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EXPERIMENTALLY-INDUCED *CESTRUM LAEVIGATUM* (SCHLECHTD.) POISONING IN SHEEP

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ABSTRACT

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Dried, milled *Cestrum laevigatum* plant material was drenched to 6 ewes at doses ranging from 2,5 to 10 g/kg/day for 1 to 47 days.

The most noticeable clinical signs were depression, anorexia and ruminal stasis. These signs were accompanied by clinical pathological changes indicative of liver involvement such as increases in the serum activities of aspartate transaminase, lactate dehydrogenase and gamma-glutamyltransferase.

Hepatosis characterized by accentuated lobulation, and centrilobular to midzonal coagulative necrosis, haemorrhage and congestion occurred in 2 of the 3 ewes given high doses of plant material. Liver lesions in the other animals included disappearance of hepatocytes and collapse of the reticulin stroma in the centrilobular areas. Spongy changes in the cerebral white matter were evident in the ewes of the high-dose group. Ultrastructural changes in the liver comprised degeneration and necrosis of hepatocytes and occasionally endothelial cells, and disruption of sinusoidal walls.

INTRODUCTION

Cestrum laevigatum (Schlechtd.), commonly known as ink-berry, is a perennial shrub or tree belonging to the Solanaceae. The plant is native to South America and was introduced into southern Africa as evergreen ornamental shrubs, hedges or windbreaks in gardens (Steyn, 1934). It has spread rapidly in South Africa and is found mostly in the moister eastern parts of the country (Vahrmeyer, 1981).

Thorburn (1934) identified *C. laevigatum* as the cause of Chase Valley disease in cattle which had occurred in the Chase Valley near Pietermaritzburg for many years. Acute inflammation and fatty degeneration to cirrhosis of the liver with oedema and petechiae in the wall of the gall-bladder were reported in steers which were dosed with toxic plant material. Macroscopical liver lesions similar in nature were produced in sheep. Döbereiner, Tokarnia & Canella (1969) reported centrilobular necrosis, congestion and haemorrhage of the liver in natural and experimental poisoning in cattle from Brazil.

Two other *Cestrum* spp., namely *C. aurantiacum* (Shone & Drummond, 1965; Mugera & Nderito, 1968) and *C. parqui* (Lavers, 1953; McLennan & Kelly, 1984) are also primarily hepatotoxic and produce clinical signs and lesions similar to those described with *C. laevigatum* poisoning. *Cestrum parqui* is found in South Africa (Watt & Breyer-Brandwyk, 1962), but poisoning with it has not been reported.

Clinical features and pathological changes in steers dosed with toxic *C. laevigatum* plant material have been described in detail (Van der Lugt, Nel & Kitching, 1991). In this paper the clinical and pathological findings in sheep dosed with the same plant material are reported.

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MATERIALS AND METHODS

The materials and methods for the collection and storage of the plant material, clinical examination, clinical pathology and the macroscopical, microscopical and transmission electron microscopical pathology were described previously (Van der Lugt *et al.*, 1991).

In this study 7 Dohne Merino ewes, 10 to 18 months old, which had not previously been exposed to the plant, were used. Milled *Cestrum laevigatum* plant material was mixed with water and administered intraruminally by means of a stomach tube to 6 ewes at dosage levels ranging from 2,5 to 10 g/kg/day and intervals of 1 to 47 days (Table 1). The seventh animal did not receive plant material and served as a control. The sheep, which were obtained from Mara Research Station (Northern Transvaal), were vaccinated against heartwater, bluetongue, enterotoxaemia and *Corynebacterium pseudotuberculosis* infection, and dewormed with a broad spectrum anthelmintic. During the day the animals were kept outside in pens with concrete floors. At night, the sheep were housed in individual pens in the stables of the Section of Toxicology, Veterinary Research Institute (VRI). The animals were given a pelleted ration (Onderstepoort formulation) and were supplied *ad libitum* milled lucerne hay (*Medicago sativa* L.). Drinking water was available at all times.

RESULTS

Clinical signs

Ewe 1 was depressed and recumbent prior to death on the morning of Day 1. Ewe 2 was euthanized on Day 1 after it developed severe depression, anorexia and ruminal stasis followed by recumbency and an increased respiratory rate. On Day 1 ruminal stasis and on Days 3 and 4 congestion of the visible mucous membranes were noted in Ewe 3. The animal was euthanized on Day 7.

Clinical signs were not observed in Ewes 4 and 5 and they were clinically normal at the time when euthanazia was performed. Ewe 6 was slightly

TABLE 1 Dosing regimen of *C. laevigatum* plant material to ewes

Group	Ewe	Initial body mass (kg)	Dose (g/kg × n*)	Dosing regimen			Duration of experiment (Day 0 to termination)	Fate of animal
				Days on which dosed	Total dose (g)	Total dose (g/kg)		
High dose	1	35	10 × 1	0	350	10	1	Died
	2	32	8 × 1	0	256	8	1	Euthanized
	3	31	6 × 1	0	186	6	7	Euthanized
Low dose	4	53	4 × 1	0	212	4	48	Euthanized
	5	56	2,5 × 48	0–47	6720	120	48	Euthanized
	6	41	2,5 × 14	0–13				
			3,5 × 23	14–36	4735,5	115,5	37	Euthanized
Control	7	51	—	—	—	—	48	Euthanized

*n = days

depressed, rumen movements decreased and the heart rate increased on Day 15. On the 2 subsequent days the ewe was recumbent, although it could rise reluctantly when stimulated. Thereafter the ewe recovered rapidly and was in good health when euthanized on Day 37. No clinical signs were observed in the control animal (Ewe 7).

