) before the calves died. There was a general trend for the LDH levels to rise within the last two or three days preceeding the death of the calf. The LDH levels increased from a low of 100 to a high of 300 Wacker units per 100 ml serum at the beginning of the feeding, to high levels of 700 to 1,000 W.U. per 100 ml of serum during the last three days preceding the death of the calves. These were particularly evident in the case of calves 6 (at 200 mg/kg/day), 9 and 10 (at 100 mg/kg/day). LDH is one of the many intracytoplasmic enzymes that gets

released readily into the bloodstream when cellular damage occurs. This dramatic increase would therefore point to some marked cellular necrosis which might mainly have been in the liver parenchyma. It would however be quite difficult to pinpoint the particular tissue damaged because there were no tests performed on organ function.

Pathological Lesions

The animals which died of the acute intoxication and those that died after a prolonged feeding with Gnidia material were all subjected to a thorough postmortem examination. The survival period of the individual animals are shown on Table I. The acute cases showed slight distension of the abdomen. There was also an eversion of the anus and little rigor mortis in animals that were dead for about 12 hours. In acute cases, gross gastrointestinal changes included haemorrhages in the submucosa of the rumen, abomasum and intestine. There was a marked degree of submucosal oedema in the more chronic cases involving especially the abomasal folds. The animals which died after a prolonged course of toxicity, lasting more than five weeks, further had a general emaciation of the body with a generalized gelatinous atrophy of the body fat and muscular atrophy. The lesions noted in the experimentally intoxicated calves are given in detail as follows:

The Peritoneal, Thoracic and the Serosal cavities:

The abdominal and thoracic cavities of the acutely intoxicated cases showed moderate fluid effusion of a serosanguinous nature. Ascites and hydrothorax were very evident in all the more chronically intoxicated

cases. The amount of the fluid varied both in quantity and colour with the acute cases having less fluid, more blood tinged in colour, while the chronically affected cases showed more accumulation of straw-coloured fluid which measured as much as two litres each in the thorax and the peritoneum. The calf fed 1 gm/kg/day of bark material for 54 days was a typical case with much fluid accumulation. About 100-150 ml. straw-coloured fluid was obtained from the pericardial sacs of all the chronically affected cases, while all the prominent joints (hips, stifle, and shoulder) had excessive serous-yellowish fluid. All the subcutaneous, peritoneal and perirenal fat was degenerated into a gelatinous mass. The meninges also grossly showed some slight oedema but histologically did not show any alteration in the brain tissue.

The Liver:

The animals which died of the acute toxicity had congestions and haemorrhages of the liver. The liver was friable and showed several subcapsular necrotic zones. The chronic cases showed some whitish subcapsular patches which were more visible on the parietal surface. The liver capsule was firmly attached to the parenchyma in some of the white zones. Histologically the liver parenchymal cells showed a widely varied picture. Figures 5 to 8 show changes observed in the liver tissue of calves fed leaf material.

In acute intoxications, the liver parenchymal cells showed vacuolation of the cytoplasm and some had eosinophilic hyaline droplets. The droplets could in some cases be seen to fill the sinusoidal spaces.

Figure 5 shows droplet formation and sinusoidal occlusion by hyaline casts. There were instances of marked cellular degeneration in the periportal areas accompanied by diffuse hepatic congestions and haemorrhages. The more subacute cases of toxicity demonstrated increased fibroblastic cell formation. These changes followed the parenchymal cellular degeneration. Figure 6 shows an area of cellular degeneration in mid-zonal parenchymal tissue. There is evidence of tissue hyperplasia by the presence of many multinucleated cells at the periphery of the necrotic zones. Figure 7 represents a subacute toxicity with an increased number of spindle shaped cells. These are precursors of fibroblastic cell formations. The hepatic parenchymal cells show marked vacuole formations accompanying the haemorrhages.

In the chronic cases, the whitish patches were demonstrated as areas of fibrous tissue formations. Figure 8 shows a histological section of chronic intoxication in the calf liver. The liver lobules showed a generalized cirrhosis with bands of fibrous connective tissue infiltrating the centrilobular and periportal areas. The fibroses were either in the form of circular rings surrounding the portal triads and central veins or they appeared as haphazard fibroblastic proliferations in the hepatic lobules. The connective tissue running from the periportal veins to the periphery of the lobule varied in amount. Some of the degenerative changes were followed by the infiltration of mononuclear inflammatory cells. There was a varied proportion of the nature and the proliferating connective tissue and some degree of bile duct proliferation was evident.

The Spleen and Lymph nodes:

The spleen appeared grossly pale in both the acutely and the chronically intoxicated calves. When sectioned the lymph nodes showed a marked gelatinous oedema with a reduced cortical tissue.

Fig. 9 represents a histological section of a lymph node from a calf fed leaf material. There were very marked lymphocytic cell degenerations in both the spleen and lymph nodes. The lymphocytic degeneration was more marked in the acute and subacute cases than in the chronic ones. These were seen as areas of cellular depletion in the centres of the lymph follicles and splenic follicles with marked cell necrosis and degeneration leaving partially empty follicular centres. The acute cases further showed areas of haemorrhages and congestions accompanied by increased infiltration of neutrophilic leucocytes. In the more chronic cases there was a relative increase in the amount of fibrous tissue in the lymph follicles following the lymphocytic depletion and an accompanying increase in macrophage cells. The lymphocytic cell necrosis was also seen in the Peyer's patches in the intestines of the acutely affected cases.

The Kidneys:

The kidneys in the acutely intoxicated cases were friable and soft and had little colour alterations. The chronic cases had kidney which looked pale and oedematous. Some cases showed slight congestion of the kidney cortex while others appeared slightly pale. Microscopically,

the kidneys showed some petechial cortical haemorrhages in the acute cases. There were also several zones of cellular necrosis and tissue degeneration in the subacute and chronic cases. There were areas of slight interstitial fibrosis with some hyaline deposits and albuminous casts in the descending tubules. The glomeruli were slightly affected in the calf and the haemorrhages were more prominent in the interstitial areas and the periglomerular space was empty in most cases. Figure 10 shows an example of the cortical changes seen in the kidneys of an acutely intoxicated animal. Several zones of congestion were observed. Haemorrhages with marked tubular degeneration were commonly observed in the acute cases.

The adrenal gland was most affected with marked haemorrhages, cell degeneration and hyaline cast depositions. The hyaline deposits were deposited in between the cells of the zona reticularis and zona fasciculata and the cells adjacent to the deposits were undergoing degenerative changes. There were several vacuolar formations within the adrenal medulla and cortex. The significance of this vacuole formation in the adrenal body could not be explained.

The Lungs:

The lungs appeared grossly congested and slightly oedematous but the changes were not noticeable on histological sections. There was a slight catarrhal bronchitis with foamy exudate in the acute cases. The tracheal and bronchial mucosa appeared congested with prominent capillaries. The chronic cases further showed slight pneumonia especially in the apical lobes but this was presumed to be due to the recumbency before death.

The Heart:

The heart showed some gross subepicardial haemorrhages and there was consequently an increase in the volume of pericardial fluid. In the chronic cases the heart appeared flabby but on histological examination did not show any significant cellular alteration.

DISCUSSION ON THE CALF FEEDING EXPERIMENTS

Calves fed Gn. latifolia material developed necrosis of the liver and kidney tubules similar to those described by Nwude and Parsons (1978) and Terblanche et al. (1966). These changes were accompanied by severe necrotic changes in the lymphocytic follicles of the cortices of the lymph nodes and the spleen. The epithelial cells of the digestive system and kidney tubules showed pathological changes similar to those observed in the liver parenchymal cells. The active principle(s) in Gn. latifolia affected the most rapidly multiplying tissue cells of the body.

The mononuclear cell series were affected the most. The lymphopaenia observed in the calves was likely due to direct injury of the lymphoblastic cells in the lymph follicles. It was not possible however, to ascertain whether the fast-multiplying cells of the bone marrow were affected because the total erythrocyte and neutrophil counts were within normal limits. Marked cellular depletion of the lymphocytic follicles in the lymph nodes and the spleen was observed in histological sections. This would account for the lymphopaenia observed.

Most of the published literature on the toxicity of Gnidia species date back to the 1962 to 1975 period when toxic compounds in plants were investigated for their potential in the treatment of cancer (Adamson et al., 1965; Farnsworth, 1966; Terblanche et al., 1966; O'Gara et al., 1971; Kupchan et al., 1975). Before that time, the bulk of the published work dealt with accidental poisoning of livestock following ingestion of Gnidia material (Nicholson, 1947; Shone and Drummond, 1958; Verdcourt and Trump, 1969). All the toxicity trials confirmed the enteritis and hepatotoxicity in the intoxicated animals; the most characteristic lesion being centrilobular hepatic necrosis. Terblanche et al. (1966) had fed sheep, rabbits, guinea pigs, pigs and rats with the ethanol extract of Gn. burchelli. They found that there were changes in other body organs such as the heart, the lungs, kidneys and the lymph nodes. They concluded that Gn. burchelli contained a toxin which acted on the body tissues by interfering with the vital functioning of the heart, the lungs, lymphatic and nervous systems. Apart from the dullness and muscular tremors observed in the calves in the present work, there were no other nervous disturbances noted.

An attempt was made to analyse the activity of the toxic principle(s) quantitatively. Calves were fed various doses and the effects of the short term feeding of high doses and the long-term feeding of smaller doses investigated. The acutely intoxicated calves showed haemorrhagic cellular destruction while the chronically poisoned ones had more cellular degeneration followed by progressive fibrous tissue proliferation.

Marked serous fluid effusion into body cavities was also demonstrated. Oedema in the body cavities and connective tissue was consistently found in the calves. There were no changes in the blood protein levels even though there were marked renal injuries of the kidney tubules. The extensive capillary damage led to haemorrhages and loss of plasma protein. Renal tubular damage permits proteins to escape through the kidney into the urine. Boyd et al. (1965) showed that plant alkaloids and tannins caused the loss of blood protein. They suspected that such loss was due to the slow bleeding of gastric ulcers. It is possible that plant macrolides also caused blood protein loss in a similar manner. This would have led to fluid effusion and oedema.

Both liver and kidneys are primary targets in many types of chemical poisonings. The exact mode of action of the active constituents in Gn. latifolia could not be precisely described. Also, the duration of time that one would need to employ them before marked toxicity was observed was not fully investigated. As yet the physiological and biochemical basis for drug interference in cellular function is poorly understood (Farnsworth, 1966; O'Gara et al. 1971). The cellular processes examined so far, do not permit identification of the fundamental alterations related to cell growth and multiplication. It has, however, been established that several plant constituents interfere with cell growth and multiplication (Tin-Wa et al., 1971; Broder and Carter, 1971; Slavik, 1974; Kupchan et al., 1975). Cellular damage in the intoxicated kidneys, liver and the adrenal bodies interfere with

the vital functioning of these organs. Cellular damage was followed by degenerative changes and the development of hyaline droplets, or hyaline inclusions in the cytoplasm of the liver and adrenal parenchymal cells. The appearance of hyaline droplets was accompanied by mononuclear cell infiltration in the liver.

The biochemical tests revealed a marked increase in LDH and BUN levels especially towards the death of the animal. These changes may have resulted from interference in liver function. Continous administration of chemical compounds such as pyrrolizidine often lead to a marked increase of enzymes involved in the metabolism of the same as well as other related and unrelated substances (Corney et al., 1960). This finding has important toxicological implication. It is conceivable that a chemically administered compound may stimulate the activity of microsomal liver enzymes to such a degree that the administered substance is metabolised and detoxified rapidly. Consequently some toxicity experiments may not show potentially important organ changes because the test compound is quickly eliminated or detoxified in the body and does not, therefore, produce a sustained toxic effect. Nwude and Parsons (1978) postulated that the active principle of Gn. kraussiana either cuased the lysis of the lymphocytes or the release of adrenocorticotropic substances which in turn caused lymphopaenia. The present experiments have demonstrated the direct effect of the toxic principle(s) of Gn. latifolia on the lymphocytic follicles in the spleen and lymph nodes. This is direct evidence for the hypothesis that the active principle of Gnidia species caused lymphocytic lysis.

On the other hand, the toxicity on the adrenocortical cells observed in the histological sections suggest the release of excess adrenocorticotropic substances from the damaged cells. Information obtained in the literature (Kupchan and Lacadie, 1972; Harborne, 1973) further stated that the diterpenoids were eliminated in the urine as glucuronides. This would further explain the role the liver plays in the detoxification of Gn. latifolia macrolides.

Table I: Dosage, feeding regimen and survival data on calves fed *Gn. latifolia* (Meisn)

Calf No.	Body weight Kg.	Material used	Dosage rate per kg.	Survival time (days)
9	74	leaves	100 mg	142
10	116	"	"	51
11	154	"	"	36
12	82	"	"	38
7	81	"	200 mg	28
8	96	"	"	31
5	98	"	500 mg	28
6	112	"	"	12
2	54	"	1 gm	19*
1	82	"	2 gm	4
4	58	Bark	1 gm	16
3	81	"	2 gm	18
		Control calves		
				Examination Period (days)
13	94	None	None	150
14	100	"	"	150
15	96	"	"	150
16	83	"	"	150

* On first regimen the calf survived for 60 days. It then recovered when feeding was suspended for one month. On second regimen it died after 19 days of feeding.

Table II: The mean ± s.d. of haematological values of calves fed *Gn. latifolia* (Meisn)

Days	PCV (%)	Hb (mg/100 ml)	Rbc (10^6/mm^3)
0	27.25 ± 5.49	9.62 ± 1.26	6.08 ± 1.30
1	27.70 ± 5.43	8.96 ± 1.66	5.35 ± 1.06
2	27.7 ± 5.86	8.86 ± 1.87	7.07 ± 1.20
3	27.65 ± 4.9	8.68 ± 1.67	5.48 ± 1.08
4	28.1 ± 5.17	8.70 ± 1.58	5.42 ± 1.13
5	28.8 ± 4.80	8.76 ± 1.74	5.31 ± 1.10
6	28.9 ± 3.59	8.68 ± 1.90	5.33 ± 1.15
7	28.95 ± 4.83	8.63 ± 1.76	5.48 ± 1.35
8	28.3 ± 4.23	8.83 ± 1.82	5.27 ± 1.44
9	27.95 ± 4.98	8.71 ± 1.63	5.00 ± 1.36
10	27.8 ± 4.67	8.21 ± 1.66	5.10 ± 1.01
11	27.69 ± 4.94	8.63 ± 1.52	5.43 ± 1.07
12	28.67 ± 4.61	8.85 ± 1.84	5.41 ± 1.60
13	28.12 ± 5.63	8.68 ± 1.87	5.34 ± 1.43
14	29.75 ± 6.25	8.65 ± 1.68	5.11 ± 1.48
15	28.5 ± 5.94	8.65 ± 1.74	5.13 ± 1.49
16	29.02 ± 4.58	8.81 ± 2.03	5.25 ± 1.79
17	28.05 ± 6.4	8.85 ± 1.44	5.28 ± 1.51
18	24.25 ± 4.37	8.43 ± 1.36	5.28 ± 1.51
19	23.6 ± 3.84	8.40 ± 1.52	5.11 ± 1.44
20	23.1 ± 4.16	8.41 ± 1.84	5.34 ± 1.51

Table II cont.

Days	PCV (%)	Hb (mg/100 ml)	Rbc ($10^6/mm^3$)
21	22.7 ± 3.25	8.10 ± 1.35	5.00 ± 1.21
22	23.5 ± 4.69	7.96 ± 1.16	5.07 ± 1.24
23	23.5 ± 4.63	8.20 ± 1.92	5.64 ± 1.49
24	23.75 ± 5.88	8.00 ± 1.55	5.07 ± 1.46
25	23.6 ± 3.66	7.85 ± 1.65	5.14 ± 1.19
26	23.25 ± 4.87	7.70 ± 1.88	5.06 ± 1.45
27	22.3 ± 5.78	8.06 ± 1.75	4.97 ± 1.30
28	23.9 ± 6.06	7.95 ± 1.75	4.84 ± 1.15
29	24.1 ± 5.05	8.60 ± 1.90	5.28 ± 1.49
30	21.25 ± 2.50	8.20 ± 1.96	5.29 ± 1.56
31	22.25 ± 2.50	8.46 ± 2.06	5.31 ± 1.38
32	23.0 ± 1.65	7.40 ± 1.10	4.46 ± 1.10
33	21.5 ± 2.06	7.47 ± 1.02	4.67 ± 0.84
34	19.75 ± 2.64	7.62 ± 0.75	4.89 ± 0.60
35	19.6 ± 3.21	7.15 ± 1.08	4.71 ± 0.91
36	19 ± 3.60	7.25 ± 0.31	4.71 ± 0.91

s.d. indicates the standard deviation

Table III: The mean ± s.d of white cell count values of c lves fed Gnidia latifolia (Meisn)

Treated calves

Days	Wbc	Lymphocytes	Neuthrophils
0	8666 ± 3155	7457 ± 3130	1500 ± 930
1	7710 ± 1079	6006 ± 1840	1600 ± 500
2	8200 ± 2900	5826 ± 1490	1900 ± 740
3	7550 ± 2300	5890 ± 1590	1600 ± 970
4	8300 ± 3120	6181 ± 1870	2300 ± 1300
5	8600 ± 3015	6159 ± 2450	2200 ± 1500
6	8300 ± 4200	6166 ± 2110	2400 ± 1400
7	7500 ± 2200	5078 ± 1840	1800 ± 600
8	8300 ± 2800	6086 ± 2420	2200 ± 1200
9	8380 ± 3000	5594 ± 2030	1700 ± 1100
10	7700 ± 2100	5605 ± 1490	1900 ± 1000
11	7100 ± 2700	5436 ± 1690	2000 ± 990
12	8200 ± 2600	5833 ± 2230	2200 ± 740
13	8100 ± 2600	5724 ± 2450	2600 ± 560
14	7400 ± 2100	5423 ± 2270	2000 ± 1040
15	8200 ± 1900	5410 ± 2040	2400 ± 1400
16	6400 ± 1100	4458 ± 1070	1400 ± 810
17	7500 ± 2600	4538 ± 1680	2300 ± 1300
18	8600 ± 2600	4857 ± 1800	1900 ± 1100
19	6100 ± 2300	4100 ± 1610	1700 ± 600
20	5400 ± 2100	3506 ± 1800	1900 ± 750

Table III. cont.

Days	Wbc	Lymphocytes	Neuthrophils
21	5400 ± 2500	4378 ± 1980	1600 ± 580
22	5300 ± 2300	3945 ± 1950	1700 ± 500
23	5600 ± 2700	3628 ± 2400	1600 ± 390
24	4900 ± 2300	3959 ± 1780	1500 ± 530
25	5100 ± 2000	3974 ± 2000	1000 ± 320
26	5300 ± 2100	3517 ± 2040	1500 ± 500
27	5100 ± 2400	4469 ± 2460	1600 ± 650
28	5600 ± 2100	4481 ± 2180	1400 ± 650
29	6100 ± 2500	4671 ± 1660	1690 ± 490
30	5800 ± 1600	4786 ± 2170	1400 ± 288
31	5900 ± 1900	4045 ± 1830	2000 ± 670
32	5600 ± 1400	4573 ± 1560	1800 ± 690
33	4800 ± 1800	2846 ± 390	1700 ± 860
34	4500 ± 600	2932 ± 660	1700 ± 650
35	3800 ± 1400	2809 ± 1140	1200 ± 530
36	3700 ± 1200	2859 ± 750	1200 ± 350
37	3500 ± 800	2480 ± 860	

s.d. indicates the standard deviation

Table IV: The mean ± s.d. of serum G.O.T. and ALK-P. values of calves fed Gn. latifolia (Meisn)

Days	S.G.O.T.	S.ALK-P
1	73.77 ± 26.5	7.35 ± 2.92
2	75.0 ± 26.4	7.66 ± 3.06
3	57.77 ± 22.8	6.71 ± 2.13
4	60.0 ± 22.5	6.80 ± 1.18
5	61.66 ± 19.5	7.18 ± 2.81
6	68.5 ± 23.4	7.51 ± 2.87
7	65.62 ± 28.3	8.03 ± 2.81
8	61.57 ± 10.8	8.90 ± 3.0
9	59.28 ± 13.2	9.04 ± 3.05
10	68.14 ± 21.3	8.48 ± 2.91
11	67.00 ± 22.9	8.75 ± 3.1
12	73.14 ± 31.2	7.01 ± 2.8
13	63.78 ± 23.2	6.48 ± 2.1
14	56.14 ± 28.2	6.32 ± 1.83
15	63.71 ± 22.5	6.92 ± 2.18
16	70.85 ± 22.1	6.49 ± 1.93
17	77.85 ± 27.1	6.67 ± 0.95
18	88.71 ± 87.2	7.42 ± 2.18
19	58.83 ± 9.3	9.63 ± 3.91
20	50.50 ± 18.5	7.53 ± 2.41
21	47.25 ± 19.1	7.40 ± 2.37
22	46.00 ± 17.7	7.48 ± 2.34
23	59.33 ± 4.7	6.82 ± 2.52
24	48.5 ± 13.8	7.13 ± 3.49
25	40.0 ± 13.8	5.84 ± 1.63
26	59.00 ± 34.3	6.78 ± 1.94
27	57.66 ± 29.0	11.02 ± 6.87
28	47.33 ± 33	11.91
29	42.5 ± 22.1	15.66
30	34.00 ± 11.1	14.28
31	43.00	12.15
32	68.00	

s.d indicates the standard deviation

Table V: The mean ± s.d. of serum biochemical values of calves fed *Gn. latifolia* (Meisn)

Days	BUN (mg/100 ml)	LDH (w/u)	ALBUMIN gm/100 ml
1	12	325.5 ± 37.32	3.38 ± 1.16
2	13.33 ± 2.88	323.5 ± 28.59	3.05 ± 0.5
3	10.0 ± 5.0	258 ± 32.69	2.92 ± 0.28
4	5.0 ± 0	182 ± 51.17	2.90 ± 0.58
5	8.33 ± 2.88	231. ± 23.88	3.00 ± 0.55
6	11.66 ± 2.88	237 ± 45.58	3.01 ± 0.57
7	10.0 ± 8.66	235 ± 48.29	2.98 ± 0.14
8	6.75 ± 3.5	231. ± 53.38	2.91 ± 0.18
9	6.25 ± 2.5	264.5 ± 47.08	3.08 ± 0.48
10	8.75 ± 7.5	303 ± 37.18	3.09 ± 0.28
11	7.5 ± 5.0	286.5 ± 52.63	3.04 ± 0.37
12	8.75 ± 4.78	371 ± 39.81	3.06 ± 0.2
13	8.0 ± 3.55	300 ± 43.55	3.00 ± 0.32
14	8.33 ± 4.08	316.66 ± 31.89	3.11 ± 0.7
15	9.16 ± 4.91	308.75 ± 31.78	3.19 ± 0.47
16	9.16 ± 3.76	268.25 ± 107.52	3.10 ± 0.32
17	11.66 ± 6.25	270.5 ± 86.88	3.07 ± 0.43
18	9.16 ± 6.64	275.75 ± 70.87	2.94 ± 0.32
19	5.83 ± 2.04	289.25 ± 48.04	2.90 ± 0.31
20	7.50 ± 2.73	274.5 ± 51.17	3.05 ± 0.38
21	8.33 ± 6.05	272.5 ± 19.20	2.82 ± 0.32
22	8.33 ± 2.58	235.5 ± 53.31	2.80 ± 0.24
23	7.50 ± 2.73	274 ± 45.58	3.02 ± 0.5
24	14.16 ± 5.84	251 ± 62.53	2.68 ± 0.53
25	12.5 ± 5.24	233 ± 71.44	2.86 ± 0.5
26	11.66 ± 7.52	245.6 ± 49.13	2.88 ± 0.41
27	11.66 ± 11.69	224 ± 61.92	2.86 ± 0.61
28	13.33 ± 8.75	205.52 ± 102.38	2.88 ± 0.38
29	13.33 ± 13.29	282.5 ± 110.51	3.96 ± 0.54
30	17.5 ± 15.08	362.66 ± 48.29	3.03 ± 0.57
31	16.66 ± 17.22	577.83 ± 298.56	3.03 ± 0.49
32	27.83 ± 37.49	585.33 ± 294.43	2.75

s.d. indicates the standard deviation

Table VI: The mean ± s.d. of white cell count values of calves used as controls in experiments with Gn. latifolia (Meisn)

Days	WBC	Lymphocytes
0	9600 ± 2700	6853 ± 2700
1	9625 ± 4800	7162 ± 4900
2	10375 ± 3200	7372 ± 3800
3	8025 ± 2400	6354 ± 1700
4	9725 ± 2800	7165 ± 2500
5	8550 ± 2000	6247 ± 2400
6	8575 ± 2300	6580 ± 2100
7	8975 ± 3500	6354 ± 2100
8	8825 ± 2600	6455 ± 2200
9	8975 ± 2500	6567 ± 2500
10	8850 ± 2900	6923 ± 2700
11	8350 ± 1900	6360 ± 1700
12	7775 ± 2100	5864 ± 1500
13	9025 ± 3100	6299 ± 2300
14	8575 ± 2500	6156 ± 2400
15	8450 ± 1600	6285 ± 200
16	8250 ± 1700	6044 ± 1700
17	8250 ± 2000	6192 ± 1900
18	9025 ± 1000	6333 ± 1700
19	8100 ± 1700	6279 ± 2000
20	7575 ± 1300	6344 ± 1800
21	8075 ± 1300	6570 ± 2400
22	8650 ± 1900	7294 ± 2400
23	8600 ± 1900	6787 ± 2100
24	8525 ± 2300	6341 ± 2000
25	8475 ± 1500	6214 ± 2000
26	9275 ± 2000	7043 ± 2000
27	8225 ± 1700	6589 ± 1500
28	8325 ± 1600	6376 ± 2000
29	8275 ± 1600	6452 ± 2000
30	7467 ± 800	5618 ± 1400

s.d. indicates the standard deviation

Table VII: The mean serum biochemical values of the calves used as controls in experiments with Gn. latifolia (Meisn)