Clinical pathology

The serum activities of the enzymes aspartate transaminase (AST) and lactate dehydrogenase (LD) rose rapidly in the sheep of the high-dose group. In Ewe 3 activities up to 30-fold predosing levels were reached after 48 h and, although gradually decreasing after 72 h, was still 2 to 3 times predosing levels at the time of euthanazia. In Ewes 4 to 6 peak activities of AST and LD of up to 10-fold predosing levels were reached after 6 days. The levels then diminished and returned to normal by Day 22 in Ewe 4, while in Ewes 5 and 6 two to 3 times predosing levels were recorded prior to death. Elevations in gamma-glutamyltransferase (GGT) activity were generally similar to those for AST and LD but of lower magnitude. The creatine kinase (CK) activities were within normal limits throughout the trial.

Macroscopical pathology

Liver and gall-bladder: The livers of Ewes 1 to 3 showed pathological changes which varied in degree from severe (Ewes 1 and 2) to mild (Ewe 3). In Ewes 1 and 2 the livers were moderately enlarged and deep red to almost purple-red. In addition, a few congested, poorly circumscribed patches, 5 to 20 mm in diameter, were distributed subcapsularly in Ewe 2. Both livers were friable on cut surface and oozed copious amounts of blood. Distinct lobulation coupled with centrilobular congestion and/or haemorrhage gave the livers a mosaic appearance (Fig. 1). The gall-bladder walls were severely oedematous, and in Ewe 1 the mucosa contained a few petechiae and ecchymoses (Fig. 2). The gall-bladders in these two animals were moderately distended with light green bile. The hepatic lymph nodes were moderately swollen and oedematous and there was mild oedema of the loose connective tissues about the hilus of the liver in Ewe 1. In Ewe 3 the liver was mildly swollen and yellowish-brown. The lobulation was slightly more distinct with light red sunken centrilobular areas surrounded by yellowish portal zones.

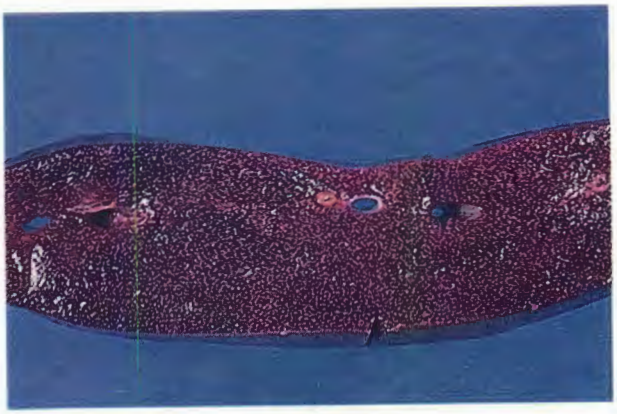


FIG. 1 Ewe 1. Cut surface of the liver with distinct lobulation

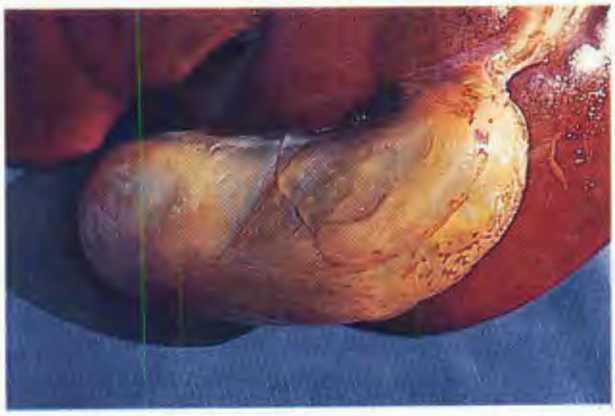


FIG. 2 Ewe 1. Severe oedema of the wall of the gall-bladder

Macroscopical lesions in Ewes 4 to 6 were limited to the liver and were mild. The livers were swollen and the lobulation slightly accentuated. In Ewe 4 this organ was greyish-brown in colour. No lesions were apparent in the gall-bladders.

Other organs: Moderate ascites occurred in Ewes 1 and 2; the abdominal cavity containing 750–1 000 mℓ of transparent light yellow fluid. The kidneys in these two animals were slightly swollen and the cortices were light brown. Numerous pete-

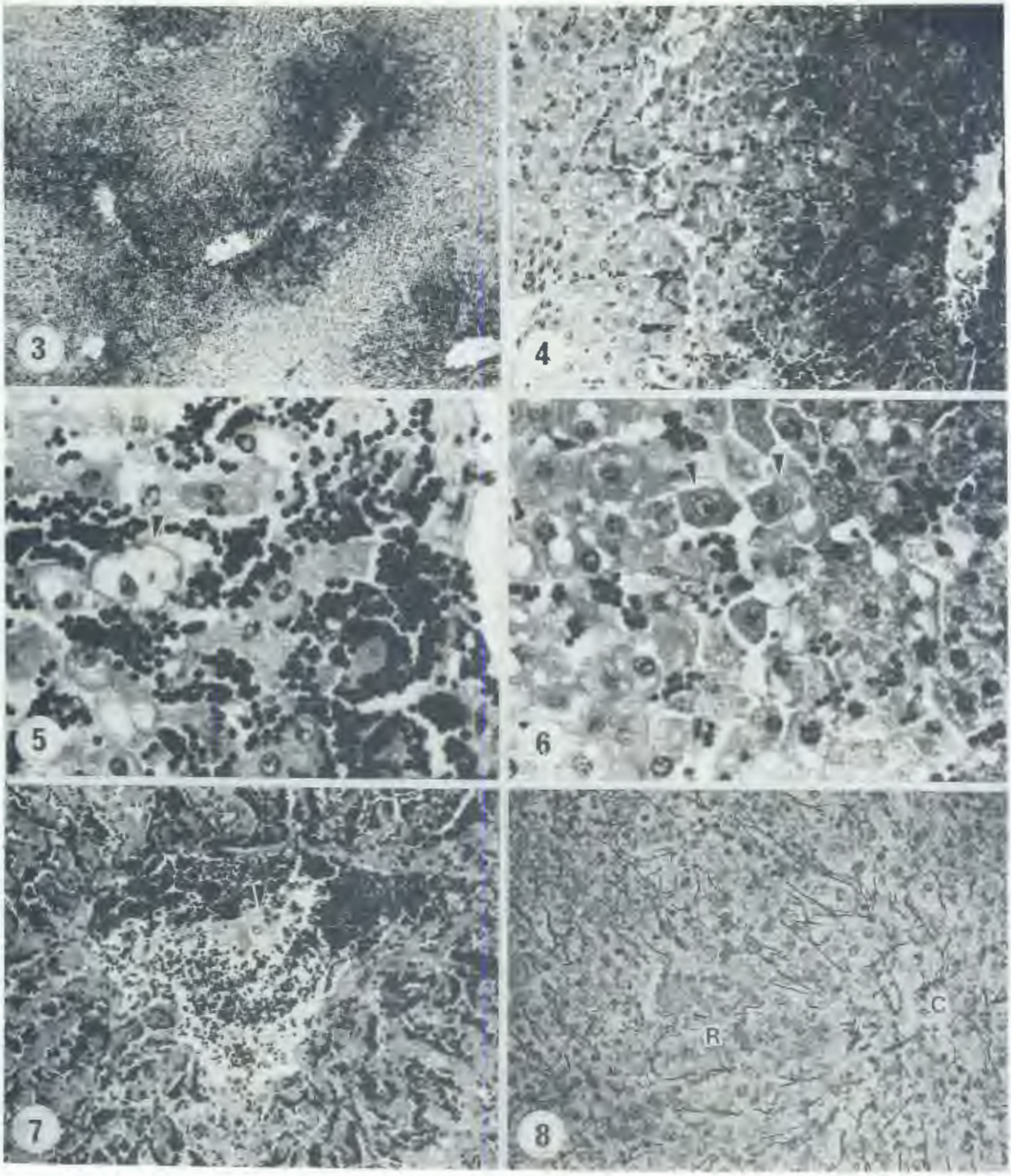


FIG. 3 Ewe 2. Centrilobular to midzonal necrosis, congestion and haemorrhage. HE × 40

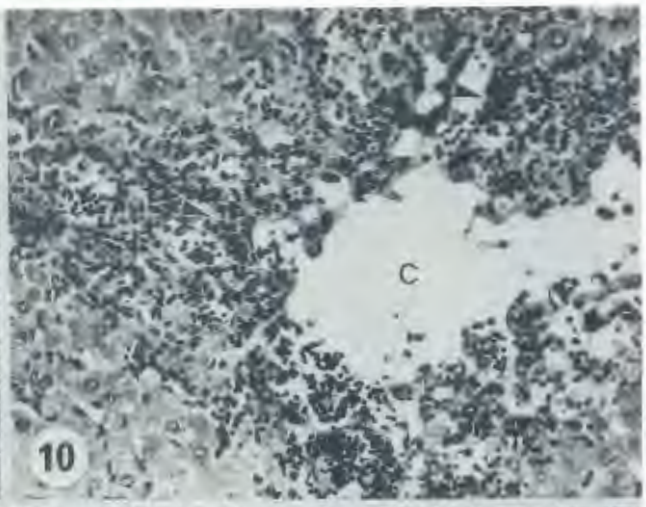
FIG. 4 Ewe 2. Central vein (at right) with surrounding necro-haemorrhagic zone. Degeneration of parenchymal cells at the periphery of the lobule (at left). HE × 200

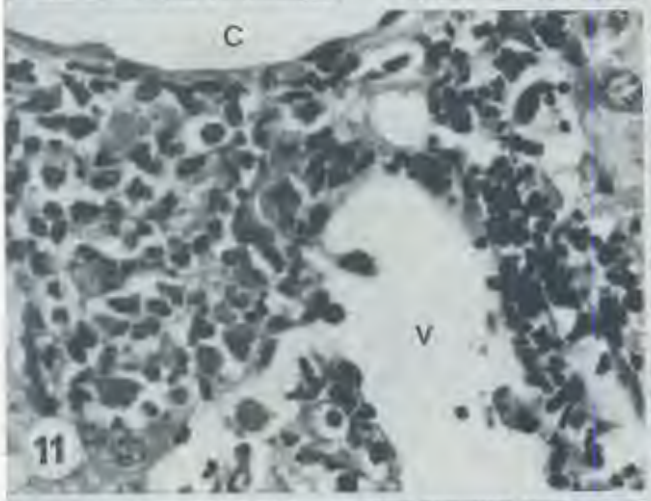
FIG. 5 Ewe 1. Degenerated peripheral hepatocytes containing hypoxic vacuoles (arrow head). Portal tract at right. HE × 600

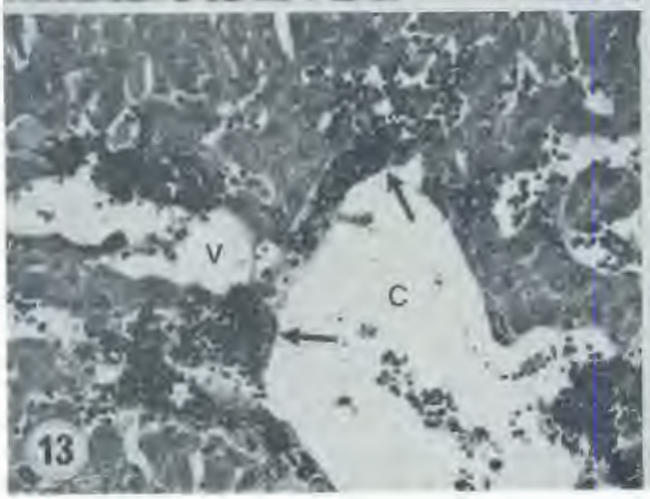
FIG. 6 Ewe 2. Necrotic midlobular parenchymal cells are separated from adjacent cells and their nuclei show karyopycnosis (arrow heads). HE × 600