Days	ALBUMIN (gm/100 ml)	S.G.O.T.	S. ALK-P
1	5.82	36.0	9.48
2	5.00	35.0	8.50
3	3.42	38.0	8.67
4	3,42	36.5	12.3
5	3.57	64.5	10.0
6	3.35	42.5	7.90
7	3.15	58.5	12.25
8	3.57	51.0	10.5
9	3.40	52.0	13.02
10	3.40	52.0	13.02
11	3.17	53.0	13.8
12	3.55	51.0	11.75
13	3.60	42.0	7.67
14	3.25	52.0	6.47
15	3.50	51.0	7.25
16	3.47	53.0	7.95
17	3.07	49.0	7.57
18	3.65	24.0	10.0
19	3.02	22.0	15.75
20	3.32	28.25	13.05
21	3.20	20.0	10.0
22	2.95	19.5	7.95
23	3.15	30.0	9.20
24	2.82	27.0	9.52
25	3.40	28	7.65
26	3.40	26.15	11.27
27	3.35	30.5	14.0
28	3.55	27.5	13.8
29	3.65	31.5	14.8
30	3.60	27.0	13.05
31	3.52		12.62
32	3.20		

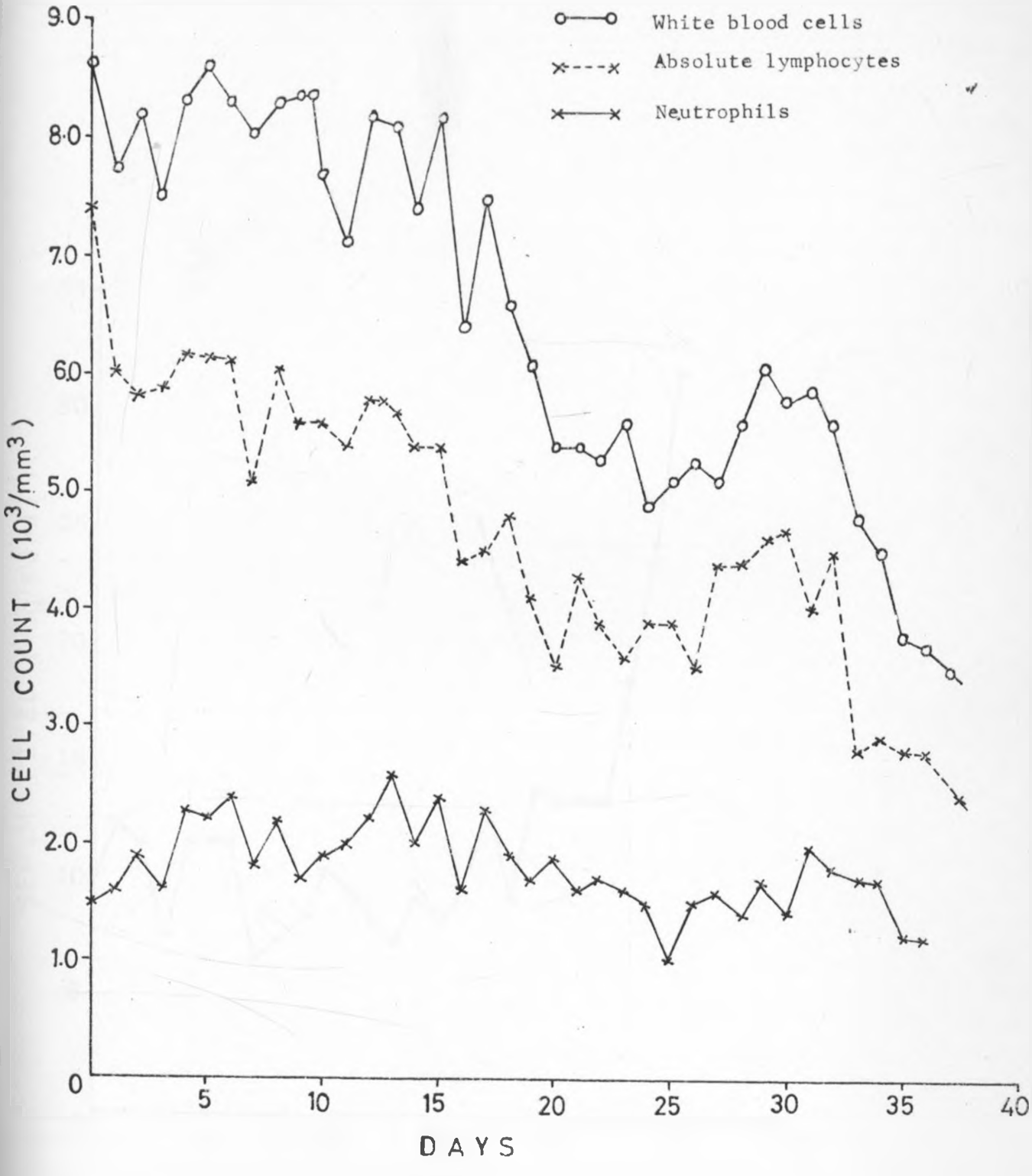

Fig. 2: Changes in the Mean Haematological parameters of calves fed Gnidia latifolia (Meisn)

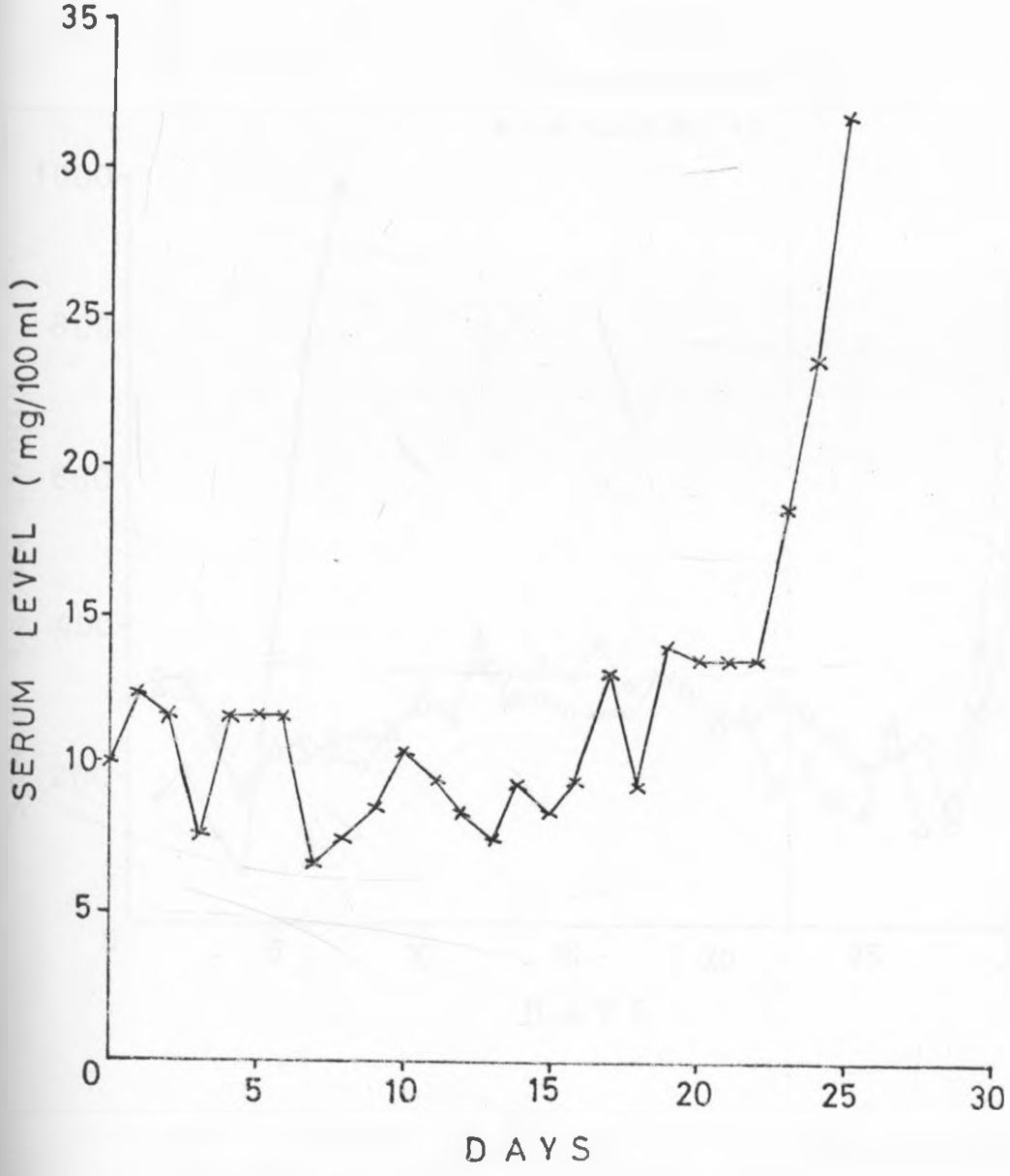

Fig. 3: The Mean levels of B.U.N. in calves fed with Gnidia latifolia (Meisn)

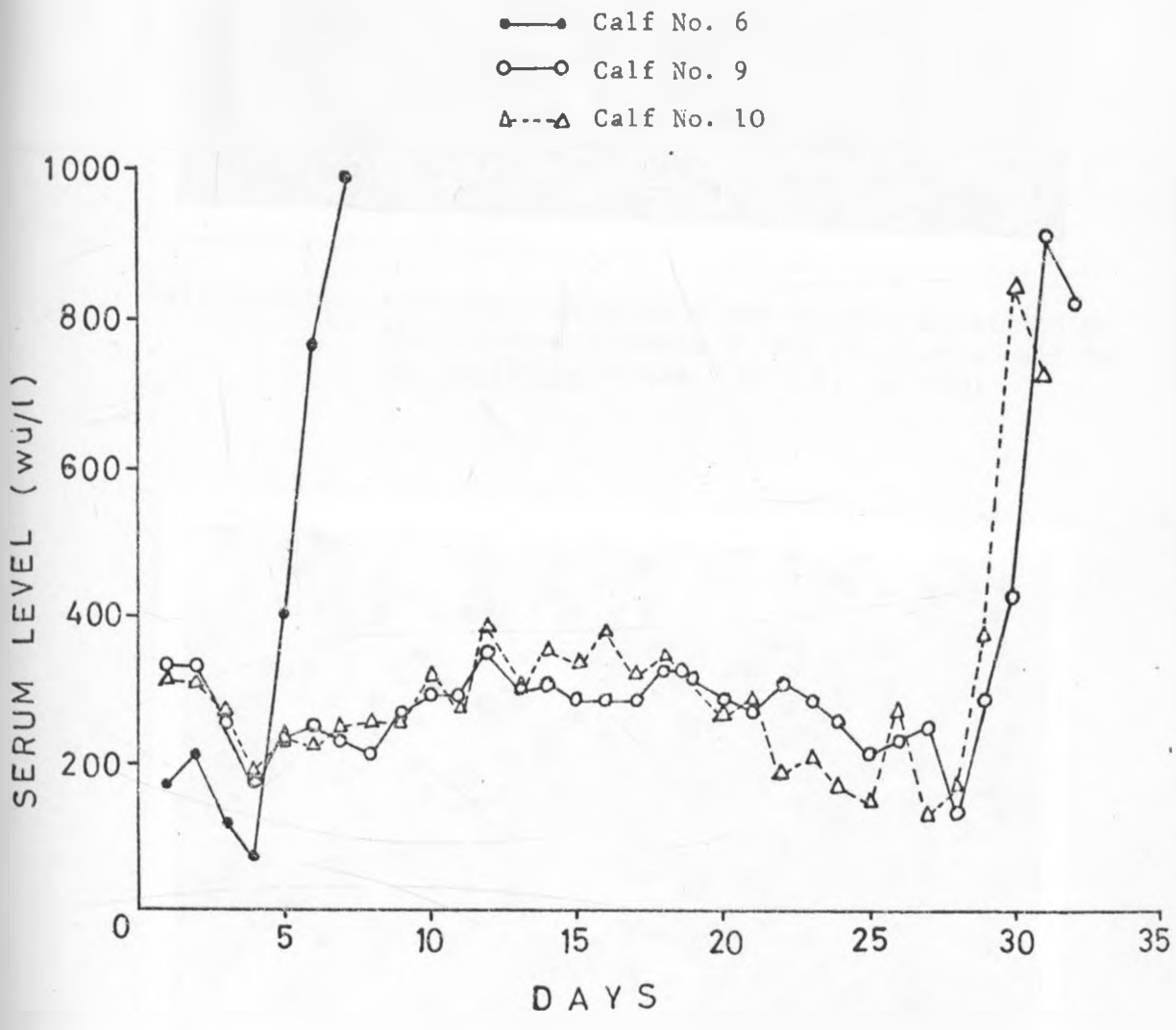

Fig. 4: LDH levels in sera of calves fed Gnidia latifolia (Meisn)

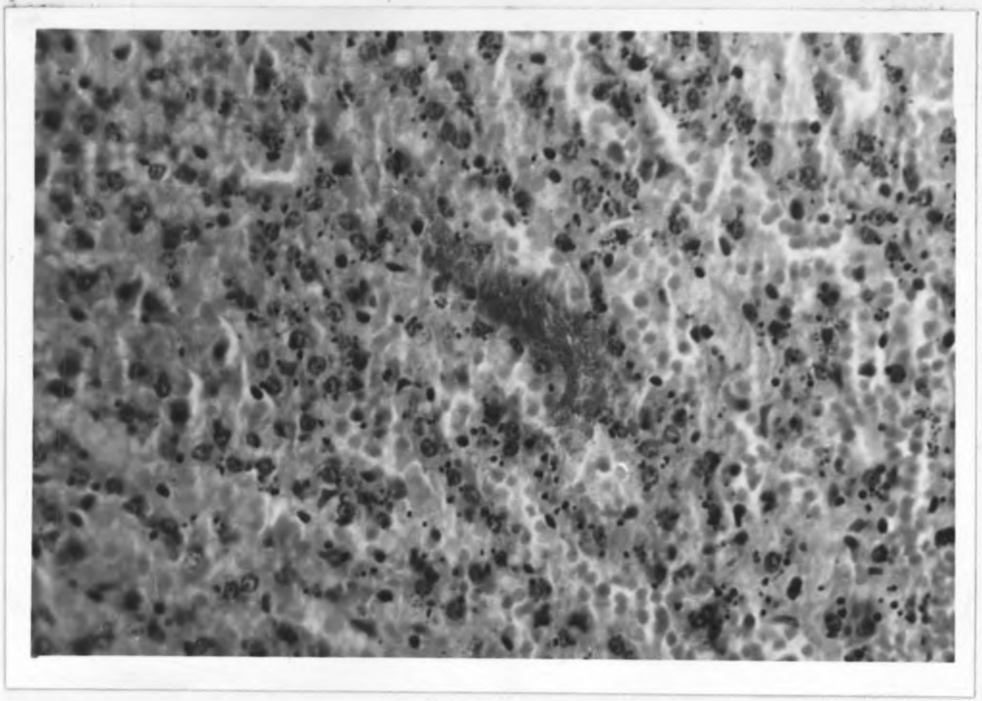

Fig. 5: Calf Liver: Cellular degeneration and hyaline deposition in the sinusoidal spaces in the liver of a calf fed Gn. latifolia leaves. (H & E; x 400)

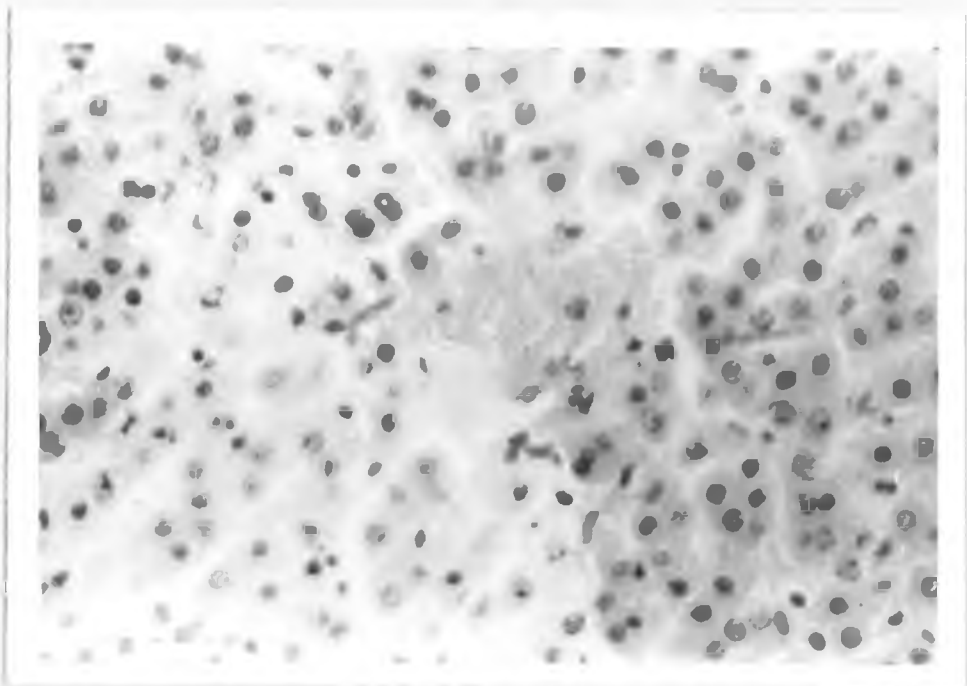

Fig. 6: Calf Liver: Cellular degeneration in mid-zonal parenchymal tissue of calf liver. Many multinucleated cells with accompanying cellular degeneration. (H & E; x 400)

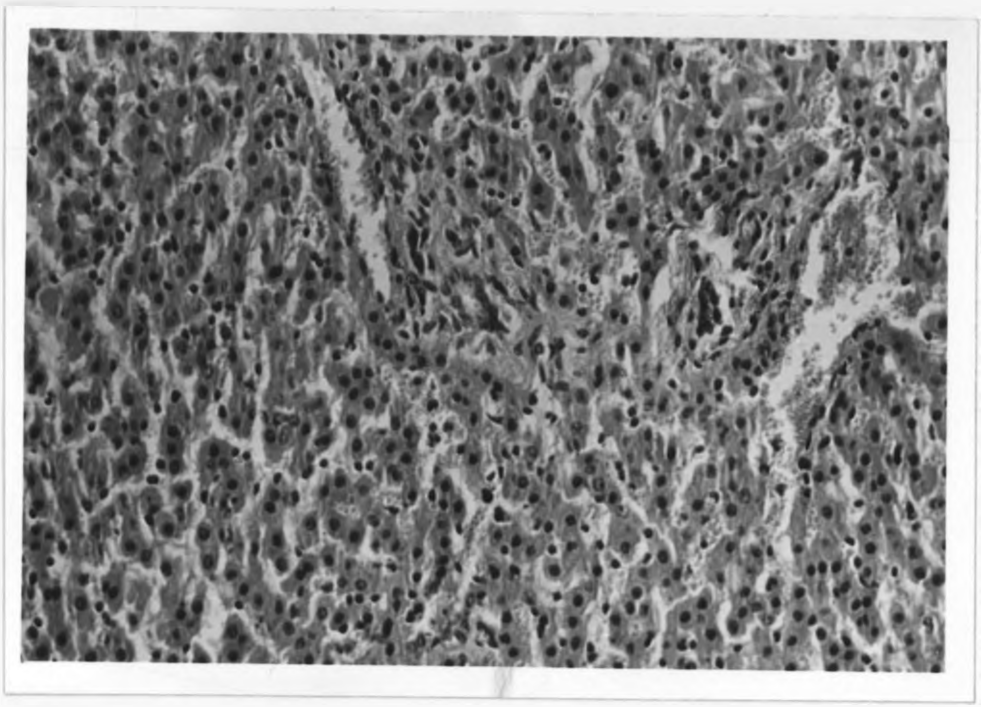

Fig. 7: Calf Liver: Haemorrhage and increased fibroblastic cell formation in subacute toxicity of calf fed Gn. latifolia (H & E; x 400)

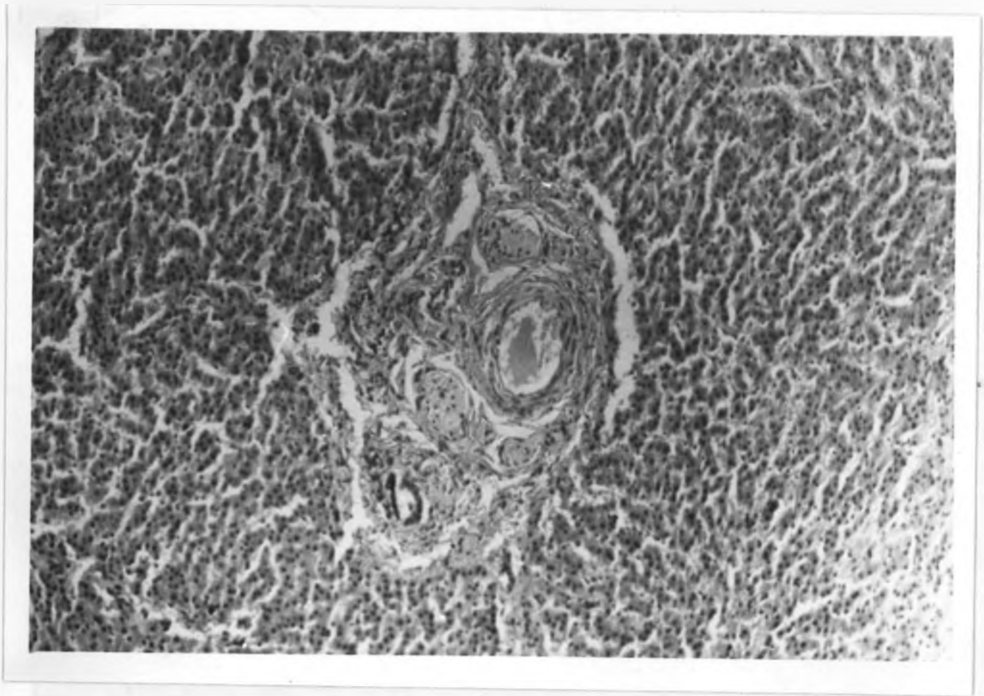

Fig. 8: Calf Liver: Extensive fibrosis in the portal triad of a hepatic lobule. The periportal zone shows increased fibroblastic cell formation in chronic Gn. latifolia poisoning

(H & E; x 250)

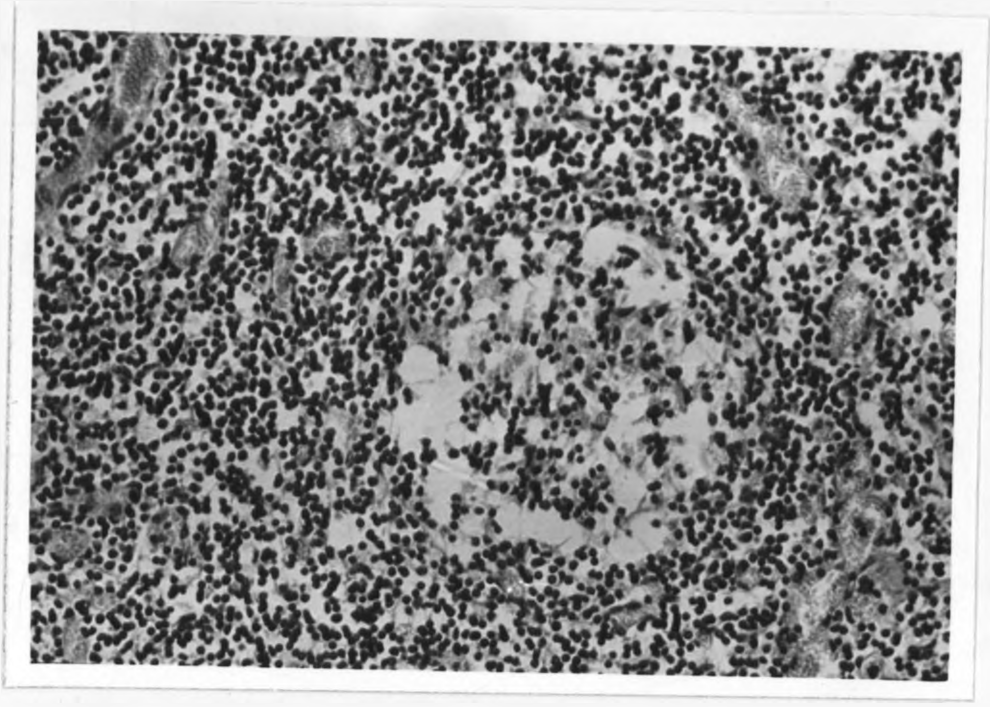

Fig. 9: Calf Lymphnode: Marked lymphocytic cell degeneration in the lymph follicle of a markedly congested lymph node of calf fed with Gn. latifolia (H & E; x 400)

Fig. 10: Calf Kidney: Congestion and haemorrhages in the kidney cortex of a calf fed Gn. latifolia (H & E; x 250)

Experiment II: (a) TOXICITY OF THE EXTRACTS OF GNIDIA LATIFOLIA (MEISN) IN RATS

MATERIALS AND METHODS

The Extract Fractions:

Five hundred grams of powdered Gnidia latifolia (Meisn) leaves and flowers were weighed and placed in a conical three litre flask. The powdered material was then flooded with an appropriate solvent and the contents stirred to mix. The flask was then left overnight in a waterbath at 45°C. The solvent was recovered after 24 hours and the soluble extract was separated and weighed. Four solvents were used for the extraction.

i) The Methanol extract:

Five hundred grams of pulverized flowers were extracted with methanol. After an overnight extraction, the methanol was recovered in a soxhlet apparatus leaving a dark gelatinous mass which was difficult to dry. The extraction was repeated on the flowers samples for another 24 hours and yielded more of the gelatinous material. The total weighed recovered after 72 hours was 6.032 grams. The stock solution of 250 mg/ml. was made by dissolving 2 grams of the methanol extract in 5.0 ml of double-distilled water. The resulting stock solution measured 6.0 ml. To make extract concentration of 50 mg/ml. (solution I), 1.0 ml. of the

stock solution was diluted to 5.0 ml. with double-distilled water. A solution of 12.5 mg/ml. of the methanol extract (Solution II) was made by taking 0.5 ml of stock solution and making it up to 10.0 ml. with double-distilled water. The solutions were injected intraperitoneally into the weaner rats.

Rats in group 1 received 0.5 ml. of the stock solution. Groups 2 and 3 received 1.20 ml. and 0.65 ml. respectively, of solution I. Groups 4 and 5 received 1.24 ml. and 0.5 ml. of solution II respectively. Details of rat treatment with the methanol extract are shown in Table VIII.