FIG. 7 Ewe 1. Central vein contains hepatocytes and cellular debris (arrow head) and erythrocytes. HE × 300

FIG. 8 Ewe 1. Disruption and fragmentation of the reticulin network in the centrilobular area (R) and loss of the reticular outline around the central vein (C). GRI × 200







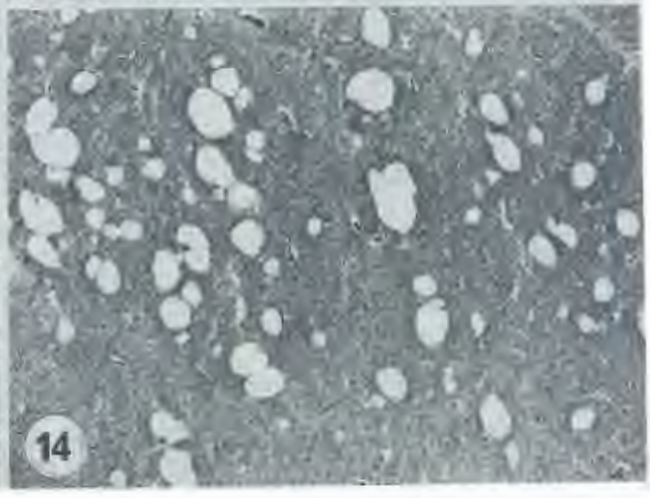


FIG. 9 Ewe 3. Central vein areas are linked by bands of collapsed reticulin (arrow). HE × 40

FIG. 10 Ewe 3. Central vein (C) is surrounded by collapsed stroma and sinusoids. Erythrocytes occupy the sites of original hepatic cords (arrow heads). HE × 200

FIG. 11 Ewe 3. Area of collapsed stroma is infiltrated by macrophages and lymphocytes (C, central vein; V, vascular channel). HE × 800

FIG. 12 Ewe 3. Reticulin stain to demonstrate reticulin collapse in the centrilobular zone and septum joining neighbouring central vein areas (arrow) (C, central vein). GRI × 200

FIG. 13 Ewe 5. Erythrocytes are evident in sites previously occupied by hepatocytes (arrows) (C, central vein; V, vascular channel). HE × 300

FIG. 14 Ewe 1. Numerous, large, round to oval spaces are present in the cerebral white matter. HE × 100

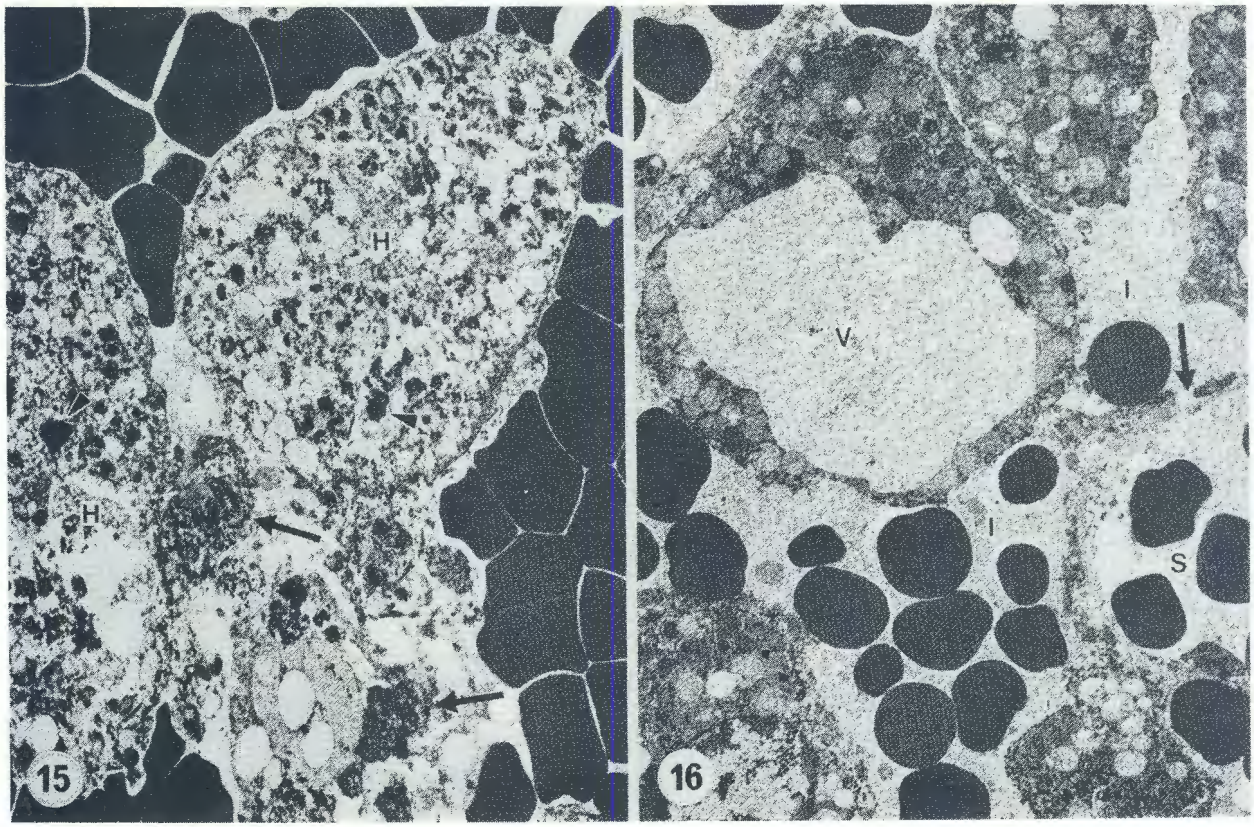


FIG. 15 Ewe 1. Coagulative necrosis of centrilobular hepatocytes (H) containing nuclear debris (arrow heads). Cellular debris (pieces of disintegrated hepatocytes) and amorphous bodies (arrows) are located in widened intercellular spaces. × 4500

FIG. 16 Ewe 1. Degenerated hepatocyte in the periphery of the lobule contains a hypoxic vacuole (V). Sinusoidal wall is indistinct (arrow). Intercellular spaces are dilated and contain granular material and erythrocytes (I) (S, sinusoid). × 4000

chiae were distributed throughout the thymus in both ewes. Other changes included several sub-endocardial ecchymoses in the left ventricular papillary muscle (Ewe 1) and mild congestion of the mucosa of the small and large intestines (Ewe 2).