2. The Water extract:

Five hundred grams of powdered leaves were suspended in double-distilled water and extracted continuously in a waterbath at 45°C for 24 hours. A dark greenish solution was obtained which was further fractionated into the acidic and the basic fractions. The solution was acidified with sulphuric acid and the acidic fraction was extracted with chloroform. The remaining solution was further made basic with ammonia and the basic fraction was extracted with chloroform. The fractions were neutralized to pH 7 by the addition of a few drops of hydrochloric acid to the basic fraction and a few drops of sodium hydroxide to the acid fraction. The fractions were dried in a vacuum drier at 22°C. The acid soluble fraction crystallized on freeze drying and was easily soluble in water. The base soluble fraction weighed less than 100 mg and was considered insufficient for injections into the rats. One gram of

the acid soluble fraction was weighed into a bottle and dissolved in 5.0 ml of double-distilled water. The resulting stock solution measured 5.3 ml. with a concentration of 188.7 mg/ml. Rats in group 1 received 1.0 ml each while group 2 received 0.5 ml each and group 3 received 0.25 ml each. A new solution (solution I) was prepared by diluting 1.0 ml of stock solution with 4.0 ml of double distilled water to give a concentration of 37.76 mg/ml. Rats in group 4 received 0.7 ml each of solution I while those in group 5 received 0.3 ml each (Table VIII). The rats were then observed for any change in their demeanour and eating habits.

3) The chloroform soluble extract:

Five hundred grams of the dry powdered leaves were suspended in one litre of pure chloroform and extracted continously in a waterbath at 45^{o}C for 72 hours. The chloroform was then recovered in a soxhlet apparatus and the residue was washed with water and freeze dried. A dark gummy substance weighing 0.662 grams was obtained. The gummy substance was suspended in 5.0 ml. of double-distilled water. It could not dissolve in the distilled water and about five drops of ethanol were added to enhance dissolution. The resulting stock solution measured 6.0 ml. and had a concentration of 110.33 mg/ml. The solution was used at this concentration for the intraperitoneal injections of the weaner rats. The rats received dosages ranging from 100 mg/kg body weight to 2 gm/kg body weight (Table VIII). The treated rats were observed for a period of 72 hours for any alteration in their demeanour and eating habits.

4) The Ethanol Extract:

Five hundred grams of powdered dried leaves were placed in a three litre flask and then flooded with ethanol. The flask was placed in a waterbath at 45°C. The contents were stirred to mix and left to stand in the waterbath for 24 hours. The solvent was decanted and evaporated in a soxhlet apparatus. A dark-green residue was obtained and the ethanol recovered. The residue was stored at 4°C while the recovered ethanol was re-cycled for a repeat extraction of 24 hours duration. The re-cycling was repeated three more times, and this extraction lasted approximately five days. The residue obtained from the ethanol extractions was pooled and then purified with three changes (portions) of petroleum spirit (B.p. 40-60°C). The portions of petroleum spirit (each 400 ml) removed pigments and fats and were discarded. A black oily substance remained which was then suspended in double-distilled water and shaken. The water soluble impurities were discarded. The remaining dark green residue which contained the active principles was lyophilized, weighed and stored at 4°C till required. The total yield from 500 gms of dried leaves was approximately 45 gms; The substance dissolved slowly in cold water and had to be warmed every time to enhance dissolution. Five drops of ethanol were frequently added to aid in dissolution.

Six hundred milligrams of this ethanol extract were weighed into a clean sterile universal bottle, and flooded with 5.0 ml double-distilled water. The dissolution was further enhanced with five drops of ethanol. When dissolution was completed the ethanol was expelled by stirring the solution in a hot water-bath (96°C) for about 30 minutes.

The suspension was reconstituted to 10 ml with more double-distilled water so that each millilitre of the solution contained 60 mg of the ethanol extract. The solution was cooled to room temperature for the intraperitoneal injections of rats. The rats received dosages ranging from 100 mg/kg to 2 gm/kg body weight. Then the treated rats were observed for 72 hours for any alteration in their demeanour (Table VIII).

The Experimental Animals:

Weaner male and female albino Wistar rats were used in the experiments. The rats were obtained from the Kenyatta National Teaching Hospital's laboratory animal unit at 21-22 days of age. They were divided into groups and weighed. They were then housed in all-metal cages which were provided with coarse mesh wire side and top. The animals were fed ad libitum on rat grain ration and water was provided in feeding bottles with a metallic tube. Each morning the rats were checked for any alteration in their demeanour and eating habits. The rats were then weighed and any deaths were recorded. A thorough postmortem examination was undertaken on the dead ones. If the rats died the experiment was repeated with another batch of weaner rats. The surviving members of the groups were observed for about 48 hours and a repeat injection was carried out using the same dosage rates as in the previous tests. The rats which recovered after the repeat injections were watched for a further period of 72 hours and then euthanized to check for any gross tissue changes.

More experiments were carried out to determine the effect of the

extract on the body weight gains of growing weaner rats when the dosages were varied. It was therefore necessary to determine the median lethal dose (LD_{50}) of the ethanol extract for the rat. Once this value was determined, it was possible to vary the dosages and to study the long term effects of the extract when rats were exposed to dosages lower than the LD_{50}. It was also possible to study the short term effects of the extract when dosages higher than the LD_{50} were administered to the rats. Emphasis was laid on the effects of the extract on the cells of tissues with fast-multiplying cells.

Determination of the Median lethal dose (LD_{50}):

Forty-two male albino Wistar rats were obtained from the Kenyatta National Teaching Hospital animal colony. They were accommodated at the Department of Veterinary Pathology animal colony in cages and provided with a rat grain ration and water ad libitum. Bedding was kept clean by changing regularly and the rats were divided into seven groups and weighed. The 7th group of rats was kept as a control group while the other six groups were used for intraperitoneal injections with the ethanol extract. Group 1 received dosages of 1 gm/kg while group 2 received 800 mg/kg body weight, Group 3 received 600 mg/kg body weight. Rats in groups 4, 5 and 6 got 400 mg/kg, 200 mg/kg and 100 mg/kg respectively (Table IX). The calculated amounts of the extract were dissolved in double-distilled water and the volume of the solution calculated for each group. The rats were then injected intraperitoneally using a 2 ml syringe and a 20G needle. The rats were then observed for a period of 24 hours to determine their mortality. The results obtained were used to compute the

percentage deaths of the groups.

The injections were repeated with a new batch of twenty male weaner rats from the same source. The rats were divided into five groups and weighed. Group 5 were kept aside as controls while groups 1 to 4 were used for intraperitoneal injections with the ethanol extract. The calculated amounts (260.8 mg) were dissolved in 10.0 ml of double-distilled water and the volume of solution calculated for each group. Group 1 received dosages of 1 gm/kg body weight while group 2 received 500 mg/kg body weight. Groups 3 and 4 received 250 mg/kg and 100 mg/kg body weight, respectively. The rats were then observed for a period of 24 hours to determine their mortality. The results obtained were also computed as percentage deaths of the groups.

The percentage figures obtained from the two tests were plotted on graph paper. Then the median lethal dose (LD_{50}) was estimated from the graph.

Effects of dosages lower than the LD_{50}

Seventy weaner albino Wistar rats of both sexes were obtained from the Kenyatta National Teaching Hospital. The rats were divided into seven groups of ten rats and weighed. Each group consisted of rats of the same sex and kept in separate cages. The animals were provided with rat grain ration feed and water *ad libitum*. The rats in group 7 were kept aside as controls while those in groups 1 to 6 were used as the test animals. All the test rats were daily injected intraperitoneally with

known amounts of the ethanol extract while the control group were injected with 1.0 ml of physiclogical saline solution.

The dry ethanol extract of Gn. latifolia leaves was calculated for each group and the total weight (203.02 mg) was transferred into a clean dry universal bottle for dissolution. It was dissolved in 5.0 ml double-distilled water and further reconstituted to 20.0 ml (stock solution) with more double-distilled water. Each millilitre of the solution contained approximately 10.15 mg of the original dry ethanol extract. Group 1 received 200 mg/kg body weight while group 2 received 100 mg/kg body weight. Each rat in group 1 and group 2 received 1.0 ml and 0.5 ml, respectively. Rats in group 3 received 0.26 ml, equivalent to 50 mg/kg. body weight. The remaining solution, measured 2.40 ml and contained approximately 24.36 mg of the ethanol extract. The solution was further reconstituted to 10 ml. with more double-distilled water and the resulting solution (soln. I) had a concentration of about 2.436 mg per ml. The solution was then used for the treatment of the rats in groups 4, 5 and 6 which were to receive 25 mg/kg, 10 mg/kg and 5 mg/kg body weight, respectively. Rats in group 4 were injected with 0.6 ml of solution I (1.51 mg/rat) while those in group 5 received 0.2 ml (0.55 mg/rat) and those in group 6 received 0.1 ml (0.299 mg/rat). The rats were then observed for 24 hours for their clinical response. Any alteration in their demeanour and eating habits were recorded. At the end of the 24 hour period, the rats weighed and then injected with a freshly weighed extract based on the day's body weight of the groups. The daily body weight gains of each group were tabulated and expressed as a percentage increase on

the original total body weight of the group. At the end of the experiment, the changes in the body weight gains were presented in a graphic form to compare the response of each group to the dose levels of the ethanol extract of Gn. latifolia which they had received. The rats which died during the experiment were subjected to a thorough postmortem examination. Gross pathological lesions were recorded and tissues for histopathological examination were taken.

Effect of dosages slightly above and below the LD_{50}

Another group of fifty male albino Wistar rats were obtained from the same source as those of the first experiment. Their weights varied from 84 grams to 166 grams. The rats were divided into five groups of ten rats each and kept in separate cages. Each group had grain ration feed and water available ad libitum. The fifth group of ten rats was set aside as a control group while the remaining four groups were used for the intraperitoneal injections with the ethanol extract from the leaves. The rats were weighed and the average body weights of each group was recorded.

The extract dosages were calculated for each group and the total amount required weighed. The total calculated weight of the ethanol extract was 1123.05 mg. The amount was transferred into a conical flask and 30.0 ml of double-distilled water was added. Dissolution of the extract was enhanced by the addition 1.0 ml ethanol and heating the solution over a hot ($36^{o}C$) waterbath. The stirring and heating were continued for

about 12-15 minutes to expel the ethanol before the solutio was cooled. The resulting solution was further reconstituted to 50.0 ml with double-distilled water, so that each millilitre contained 22.46 mg. The solution showing the highest possible dissolution was cooled in a waterbath to room temperature (22°C) before it was injected into the rats. The control group was each injected with 1.0 ml physiological saline into which 0.1 ml ethanol was added to check the effect of the ethanol used in the dissolution. The rats were subsequently weighed daily and any change in their body weights was expressed as a percentage gain or loss (Fig. 13). The immediate clinical responses of the groups were recorded and any rats which died were subjected to a thorough postmortem examination. Gross pathological lesions were recorded and tissues for histopathological examination were taken.

c. The Effect of dosages much higher than the LD_{50}

Ninety female weaner albino Wistar rats were obtained and divided into nine groups of ten rats each. All the rats were obtained at 22 days old from the Kenyatta National Teaching Hospital. Their body weights varied from 36 to 73 grams. Each group had water and grain ration feed available ad libitum. Group 1 to 4 were injected with dosages between 900 and 400 mg/kg body weight per day while groups 5 to 8 were injected with dosages of 300 to 100 mg/kg body weight per day. Group 9 was fed a similar grain ration feed and kept in a separate cage but they were not given injections of the ethanol extract. It served as a control group and received a daily intraperitoneal injection of 1.0 ml physiological saline solution.

The ethanol extract was calculated for each group according to the determined dosage rates. The total amount of the dry extract was weighed out (1895.3 mg) into a conical flask for dissolution. About 30.0 ml. of double-distilled water was added to the extract and dissolution was enhanced by stirring the mixture in a hot (96^{o}C) waterbath for about 15 minutes. The solution was further retained in the waterbath for ten minutes to evaporate the ethanol used to enhance dissolution. The remaining solution was reconstituted to 40.0 ml so that each millilitre contained 47.38 mg. The calculated dosage for each rat in the groups was injected intraperitoneally. After the injections the rats were observed for any clinical signs related to the injections and the observation maintained for 24 hours. At the end of this period, the rats were weighed and the change in their total weight was expressed as a percentage body weight gain or loss on the original total weight of the group (Table XI) Daily injections were carried out for about one month and then discontinued. The rats were watched for two months to observe the long term effects of the extract. During the period of injection, deaths occurring were noted and daily weights of the surviving rats were recorded. Those which died were autopsied and the gross pathological changes recorded.

Experiment II (b): Investigation of the Chemical fractions of the Ethanol extract of Gn. latifolia (Meisn) obtainable on Thin Layer Chromatography

Thin Layer Chromatography (TLC) on the crude Ethanol Extract:

Thin layer chromatography was done on the crude ethanol extract using

aluminium and glass plates pre-coated with "silica-gel 60 F-254"[a] to a thickness of 0.5 mm. Equal volumes of n-butanol and 5% acetic acid (20.0 ml of each) were shaken vigorously in a separating funnel and left to stand for 10 minutes. They were then separated into two layers, the upper phase being the part required for the solvent system for this test. Five milligrams of the dried ethanol extract were dissolved in one drop of the solvent. Spotting was done using a plain capillary tube (75 mm long, 1 mm bore). The spots were kept less than 2.0 mm in diameter along a pencil line drawn 2.0 cm. from the edge of the bottom of the plate. The chromatogram was then either placed in the chromatography chamber[b] for 4 hours, or allowed to develop until the solvent front reached 13.0 cm from the point of origin. The separated spots were then viewed under the long ultra-violet light (365 nm).

Chromatography was repeated several times to determine the R_fs of the fractions and their mean values calculated. The spots were further eluted separately for a further secondary run to obtain pure fractions from each spot.

Elution of Spots and Secondary run:

Material from the TLC spots obtained were recovered from the plates. The spots were outlined with pencil under the u.v. light. The predominantly bright blue spot (Fraction I) was scraped off and re-suspended in ethanol. The suspension was then shaken gently and then centrifuged. The supernatant was recovered and freeze-dried to remove the water component. There was poor resolution between the second and

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a - E. Merck, Darmstadt, Germany

b - Shandorn Southern Ltd., England.

third spots. The two spots were scraped off together and the supernatant recovered in a similar manner. The supernatant was freeze-dried. About 5.0 milligrams of each of the freeze-dried fractions were re-dissolved in a few drops of ethanol and spotted on a new plate. The chromatogram of the fractions showed a marked exclusion of the run and the fractions were therefore considered pure enough for experimental injections into weaner rats.

The freeze-dried fractions were weigned Fr. I: 0.164 gms; Fr. II & III: 0.10 gm). About 100 mg of each of the freeze-dried fractions were weighed and prepared for intraperitoneal injection to weaner rats. The fractions were dissolved in double-distilled water. Four weaner albino Wistar rats were obtained, weighed and prepared for intraperitoneal injections with the fractions. Two rats were injected with Fr. I at dosage rate of 1.0 gm/kg. body weight. The other two rats were injected with a solution of Fr. II and III at dosage rates of 1.0 gm/kg. body weight. The rats were then observed for a period of 24 hours for signs of toxicity.

The Histogram of the Components of Gn. latifolia (Meisn) from the paper Chromatogram

The support used here was Whatman No. I chromatography paper. The solvent used was the same n-butanol/acetic acid system described in the previous experiment. The upper phase was obtained by vigorously shaking equal volumes of n-butanol and 5% acetic acid. The bottom layer was used

at the bottom of the tank for saturation.

The application of the material on the paper was done by streaking a thin line of the dissolved extract on a thin line across the entire origin mark on the paper. After the 20 hour run, the paper strip was dried and viewed under the long ultra-violet light (365 nm).

The development in this case was descending and it was allowed to run for exactly 20 hours. The solvent front was allowed to overrun the paper length while the components separated with a good resolution without approaching the end of the paper.

RESULTS

Response to the injection of the extract fractions:

The results observed in rats injected with the extracts of *Gn. latifolia* varied for each group and depended on the extract used. Following the intraperitoneal injections the rats were observed for four to six hours following the injections. Then their response was followed up for 24 hour and again after 36 hours. Their general responses are summarized in Table VIII.

TABLE VIII

The Response of Weaner albino Wistar rats to an intreperitoneal injection of the extracts of Gn. latifolia (Meisn)

Rat groups

		1	2	3	4	5
EXTRACT	DOSAGE RATE per kg.	2.0 gm	1.0 gm	500 mg	250 mg	100 mg
Methanol	Average weight (gms)	60	61	61.5	62	60
	Average Dose (mg/rat)	120	61	31	15.5	6.0
	RESPONSE (mortality in 24 hrs)	dull 1/1	dull	normal	normal	normal
	Average wt. (gm)	98	98.5	98	97	99
Water	Average Dose (mg/rat)	196	98.5	49	24.3	10
	Response (Mortality in 24 hrs)	excess urination Normal	Excess urination Normal	Excess urination Normal	Normal	Normal
Chloro-form	Average wt (gm)	93	102	108.5	93.5	88
	Average dose (mg/rat)	186	102	54.25	23.4	8.8
	Response (Mortality in 24 hrs.)	Normal	Normal	Normal	Normal	Normal

Table VIII cont.

		1	2	3	4	5
EXTRACT						
	Average wt (gm)	35	35.25	35.25	34.75	35.75
Ethanol	Average dose (mg/rat)	70	35.25	17.63	8.69	3.56
	Response (Mortality in 12 hrs)	4/4	3/4	1/4	1/4	2/4
Repeat ethanol	(Mortality in 72 hrs.)	-	1/1	1/3	1/3	None

a) Response to the methanol extract:

The rats were observed for any disturbances in their demeanour and eating habits. After three hours, the rats in group 1 became dull, inappetent and also passed excess urine. Those in group 2 had a raised haircoat but were eating a little. The rats in groups 3, 4 and 5 went about eating and behaving normally. One rat in group 1 died within 36 hours following the injections. The remaining rats recovered uneventfully after 72 hours (Table VIII).

The rat that died in group 1 showed severe congestion of the

subcutaneous blood vessels, the thymus, liver and lungs. Microscopically, there were no significant pathological changes in those organs apart from vascular congestion. The repeat injections with a new batch of rats showed a similar clinical response with all the rats recovering within 72 hours after the injections. It was then concluded that the methanol extract of Gn. latifolia was not toxic to rats at dosage rates below 2 gm/kg body weight.

b) Response to the water extract:

The rats showed excessive urination for the first three to four hours following the intraperitoneal injections of the acid soluble fraction. Recovery, however, followed uneventfully within 72 hours (Table VIII). It was also concluded that the acid soluble fraction of the water extract was not toxic to rats at dosages below 2 gm/kg body weight.

c) Response to the Chloroform extract

All the rats recovered after 72 hours without any significant change in their demeanour and feeding habits (Table VIII). There were small unhealed wounds at the site of injection.

d) Response to the ethanol extract:

The rats injected intraperitoneally with the ethanol extract showed clinical response within three hours after injection. The most conspicuous clinical signs included a raised haircoat and a tendency to walk with an arched back. Several rats showed nervous disturbances including walking in circles, drowsiness and posterior leg paralysis.

Rats which received more than 500 mg/kg body weight stopped eating and drinking water. Those which received lower doses showed increased thirst and loss of appetite. Several rats further had muscular tremors and crying when they attempted to move. After the initial signs the rats passed excessive urine and the faeces became loose. These clinical disturbances lasted till the death of the animal, while those which recovered showed a gradual return to normal after 48 hours.

Of the twenty rats injected with the ethanol extract, eleven died within 12 hours (Table VIII). All the four rats in group 1 receiving dosages of 2 gm/kg body weight and three out of four in group 2 receiving 1 gm/kg body weight died. One rat each in group 3 and 4 died and two more rats died in group 5. The surviving rats in group 2, 3, 4 and 5 were very dull and anorexic. Their haircoat was raised. They tended to remain motionless at one part of the cage and cried whenever they were forced to move. Their faeces were watery and their abdomen appeared cyanotic. The eleven rats which died were subjected to a thorough post mortem examination and the results are summarized below as follows:

Group I (2 gm/kg): All(4/4) rats died and when opened showed congestion of the intestines and haemorrhagic enteritis in three rats. All had extensive haemorrhages of the thymus, lungs and heart. The liver, kidneys, spleen and the subcutaneous vessels were markedly congested.

Group 2 (1 gm/kg): Three (3/4) rats died and showed a marked congestion of the intestines, lungs, liver and spleen. Two rats

further showed extensive haemorrhages of the thymus and heart.

Group 3 and 4 (500 mg and 250 mg/kg. respectively):

One rat died in each group and showed marked congestion of the thymus, heart, intestines, liver and lungs. There were no gross haemorrhages in these groups.

Group 5 (100 mg/kg): Two (2/4) rats died and showed extensive haemorrhages of the thymus and marked congestion of the intestines, lungs, liver, heart and spleen.

Tissues for histological examination were taken from the organs that showed gross changes in the rats which died and the tissues were processed accordingly. On repeat injections, only one rat died in each of groups 2, 3 and 4 (Table VIII). When examined for gross pathological changes, all dead rats showed a marked atrophy of the thymus gland and spleen. The liver appeared very pale while the kidneys were slightly congested. Tissues for histological examination were also taken from the liver, spleen, thymus, kidneys, lung, heart, duodenum and pancreas. Tissue samples were obtained and fixed in 10% formalin for at least 48 hours and then processed for histopathological examination.

When the repeat injection was carried out, the only surviving rat in group 2 and one of three in each of groups 3 and 4 died. The two rats in group 5 which received 100 mg/kg body weight survived the repeat injections. After 72 hours the surviving rats had started moving again

and eating normally before they were euthanized. On postmortem, they grossly showed adhesions of the abdominal viscera, congestion of the liver and kidneys, but nothing more of pathological significance.

The median lethal dose (LD_{50}) of the Ethanol Extract

The responses of the rats was similar to the previous observation of the ethanol extract. They showed a raised haircoat and walking with an arched back. Nervous disturbances were present including walking in circles, posterior leg paralysis and muscular tremors. Rats which received more than 500 mg/kg body weight also stopped eating and drinking water. After the initial signs the rats passed excessive urine within three hours following the injections. The faeces became loose and the rats tended to cry whenever they were forced to move. After 24 hours, however, there was a marked mortality in those rats which received high doses while the mortality progressively decreased in those which received lower doses (Table IX).

All the six rats in group 1 which received 1 gm/kg body weight died within 24 hours. Five rats also in group 2 which received 800 mg/kg body weight died within the same period. Several other rats died in groups 3, 4 and 5 as shown in Table IX, while none died in group 6. Three rats in group 3 had the solution ooze out through the injection site and might have affected the mortality in that group. The LD_{50} for this preparation was estimated at 278 mg/kg. All the rats which died were then subjected to a thorough postmortem examination and their gross pathological changes were recorded.

Table IX: The response of weaner albino Wistar rats to an intraperitoneal injection of the ethanol extract of Gn. latifolia (Meisn). The estimation of the LD_{50}

	Rat groups						
	1	2	3	4	5	6	7
Dosage rate (mg/kg)	1000	800	600	400	200	100	NIL
Average weights (gm)	40.3	45.5	140.4	120.2	131.0	120.0	47.0
Dosage/rat (mg)	40.33	32.3	84.25	48.4	26.2	12.0	-
Mortality (in 24 hours)	6/6	5/6	3/5	3/5	2/5	None	None
	(100%)	(83.3%)	(60%)	(60%)	(40%)	(0%)	(0%)

Repeat injections:

	1	2	3	4	5 Control
Dosage rate mg/kg	1000	500	250	100	None
Average weights (gm)	35.25	35.25	34.75	35.75	35.0
Dose per rat (mg)	35.25	17.65	8.7	3.6	-
Mortality (in 24 hours)	4/4	3/4	3/4	1/4	NONE
	(100%)	(75%)	(75%)	(25%)	

The repeat injections with twenty male rats were also used for the determination of the LD_{50}. All rats in group 1 which received 1 gm/kg died while three rats each died in group 2 and 3, and one rat died in group 4. These mortalities were then expressed as percentages and plotted on graph paper. The LD_{50} for this preparation was then estimated at 243 mg/kg body weight. The rats were then examined for any pathological changes in the normal way.

(a) The response to dosage lower than the LD_{50}

The rats in treatment groups 1, 2 and 3 showed excessive urination, looked dull and had a staring hair coat within four hours following the injections. The rats in groups 4, 5 and 6 passed less urine and looked slightly depressed. The control group did not show any clinical disturbances during the same time. Information on the responses of rats is given in Table X (a).

The rats injected with dosages of 5 mg/kg, 10 mg/kg and 25 mg/kg body weight showed essentially the same body weight changes as the controls. Those which received 50 mg/kg, 100 mg/kg and 200 mg/kg body weight showed a slower growth rate than was recorded for the controls. One rat died in the group injected with 200 mg/kg body weight and the death occurred on the 5th day of injection. The changes observed in the body weights were expressed as a percentage gain or loss on the initial total weight of the group.

Table X: Response of weaner albino Wistar rats to an intraperitoneal injection of an ethanol extract of Gn. latifolia at low dosages:

(a)

	Rat groups						
	1	2	3	4	5	6	7 Control
Dosage rate (mg/kg)	200	100	50	25	10	5	Nil
Average wt. (gm)	51.1	49.9	52.2	57.4	55.0	54.3	60.5
Dose/rat (mg)	10.35	4.99	2.61	1.51	0.55	0.299	Nil
%wt. gain day 5	-2.70	2.70	7.66	12.54	21.00	19.98	21.51
Day 10	20.59	12.02	18.48	25.00	28.9	36.92	36.03
Day 15	36.36	24.94	27.58	38.76	49.27	46.4	47.76

(b)

	1	2	3	4	5 Control
Dosage rate (mg/kg)	.400	300	200	100	Nil
Average weight (gm)	100.3	107.85	138.6	121.1	126.7
Dosage/rate (mg)	40.12	32.36	27.72	12.11	None
Mortality (in 24 hours)	4/10	3/10	None	1/10	None
(%)	40	30	0	10	0

(b) The Response of injections with dosages slightly above and below the LD_{50}

There was excessive urination in all the test groups within four hours following the injections. The rats in groups 1 to 3 had very poor appetite. Several rats in the groups walked with arched backs and tended to cry everytime they were touched or when they attempted to turn. The haircoat was stary in all the test groups and group 3 and 4 showed excessive thirst. The groups 1 and 2 injected at 400 mg/kg and 300 mg/kg. respectively, showed the most dramatic response. Four rats in group 1 and three in group 2 died within 18 hours. One rat died in group 4 (100 mg/kg) while none died in group 3 (200 mg/kg.). The control group did not show any clinical disturbances during this period and they continued eating and moving about normally (Table X,b). The rats which died in group 1 and 2 were examined by standard postmortem procedure.

Injections with the ethanol extract were suspended after daily injections for three weeks. The rats were then observed for two months for any effects on their development. The rats gained body weights when the injections were suspended and they recovsred without any other disturbances. At the end of the two months' period the rats were euthanized and checked for any pathological changes.

c) The Response of injections with doseges much higher then the LD_{50}

Following the first injections, one rat died in group 3 which

received 500 mg/kg per day and the death was attributed to murine pneumonia complication. The first deaths due to toxicity occurred on day 7 in group 1 which received 900 mg/kg body weight. All the rats in this group died by the 8th day of injection. The other groups started showing mortalities on days 10 and 11 which were distributed as follows:- One rat each died in group 4 (400 mg/kg) and 6 (200 mg/kg) on day 10; and one each in group 5 (300 mg/kg) and 8 (100 mg/kg) on day 12.

There was generally a slower body weight gain in the first four groups which received dosages of 400 mg/kg and higher. The groups which received dosages of 300 mg/kg body weight and lower showed depressed demeancur but continued gaining weights nearly as fast as the control group. The body weight changes were then calculated and expressed as percentage gains on the original total body weights of the groups (Figures 13 and 14). Several rats had staring haircoat and walked with arched backs. The dying ones developed diarrhoea with loose brown faeces prior to death. Throughout the period, no deaths occurred in the control group which received a daily injection of 1.0 ml. physiological saline solution per rat. The control group was weighed daily and their weight gains treated in similar manner as the weights in the test groups.

Gross pathological Lesions:

The rats which died were subjected to a thorough postmortem examination. All the rats which died during the day were autopsied at 4.30 p.m. in the afternoon while those which died during the night were done at 8.30 a.m. the next morning. The rats were dissected and their organs scrutinized for visible pathological changes. Tissue samples were

obtained and fixed in 10% formalin for at least 48 hours and then processed for histopathological examination.

Gross pathological lesions were seen in the thymus which in the acute cases was grossly congested and showed several petechiae and acchymoses on its surface. In the experiment to determine the LD_{50}, 19 rats died. 17 (90%) showed haemorrhages on the thymus and all the dead showed a marked congestion of the liver, kidneys and the adrenals which looked dark red (Fig. 12). Nine of the 19 rats further showed haemorrhagic myocarditis while two showed petechial haemorrhages of the pancreas. The spleen was pale and appeared contracted in the acutely intoxicated rats. The lungs showed congestion with a few petechial haemorrhages. The small intestines on the other hand were very congested. The mesenteric blood vessels stood out prominently. The duodenum and the ileum were filled with blood-tinged ingesta while the colon, caecum and rectum were filled with loose faeces of normal colour. The grossly affected tissues were taken for further histopathological examination.

Most of the rats that died in the subsequent tests showed similar gross pathological changes. There were marked haemorrhages of the thymus, intestines, pancreas and heart. These haemorrhages were further accompanied by congestion of the duodenum, liver, lungs and pancreas. There were also congestions of the spleen and kidneys.

In the rats that survived the injection for a bit longer period, there were extensive fibrous adhesions of the liver to the abdominal

wall. The intestines were covered by some whitish patches on their serosal surface which also involved the liver surface in one case. The chronic cases which had tissue adhesions did not show any haemorrhages. The spleen and the thymus however looked smaller in size and appeared quite pale. Two of the rats showed the accumulation of excessive fluid and a fibrosis of the spleen with marked adhesion of the organ to the intestines. Another rat showed extensive adhesion of the pancreas to the intestines and the liver. Tissues for histopathological examination were obtained from the organ which showed gross changes and processed accordingly. Tissues were also routinely collected from the liver, spleen, pancreas, kidney, heart, lungs, duodenum, thymus and the adrenal body.

HISTOPATHOLOGICAL EXAMINATION OF THE TISSUES:

Histological section from organs of the dying rats were examined for pathological alterations. In the tissue sections, the main pathological changes observed were the extensive cellular degeneration especially in the parenchymatous organs with fast multiplying cells. Areas showing cell necrosis were accompanied in the acute cases by haemorrhages and excessive deposition of cellular debris. The more chronic cellular changes were accompanied by the proliferation of fibrous connective tissue to replace the damaged parenchymal cells. Cellular degeneration was preceeded in most cases by internal cellular changes such as cloudy swelling and vacuolation, followed by the lysis of the cell nuclei. The tissue necrosis was very marked in the secretory organs especially the pancreas, the adrenal and the kidney tubules.

The alimentary canal of the rat showed areas of submucosal haemorrhages with tissue necrosis. In the acute intoxication, there was little epithelial alteration and the intestinal villi appeared intact (Fig. 15). In the more subacute cases, however there was very extensive epithelial erosion and submucosal cellular necrosis as shown in Fig. 16. The intestinal changes were accompanied by some mononuclear cell infiltrations while the Peyer's patches showed cellular degeneration in the lymphoid follicles.

Although the liver appeared grossly congested, it was difficult to detect any haemorrhages in the parenchyma. The liver cells showed many degenerative changes especially around the portal veins. The parenchymal cells showed cloudy swelling, fatty degeneration and karryolysis of the nucleus. The sinusoidal cells showed engorgement with hyaline casts and some deposition of casts in the sinusoidal spaces.

The pancreas was most affected with extensive interlobular haemorrhages. The haemorrhages were further accompanied by parenchymal cell degeneration (Fig. 17). The cellular degenerations involved both the acinar and the islet cells. The cell necrosis accurred either separately or together with haemorrhages. The changes in the pancreatic acinar cells were liquefactive necrosis which in a few cases were preceeded by the individual cells swelling and losing the cytoplasmic membranes (Fig. 18).

The kidneys first showed histological changes in the cortical areas.

There were petechial haemorrhages in the kidney cortex of the rats that died acutely. The haemorrhages were followed or accompanied by diffuse tubular degeneration with erosion of the tubular epithelium. The adjacent intact tubular epithelial cells showed a marked degree of swelling and hydropic degeneration. Some tubular cells seemed to swell and occlude the tubular lumen and this may be due to an increase in the cytoplasmic water component. These tubular cell degenerations were further accompanied by the formation and an uneven deposition of albuminous casts in the tubules.

Figure 19 demonstrates the earlier stage of haemorrhagic nephritis in the rat kidney following toxicity with Gnidia extract. The interstitial haemorrhages are accompanied by extensive tubular epithelial damage. Also shown are early stages of hyaline degenerations and the deposition of cell debris. Figure 20 is a later stage of the degeneration with a marked loss of tubular integrity and the deposition of frank albuminous casts in the tubules. There is a proliferation of fibroblastic cell cords. The albuminous casts pass by filtration from the glomeruli so that they appear massively deposited in the periglomerular spaces and the proximal tubules. The glomerular tufts themselves did not show any albuminous deposition within their tissues. The albuminous casts in many instances induced a mononuclear inflammatory cell infiltration as if they acted as foreign body, causing an acute inflammation as shown in Fig. 22. It was difficult to explain the origin of these albuminous casts in the glomerular and tubular spaces. Fig. 21 shows an earlier space when the albuminous casts are filling the proximal tubule next to

the periglomerular space. These nephrotic changes were further accompanied by coagulative necrosis of the proximal tubules so that the cellular integrity disappeared and were replaced by cell debris.

Following the mononuclear cell infiltration in the periglomerular spaces, the tubular cells surrounding the periglomerular space underwent degeneration due to interspatial pressure. The internal structure of the albuminous casts were granular suggesting that they crystallize after filtration and deposition. This observable periglomerular damage would explain the origin of the increase in BUN in the intoxicated cattle at the terminal stages of the toxicity. It is difficult to explain why there were no cast deposition in the bovine kidney.

The adrenal body showed many pathological alterations involving the zona fasciculata and the zona reticularis. There were cortical haemorrhages and extensive cellular necrosis. Figure 23 demonstrates the changes observed in the adrenal cortex with marked necrosis of the zona fasciculata. Fig. 24 further shows zones of haemorrhage and cellular necrosis in the zona reticularis of the adrenal body. The cellular damage in the adrenal body is seen as nuclear disintegration followed by haemorrhages. The medulla showed more cellular vacuolations.

The thymus was most severely affected by haemorrhages and lymphocytic cell necrosis. Several areas of thymic sub-capsular tissue showed vacuolation and lymphocytic cell degeneration as shown in Fig. 25. There were further several areas of haemorrhages involving both the lobules

and the interlobular connective tissue. Haemorrhages were accompanied by cellular vacuolation and lymphocytic cell pyknosis and karyorrhexis as shown in Fig. 26. The petechiae that were grossly visible were shown histologically to be due to capillary damage in the follicles accompanied by lymphocytic cell necrosis.

The spleen demonstrated similar changes to those in the thymus. There were very extensive lymphocytic cell necrosis and haemorrhages. The changes in the spleen were similar to those observed in the lymph nodes. The lymphocytic cell degeneration was more marked in the chronic cases of rat intoxication. The lymphocytic follicles were depleted of the lymphoid cell especially in their lymphopoietic centres. Fig. 27 demonstrates the lymphocytic cell changes in a spleen follicle with partially empty follicular centres. The lymphocytic cells show pyknosis, degeneration and necrosis.

Fig. 28 is a more acute follicular change with depletion of lymphocytic cells and their replacement with follicles partially filled with debris. The spleen shows many areas of subcapsular haemorrhages and little tissue of lymphopoietic function. Many fibroblastic cells are scattered in the spleen parenchyma. The few follicles still visible have little lymphocytic cellular tissue. There is further a slight infiltration of neutrophilic cell types.

Thin layer Chromatography (TLC) of the Crude Ethanol extract:

The chromatogram was run for four hours and then the spots were viewed under the long u.v. light (365 nm). There were four distinct fractions consisting of the fast moving pigment that glowed bright purple, the two light blue spots of Fr. III and Fr. II and the slow moving bright blue fraction 1 (Fr. I). Determination of the mean R_fs gave the following values:-

Fraction No.	Mean R_f Value	Range
1	0.40	(0.37 - 0.44)
2	0.62	(0.58 - 0.64)
3	0.69	(0.63 - 0.74)

Elution of spots and secondary run

The chromatogram of the re-spotted fractions showed a marked exclusion of the run (Fig. 29). These were then considered pure enough and used for rat injections. The clinical responses observed in the rats injected with Fr. I were similar to those observed in the rats injected with the crude ethanol extract. Both rats injected with Fr. I died within 18 hours while those injected with Fr. II and III survived after a transitional dullness lasting about four hours after the injection. The rats injected with Fr. I were autopsied and revealed toxicological changes similar to those observed in the rats injected with the crude ethanol extract (Fig. 30). The changes observed were petechial haemorrhages of the thymus, adrenal glands, heart, intestines, pancreas and congestion of the liver, lungs and the kidneys. They also

showed the accumulation of blood-tinged fluid in the abdominal and thoracic cavities. The histopathological changes were also similar to those already described for rats injected with the crude ethanol extract.

Results of the Histogram of the components recovered from paper chromatography:

The components separated with a good resolution without approaching the end of the paper. Fr. I moved about 20 cm from the origin with a band thickness of about 5.6 cm. There were small amounts of Fr. II with a band thickness of 2.3 cm which migrated about 25 cm from the point of origin. Fr. III moved to about 34 cm from the point of origin with a band thickness of about 5.0 cm. Their band thicknesses and the total weights obtained were used to estimate the ratio of the three fractions obtainable from the crude ethanol extract. Their ratio by weights were found to be approximate 8:1:3 for Fr. I, Fr. II and Fr. III respectively. There were also some very slow moving fractions (A and B) which were not present in the TLC because they had not left the origin within the four hours of the run. They were very volatile and evaporated (no longer visible with the long u.v. light) from the strips within 24 hours of drying at room temperature ($22^{o}C$).

DISCUSSION ON RAT INJECTIONS AND THE EXTRACT FRACTIONS OF GNIDIA LATIFOLIA (MEISN)

In the second part of the experiment extracts were prepared from the various fractions of Gn. latifolia (Meisn). Several solvents, namely water, ether, methanol and chloroform were used. The fractions were tested for their toxicities on male and female weaner albino Wistar rats. It was found that the ethanol fraction purified in petroleum spirit was highly toxic at dosage levels below 2 gm/kg. body weight. Other fractions extracted by water, ether, methanol and chloroform were non-toxic to the rats at dosages below this level and for practical purposes were considered non-toxic in rats.

One part of the experiment dealt with the direct toxicity of Gn. latifolia extract on the rats. The rats were injected intraperitoneally with the ethanol extract. The ethanol-soluble fraction had a median lethal dose (LD_{50})which was estimated at 262 mg/kg. body weight (range: 220 mg - 340 mg/kg).

The other part of the experiment dealt with the physiological interference in the normal rat metabolism when dosages were reduced and the rats exposed to the toxicity over a long period of time.

Pathological changes were noted in rats which succumbed to the intoxication. High doses of the ethanol extract caused acute toxicities accompanied by tissue necroses and haemorrheges. Histopathological

alterations were observed in the liver, kidneys, adrenals and pancreas. Rats injected intraperitoneally with the ethanol extract showed changes similar to those reported by Terblanche et al. (1966) in animals drenched orally with an acetone extract of Gn. burchelli. Lesions observed in the vital organs included haemorrhages and necrosis of the thymus, pancreas and kidneys. The liver, adrenals and spleen showed various degrees of degeneration. The haemorrhages in acute cases were probably due to an irritative nephritis, pancreatitis and thymitis. The central follicular necrosis in the thymus and epithelial desquamation in the kidney tubules might have deepened resulting in capillary injury. It appeared that the ethanol extract of Gnidia impaired the integrity of the mucosal epithelium. The marked lymphocytic necrosis in the lymph nodes and thymus were similar to those observed in calf lymph nodes. These are tissues which are known to have a high rate of cellular regeneration. This finding gives a clue as to why Gnidia plants are commonly used in traditional medicine, especially in the treatment of ailments which involve swelling. The results obtained in experiments done with rats were consistent with this expectation. There was a marked drop in the lymphocytic counts in calves and the histological sections revealed a marked lymph follicular necrosis in the calves and rats. It was therefore considered that the ethanol extract of Gn. latifolia (Meisn) might be highly toxic to the multiplying cells and especially in view of the fact that haemorrhages were mostly located at the central follicular areas of lymph nodes.

Pathological development of hyaline droplets, or hyaline

inclusions were also observed in the cytoplasm of parenchymal cells. The hyaline droplets were observed in the kidneys, liver and adrenals. They stained similarly in the various tissues but differed in their distribution and quantities. The liver showed the droplets filling the sinusoidal spaces while the adrenals showed the droplets in the cytoplasm of cell cords in the zona reticularis and zona fasciculata. The kidneys showed the hyaline droplets filling the periglomerular spaces and extending into the proximal tubule and the descending loop of Henle. Mugera (1965) reported that hyaline droplets represented a degenerative change, and preceeded coagulative cellular necrosis in the regions of their earliest apperance in the organ. The appearance of hyaline casts in the rat kidney was accompanied by mononuclear cell infiltration. The distributions of the cellular infiltrations followed those of the hyaline droplets in this organ.

Chronically-treated rats showed degenerations of the parenchymatous organs and an increase in the amount of fibrous tissue proliferation in the liver, kidneys, lymph nodes and the spleen. The rats injected with the ethanol extract for prolonged period showed centrilobular cell degeneration. The periportal hepatic zones showed a marked proliferation of fibroblastic cells and an alteration of the parenchymal cells. The zone of necrosis was accompanied by extensive hyperplasia of oval basophilic multinucleated cells. Leukocytic cell infiltration were present but not very marked in the liver tissue. Regenerating hepatic cells were characterized by their large size, with nuclei which were occasionally binucleated. There were many instances of cytoplasmic vacuolation. The fibrosis observed in the liver could probably be due

to chronic toxic irritations by the active principle(s).

Renal tubular changes were accompanied by interstitial cellular changes. On microscopic examination several rats showed petechial haemorrhages of the kidney-cortex - possibly as a result of increased capillary permeability. Interstitial haemorrhagic nephritis was further accompanied by degeneration of the kidney tubular epithelium in the acutely intoxicated rats. The tubular epithelial damage was accompanied by the formation of hyaline inclusions. The hyaline casts could be presumed to result from excessive cellular damage and crystallization of the proteins in the urine.

There were also several rats which showed petechial haemorrhages in the heart. Raab (1960) suggested that cardiac lesions occur when foreign compounds (amines) interfere with energy generation and utilization at the cellular level. Another theory is that these compounds impair the function of the myocardium through myogenic mechanisms. These are manifested as a reduction in the myocardial contractile force (Rosenbleum et al., 1965). Theories advanced by various workers to explain the pathogenesis of cardiac lesions do not exclude the possibility of pleuricausal origin of these lesions (Handler and Baker, 1944; Rona, Chappel, Balazs and Gaudry, 1959; Fairchild and Alles, 1967).

The rats injected with the ethanol extract developed ascites of varying amounts and oedema of the abdominal organs. Many of the rats; showed generalized degeneration of the acinar and islet tissue of the

pancreas accompanied by extensive haemorrhegic pancreatitis. No definitive conclusion can be drawn as to the pathogenesis of pancreatic lesions in this toxicity. Mugera (1965) postulated that the same factors which produced hepatic injury were also responsible for the pancreatic lesions. He further stated that the damaged liver pre-disposed the pancreas to a deficiency of particular amino acids necessary for the maintenance and integrity of the pancreatic tissue.

Rats injected with the ethanol extract of Gn. latifolia generally showed systemic disturbances within 12 hours following administration of the extract. They had raised haircoat, changed behaviour and partial loss of appetite. It was thought that the anorexigenic effect and altered behaviour were due to pain. The extracts however acted directly on organs with fast-multiplying cells including digestive glands and this might have contributed to the partial loss of appetite.

Rats which received sub-lethal doses for more than two weeks progressively showed marked retardation in body weight gains. The dose-effect of the ethanol extract was correlated directly with the weight gains. Animals receiving higher dosages gained less weight per unit time than those receiving lower than 300 mg/kg. body weight. As such it took the more intoxicated rats longer to mature and gain sufficiently in their body weights.

Among the chemical compounds isolated from Gn. latifolia (Meisn) material were tannins and volatile acids but the toxicological principle(s)

seemed to be the macrolide fractions. Although macrolides were distributed throughout the various parts of the plant, they were more concentrated in the young green leaves. Test animals showed more dramatic responses when the material used came from the young leaves. The distinct extract fractions were obtained after fractionation of the crude extract by thin layer chromatography. When the three fractions were recovered separately and injected into rats, it was found that Fr. 1 was highly toxic to the rats while Fr. 2 and Fr. 3 had marginal toxic activity. It was found that the ratio of the three fractions obtained from the crude ethanol extract was on weight basis, 6:1:3 for Fr. 1, Fr. 2 and Fr. 3, respectively. Fr. 2 and Fr. 3 had nearly the same R_f values and it was difficult to obtain either fraction in pure form due to contamination of one with the other. Kupchan et al. (1975) isolated three active chemical fractions (Macrolides) from Gn. lamprantha (Gilg). The three fractions obtained from Gn.latifolia (Meisn) may be similar to those obtained by Kupchan et al. (1975). The active principles have related heterocyclic diterpenoid structures with the modification on their active moiety (alkyl). The author could find limited published work on the toxicity of Gn. latifolia even though it was reported to be widely used in traditional medicine (Kupchan et al., 1975; Kokwaro, 1976). Gn. latifolia was extensively used as medicine in ailments that involved a swelling or tenseness. The lympholytic activities of Gn. latifolia principle(s) were of primary pharmacological interest in connection with its usage. A study of Gnidia material without a consideration of this aspect would therefore be incomplete. Kupchan et al. (1975) attributed the lympholytic activity of the extract to the presence of

similar fractions from Gn. lamprantha. They thought that the reactivity of the fractions of the ethanol extract was dependent on the alkyl moiety attached at position C-12 in the heterocyclic diterpenoid ring of the macrolide. They further suggested that "the ester affixed at C-12 may act as a carrier moiety, e.g. in processes concerned with cell penetration or selective molecular complex formation". Results obtained in the present work using TLC showed that there were basically three compounds in the Gn. latifolia ethanol extract similar to those obtained from Gn. lamprantha. Kupchan et al. (1975) further demonstrated that both the antileukemic and piscicidal activities of the extract were concentrated on the three diterpenoid fractions. The same workers stated that the compounds which bore no function at C-12 showed no antileukemic activity. In the present work, it was not possible to quantitatively evaluate the lympholytic activity of Gn. latifolia material due to lack of appropriate laboratory equipment. It was however established that 100 mg/kg body weight was adequate to give a definite lympholytic effect in the calf. This amount (100 mg) is present in approximately 10 grams of the dry leaf or bark of Gn. latifolia material, depending on the stage of their plumage.

Fr. 1 which was recovered last in the TLC contained the more active principle. Terminal cases of rats injected with Fr. 1 showed similar pathological changes as rats injected with the original ethanol extract suggesting that the toxicity in the Gn. latifolia extract was due to the presence of Fr. 1. Kupchan et al. (1975) employed TLC to recover the last fraction (B) which they named Gnidicin.

This last fraction had a molecular weight (M.W.) of 21,000. They did not however indicate whether they tested the fraction separately for any toxicity. Fr. 2 and Fr. 3 were recovered faster than Fr. 1. The two fractions were not as markedly toxic as Fr. 1. Kupchan *et al.* (1975) isolated the first fraction (A) which they said was a mixture of two closely related compounds, *Gnididin* (M.W. 27,750) and *Gniditrin* (M.W. 31,8000). It would therefore be conceivable to postulate that the three fractions (macrolides) isolated from *Gn. latifolia* (Meisn) may be similar or related to those obtained by Kupchan *et al.* (1975) from *Gn. lamprantha* (Gilg).

Table XI: The daily percentage (%) body weight gains in groups of rats injected with the ethanol extract of Gn. latifolia (Meisn)

	Rat groups						
Days	1	2	3	4	5	6	7
0	51.7	49.9	52.2	57.4	55.0	54.3	60.5
1	1.35	1.00	1.53	2.17	8.00	7.18	9.09
2	4.44	6.01	2.60	2.43	12.36	10.31	9.09
3	5.12	6.18	4.59	5.66	13.09	11.60	14.38
4	6.09	7.81	6.80	10.71	16.00	13.44	16.77
5	(-2.70)	2.70	7.66	12.54	21.00	19.98	21.51
6	6.09	4.00	3.92	12.02	35.72	22.46	19.17
7	11.41	12.32	8.04	17.77	19.63	16.39	23.05
8	14.89	11.82	18.86	19.25	28.00	31.49	31.48
9	17.60	12.62	19.34	21.60	28.72	34.06	32.89
10	20.59	12.02	18.48	25.00	28.90	36.92	36.03
11	23.59	15.43	19.54	27.43	33.72	39.87	43.55
12	22.34	20.74	20.59	30.48	43.18	35.72	46.03
13	26.49	20.64	24.13	34.83	43.45	43.83	46.77
14	32.88	23.041	26.24	36.41	46.18	45.48	47.76
15	36.36	24.94	27.58	38.76	49.27	46.40	45.45
16	35.29	28.15	27.49	39.98	46.54	46.22	42.31

Table XII: The daily percentage (%) bodyweight gains in groups of rats injected with the ethanol extract of *Gn. latifolia* (Meisn)

	Rat Groups								Control
Days	1	2	3	4	5	6	7	8	9
0	59.65	52.7	55.9	54.5	48.5	49.3	47.4	57.2	49.55
1	5.04	13.47	6.25	3.66	5.97	1.82	6.86	3.49	2.72
2	-15.29	0.75	6.35	6.42	8.45	9.93	7.80	5.41	16.75
3	7.22	20.11	2.95	1.10	20.20	12.57	17.97	9.26	23.00
5	19.32	22.96	10.32	10.27	22.06	23.93	32.70	19.93	30.97
8	20.33	39.65	24.63	29.35	46.39	40.46	38.18	38.28	56.00
9	23.02	46.67	34.97	42.56	58.76	58.62	35.97	49.30	67.50
10	8.20	41.93	34.18	41.83	52.57	59.43	46.41	41.78	67.91
11		48.19	40.73	48.62	65.97	66.53	49.15	55.24	65.06
12		45.54	46.5	54.31	68.24	72.61	44.51	59.26	65.99
15		39.08	35.36	44.95	74.22	77.89	64.13	58.74	68.11
16		24.09	40.14	26.60	62.26	42.19	67.08	56.29	66.09
18		49.90	59.42	57.06	62.47	90.06	98.57	89.16	97.37
19		53.32	7.94	62.93	80.41	102.83	101.89	97.72	104.43
20		57.11	72.54	69.35	86.52	110.34	116.03	103.36	129.46
21		69.25	83.86	79.44	89.69	120.89	137.55	109.35	145.61
22		73.24	97.17	84.40	103.77	128.39	143.03	121.36	160.94
33		69.63	99.55	88.25	109.60	117.03	141.98	121.80	165.59
24		68.50	95.97	78.16	115.79	123.32	146.83	117.76	170.43
25		75.14	95.97	84.22	116.14	125.55	146.20	106.73	177.69
26		70.20	88.42	77.98	115.46	104.46	147.89	100.82	153.68
27		65.46	98.15	83.66	127.83	125.96	136.49	104.10	180.32

Fig. 11: Weaner Rats: Showing stary haircoat and posterior leg paralysis after injection with the crude ethanol extract of Gn. latifolia.

Fig. 12: Haemorrhages of the pancreas and thymus (arrowed) in rat injected with Gn. latifolia extract.