No lesions were encountered in the liver or in any other organ or tissue in Ewe 7.

Microscopical pathology

Liver: In Ewes 1 and 2 centrilobular coagulative necrosis and haemorrhage, often extending into the midlobular zones, bridged adjacent lobules. Congestion was evident throughout the liver but was most pronounced in the centrilobular and midlobular areas where it was accompanied by haemorrhage (Fig. 3 & 4). In Ewe 2 the necrosis was less extensive and the haemorrhage more severe than in Ewe 1. Hepatocytes in the remainder of the lobules in both sheep were variably degenerated (Fig. 5 & 6). Some degenerated hepatocytes contained poorly demarcated vacuoles of varying size and shape or small, irregular, often elongated clumps of acidophilic material. The larger vacuoles sometimes contained finely fibrillar, pink material (Fig. 5). Bright acidophilic, homogeneous and well-delineated hyaline globules of different sizes were also noted in the cytoplasm of some of the degenerated hepatocytes. Sinusoidal lining cells were prominent and frequently moderately enlarged. In Ewe 2 a few small groups of neutrophils were scattered amongst the degenerated and necrotic parenchymal cells. In several lobules in the two animals the endothelial cells of the central veins were moderately swollen, had vesicular nuclei and finely vacuolated cytoplasm, while in other lobules the veins were denuded of endothelial cells. Small groups of dissociated hepatocytes and cellular debris were evident in the lumen of some central veins (Fig. 7) as well as in a few portal vessels. The reticular outline of several central veins was disrupted and fragmentation of the reticulin network lining the adjacent sinusoids was obvious in sections stained with Gomori's reticulin impregnation (GRI) (Fig. 8). Mild bile ductular proliferation and oedema were present in some portal triads.

Significant changes in Ewe 3 were restricted to the centrizonal areas (Fig. 9–12). Hepatocytes bordering the central veins were necrotic and cell cords were often replaced by groups of red blood cells (Fig. 10). The reticulin stain (GRI) demonstrated collapse of the remaining reticulin framework with only delicate bands of reticulin joining neighbouring central vein areas (Fig. 12). Dilated sinusoids, surrounded by fine trabeculae of immature connective tissue, were evident in the immediate vicinity of the central veins (Fig. 10). Central veins were usually