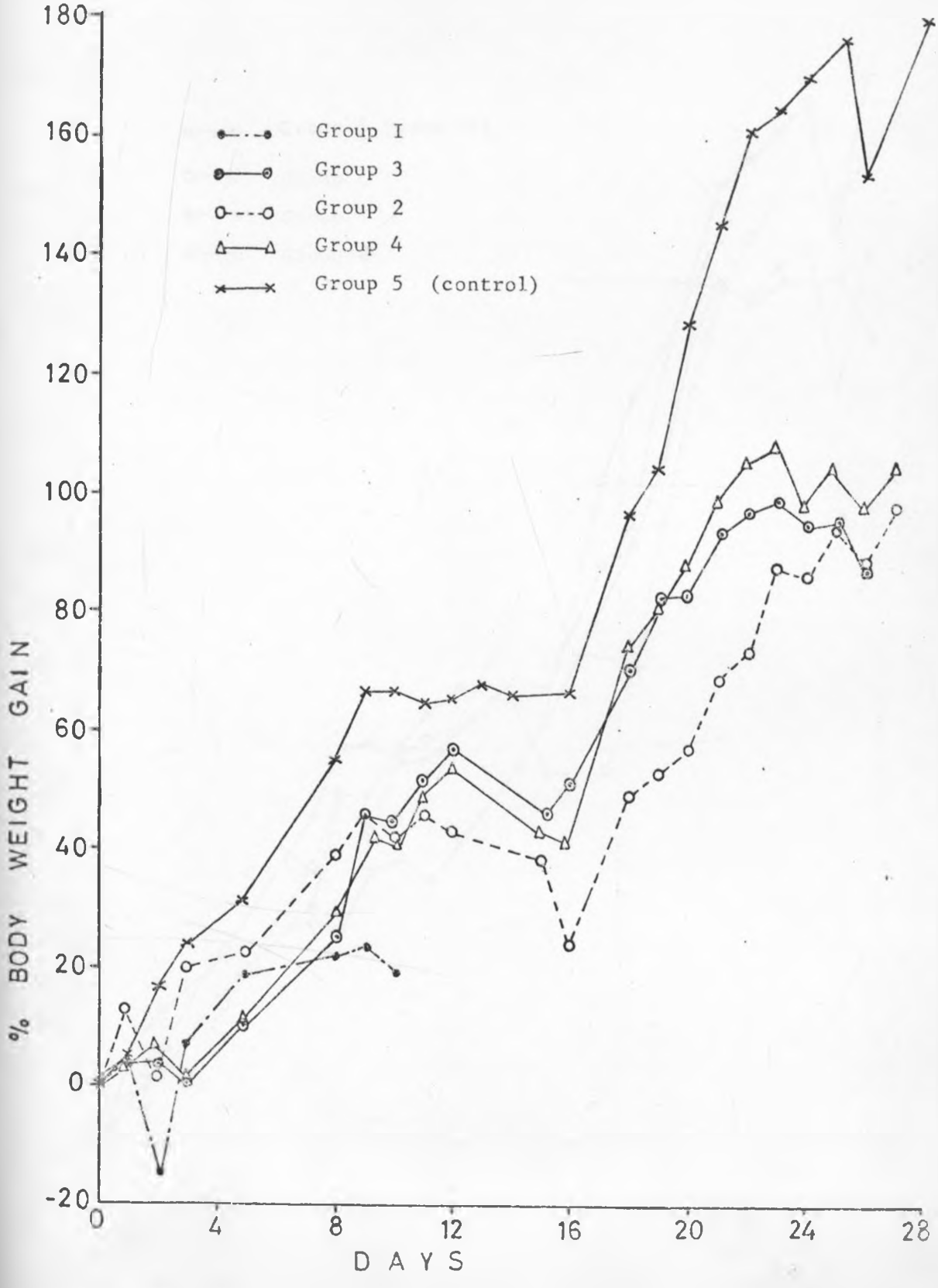


Fig. 13: (%) body weight gain in rats injected with extract of Gnidia latifolia (Meisn)

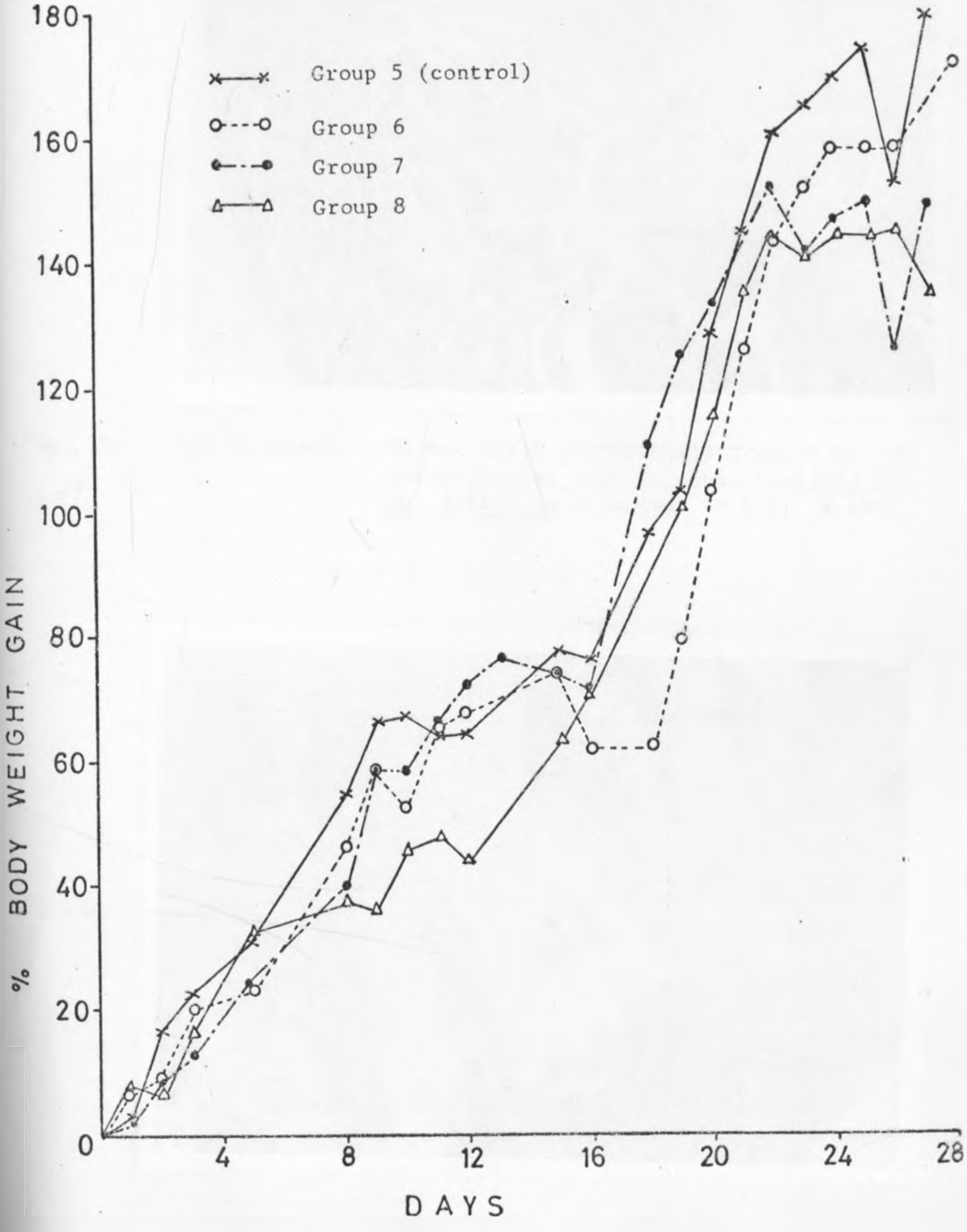


Fig. 14: Body weight gains (%) of rats injected with extract of Gnidia latifolia (Meisn)

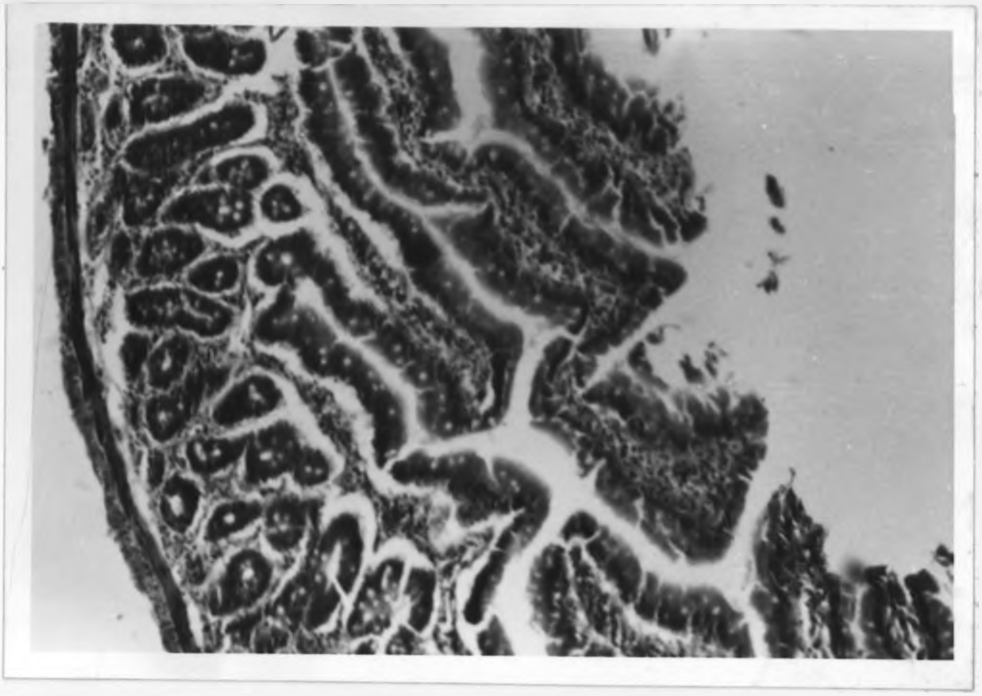


Fig. 15: Rat Duodenum: Normal villi and epithelial cells of rat duodenum that died in acute toxicity of Gn. latifolia injection (H & E; x 100)

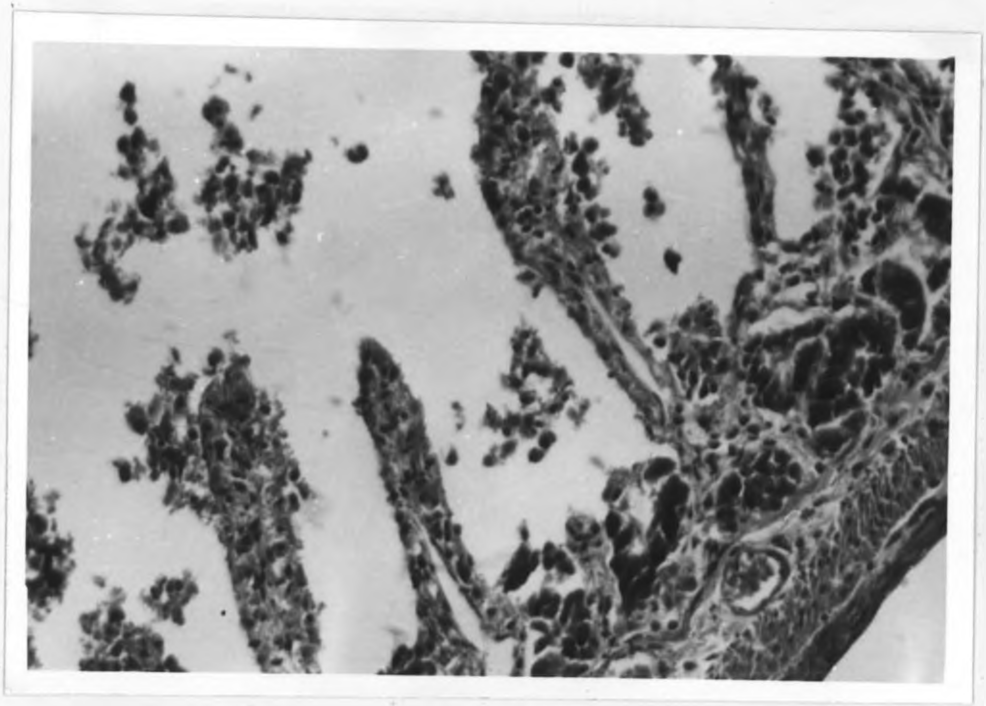


Fig. 16: Rat Duodenum: Epithelial desquamation and erosion in rat that died 24 hours after injection with Gn. latifolia extract (H & E; x 250)

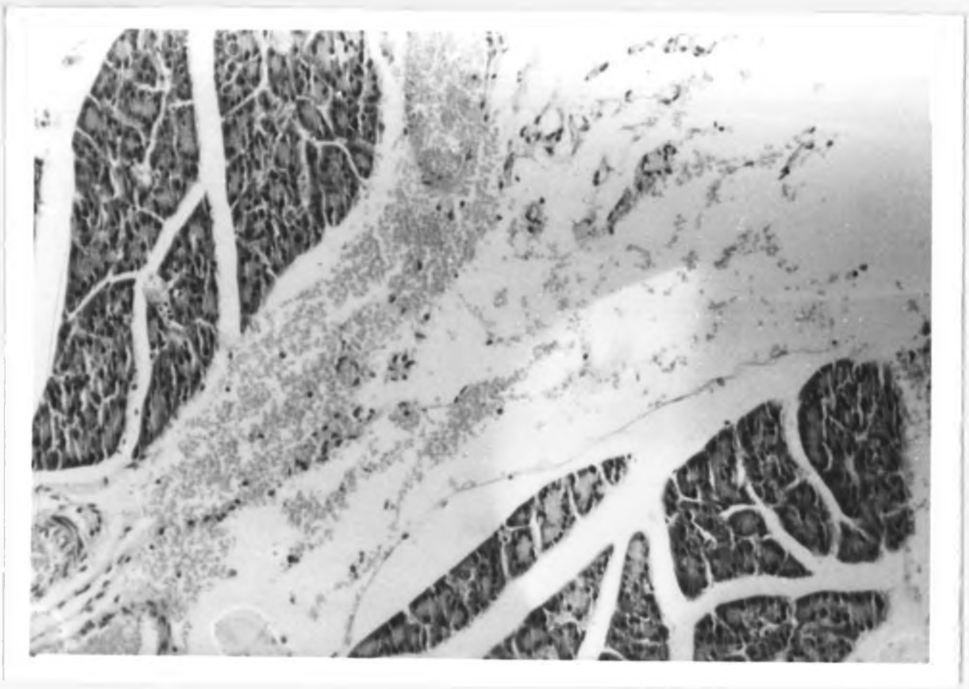


Fig. 17: Rat Pancreas: Extensive sub-capsular haemorrhage of the pancreas following acute toxicity with ethanol extract of Gn. latifolia injection (H & E; x 100)

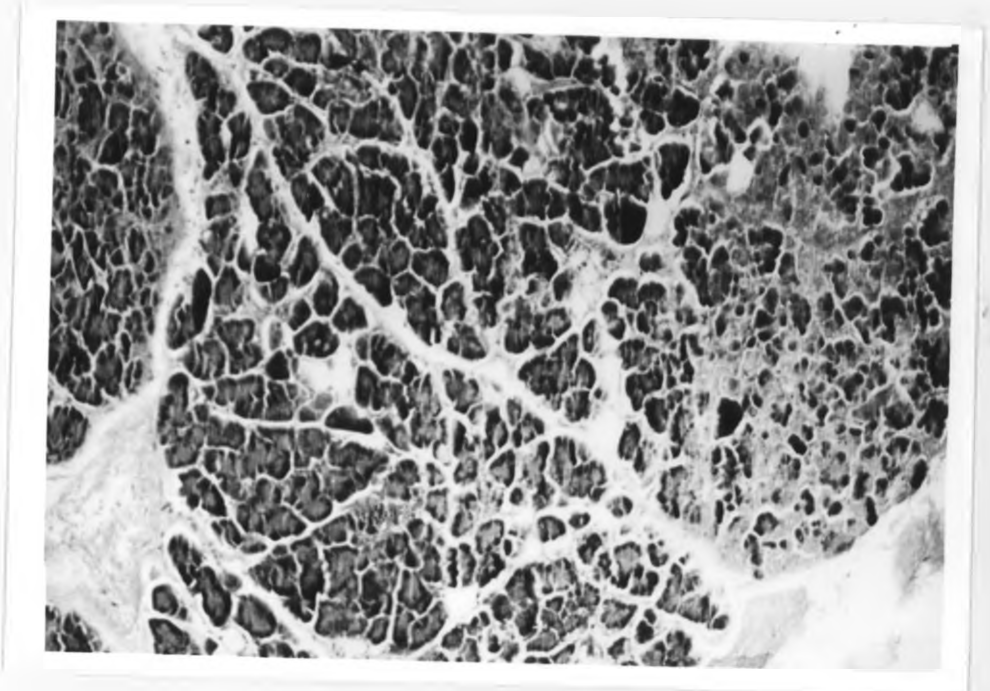


Fig. 18: Rat Pancreas : Marked pancreatic cell degeneration and haemorrhage in subacute Gn. latifolia toxicity. (H & E; x 250)

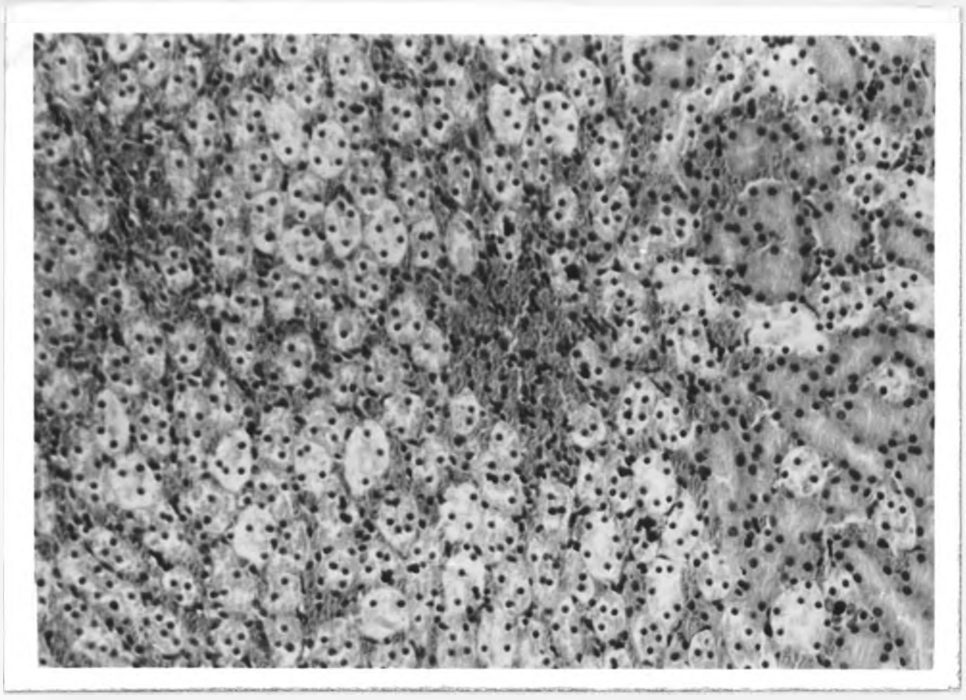


Fig. 19: Rat Kidney: Sub-cortical haemorrhages and nephrosis in rat kidney resulting from Gn. latifolia toxicity: (H & E; x 250)

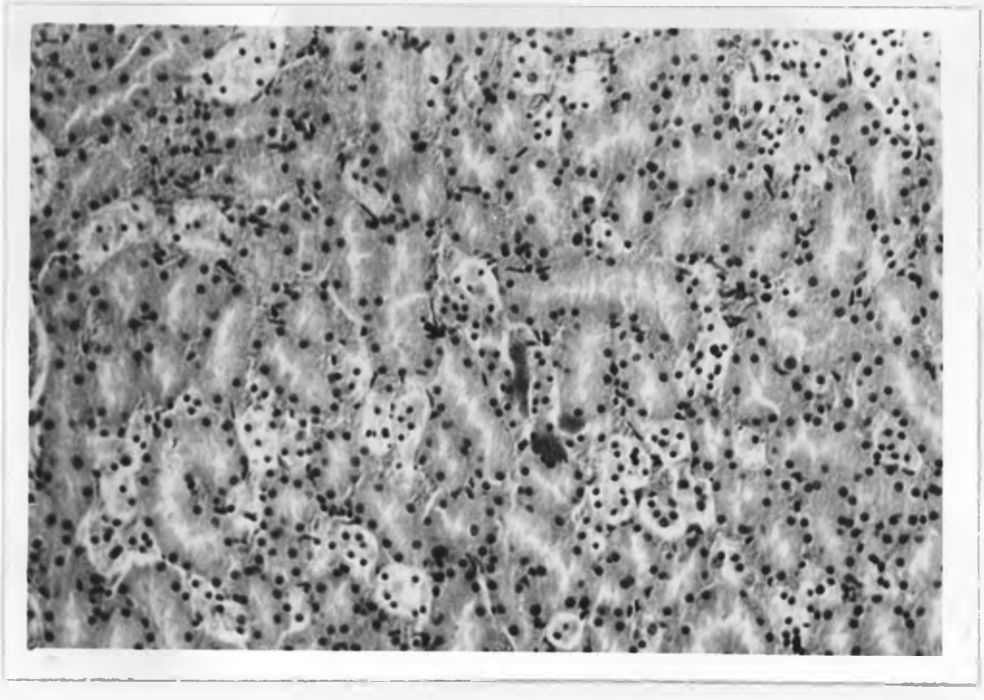


Fig. 20: Rat Kidney: Marked sub-cortical nephrosis with the deposition of Albuminous casts in the tubules: (H & E; x 250)

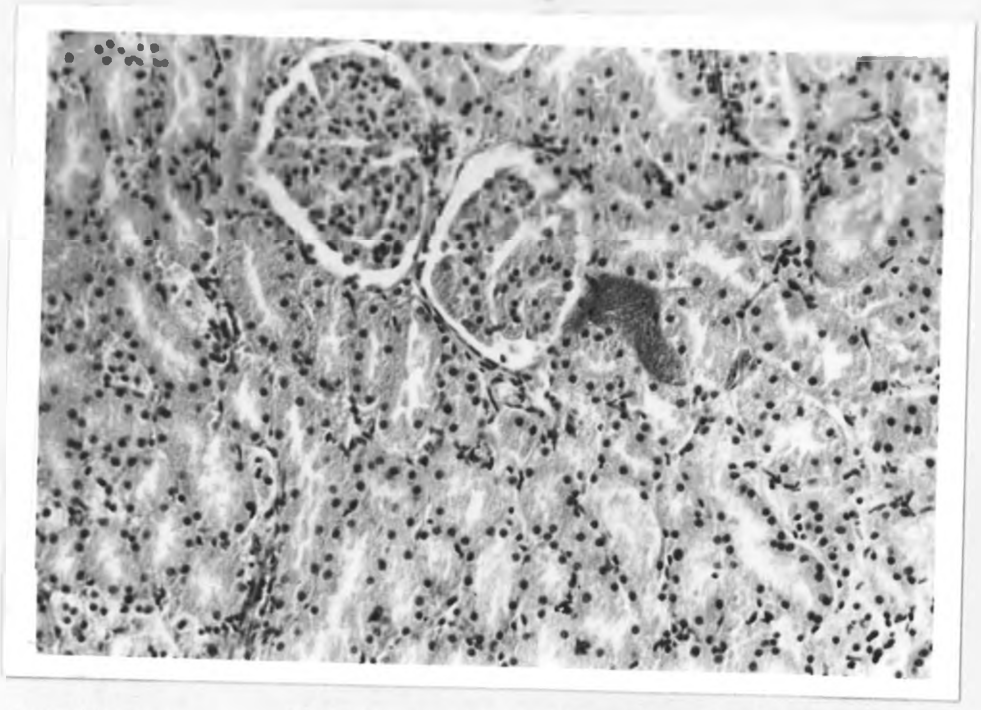


Fig. 21: Rat Kidney: Cortical nephrosis with albuminous cast deposition in the proximal tubule (H & E; x250)

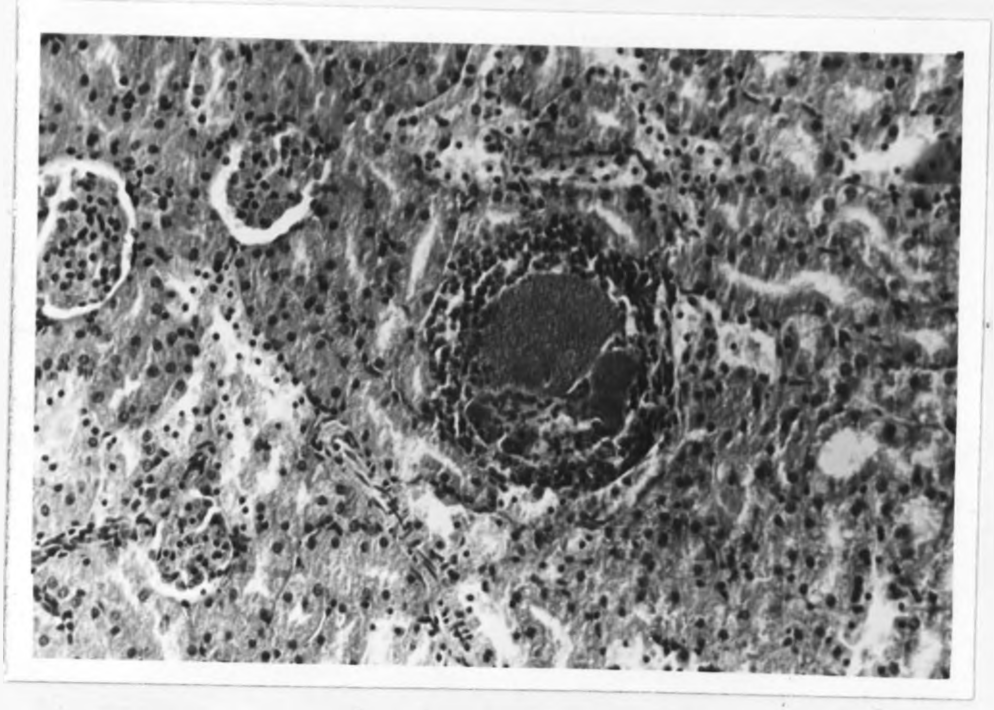


Fig. 22: Rat Kidney: Albumincus casts deposited in the periglomerular space causing glomerular displacement and eliciting inflammatory cell reaction: (H & E; x 250)

Fig. 23: Rat Adrenal: Marked cortical degeneration in the zona fasciculata of the adrenal gland of rat injected with ethanol extract of Gn. latifolia

(H & E; x 40)