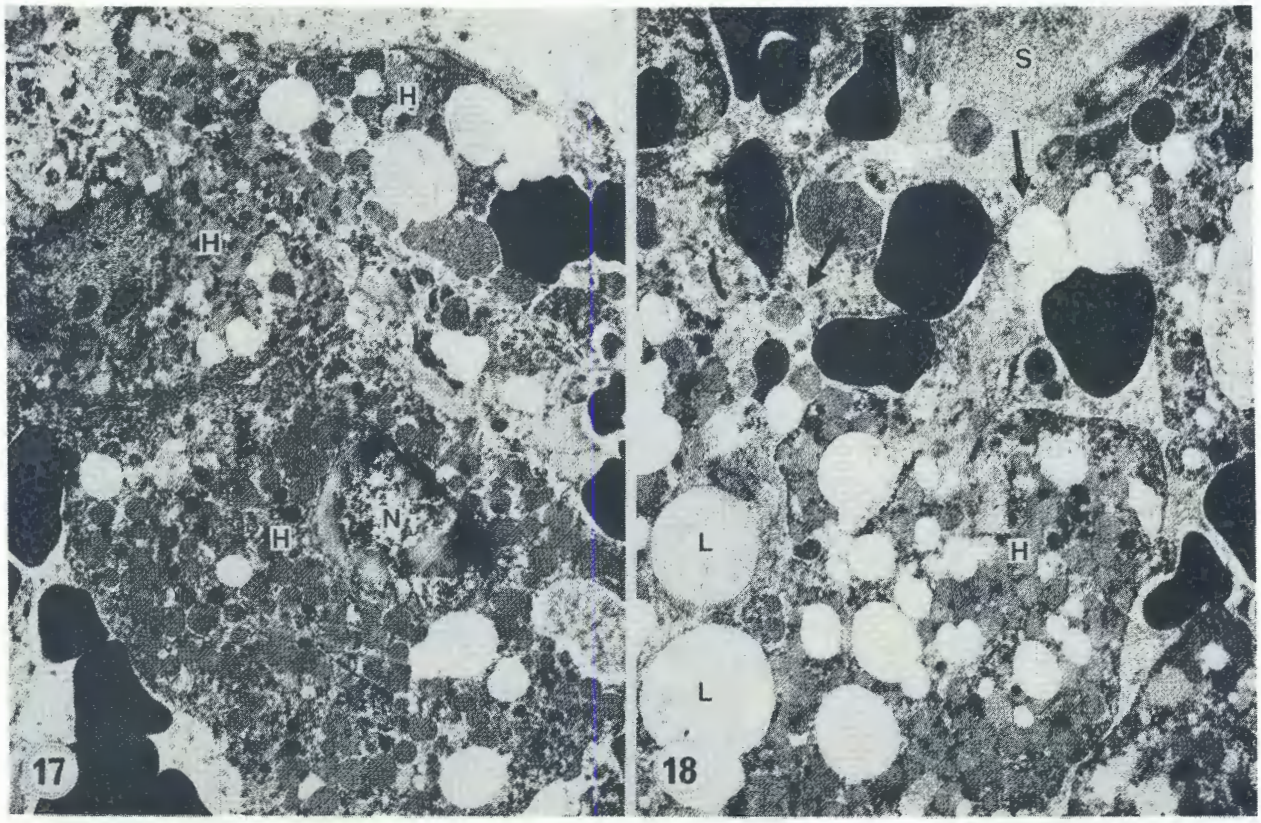


FIG. 17 Ewe 2. Centrilobular hepatocytes (H) showing various stages of degeneration and necrosis. Nucleus of one cell has a faded appearance (N). × 4500

FIG. 18 Ewe 2. Necrotic hepatocyte (H) in the centrizonal area is vacuolated and contains lipid globules (L). Sinusoidal walls are disrupted (arrows) allowing erythrocytes into the spaces of Disse (S, sinusoid). × 4500

moderately dilated and irregular in shape, and a slight thickening of the intima due to the deposition of collagen was frequently seen. A few focal accumulations of lymphocytes, admixed with plasma cells and macrophages, infiltrated some affected centrilobular areas (Fig. 11). Several of these macrophages contained varying proportions of haemosiderin and lipofuscin. Hepatocytes bordering the affected centrilobular areas revealed a slight increase in mitotic activity, and liver cells throughout the lobules showed mild anisokaryosis and binucleation.

Lesions in Ewe 4 were minimal and characterized by dilation of the sinusoids in the centrilobular areas and infiltrations of small numbers of lymphocytes in some portal triads.

In the livers of Ewes 5 and 6 necrosis, followed by lysis of hepatocytes bordering central veins, resulted in pooling of erythrocytes in areas previously occupied by cell cords (Fig. 13). Dilated sinusoids were present in some affected areas and were usually congested. Central veins were mildly dilated and communicated with the sinusoids in the surrounding parenchyma. The hepatocytes in the remainder of the lobule were moderately swollen, had a granular eosinophilic cytoplasm and showed marked nuclear anisonucleosis. Isolated necrotic hepatocytes were dispersed throughout the parenchyma. A few small aggregates of macrophages admixed with lymphocytes occurred in the centrilobular areas. These macrophages often contained moderate amounts of lipofuscin. This pigment also occurred in Kupffer cells throughout the parenchyma and in macrophages in the portal triads. Changes in the portal triads were mild and included bile ductular proliferation, fibrosis and mononuclear cell infiltration.

No microscopical lesions were noticed in the liver of the control animal (Ewe 7).

Other organs: The walls of the gall-bladders in Ewes 1 and 2 were severely oedematous and in Ewe 1 contained several serosal petechiae and ecchymoses. Tubular epithelial cells in the cortex of the kidneys in Ewes 1 and 2 were mildly to moderately degenerated and vacuolated and frequently contained fine, golden-brown pigment granules. In Ewes 1 and 2 nuclear pycnosis and karyorrhexis of lymphocytes were marked in the germinal centres of the hepatic and superficial lymph nodes and spleen but was less prominent in the Peyer's patches. Numerous macrophages in the affected germinal centres contained nuclear debris and variable amounts of lipofuscin. The medulla of the hepatic lymph node in Ewe 1 was moderately oedematous. Scattered small haemorrhages were noted in the thymus in Ewe 1.

Mild to moderate status spongiosis occurred in the brains of Ewes 1 to 3. The lesions in Ewes 1 and 2 were characterized by round to ovoid, often large, empty spaces in the white matter (mostly at the junction of the white and grey matter) of the midbrain (Fig. 14). The vacuoles were independent of blood vessels, and perivascular spaces were not affected. In Ewe 3 the vacuoles were more irregular in outline, occurring singly or in rows primarily along the axis of myelinated fibres in the midbrain and brain stem. The vacuoles were empty or contained small septae which appeared to be myelin lamellae.

Light microscopic examination of a range of organs from the control sheep revealed no lesions.

Transmission electron microscopical pathology

A variety of degenerative and necrotic changes were discernible in the hepatocytes in Ewes 1 and 2 (Fig. 15–18). Necrotic parenchymal cells were shrunken, showed loss of internal structure and contained condensed remnants of broken down organelles, nuclear debris and numerous small vacuoles with finely granular material (Fig. 15). Changes in degenerated hepatocytes included depletion of glycogen, swelling of mitochondria and endoplasmic reticulum and the presence of variably sized lipid globules in the cytoplasm (Fig. 18). The intercellular spaces were widened and often contained sequestered portions of necrotic cyctoplasm and amorphous bodies (Fig. 15). In addition, hypoxic vacuoles were evident in the cytoplasm of several degenerated and necrotic parenchymal cells which corresponded to the vacuoles containing fibrillar material seen with the light microscope (Fig. 16). The vacuoles consisted of large well-delineated, irregular areas limited by a single membrane and containing mildly electron-dense, granular material. Alterations seen in the nuclei included clumping and margination of chromatin, pycnosis, karyorrhexis and karyolysis. Some nuclei revealed a variety of these changes.

The sinusoids in Ewes 1 and 2 were usually intact, but in some centrilobular areas the sinusoidal walls appeared indistinct or were not discernible (Fig. 16 & 18). Spaces of Disse were either narrowed or widened and occupied by erythrocytes. Cells showing degeneration or necrosis, interpreted to be endothelial cells, were observed between degenerated and necrotic hepatocytes in the centrizonal areas. No morphological alterations were observed in the Kupffer cells in Ewes 1 and 2.