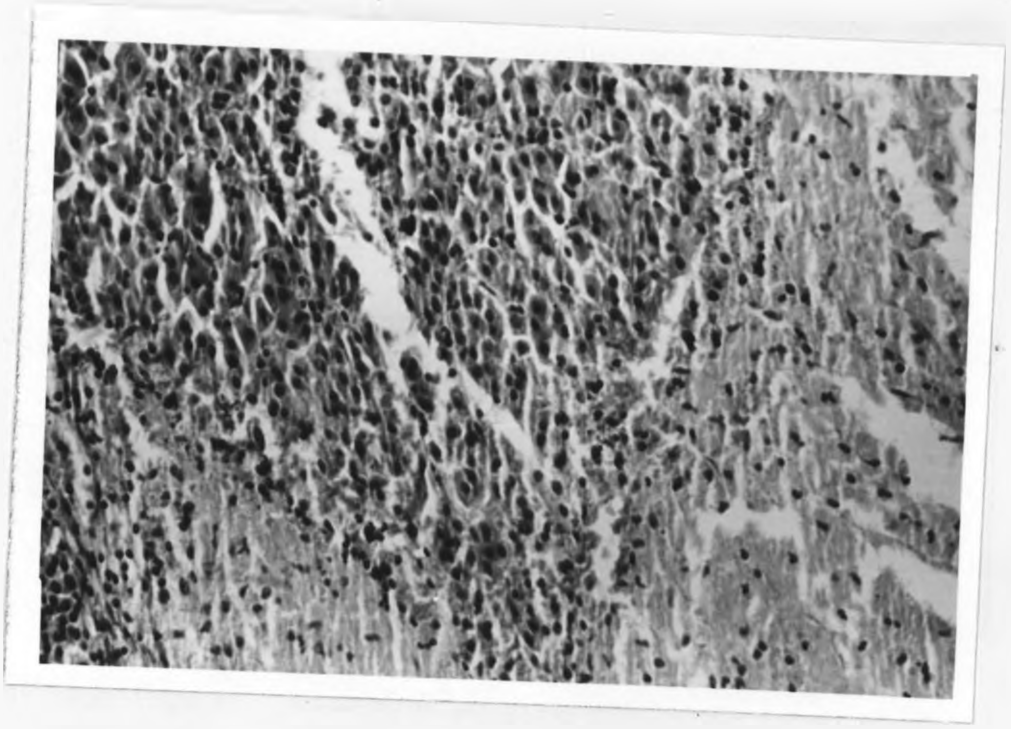


Fig. 24: Rat Adrenal: Marked haemorrhage and cellular degeneration in the zona reticularis of the adrenal gland resulting from intoxication with Gn. latifolia:

(H & E; x 250)

Fig. 25: Rat Thymus: Marked sub-capsular cell degeneration (lymphocytic pyknosis) in the lymphopoietic zones: (H & E; x 100)

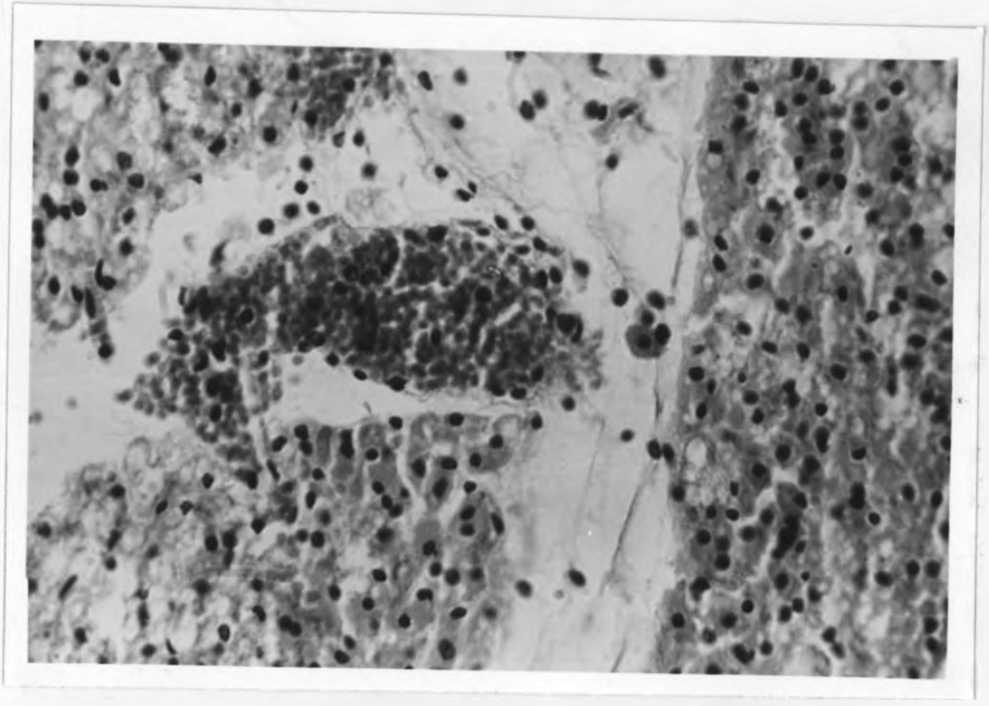


Fig. 26: Rat Thymus: Extensive interlobular haemorrhage in the thymus of rat injected with Gn. latifolia extract (H & E; x 400)

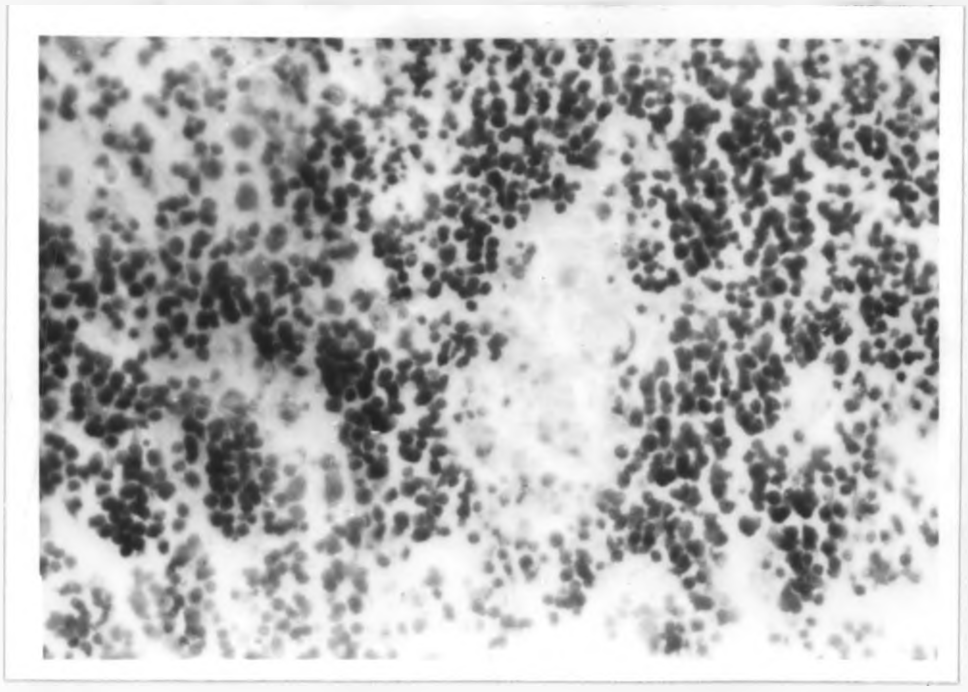


Fig. 27: Rat Spleen: A zone of lymphocytic cell degeneration in the lymph follicles of rat injected with Gn. latifolia extract: (H & E x250)

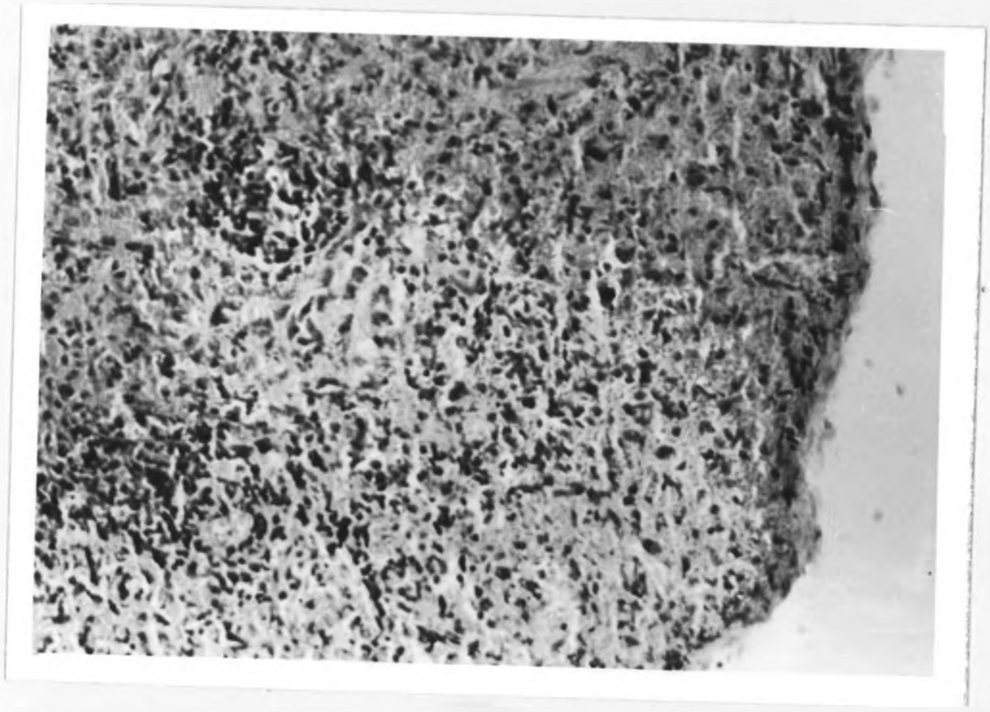


Fig. 28: Rat Spleen: Chronic lymphocytic cell depletion and replacement with fibroblastic cell types. Reduced lymphopoietic activity and cellular degeneration: (H & E; x 100)

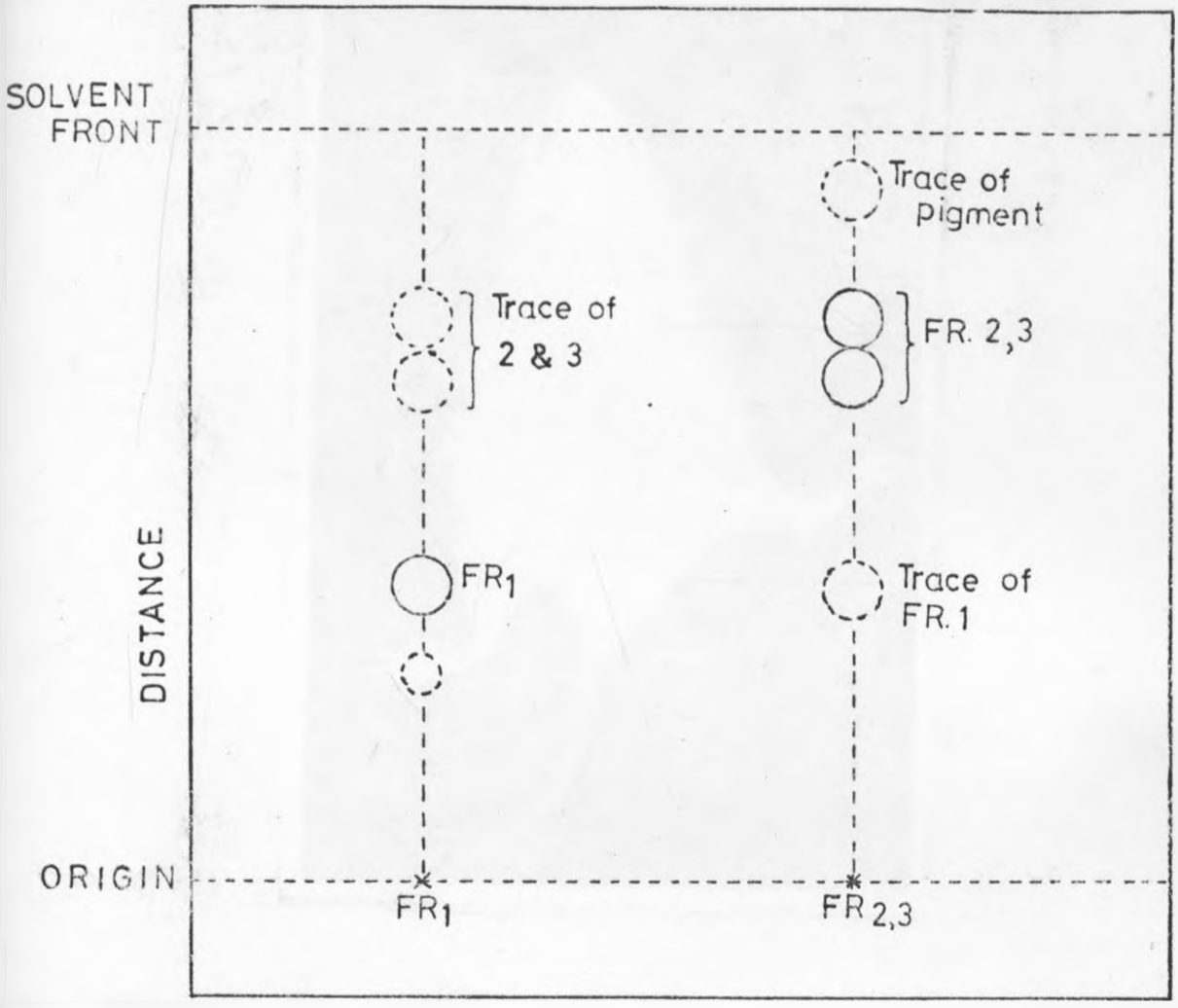


Fig. 29: Secondary run of the Thin layer chromatogram of the ethanol extract of Gnidia latifolia (Meisn)

Fig. 30: Weaner Rat: Injected intraperitoneally with Fr. I of TLC separation showing stary hair coat and posterior leg paralysis like rat injected with whole ethanol extract of Gn. latifolia (Meisn)

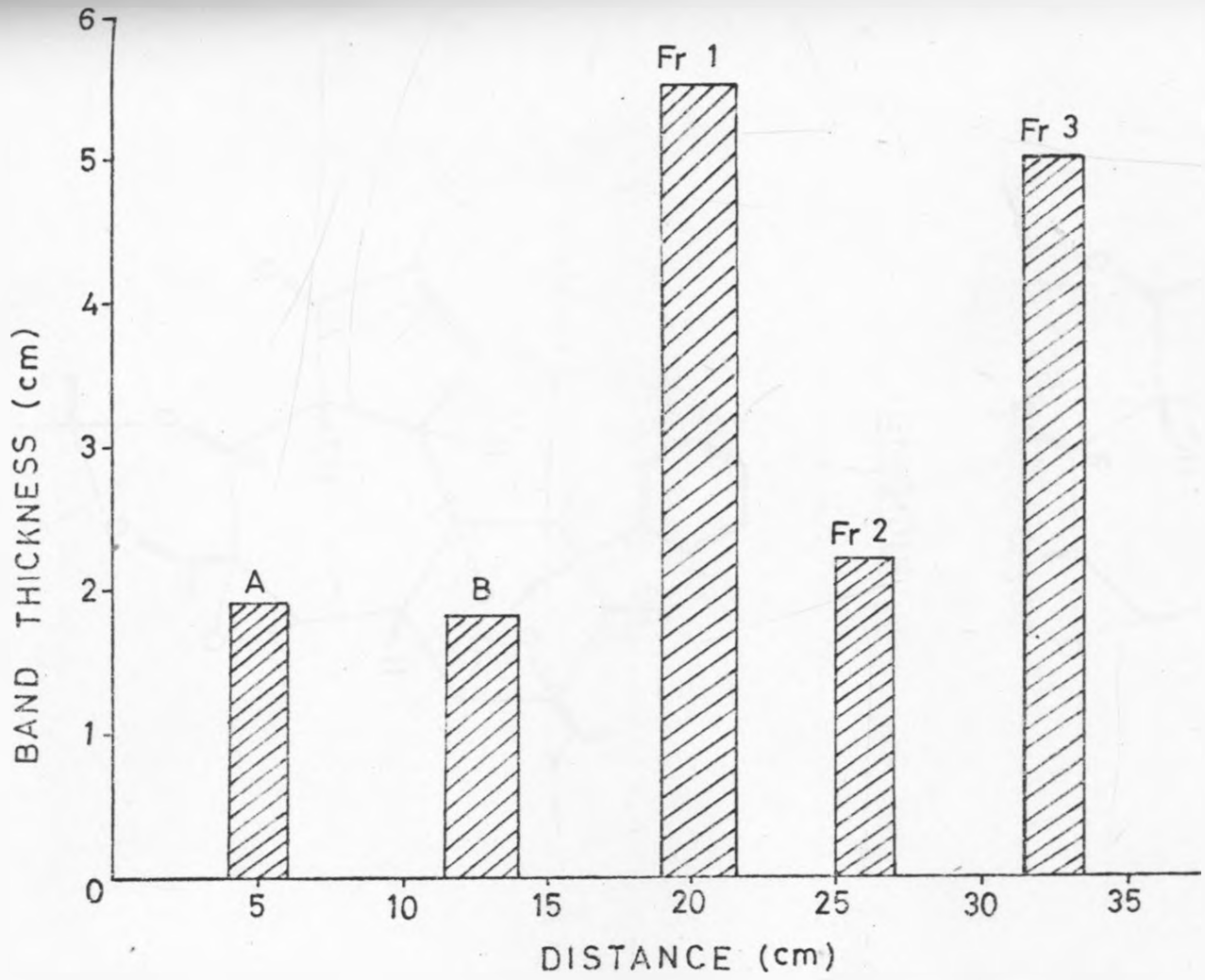
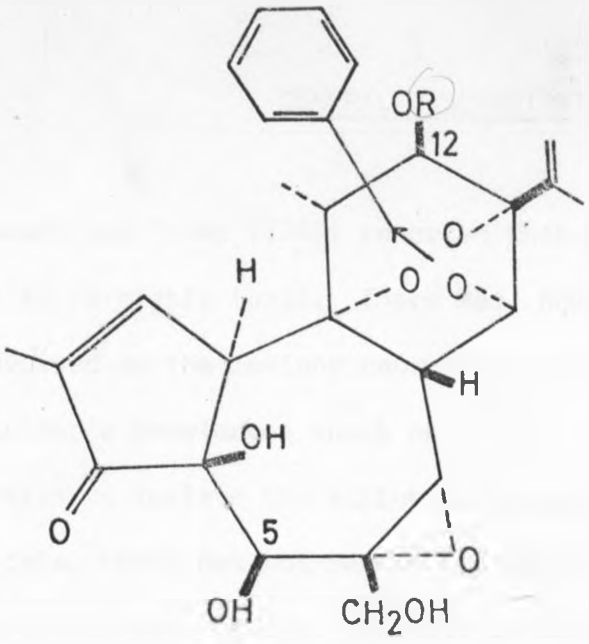
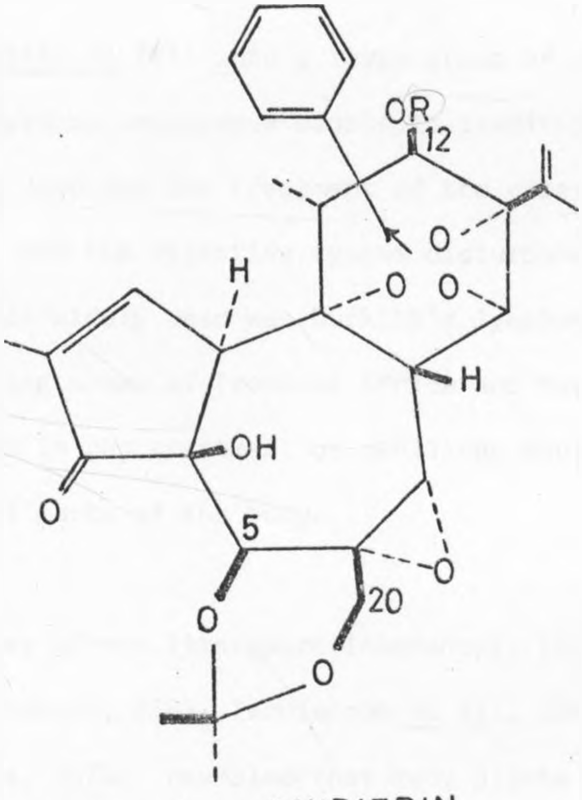


Fig. 31: Histogram of the ethanol extract of Cnidia latifolia paper chromatography as viewed under the long UV light (365 nm)



GNIDIDIN



GNIDITRIN

Fig. 32: Chemical configuration of the Daphnetoxins of Gnidia species (Kupchan et al, 1975)

GENERAL DISCUSSION

Verdcourt and Trump (1969) reported that Gn. latifoli (Meisn) was known to be highly toxic. There was, however, no indication of the organs involved or the lesions caused in the poisoned animal. To the present author's knowledge, there has been no previous attempt by other workers to isolate the active principle(s) in Gn. latifolia. Also, to date, there has not been a systematic study carried out to determine its (their) mode of toxicity on tissue cells. Watt and Breyer-Brandwijk (1962) had also reported the toxicity of Gn. latifolia. They indicated that there had been no previous attempt to identify the active principle(s) and its (their) mode of toxicity.

Gn. latifolia fell into a large group of poisonous plants which were employed by indigenous people in traditional medicine. The plant was widely used for the treatment of the upper respiratory tract infections and the digestive system disturbances. One such disease where it was widely used was Burkitt's lymphoma, a disease prevalent in the low-lying areas of Tropical Africa and the Carribean. It was also widely used in the treatment of swellings and tense enlargements of superficial parts of the body.

A survey of the literature (Alexander, 1928, Watt and Breyer-Brandwijk, 1962; Terblanche et al., 1966; Kokwaro, 1976, Nwude and Parsons, 1978) revealed that many plants of the genus Gnidia were

poisonous. A few species, namely Gn. anthylloides, Gn. burchelli, Gn. kraussiana and Gn. lamprantha had been tested for their toxicity in animals. Gn. latifolia (Meisn) had not been investigated either for its toxicity or the active principle(s) identified and quantitated. In the present investigation the toxicity of Gn. latifolia in the bovine and albino Wistar rats has been reported. The effect of its active principle(s) on the parenchymatous organs has also been recorded, especially the effect(s) it has on the lymphatic system. Results obtained have supported those recorded by other workers on Gnidia plants (Terblanche et al., 1966; Nwude and Parsons, 1978).

Feeding Gn. latifolia material to calves produced lesions in their livers, kidneys, lymph nodes and adrenals. The lesions in the acute cases consisted primarily of portal and centrilobular liver necrosis. There have also been marked renal tubular nephrosis. Varying degrees of lymphocytic degeneration were noticed in the lymph node and spleen follicles. The parenchymatous glandular organs (liver, kidney and adrenals) demonstrated petechial haemorrhages resulting from severe toxicity. Gross and histopathological lesions were also seen in rats injected intraperitoneally with an ethanol extract of Gn. latifolia. Severe necrotic lesions were observed in the thymus, pancreas and lymph nodes. The kidney and liver lesions were more marked than in the bovine. Haemorrhages and the development of hyaline depositions were more pronounced. The active principle(s) seemed to affect the lymphocytic cells at the lymphoblastic follicular zones in the thymus, lymph nodes and the spleen. This is an important discovery of biomedical

significance.

Gibb (1973) reported that terpenoids were synthesized by plants from the condensation of isoprene units into structures similar to agarthic acid and several other cyclization stages into the heterocyclic terpenes. The terpenoid compounds have many and varied functions in the plant, the main of which are to act as plant protectants and insect attractants. They have variations in their active terminals but the heterocyclic moiety is nearly similar in all the toxic terpenoids. Kupchan et al. (1975) reported that the reactivity of Gnidia diterpenoids depended on the alkyl moiety attached at position C-12 in the heterocyclic ring.

Most reports on the use of TLC on plant extracts have been restricted to the identification of the plant fractions (Stock and Ric, 1974; Stahl 1969). So far little information has been reported on the testing of the fractions for their toxicity in animals. There is, thus, a scarcity of information on the mode of toxicity of Gn. latifolia (Meisn). This necessitates that more work on the purification and identification of the extracts needs to be carried out. The semi-quantitative parameters adopted in assessing toxicity in this work are not adequate to enable one to make any definitive evaluation of the dose-response findings. Even where quantitative measures have been used, results are difficult to pinpoint as resulting from interference of the vital function of a particular organ.

This research suggests the need for additional studies on the preparation and purification of the extracts. The purified extracts would then be used to pinpoint definitively the mode of toxicity of Gn. latifolia (Meisn) to man and animals.

CONCLUSION

Feeding Gnidia material to calves produced lesions in their liver, kidneys and lymphatic system. The lesions consisted primarily of portal and centrilobular necrosis of the liver and kidney tubular damage in the kidney cortex. Chronic liver changes were followed by fibroblastic cell proliferations. The lymph node follicles and splenic parenchyma showed a marked lymphocytic cell degeneration and depletion. The lymphopoietic centres appeared to be more susceptible to the toxic principle(s) than the other organs, and the severity of lesions was more marked in animals fed higher doses.

Haematological examinations revealed a marked lymphopaenia in Gnidia toxicity. This finding was consistent with the histopathological lesions observed in the spleen and lymph nodes. Blood biochemical examinations revealed increased LDH and BUN levels which were more marked in the terminal stages of the intoxication. It appears,

therefore, that the toxic principle(s) of Gn. latifolia are highly toxic to parenchymatous tissues and especially lymphocytic tissue. This is an important discovery of biomedical significance.

Histopathological lesions were also noted in weaner albino Wister rats injected with the ethanol extract of Gn. latifolia (Meisn). The lesions were similar to those noted in the bovine and were more severe in the rats receiving high dosages. Rats injected with sub-lethal doses of the ethanol extract for a long time showed retarded growth and general tissue wasting. Their lymphocytic tissue showed degenerations in the thymus, spleen and lymph nodes. Degenerative changes were also seen in the kidneys and the adrenal body. The acute and chronic liver changes were also similar to those observed in the bovine.

The ethanol extract was further separated by TLC into three district fractions. Rats injected with Fr. 1 showed similar pathological responses as the rats injected with whole ethanol extract.

This research suggests the need for additional studies on the implication of Gnidia material used as medicine in relation to changes caused in the body of man and animals.