Mild degenerative changes were evident in the hepatocytes in Ewes 3 to 6. Proliferation of the smooth endoplasmic reticulum was not apparent in these animals. Other ultrastructural changes observed in Ewes 3 to 6 could be correlated with those seen with the light miroscope and were found in the vicinity of central veins. In some areas erythrocytes filled the spaces of Disse and replaced the liver cell cords. Bundles of collagen fibres were noticeable in the spaces of Disse.

Discussion

The lesions in the livers of Ewes 1 and 2 which received high doses of *C. laevigatum,* namely centrizonal to midzonal necrosis and haemorrhage with bridging between adjacent lobules, were similar to those described in cattle given the same plant material (Van der Lugt *et al.,* 1991).

Centrilobular necrosis of the liver is a relatively common finding in sheep in South Africa (Kellerman, Coetzer & Naudé, 1988). This change has been associated with the acute poisoning by several hepatotoxic plants, including *Senecio* spp. (Kellerman *et al.,* 1988) as well as with other plants which belong to the family Asteraceae, namely, *Asaemia axillaris* (Coetzer & Bergh, 1983), *Athanasia trifurcata* (Kellerman, Coetzer, Schneider & Welman, 1983), *Hertia pallens* (Prozesky, Kellerman, Jordaan, Welman & Joubert, 1985), *Lasiospermum bipinnatum* (Williams, 1991), *Pteronia pallens* (Prozesky, Kellerman & Welman, 1986) and *Nidorella foetida* (Schneider, Green & Collett, 1987). Other causes of centrilobular necrosis in sheep are intoxication with the blue-green alga *Microcystis aeruginosa* (Jackson, McInnes, Falconer & Runnegar, 1984) and anaemic conditions, e.g. haemonchosis.

The liver lesions caused by plants of the Asteraceae, which may range from distinct zonal necrosis to diffuse degeneration and hepatocellular unrest, scattered single cell or focal necrosis, however, depend on several factors including the level of dosing and toxicity of the plant (Kellerman *et al.,* 1988). A variation in the dosing regimen does not seem to lead to different patterns of zonal necrosis with either *Senecio* spp. (Kellerman *et al.,* 1988) or in acute poisoning by *Cestrum* spp.

The toxic principle(s) and their mechanism of action in *Cestrum* spp. remain unknown. A saponin as well as a substance called cestrumid was initially extracted from *C. laevigatum* by Wehner (Steyn, 1934). Canham & Warren (1950a; 1950b) reported the presence of saponins and sapogenins (gitogenin and digitogenin) in *C. laevigatum* and *C. parqui,* while Lopez, Keeler, Sharma & Shupe (1984) concluded that saponins and various cardiac glycosides accounted for most of the toxic effects of the latter plant. On the other hand Kudo, Kelly & Oelrichs (1978), regarded the saponins and glycosides not to be the active principles of *C. parqui* and induced centrilobular hepatic necrosis in mice and sheep with a chromatographically pure, highly water-soluble, but as yet unidentified toxin.

Most lipid-soluble drugs and toxins are metabolized by the mixed function oxidase (MFO) system, a chain of enzymes that includes NADPH cytochrome C reductase and cytochrome P450. The MFO system is most abundant in the smooth endoplasmic reticulum (SER) in hepatocytes in the periacinar zone of the hepatic acinus, which roughly corresponds with the centrilobular zone of the classic lobule. This explains the relative susceptibility of the centrilobular hepatocytes to injury by toxic metabolites formed during detoxification of many chemical agents (Rappaport, 1979). The mixed function oxidases are important in the metabolism of lipid-soluble substances, the essential effect being the conversions of lipophillic compounds into more polar and water-soluble metabolites that are more easily excreted via the bile or urine (Swick, 1984). If

the toxin of *Cestrum* spp. is indeed highly water-soluble, as reported by Kudo *et al.,* (1978), it follows that one would not expect the MFO system to be involved in its metabolism, yet the pattern of necrosis in acute poisoning with *Cestrum* spp. is consistently centrilobular. The lack of proliferation of SER in the sheep in this study as well as in cattle dosed with the same plant material (Van der Lugt *et al.,* 1991), on the other hand, suggest that the toxic substance of *C. laevigatum* is water-soluble and therefore not metabolized by the SER. An explanation for this apparent contradiction, however, will have to wait until the toxic principle(s) has been identified.

Various classifications of hepatotoxins have been proposed, and based upon mechanism of injury and morphology of the lesions in humans, two main categories of hepatotoxic substances have been identified (Zimmerman, 1978). One group are the intrinsic, true or predictable hepatotoxins which are recognized by the high incidence of hepatic injury in individuals exposed to them and by the production of similar lesions in experimental animals. Here the degree of hepatic injury depends on the dose of the agent. The other group are the idiosyncratic or unpredictable hepatotoxins. Their toxic effects result from the specific vulnerability of affected individuals rather than intrinsic toxicity of the incriminated agent. The incidence of hepatic injury caused by these substances is low, liver lesions are not produced in experimental animals and the development of injury to the liver in humans is independent of the dose of toxin.

Intrinsic hepatotoxins may lead to cytotoxic or cholestatic injury (Zimmerman, 1978). It appears that some cytotoxic agents damage the liver by direct physicochemical destruction of the membranes of hepatocytes and their organelles, with consequent interference with cell metabolism. Others produce hepatic injury indirectly by interference with specific metabolic pathways and processes essential for the maintenance of parenchymal cell integrity, resulting in morphological alterations. Cholestatic agents, on the other hand, interfere with hepatic excretory pathways leading to bile stasis, without producing other injury to hepatocytes (Zimmerman, 1978).