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Appendix I

Packed Cell Volume

Calf Numbers

Days of Treatment	1	2	3	4	5	6	7
1	28.0	38.0	22	33	26	30	26
2	25.0	43	27	36	25	27	22
3	28.0	41	21	36	27	30	23
4	26.0	43	24	15	26	32	25
5		41	24	35	26	30.5	24.5
6		43	29	33	27	31	26
7		44	27.5	33	27	31	25
8		45	27	36	25	29	27
9		44	25	33	27	29	27
10		43	24	32	25	33.5	27.5
11		42	25	33	24	30	26
12		43	24.4	33	24	25	27
13		46	27	32	24		27
14		48	22	34	24		25.5
15		50	25	37	25		26.5
16		46	23	36	26		25.5
17		47	25	33	26		26
18		41	28		25		27
19		47			23		25.5
20					22		24.6
21					22		24
22					22		23
23					22		22
24					21		21
25					22.5		18.5
26					24		19
27					20		19.5
28					15		20
29							
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Packed Cell Volume

Calf numbers

8	9	10	11	12	Mean S.D.
21	18	27	29	30.5	25.25 ± 4.81
25	16	25	27.5	34	24.9 ± 5.95
27	16	24	27	32.5	24.9 ± 5.48
23	16	26	26	30	24.3 ± 4.68
24	16	26	26	33	24.9 ± 5.44
22	15	28	29	30	25 ± 5.65
26	17	28	27	29	25.3 ± 4.32
26	16	26	28	30	25.5 ± 4.88
25	15	25	27	30	25 ± 5.05
24	15	24	26	30	24.4 ± 5.14
27	15	25	26	30	24.8 ± 5.11
27	15	24	30	30	25.5 ± 5.61
27	15	26	26	30	25.1 ± 5.19
25	15	24	27	29	24.25 ± 4.35
29	15	23	28	30	25.25 ± 5.58
29	15	22	27	27	24.25 ± 5.09
28	16	25	29	28	25.3 ± 4.80
29	23	17	26	27	24.8 ± 4.30
29	16	24	25	26	24.25 ± 4.37
29	17	24	24	23.5	23.6 ± 3.84
27	15	25	24	24	23.1 ± 4.16
27	17	23	24	22.4	22.7 ± 3.25
30	16	23	22	28	23.5 ± 4.69
31	17	23	25	24	23.5 ± 4.63
31	16.5	22.5	24	30	23.75 ± 5.88
29	23	22	22	27	23.6 ± 3.66
32	18	23	24	23	23.25 ± 4.87
31	19	20	20	24	22.3 ± 4.58
33	18	21	22	26	24 ± 5.78
34	18	21	22.5	24	23.9 ± 6.06
32	18	24	22.5	24	24.1 ± 5.05
	18	21	22	24	21.25 ± 2.50
	19	25	23	22	22.25 ± 2.50
	21	25	23.5	23	23 ± 1.65
	18	21	22	18	19.75 ± 2.06
	18	24	23	21	21.5 ± 2.64
	16	22		21	19.6 ± 3.21
	15	20		22	19 ± 3.60
	17	20.5			18.25
	17	19.5			18.25
	16.5	20			18.5
		19			17.75
		18.6			18.6
		18			18
		18.5			18.5
		22			22
		27			27
		22			22
		22			22
		22			22
		23			23

Appendix II

Total Protein (gm/100 ml)

Calf numbers.

Days of Treatment	1	2	3	4	5	6	7
1	5.6	5.6	6.0	6.6	5.8	4.8	6.2
2	5.6	5.8	6.4	6.7	5.4	4.6	5.6
3	6.0	5.8	5.8	7.2	5.2	7.2	5.6
4	5.4	6.0	5.6	-	6.4	7.4	6.0
5		5.3	6.0	6.4	5.8	7.0	6.0
6		6.2	6.8	6.7	6.0	7.4	5.8
7		5.8	6.6	6.7	5.8	7.8	6.0
8		6.3	7.8	6.4	6.8	7.2	6.1
9		5.2	7.6	6.0	6.4	4.6	6.0
10		6.0	6.0	6.5	5.8	5.8	5.8
11		5.8	5.2	6.4	5.0	5.2	5.8
12		6.2	6.8	6.2	5.8	5.4	5.8
13		6.1	5.2	6.4	5.2		6.2
14		6.0	5.2	6.6	5.2		5.6
15		6.4	5.8	7.1	5.6		6.4
16		6.4	5.4	7.6	6.2		5.2
17		6.5	6.4	7.2	5.6		6.0
18		6.2	6.4		6.2		6.4
19		6.2			7.6		6.2
20					5.6		6.1
21					5.6		6.8
22					6.2		6.0
23					5.4		5.8
24					6.2		6.0
25					6.0		5.6
26					6.4		5.6
27					4.8		5.4
28					6.4		5.4
29							
30							
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Total Protein (gm/100 ml)

Calf numbers

8	9	10	11	12
6.4	4.8	5.8	6.4	6.8
6.6	4.6	6.0	5.8	6.0
7.3	4.0	5.4	6.0	6.4
7.0	5.4	6.2	6.0	5.4
6.6	4.2	5.4	6.2	6.4
6.8	4.8	5.8	6.4	6.2
7.0	5.6	6.2	6.2	6.0
6.8	4.8	6.0	6.3	6.4
6.0	5.2	6.6	6.6	6.5
7.0	5.0	6.0	6.2	6.3
7.2	4.6	5.8	6.5	6.4
6.6	5.2	6.4	7.0	6.2
6.8	4.8	6.2	6.0	6.4
7.0	5.0	5.8	6.4	6.0
8.0	5.0	6.2	6.3	6.4
7.8	6.5	6.6	6.2	7.0
7.0	5.4	6.6	7.1	7.0
7.0	4.4	5.6	6.4	6.0
7.1	5.4	6.0	6.2	6.4
7.6	5.8	6.6	6.2	-
6.5	5.4	6.6	6.0	
6.0	5.6	6.4	6.1	
7.0	6.4	6.2	6.0	
7.2	5.4	6.2	6.4	
7.0	5.2	6.2	6.0	
7.2	6.0	6.0	6.2	
7.0	5.8	6.8	6.0	
6.6	5.2	7.4	6.4	
7.2	7.6	8.0	6.5	
7.0	7.6	7.6	6.6	
7.6	7.6	7.7	6.4	
	7.2	8.0	6.0	
	7.8	8.2	6.4	
	7.8	6.2	6.6	
	5.8	6.6	6.0	
	6.2	5.2	5.8	
	5.4	5.8		
	5.2	5.0		
	4.8	5.4		
	5.4	5.2		
	5.2	5.6		
	5.4	5.4		
		5.8		
		5.8		
		5.4		
		6.0		
		6.0		
		5.6		
		6.0		
		6.0		
		5.6		

Appendix III Hemoglobin Concentration (mg/100 ml)

Calf Numbers

Days of Treatment	1	2	3	4	5	6
1	10.5	12.7	7.8	10.9	8.9	10.0
2	9.6	14.1	9.3	11.4	9.1	9.1
3	10.9	13.3	8.8	11.4	10.4	10.7
4	9.4	13.6	8.5	10.7	9.8	11.1
5		13.3	8.5	10.8	9.7	10.6
6		13.6	9.4	10	9.4	10.2
7		14.6	9.3	10.7	8.9	10
8		14.6	9.6	11.4	8.8	10.3
9		14	8.8	10.5	9.8	8.9
10		14.4	8.6	10.5	9.0	11.0
11		14	9.2	10.7	9.1	9.2
12		14.1	8.5	10.2	8.6	11.1
13		15	9.1	10.2	9.0	
14		15.8	8.1	11.2	9.4	
15		16.4	8.8	11.8	8.8	
16		15.5	8.3	11.7	10.4	
17		15.2	12.7	10.5	8.7	
18		14	11.9		8.7	
19		15.2			7.8	
20					8.1	
21					8.0	
22					8.0	
23					7.7	
24					7.4	
25					7.4	
26					9.3	
27					7.2	
28					5.7	
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Haemoglobin concentration (mg/100 ml)

Calf numbers

7	8	9	10	11	12	Mean	SD
9.4	7.4	6.4	9.8	10.2	10.6	8.96	± 1.68
7.9	8.4	6.2	9.7	9.1	11.8	8.85	± 1.87
7.8	8.9	5.9	9.3	9.4	10.8	8.68	± 1.67
8.7	7.6	6.2	10.0	9.2	10.5	8.7	± 1.58
8.5	8.2	6.0	9.6	9.0	11.3	8.76	± 1.74
8.6	7.5	5.6	10.3	9.4	10.7	8.68	± 1.90
8.2	8.8	5.5	9.4	9.1	10.8	8.63	± 1.76
8.7	8.8	5.5	9.4	9.6	11.0	8.83	± 1.82
9.4	8.3	5.6	9.6	9.3	10.1	8.71	± 1.63
9.2	8.1	5.2	8.1	8.6	10.1	8.21	± 1.66
8.9	9.2	5.8	8.7	8.8	10.4	8.63	± 1.52
8.8	9.5	5.4	8.7	10.0	10.7	8.85	± 1.84
9.0	8.7	5.3	9.7	8.5	10.9	8.68	± 1.87
8.6	8.6	5.5	9.5	9.3	10.4	8.65	± 1.68
8.9	9.9	5.5	7.9	9.5	10.2	8.65	± 1.74
8.6	11.7	5.8	7.5	9.4	9.9	8.81	± 2.03
8.8	9.6	6.1	8.9	9.5	10.2	8.85	± 1.44
9.3	9.5	7.8	5.9	8.8	9.3	8.43	± 1.38
8.7	9.9	5.5	8.4	8.6	9.3	8.40	± 1.52
8.8	11.4	5.6	8.2	8.2	8.3	8.41	± 1.84
8.2	9.2	5.5	8.7	8.0	9.0	8.10	± 1.35
7.5	9.3	5.9	8.3	8.3	8.5	7.96	± 1.16
8.3	10.5	5.4	7.9	7.8	9.4	8.20	± 1.92
7.1	10.6	5.9	7.9	8.1	8.4	8.0	± 1.55
6.6	10.2	5.6	7.9	7.7	9.1	7.85	± 1.65
6.3	10.4	5.4	7.6	7.1	9.4	7.70	± 1.88
7.2	11.3	6.1	7.8	7.7	8.3	8.06	± 1.75
7.4	11.0	6.1	7.5	6.8	8.9	7.95	± 1.75
	11.5	6.9	7.9	7.2	9.5	8.60	± 1.90
	11.3	6.3	7.3	7.2	8.9	8.20	± 1.96
	11.7	6.1	8.4	7.5	8.6	8.46	± 2.06
		6.1	7.3	7.4	8.8	7.40	± 1.10
		6.1	8.3	7.3	8.2	7.47	± 1.02
		6.6	8.2	7.5	8.2	7.62	± 0.75
		5.9	8.2	7.9	6.6	7.15	± 1.08
		6.8	7.5	7.4	7.3	7.25	± 0.31
		5.1	8.6		8.3	7.33	± 1.93
		5.0	6.9		7.7	6.53	± 1.38
		5.4	7.1			6.25	
		5.9	7.1			6.50	
		6.0	7.2			6.60	
		5.9	6.9			6.40	
			6.6			6.6	
			6.5			6.5	
			6.4			6.4	
			7.9			7.9	
			9.3			9.3	
			7.6			7.6	
			7.5			7.5	
			8.0			8.0	
			8.3			8.3	

Appendix IV Red Blood Cell count ($10^6/mm^3$)

Days of Treatment	Calf numbers 1	2	3	4	5	6
1	6.00	7.80	4.57	7.47	5.65	4.99
2	4.84	9.51	5.49	7.32	5.66	5.41
3	5.74	9.15	4.83	7.39	6 23	5.87
4	5.14	9.67	4.96	7.24	5.64	5.88
5		8.74	4.98	7.08	6.03	5.27
6		9.34	5.58	7.00	5.83	5.55
7		9.69	5.65	7.22	5.55	5.36
8		9.76	5.85	7.32	4.91	5.90
9		9.33	5.41	6.89	5.04	5.26
10		9.73	5.15	6.60	5.03	6.01
11		8.70	5.42	6.66	5.40	5.35
12		9.07	5.36	6.33	4.78	4.83
13		9.26	5.38	6.44	4.55	
14		9.76	4.64	7.07	5.48	
15		10.54	5.32	7.02	4.61	
16		9.82	5.15	6.81	5.09	
17		10.05	5.20	6.41	4.74	
18		9.76	5.88		4.78	
19		9.93			4.19	
20					5.09	
21					4.14	
22					5.03	
23					4.87	
24					4.41	
25					3.88	
26					4.55	
27					3.88	
28					2.92	
29						
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Red Blood cell count (10^6/mm^3)

Calf numbers

7	8	9	10	11	12	Mean SD
5.27	4.92	3.62	5.42	6.40	6.51	5.35 ± 7.06
4.38	5.64	3.68	5.98	5.82	7.07	5.41 ± 1.20
5.13	5.99	3.47	5.88	5.84	6.58	5.48 ± 1.08
5.58	5.74	3.23	5.40	6.12	6.45	5.42 ± 1.13
5.36	5.68	3.12	5.71	5.78	6.21	5.31 ± 1.10
5.36	5.35	3.12	5.74	6.18	6.26	5.33 ± 1.15
5.40	6.13	2.83	5.82	6.55	6.19	5.48 ± 1.35
5.83	5.94	2.51	4.91	6.10	6.38	5.27 ± 1.44
6.00	5.42	2.41	4.65	6.08	5.44	5.00 ± 1.36
5.93	5.24	3.44	4.32	5.78	5.89	5.10 ± 1.01
5.99	6.28	3.40	5.10	5.96	5.89	5.43 ± 1.07
5.85	6.50	2.67	4.29	6.65	6.51	5.41 ± 1.60
5.91	6.17	2.70	4.75	5.84	6.67	5.34 ± 1.43
5.48	6.09	2.53	4.19	6.22	6.18	5.11 ± 1.48
5.42	6.63	2.78	3.88	5.91	6.20	5.13 ± 1.49
5.27	7.48	2.50	3.88	6.28	6.09	5.25 ± 1.79
5.66	6.39	2.67	4.29	6.40	6.28	5.28 ± 1.51
6.28	7.02	3.82	2.92	5.81	5.88	5.28 ± 1.57
5.74	6.61	2.75	3.99	5.81	5.76	5.11 ± 1.44
5.75	7.68	2.98	4.84	5.52	5.32	5.34 ± 1.51
5.34	6.14	2.85	4.39	5.84	5.47	5.00 ± 1.21
5.47	6.64	3.01	4.41	5.76	5.18	5.07 ± 1.24
-	7.08	3.28	5.31	5.83	6.71	5.64 ± 1.49
4.55	7.49	3.23	4.22	5.74	5.19	5.07 ± 1.46
4.22	6.84	3.07	4.60	5.70	5.40	4.97 ± 1.30
4.29	7.27	4.40	4.16	4.98	5.74	5.14 ± 1.19
4.41	7.64	3.40	4.28	5.48	5.18	5.06 ± 1.45
4.61	6.66	3.30	4.18	4.80	5.54	4.84 ± 1.15
	7.97	3.61	4.29	4.94	5.61	5.28 ± 1.49
	8.05	3.52	4.07	5.27	5.58	5.29 ± 1.56
	7.68	3.34	5.04	5.18	5.34	5.31 ± 1.38
		3.21	3.88	5.21	5.56	4.46 ± 1.10
		3.45	4.86	5.36	5.02	4.67 ± 0.84
		4.06	4.84	5.46	5.20	4.89 ± 0.60
		3.29	4.20	5.48	4.03	4.25 ± 0.91
		3.40	5.25	5.43	4.77	4.71 ± 0.91
		2.63	4.34		5.23	4.06 ± 1.32
		2.67	3.77		4.84	3.76 ± 1.08
		3.17	3.93			3.55
		3.23	3.75			3.49
		3.43	4.88			4.15
		4.28	4.91			4.59
			4.44			4.44
			4.68			4.68
			4.51			4.51
			5.05			5.05
			3.31			3.31
			5.10			5.10
			4.88			4.88
			5.27			5.27
			4.93			4.93

Total White Cell counts (per cmm)

Calf numbers

Days of Treatment	1	2	3	4	5	6
1	9600	7500	1000	13800	5600	5500
2	7800	8700	10700	14400	7100	5300
3	9200	9200	8800	10900	8000	4100
4	5600	8800	9400	-	5000	4000
5		8400	6700	10500	5300	5800
7		7000	7000	11100	3200	4900
8		8600	6800	9400	3100	3000
9		8500	5800	8900	4200	5700
10		9500	5000	10500	6300	4700
11		8200	7600	12900	9600	3000
12		6600	4900	9000	6400	5500
13		6500	7000	8500	5180	
14		6500	6000	8300	6200	
15		5400	7500	12300	4100	
16		3600	7700	7400	5500	
17		3700	8600	6700	9200	
18		5600	6500		2800	
19		2300			4300	
20					5100	
21					4200	
22					2300	
23					4400	
24					2700	
25					3800	
26					5000	
27					3900	
28					3300	
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Total white cell counts (per cmm)

Calf Numbers

7	8	9	10	11	12	Mean SD
6900	5500	6400	9500	7300	10700	7710 ± 1978
10700	5500	6000	8600	6700	11800	8200 ± 2900
9400	5500	6000	6700	6300	11400	7550 ± 2300
13200	5500	5700	8000	6500	10900	8300 ± 3120
11100	5700	9100	7600	6000	12300	8600 ± 3015
14800	5000	4700	6000	7200	12300	8300 ± 4200
8700	6100	5800	7600	5500	11300	7500 ± 2200
9300	6300	4500	12400	7400	10000	8300 ± 2800
13400	5300	5800	9400	6600	9800	8380 ± 3000
10000	6600	5200	7500	6500	10500	7700 ± 2100
10000	5500	3700	7100	5800	10600	7100 ± 2900
10500	6100	5300	10300	6200	10900	8200 ± 2600
8800	6800	5300	11700	5600	10500	8100 ± 2600
7300	6300	5800	9100	5200	10900	7400 ± 2100
9300	7800	4800	9300	7900	10500	8200 ± 1900
7900	5700	5800	7500	5000	6600	6400 ± 1100
7400	5400	10400	7700	3900	10600	7500 ± 2600
10000	5400	7700	5000	3000	9000	6600 ± 2600
7200	5500	4700	9800	2900	6700	6100 ± 2300
6000	5200	4500	7200	2000	8000	5400 ± 2100
6500	4500	7700	3100	2300	8700	5400 ± 2500
7100	5500	3800	5000	2000	8500	5300 ± 2300
7400	4800	3500	6200	2200	9800	5600 ± 2700
7800	4800	3000	4200	2400	7700	4900 ± 2300
6000	4900	3600	4500	2900	8700	5100 ± 2000
2900	5000	7000	6200	2700	8100	5300 ± 2100
2350	4600	4300	6400	3900	9400	5100 ± 2400
3700	4500	6600	6700	3400	9100	5600 ± 2100
	4000	4800	8300	4200	9400	6100 ± 2500
	4000	5600	6200	5100	8500	5800 ± 1600
		4200	7500	4300	7700	5900 ± 1900
		5500	5000	4200	7700	5600 ± 1400
		3700	7500	4400	3600	4800 ± 1800
		4700	4500	5400	3700	4500 ± 600
		3000	3300	6100	3100	3800 ± 1400
		3200	2800	5600	3200	3700 ± 1200
		3300	4500		2900	3500 ± 800
		3400	4900		2700	3600 ± 1100
		6400	7500			
		6500	7000			
		5500	7300			
		5800	11300			
			8000			
			86550			
			6800			
			8000			
			7900			
			5600			
			5700			
			6000			
			8600			

Appendix VI

Absolute Lymphocyte count ($10^3/mm^3$)

Calf Numbers

Days of Treatment	1	2	3	4	5	6
1	8160	7125	9000	12282	4480	3696
2	6708	7134	9202	11232	5224	4140
3	7084	7912	7744	9810	4640	3600
4	4088	7568	6768	-	2950	3074
5		6720	5695	8610	4028	3381
6		6440	4900	9324	2304	2190
7		8084	3876	8460	-	2451
8		6970	4350	7654	3528	2820
9		8170	4400	8820	5733	2117
10		6630	4134	9240	4060	1874
11		7216	6232	11352	7104	2915
12		5808	4214	7560	5312	
13		6110	5810	7140	3522	
14		5785	5100	6474	4588	
15		3564	6150	6765	3360	
16		2520	6314	4588	2695	
17		2368	7310	3618	6440	
18		4480	5525		1760	
19		2162			2752	
20					3825	
21					2940	
22					1340	
23					2464	
24					1215	
25					1748	
26					1450	
27					1092	
28					1914	
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Absolute Lymphocyte count ($10^3/mm^3$)

Calf numbers

7	8	9	10	11	12	Mean	SD
4761	4235	4416	8075	6205	8346	6006	± 18.4
-	4290	4620	6364	5829	8030	5826	± 14.9
5828	4730	5100	5829	4851	9006	5890	± 15.9
8316	4640	4161	6640	4940	8393	6181	± 18.7
5772	4290	6643	5168	4260	10824	6159	± 24.5
8732	4674	3666	5580	5616	8733	6166	± 21.1
-	3900	3480	5700	4290	8023	5078	± 18.4
-	5124	3285	9240	4884	7900	6086	± 24.2
8442	3969	4060	4606	4554	7938	5594	± 20.3
6300	4770	4316	6000	4160	8085	5605	± 14.9
5900	5676	3071	5964	4060	7950	5436	± 16.9
6615	3630	3816	8549	4216	8175	5883	± 22.3
5544	3172	4346	9126	3864	8295	5724	± 24.5
4599	3672	4814	7826	3016	8611	5423	± 22.7
6696	5040	2160	7626	5530	-	5410	± 20.4
5293	3888	3828	5625	2900	5214	4458	± 10.7
5476	3520	6136	2900	2730	6466	4538	± 16.8
6100	3432	5852	5272	1920	6570	4857	± 18.0
5040	2610	3478	5616	2030	5829	4100	± 16.1
3780	3520	3285	2387	1345	6720	3506	± 18.0
4615	3024	5775	4200	1523	7134	4378	± 19.8
4899	3168	2638	5332	1176	6460	3945	± 19.5
-	3528	2275	3612	1183	7546	3628	± 24.0
5382	3650	2400	4005	1776	6545	3959	± 17.8
3320	3220	2340	5456	2204	7308	3974	± 20.0
-	3555	2170	-	1944	6399	3517	± 20.4
	3200	3010	4757	2730	8648	4469	± 24.6
	2880	-	5146	2618	7280	4481	± 21.8
		3792	4526	3318	7050	4671	± 16.6
		2912	5100	3400	7735	4786	± 21.7
		2982	3500	2924	6776	4045	± 18.3
		3355	5475	3150	6314	4573	± 15.6
		2331	3150	3168	2736	2846	± 3.9
		2350	2836	3880	2664	2932	± 6.6
		1860	-	4087	2480	2809	± 11.4
		1920	3645	3248	2624	2859	± 7.5
		1749	3430		2262	2480	± 8.6
		2040	6450		2187	3559	± 25.0
		4736					
		4810	5621				
		4292	6000				
			6660				
			5372				
			6794				
			4704				
			5280				
			6794				

Percentage (%) absolute lymphocyte changes in calves fed with Gnidia latifolia (Meisn)

Calf Number

12	11	10	9	8	7
100 mg	100 mg	100 mg	100 mg	200 mg	200 mg
96.21	93.94	78.81	104.61	101.29	-
107.90	78.17	72.18	115.48	111.69	122.41
100.56	79.61	82.22	94.22	109.56	174 66
129.69	68.65	64.00	150.43	101.29	121 23
104.63	90.50	69.10	83.01	110.36	183.40
96.12	69.13	70.58	78.80	92.08	-
94.65	78.71	114.42	74.38	120.99	-
95.11	73.39	57.04	91 93	93.71	177 31
96.87	67 04	74.30	97.73	112.63	132 32
95.25	65.43	73.85	69.54	134.02	123.92
97.95	67.94	105.86	86.41	85.71	138.94
99.38	62.27	113.01	98.41	74.89	116 44
103.17	48.60	96.91	109.01	86.70	96 59
-	89.12	94.43	48.91	119.00	140 64
62.47	46.73	69.65	86.68	91.80	111 17
77.47	43.99	35.91	138.94	83.11	115 01
78.72	30.94	65.28	132.51	81.03	128 12
69.84	32.71	69.54	78.75	61.62	105.86
80.51	21.67	29.56	74.38	83.11	79.39
85.47	24.54	52.01	130.77	71.40	96 93
77.40	18.95	66.03	59.73	74.80	102.89
90.41	19.06	44.73	51.51	83.30	-
78.42	28.62	49.59	54.34	86.18	113 04
87.56	35.51	67.56	55.98	76.03	69.73
76.67	31.32	-	49.13	83.94	
103.61	43.99	58.91	68.16	75.56	
87.22	42.19	63.72	-	68.00	
84.47	53.47	56.04	85.86		
92.67	54.79	63.15	65.94		
81.18	47.12	43.34	67.52		
75.65	50.76	67.80	75.97		
32.78	51.05	39.00	52.78		
31.91	62.53	35.12	53.21		
29.71	65.86	-	42.11		
31.44	52.34	45.13	43.47		
27.10		42.47	39.60		
26.20		79.87	46.19		
		-	107.24		
		69.60	108.92		
		-	-		
		74.30	97.19		
		82.47			
		66.52			
		-			
		84.13			
		58.25			
		-			
		65.38			
		84.13			
		-			

Percentage (%) Drop in Absolute lymphocyte counts

Calf Numbers

Dose per Kg	6	5	4	3	2	1
	500 mg	500 mg	1 gm	1 gm	2 gm	2 gm
2	112.0	116.6	91.45	102.24	100.12	82.22
3	97.4	103.57	79.87	86.04	111.04	86 81
4	83.17	65.84	-	75.20	106.47	50 09
5	91.47	89.91	70.10	63.27	94.31	
6	59.25	51.42	75.91	54.44	90.38	
7	66.31	-	68.88	43.06	113.45	
8	76.29	78.75	62.31	48.33	97.82	
9	57.27	127.96	71.81	48.88	114.66	
10	50.70	90.62	75.23	45.93	93.05	
11	78.86	158.57	92.42	69.24	101.27	
12		118.57	61.55	46.82	81.51	
13		78.61	58.13	64.55	85.75	
14		102.41	52.71	56.66	81.19	
15		75.0	55.08	68.33	50.02	
16		60.15	37.35	70.15	35.36	
17		143.75	29.45	81.22	33.23	
18		39.28		61.38	62.87	
19		61.42			30.34	
20		85.37				
21		65.62				
22		29.91				
23		55.0				
24		27.12				
25		39.01				
26		32.36				
27		24.37				
28		42.72				
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Absolute neutrophil count of calves fed Gnidia latifolia (Meisn)

1	2	3	4	5	6
1248	300	900	966	1120	3180
1092	1479	1498	3168	1846	360
2024	1288	880	1090	3360	360
1512	1144	2632	-	2050	2668
	1680	1005	1680	1272	1470
	490	2100	1776	896	780
	516	2856	940	(x)	3249
	1530	1392	1246	630	1739
	1330	550	1680	567	1243
	1870	1166	945	2870	(x)
	984	1368	1548	2496	2365
	792	637	1440	1088	
	390	1190	1190	1428	
	715	900	1826	1612	
	1836	1350	5412	640	
	1080	1309	2812	2805	
	1332	1204	3082	2760	
	1120	910		(x)	
	368			1548	
				1173	
				1260	
				(x)	
				1936	
				1485	
				2052	
				2550	
				2730	
				1353	

Absolute neutrophil count of calves fed Gnidia latifolia (Meisn)

7	8	9	10	11	12	Mean SD
2001	1210	1856	1425	949	2247	1600 ± 500
2889	1155	1380	2236	1260	2596	1900 ± 740
3384	770	900	1360	1430	2166	1600 ± 970
4752	1102	1539	2432	1680	2507	2300 ± 1300
5217	1100	2457	1900	1584	1476	2200 ± 1500
5772	1026	2320	2728	2442	3567	2900 ± 1500
2883	900	1170	4700	1848	3051	2400 ± 1400
2871	976	1624	1751	1950	2100	1800 ± 600
4556	2268	884	2457	1508	1764	2200 ± 1200
3700	530	629	1274	1984	2310	1700 ± 1100
3800	858	1431	1581	1680	2226	1900 ± 1000
3780	1650	901	1540	2184	2507	2000 ± 990
2992	2928	928	2100	2212	2205	2200 ± 740
2482	2992	2640	3528	1950	2180	2600 ± 560
24188	1260	1972	1440	1170	3922	2000 ± 1040
2607	3822	4264	750	812	2340	2400 ± 1400
1702	1824	1694	868	(x)	1200	1400 ± 810
3500	1782	1215	3612	(x)	1392	2300 ± 1300
2016	1296	1925	4005	624	1870	1900 ± 1100
2220	1925	1225	1943	696	2156	1700 ± 600
1820	1664	2400	3154	1131	1305	1900 ± 750
2059	1890	1224	2325	782	1296	1600 ± 580
2340	1870	1400	1950	882	1729	1700 ± 500
1680	1680	1247	1305	1581	2350	1600 ± 390
(x)	1380	2520	1470	1204	1309	1500 ± 530
(x)	1372	1218	1679	966	864	1000 ± 320
	1120	2145	2000	1404	1036	1500 ± 500
	1350	2256	1989	1952	620	1600 ± 650
		1452	1462	2240	638	1400 ± 650
		1690				1690

Appendix IX Total White Cell counts - Control calves

Days	Calf numbers 13	14	15	16	Mean SD
1	12600	8000	6600	11200	9600 ± 2700
2	16800	7600	6300	7800	9625 ± 4800
3	14200	11200	6300	9800	10375 ± 3200
4	11700	7000	6500	6900	8025 ± 2400
5	13600	9400	6800	9100	9725 ± 2800
6	10400	10300	6400	7100	8550 ± 2000
7	8300	11600	5900	8500	8575 ± 2300
8	8300	13900	5500	8200	8975 ± 3500
9	9500	11400	5200	9200	8825 ± 2600
10	11200	10500	5600	8600	8975 ± 2500
11	12600	9500	5500	7800	8850 ± 2900
12	9400	9800	5500	8700	8350 ± 1900
13	10400	8400	5400	6900	7775 ± 2100
14	9600	13100	5700	7700	9025 ± 3100
15	11200	9900	5300	7900	8575 ± 2500
16	10400	9500	6500	9400	8450 ± 1600
17	9400	8500	5700	9400	8250 ± 1700
18	9600	9800	5400	8200	8250 ± 2000
19	9100	10500	7900	8600	9025 ± 1000
20	9900	9000	5900	7600	8100 ± 1700
21	7700	9400	6100	7100	7575 ± 1300
22	8800	9400	6200	7900	8075 ± 1300
23	10600	9600	6100	8300	8650 ± 1900
24	10300	9700	5900	8500	8600 ± 1900
25	10700	9900	5400	8100	8525 ± 2300
26	10300	8500	6500	8600	8475 ± 1500
27	11500	10400	6800	8400	9275 ± 2000
28	9400	9700	5900	7900	8225 ± 1700
29	9600	9900	6400	7400	8325 ± 1600
30	9400	9800	6200	7700	8275 ± 1600
31	7700		6500	8200	7467 ± 800

Absolute lymphocyte counts - control calves

Calf numbers					
13	14	15	16	Mean	SD
10769	5920	4340	6384	6853	± 2700
14616	4864	4788	4378	7162	± 4900
12780	7616	4725	4366	7372	± 3800
8658	6580	4680	5498	6354	± 1700
10880	6580	5236	5964	7165	± 2500
9776	5974	4672	4569	6247	± 2400
7802	8584	3717	6219	6580	± 2100
7470	8757	4070	5120	6354	± 2100
8930	7752	3900	5240	6455	± 2200
10080	6720	4032	5438	6567	± 2500
10458	7600	3905	5729	6923	± 2700
8084	7644	4345	5368	6360	± 1700
7416	6888	4374	4780	5864	± 1500
8832	7729	3640	4996	6299	± 2300
8736	7623	3656	4610	6156	± 2400
8112	7980	3900	5148	6285	± 2000
7896	6800	3792	5690	6044	± 1700
8256	7252	3920	5343	6192	± 1900
7644	7665	3886	6138	6333	± 1700
9009	6480	4108	5520	6279	± 2000
6622	8742	4354	5660	6344	± 1800
7744	8272	4266	6000	6570	± 2400
9964	8736	4819	5658	7294	± 2400
9064	8148	4956	4983	6787	± 2100
8346	7722	4140	5158	6341	± 2000
8549	7055	3952	5300	6214	± 2000
9200	8320	5185	5468	7043	± 2000
7426	8342	4956	5634	6589	± 1500
8160	7920	3996	5430	6376	± 2000
8178	8232	4263	5136	6452	± 2000
7084		4287	5484	5618	± 1400

Appendix X Serum Albumin Levels (gm/100 ml)

Treated calves

Days of Treatment	1	2	3	4	5	6
1	3.0	4.90	2.35	3.35	2.20	2.60
2	2.85	4.65	2.70	3.75	2.65	2.35
3	2.85	3.25	2.35	3.10	3.10	2.60
4	3.0	3.10	2.50	4.10	2.85	2.35
5	2.90	3.60	2.58	3.90	2.85	2.60
6		3.10	2.75	3.90	2.90	2.50
7		3.25	2.85	3.10	2.95	2.60
8		2.75	2.95	2.90	2.95	
9		3.60	2.75	3.90	2.85	
10		3.60	3.40	2.90	3.00	
11		3.40	2.70	3.10	2.75	
12		3.40	2.75	3.10	3.00	
13		2.90	2.65	2.85	3.10	
14		3.10	2.50	3.60	3.25	
15		3.40	2.50	3.75	2.95	
16		3.40	2.65	3.40	3.10	
17		3.25	2.75	3.90	3.00	
18		2.75	2.75	3.40	2.95	
19		2.75		2.90	3.00	
20					3.15	
21					2.85	
22					3.00	
23					3.10	
24					3.10	
25					2.60	
26						
27						
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Serum Albumin levels (gm/100 ml)

Treated Calves					Control Calves		
8	9	10	Mean	SD	13	14	Mean
5.85	3.50	2.75	3.38	± 1.16	5.25	6.40	5.82
3.40	2.25	2.90	3.05	± 0.50	4.25	5.75	5.00
3.10	2.90	3.05	2.92	± 0.28	3.35	3.50	3.42
3.10	2.25	2.90	2.90	± 0.58	3.40	3.25	3.42
3.10	2.40	3.10	3.00	± 0.55	3.75	3.40	3.57
3.75	2.25	3.00	3.01	+ 0.57	3.60	3.10	3.35
3.25	2.80	3.10	2.98	± 0.14	3.40	2.90	3.15
3.10	2.58	3.15	2.91	± 0.18	3.75	3.40	3.57
3.10	2.30	3.10	3.08	± 0.48	3.40	3.40	3.40
3.10	2.50	3.15	3.09	± 0.28	3.40	3.40	3.40
3.60	2.50	3.25	3.04	± 0.37	2.75	3.60	3.17
3.20	2.75	3.25	3.06	± 0.20	3.40	3.70	3.55
3.25	2.50	3.40	3.00	± 0.32	4.60	3.60	3.60
3.25	2.60	3.50	3.11	± 0.70	3.00	3.50	3.25
3.75	2.75	3.25	3.19	± 0.47	3.75	3.25	3.50
3.40	2.60	3.20	3.10	± 0.32	3.60	3.35	3.47
3.00	2.50	3.10	3.07	± 0.43	3.25	2.90	3.07
2.90	2.50	3.35	2.94	± 0.32	2.90	4.40	3.65
3.25	2.35	3.15	2.90	± 0.31	3.30	2.75	3.02
3.10	2.45	3.50	3.05	± 0.38	3.75	2.90	3.32
3.25	2.35	2.85	2.82	± 0.32	2.90	3.50	3.20
2.80	2.40	3.00	2.80	± 0.24	2.80	3.10	2.95
2.90	2.35	3.75	3.02	± 0.50	3.20	3.10	3.15
1.90	2.50	3.25	2.68	± 0.53	2.40	3.25	2.82
3.60	2.25	3.00	2.86	± 0.50	3.35	3.45	3.40

Appendix XI

Serum Alkaline Phosphatase

(King/Armstrong units per ml)

Treated calves

Days of Treatment	1	2	3	4	5	6
1	4.5	7.35	5.14	7.66	6.25	13.5
2	4.55	9.65	7.05	6.70	6.78	8.10
3	4.70	7.0	6.25	5.67	5.95	9.70
4	3.73	10.0	7.12	6.70	5.15	6.60
5	3.62	9.0	2.61	6.50	9.15	7.80
6		7.75	5.0	6.50	7.35	7.0
7		13.40	5.85	7.20	4.95	12.85
8		12.0	5.95	6.70	7.60	
9		13.35	4.60	10.65	6.95	
10		13.35	4.85	10.65	5.78	
11		11.36	4.85	9.35	6.65	
12		7.66	5.25	5.34	6.25	
13		5.35	6.67	4.00	6.75	
14		6.35	8.35	4.00	5.95	
15		7.25	7.50	5.50	4.60	
16		5.87	8.64	5.05	4.85	
17		6.95	7.75	5.50	5.85	
18		8.20	8.90	6.65	4.85	
19		15.80		9.70	11.10	
20					5.70	
21					5.20	
22					5.70	
23					5.95	
24					4.33	
25					5.67	
26						
27						
28						
29						
30						
31						
32						

Serum Alkaline Phosphatase
(King/Armstrong Unit/ml)

Treated Calves				Control calves		
8	9	10	Mean	13	14	Mean
6.00	6.20	9.60	7.35 ± 2.92	10.30	8.67	9.48
8.67	5.90	7.05	7.16 ± 3.06	11.0	6.00	8.50
6.00	4.85	10.30	6.71 ± 2.13	7.35	10.00	8.67
8.00	5.75	8.20	6.80 ± 1.18	11.3	13.3	12.3
7.10	7.95	10.90	7.18 ± 2.81	8.70	11.3	10.0
6.50	6.75	13.30	7.51 ± 2.87	7.10	8.70	7.90
8.50	4.55	6.97	8.03 ± 2.81	12.0	12.5	12.25
11.00	6.05	13.00	8.90 ± 3.00	10.00	11.0	10.5
8.00	5.25	7.50	8.04 ± 3.05	13.35	12.70	13.02
8.0	5.14	11.60	8.48 ± 2.91	13.35	12.70	13.02
12.0	5.15	12.00	8.75 ± 3.10	11.0	16.6	13.80
7.35	4.85	12.40	7.01 ± 2.8	12.50	11.0	11.75
6.70	5.15	10.80	6.48 ± 2.1	6.00	9.35	7.67
6.0	4.60	9.05	6.32 ± 1.83	5.65	7.30	6.47
10.00	5.00	8.65	6.92 ± 2.18	7.60	6.90	7.25
6.55	4.85	9.65	6.49 ± 1.93	6.90	9.00	7.95
6.95	5.85	7.85	6.67 ± 0.95	6.20	8.95	7 57
8.50	4.85	10.0	7.42 ± 2.18	10.0		10.0
10.40	5.40	5.40	9.63 ± 3.91	14.50	17.0	15.75
9.20	5.25	10.0	7.53 ± 2.41	10.90	15.20	13.05
8.45	5.70	10.25	7.40 ± 2.37	11.50	8.50	10.0
7.50	5.95	10.80	7.48 ± 2.34	7.80	8.10	7.95
5.30	5.40	10.56	6.82 ± 2.52	6.55	11.85	9.20
8.75	4.16	11.30	7.13 ± 3.49	9.70	9.35	9.52
7.35	3.62	6.75	5.84 ± 1.63	6.65	8.65	7.65
7.10	4.70	8.55	6.78 ± 1.94	7.35	15.2	11.27
18.0	4.26	10.80	11.02 ± 6.87	11.00	17.00	14.0
24.80	3.78	7.15	11.91	12.4	15.2	13.8
28.0	4.30	14.70	15.66	26.30	17.00	14.8
22.80	7.40	12.65	14.28	11.2	14.9	13.05
21.0	4.66	9.80	12.15	12.62		12.62
	4.85					

Appendix XII Serum G.O.T. Levels (Reitman-frankel Units/ml)

Teated calves

Days of Treatment	1	2	3	4	5	6
1	62	82.0	41.0	55.0	103.0	105.0
2	58	60.0	60.0	60.0	103.0	86.0
3	70	100.0	36.0	47.0	60.0	81.0
4	66	41.0	88.0	39.0	62.0	60.0
5	53	41.0	46.0	100.0	61.0	41.0
6		100.0	97.0	70.0	56.0	42.0
7		100.0	103.0	47.0	47.0	78.0
8		70.0	75.0	70.0	56.0	
9		51.0	79.0	55.0	60.0	
10		59.0	110.0	55.0	70.0	
11		60.0	122.0	88.0	70.0	
12		70.0	140.0	64.0	60.0	
13		41.0	110.0	51.0	65.0	
14		44.0	120.0	51.0	46.0	
15		47.0	107.0	44.0	65.0	
16		41.0	92.0	55.0	87.0	
17		66.0	100.0	64.0	92.0	
18		47.0	88.0	280.0	46.0	
19		51.0		70.0	69.0	
20					60.0	
21					43.0	
22					56.0	
23					56.0	
24					79.0	
25						
26						
27						
28						
29						
30						
31						
32						

Serum G.O.T. Levels (Reitman-Frankel units/cm^6)

Treated Calves					Control Calves		
8	9	10	Mean	SD	13	14	Mean
34.0	88.0	94.0	73.77	± 26.5	32.0	40.0	36.00
34.0	107.0	107.00	75.00	± 26.4	34.0	36.0	35.00
29.0	56.0	41.0	57.77	± 22.8	32.0	44.0	38.00
22.0	88.0	74.0	60.00	± 22.5	41.0	32.0	36.50
70.0	80.0	63.0	61.66	± 19.5	64.0	103.0	33.50
39.0	84.0	60.0	68.50	± 23.4	41.0	44.0	42.50
22.0	76.0	52.0	65.62	± 28.3	47.0	70.0	58.50
47.0	63.0	50.0	61.57	± 10.8	51.0	51.0	51.00
47.0	76.0	47.0	59.28	± 13.2	60.0	44.0	52.00
47.0	80.0	56.0	68.14	± 21.3	60.0	44.0	52.00
55.0	74.0	63.0	76.00	± 22.0	55.0	51.0	53.00
41.0	63.0	74.0	73.14	± 31.2	47.0	55.0	51.00
44.0	69.0	63.0	63.28	± 23.2	47.0	37.0	42.00
42.0	44.0	46.0	56.14	± 28.2	49.0	55.0	52.00
44.0	74.0	65.0	63.71	± 22.5	51.0	51.0	51.00
47.0	87.0	87.0	70.85	± 22.1	51.0	55.0	53.00
27.0	98.0	98.0	77.85	± 27.1	34.0	64.0	49.00
24.0	76.0	60.0	88.71	± 87.2	24.0	-	24.0
47.0	60.0	56.0	58.83	± 9.3	22.0	22.0	22.00
24.0	66.0	52.0	50.50	± 18.5	19.5	37.0	28.25
22.0	64.0	60.0	47.25	± 19.1	27.0	13.0	20.00
21.0	61.0	46.0	46.00	± 17.7	19.5	-	19.5
-	66.0	56.0	59.33	± 4.7	-	30.0	30.00
18.0	-	-	48.50	±	25.0	29.0	27.00
24.0	48.0	48.0	40.00	± 13.8	24.0	32.0	28.00
22.0	90.0	65.0	59.00	± 34.3	24.0	29.5	26.15
28.0	86.0	59.0	57.66	± 29.0	25.0	36.0	30.5
14.0	80.0	48.0	47.33	± 33.0	21.0	34.0	27.5
22.0	66.0	39.5	42.50	± 22.1	27.0	36.0	31.5
24.0	46.0	32.0	34.00	± 11.1	27.0		27.0
	43.0		43.00				
	68.0		68.00				

Levels of Blood Urea Nitrogen (mg/100 ml)

Treated Calves

Days of Treatment	2	4	5	8	9	10	Mean ± SD
1	15	10	12	10	12	15	12.33 ± 2.28
2	15	10	10	5	15	15	11.66 ± 4.08
3	10	10	5	5	10	5	7.50 ± 2.73
4	15	20	15	10	5	5	11.66 ± 6.05
5	20	5	15	10	10	10	11.66 ± 5.16
6	10	5	12	5	15	20	11.66 ± 5.84
7	10	10	5	5	5	5	6.66 ± 2.58
8	5	20	5	5	5	5	7.50 ± 6.12
9	10	10	15	5	5	5	8.33 ± 4.08
10	10	10	15	5	20	5	10.83 ± 5.84
11	15	15	5	10	5	5	9.16 ± 4.91
12	15	10	5	10	5	5	8.33 ± 4.08
13	10	10	5	10	5	5	7.50 ± 2.73
14	10	5	5	15	5	5	9.16 ± 4.18
15	10	15	5	10	5	5	8.33 ± 4.08
16	25	5	5	10	5	5	9.16 ± 8.01
17	20	30	5	15	5	5	13.33 ± 10.32
18	20	20	10	5	5	5	9.16 ± 5.84
19	10	30	25	10	5	5	14.16 ± 10.68
20			35	10	5	5	13.75 ± 14.36
21			30	10	5	10	13.75 ± 11.08
22			35	5	5	10	13.75 ± 14.36
23			40	10	5	20	18.75 ± 15.47
24			50	5	20	20	23.75 ± 18.87
25			102	5	15	5	31.75 ± 47.06
26				10	15	5	10.0 ± 5.0
27				10	5	10	8.33 ± 2.88
28				5	5	5	5.00 ± 0
29				5	5	5	5.00 ± 0
30				10	5	5	6.66 ± 2.88
31				15	5	5	8.33 ± 8.41
32					5		5.0

The Measurements of Lactate Dehydrogenase (LDH) in Serum of Calves fed Gnidia latifolia (Meisn) (Wacker Units)

Days of Treatment	Calf Numbers 1	3	5	6	9	10	Mean SD
1	333	274	290	167	331	320	285 ± 62.7
2	318	126	331	211	331	316	272 ± 84.9
3	296	334	274	118.2	252	264	256 ± 73.5
4	361	223	143	71	178	186	193 ± 96.7
5		341	207	405	230	232	235 ± 85.7
6		323	230	768	247	227	359 ± 231.9
7		276	202	988	227	243	387 ± 322.2
8		248	246		210	252	239 ± 19.4
9		318	204		271	258	262 ± 46.8
10		274	283		286	320	290 ± 20.1
11		288	311		295	278	293 ± 13.8
12		267	295		346	396	326 ± 56.9
13		253	290		300	300	285 ± 22.3
14		365	286		308	352	327 ± 37.0
15		357	248		290	340	308 ± 49.4
16		358	276		386	387	326 ± 54.2
17		326	298		290	315	307 ± 16.2
18		330	305		327	346	327 ± 16.8
19			295		311	315	307 ± 10.5
20					295	278	286.5
21					276	292	284
22					305	185	245
23					285	210	247.5
24					257	164	210.5
25					206.5	145	175.7
26					230	274	252
27					246	124.0	185
28					132.4	164.0	148.2
29					285	373.0	329
30					422	852	637
31					920	724	822
32					814		814

Daily total weights (gm) of rat groups treated with *Gnidia latifolia* (Meisn) extract

Days of Treatment	Rat Groups 1	2	3	4	Control
0	595.0	527.0	559.0	545.0	495.5
1	625.0	598.0	594.0	565.0	509.0
2	504.0	531.0	594.5	580.0	578.0
3	638.0	633.0	*518.0	551.0	609.0
5	710.0	648.0	611.0	601.0	649.0
8	716.0	736.0	683.0	705.0	773.0
9	732.0	773.0	735.0	777.0	830.0
10	*639.0	748.0	731.0	773.0	832.0
11	(D.8)	781.0	764.0	810.0	818.0
12	(D.1)	767.0	793.0	841.0	822.0
15	-	733.0	737.0	790.0	833.0
16	-	654.0	761.0	690.0	823.0
18		790.0	858.0	856.0	978.0
19		808.0	916.0	888.0	1013.0
20		828.0	924.0	923.0	1137.0
21		892.0	981.0	978.0	1217.0
22		913.0	992.0	1005.0	1293.0
23		894.0	1004.0	1026.0	1316.0
24		888.0	986.0	971.0	1340.0
25		923.0	986.0	1004.0	1376.0
26		897.0	948.0	970.0	1257.0
27		940.0	997.0	1001.0	1389.0

* = one rat died in the group

D-X = number of rats dying in the group

Rat Groups

6	7	8	9
485.0	493.0	474.0	572.0
514.0	502.0	516.0	592.0
526.0	542.0	511.0	603.0
583.0	555.0	559.0	625.0
592.0	611.0	629.0	686.0
710.0	692.5	655.0	791.0
770.0	782.0	645.0	854.0
740.0	786.0	694.0	811.0
805.0	821.0	707.0	888.0
816.0	851.0	685.0	911.0
845.0	877.0	778.0	908.0
787.0	*762.0	792.0	894.0
788.0	937.0	946.0	1082.0
875.0	1000.0	957.0	1131.0
(D.4)	1037.0	1024.0	(D.2)
552.0	1089.0	1126.0	958.0
593.0	1126.0	1152.0	1013.0
610.0	1070.0	1147.0	1015.0
628.0	1101.0	1170.0	996.0
629.0	1112.0	1167.0	946.0
627.0	1008.0	1175.0	919.0
663.0	114. 0	1121.0	934.0

The mean body weight gains (gm) per rat group

Rat Groups

Days of Treatment	1	2	3	4	Control
0	595.5	52.7	55.9	54.5	46.55
1	3.00	7.10	3.50	2.00	1.35
2	9.10	0.40	3.55	3.50	8.30
3	4.30	10.60	1.65	0.60	11.30
5	11.50	12.10	5.77	5.60	15.35
8	12.60	20.90	13.77	16.00	27.15
9	13.70	24.60	25.76	23.20	33.45
10	11.50	22.10	25.32	22.80	33.65
11	(D.8)	25.40	28.98	26.50	32.25
12	(D.1)	24.00	32.21	29.90	32.65
15		20.60	25.98	24.50	33.75
16		12.70	28.65	22.16	32.75
18		26.30	39.43	40.61	48.25
19		28.10	45.87	44.16	51.75
20		30.10	46.76	48.05	64.15
21		36.50	53.10	54.16	72.15
22		38.60	54.32	57.16	79.75
23		46.63	55.65	59.50	82.05
24		45.95	53.65	53.38	84.45
25		49.85	53.65	57.05	88.05
26		46.96	49.43	53.27	76.15
27		51.74	54.87	56.72	89.35

Rat Groups

6	7	8	9
48.5	49.3	47.4	57.2
2.90	0.90	4.20	2.00
4.10	4.90	3.70	3.10
9.80	6.20	8.50	5.30
10.70	11.80	15.50	11.40
22.50	19.95	18.10	21.90
28.50	28.90	17.10	28.20
25.50	29.30	22.00	23.90
32.00	32.80	23.30	31.60
33.10	35.80	21.10	33.90
36.00	38.40	30.40	33.60
30.20	35.36	33.80	32.20
30.30	54.81	47.20	51.00
39.00	61.81	48.30	55.90
61.90	65.92	55.00	(D.2)
61.90	71.70	65.20	62.55
70.10	75.81	67.80	69.42
73.50	69.58	67.30	69.67
77.10	73.03	69.60	67.30
77.30	74.25	69.30	61.05
76.90	62.70	70.10	57.67
84.10	74.47	64.70	59.55

Percentage (%) gain in Mean Body weights of rats
Treated with Gnidia latifolia (Meisn) extract

Days of Treatment	Rat Groups 1	2	3	4	Control
0	59.5	52.7	55.9	54.5	49.55
1	5.04	13.47	6.26	3.66	2.72
2	-15.29	0.75	6.35	6.42	16.75
3	7.22	20.11	2.95	1.10	22.80
5	19.32	22.96	10.32	10.27	30.97
8	21.17	39.65	24.63	29.35	54.78
9	23.02	46.67	46.08	42.56	67.50
10	19.32	41.93	45.29	41.83	67.91
11	(D)	48.19	51.84	48.62	65.08
12		45.54	57.62	54.86	65.89
15		39.08	46.47	44.95	68.11
16		24.09	51.25	40.66	66.09
18		49.90	70.53	74.51	97.37
19		53.32	82.05	81.02	104.43
20		57.11	83.64	88.16	129.46
21		69.25	94.99	99.37	145.61
22		73.24	97.17	104.88	160.94
23		88.48	99.55	109.17	165.59
24		87.19	95.97	97.94	170.43
25		94.59	95.97	104.67	177.69
26		89.10	88.42	97.74	153.68
27		98.17	98.15	104.07	180.32

Rat Groups

6	7	8	9
48.5	49.3	47.4	57.2
5.97	1.82	8.86	3.49
8.45	9.93	7.80	5.41
20.20	12.57	17.93	9.26
22.06	23.93	32.70	19.93
46.39	40.46	38.18	38.28
58.76	58.62	36.07	49.30
52.57	59.43	46.41	41.78
65.97	66.53	49.15	55.24
68.24	72.61	44.51	59.26
74.22	77.89	64.13	58.74
62.26	71.72	71.30	56.29
62.47	111.17	99.57	89.16
80.41	125.37	101.89	97.72
	133.71	116.03	
127.62	145.43	137.55	109.35
144.53	153.77	143.03	121.36
151.54	141.13	141.93	121.80
158.96	148.13	146.83	117.65
159.38	150.60	146.20	106.73
158.55	127.18	147.89	100.82
173.40	150.44	136.49	104.10