Degeneration, necrosis and fatty change of hepatocytes may occur in various combinations in cytotoxic injury. Hepatic necrosis may be focal, massive or zonal, and the latter may be distributed centrizonally, midzonally or peripherally in the lobules (Zimmerman, 1978). In general, the necrosis produced by intrinsic hepatotoxins is zonal, while that produced by idiosyncratic injury usually involves entire lobules throughout the liver (Zimmerman & Ishak, 1979). The specific mechanisms of action of the cytotoxic agents in causing necrosis remains poorly understood. Organelles, including mitochondria, lysosomes and smooth and rough endoplasmic reticulum have been proposed as the primary sites of injury (Zimmerman, 1978). The role of the plasma membrane in the pathogenesis of necrosis was reviewed by Farber (1979). He suggested that toxic liver cell necrosis follows a disorder of intracellular calcium homeostasis. According to him, injury to the functional integrity of the plasma membrane results in entry and intracellular accumulation of calcium ions, leading to inactivation of mitochondria, inhibition of enzymes and denaturation of structural proteins. Recently, the role of peroxidation of membrane lipids in the production of cellular damage by various hepatotoxins has been emphasized (Comporti, 1985). Lipid peroxidation can affect cellular function directly, by inducing loss of membrane structure, or indirectly, by the formation of toxic intermediates.

Ultrastructural observations in this study confirmed that the toxic substance of *C. laevigatum* primarily affects hepatocytes. Injury to hepatocytes was of the cytotoxic type, and changes in the bile secretory apparatus or biliary retention indicative of cholestasis were not evident. The large vacuoles in the cytoplasm of degenerated and necrotic hepatocytes in Ewes 1 and 2 resembled hypoxic vacuoles ultrastructurally. In humans, hypoxic vacuoles in hepatocytes are found in acute and chronic congestion and hypoxia of the liver, particularly in patients with cardiac and pulmonary failure, and in the Budd-Chiari syndrome (Uchida & Shikata, 1986; Phillips, Poucell, Patterson & Valencia, 1987). These vacuoles, originating from invagination of the plasma membrane of the hepatocyte, are enclosed by a single membrane that may be continuous with the plasma membrane, and contain serum components. It is suggested that these vacuoles develop due to hypoxia and/or elevation of sinusoidal pressure (Uchida & Shikata, 1986).

In Ewes 1 and 2 sinusoidal endothelial cells appeared degenerated or necrotic and the sinusoidal walls were often disrupted and indistinct. These lesions suggest that the centrilobular haemorrhage which accompanied hepatocellular necrosis in the two animals, probably resulted from damage to endothelial cells and breakdown in sinusoidal architecture.

Haemorrhagic diathesis, characterized by the occurrence of haemorrhages in various tissues, has been reported in acute poisoning with *Cestrum* spp. (Thorburn, 1934; Mugera & Nderito, 1968; Döbereiner *et al.,* 1969; McLennan & Kelly, 1984; Van der Lugt *et al.,* 1991), but was not evident in this study. Oedema (Ewes 1 and 2) and haemorrhage (Ewe 2) of the gall-bladder wall commonly occurs in acute, fatal hepatotoxic conditions in ruminants (Kelly, 1985), including those caused by *Cestrum* spp. (McLennan & Kelly, 1984; Mugera & Nderito, 1968; Van der Lugt *et al.,* 1991). These lesions are indicative of haemodynamic disturbances in the liver (Kellerman *et al.,* 1988) and may also be related to a direct effect by toxic metabolites excreted in the bile (Kelly, 1985).

The spongy changes in the brain of Ewes 1 to 3 resembled the description of hepatic encephalopathy, a neurologic manifestation of liver failure reported in a number of domestic animals (Hooper, 1975; Kellerman *et al.,* 1988) and man (Black, 1982). Hepatic encephalopathy is often encountered in ruminants suffering from chronic liver disease (e.g. chronic pyrrolizidine alkaloid poisoning) (Kellerman *et al.,* 1988). Clinical signs asso-

ciated with hepatic encephalopathy as a result of acute liver damage in sheep have been reported by Seawright (1982, cited by Jackson *et al.,* 1984). The precise pathogenesis of hepatic encephalopathy remains undetermined, although it appears to involve the inadequate hepatic removal of, predominantly, nitrogenous compounds (the most important being ammonia) ingested or formed in the gastrointestinal tract. The compounds gain access to the central nervous system, where they probably interact with cellular oxidative metabolism or other metabolic pathways (Hooper, 1975; Cho & Leipold, 1977; Black, 1982). Transmission electron microscopy of these lesions reveals vacuolation of the myelin sheath and separation of the myelin spiral at the intraperiod lamellae (Cho & Leipold, 1977). According to Kellerman *et al.* (1988), sheep suffering from hepatic encephalopathy as a result of plant poisoning seldom show overt nervous signs despite the presence of marked spongy changes in the brain. This observation was confirmed in Ewes 1 to 3, in which depression was the most noticeable sign.

The activities of AST and LD were greater in the sera of sheep of the high-dose group than in the low-dose group. Similar clinical pathological findings were reported in cattle dosed with the plant (Van der Lugt *et al.,* 1991). In the absence of injury to the myocardium and skeletal muscles, and with normal levels of CK activity, increased activities of AST and LD are indicative of hepatocellular injury (Coles, 1986; Duncan & Prasse, 1977). Involvement of the liver was in the current trial substantiated by elevations of GGT, an enzyme more specific for that organ than AST. The elevations in GGT activity were generally similar to those for AST and LD but of lower magnitude. This observation is consistent with the findings of Malherbe, Kellerman, Kriek & Haupt (1977) that in ovine lupinosis GGT activity was more sensitive than AST for early detection of low grade acute intoxication; while AST, in turn, gave better information on the development of severe acute hepatocellular damage. According to Schmidt & Schmidt (1984), the variable pattern of GGT activity in acute toxic liver damage results from various factors including disturbed cellular permeability, inhibition of protein synthesis and cholestatic injury to hepatocytes, superimposed one upon the other.

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