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GALENIA AFRICANA L. POISONING IN SHEEP AND GOATS: HEPATIC AND CARDIAC CHANGES

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ABSTRACT

VAN DER LUGT, J. J., SCHULTZ, R. ANITRA, FOURIE, N., HON, L. J., JORDAAN, P. & LABUSCHAGNE, LEONIE, 1992. *Galenia africana* L. poisoning in sheep and goats: hepatic and cardiac changes. *Onderstepoort Journal of Veterinary Research*, 59, 323–333 (1992)

Lesions in 4 field cases (3 sheep and 1 goat) of 'waterpens' or water belly, caused by the plant *Galenia africana,* are described. The clinical pathological and pathological findings in 7 sheep which were drenched with toxic plant material are also reported. Inappetence, ruminal stasis and apathy as well as tachycardia were noticed in some of the sheep towards the end of the dosing period.

The most prominent clinical pathological change in the experimental animals was an increase in the activity of gamma-glutamyltransferase which in some animals occurred within days after commencement of dosing. This indicates liver involvement in the early stages of the intoxication, and at this stage no heart abnormalities were detected clinically, clinical pathologically or with cardiac function tests. Decrease in cardiac function were recorded in 2 sheep towards the end of the dosing period.

Liver and heart lesions were present in all the animals. In some cases hepatic changes were mild and characterized by dilation of central veins and sinusoids and, less commonly, centrilobular fibrosis. More advanced lesions included centrilobular fibrosis and bridging between neighbouring lobules with adjacent areas of coagulative necrosis, lysis and ballooning degeneration of hepatocytes. Myocardial changes occurred in the free ventricular walls and interventricular septum and comprised hypertrophy of myocytes with consequent degeneration and necrosis and fibrosis. In cases of longer duration myocytes were diffusely atrophic with scattered groups of remaining hypertrophic fibres.

The clinical pathological and pathological features suggest that *G. africana* is primarily hepatotoxic with myocardial involvement occurring only in the terminal stages of the intoxication.

INTRODUCTION

Galenia africana L., commonly known as kraalbos or geelbos, is a perennial sub-shrub belonging to the Aizoaceae. The plant is mostly found in the western and southern Karoo and in recent years has become more widespread than before. *Galenia africana* is an active invader, and is especially abundant in disturbed areas around kraals, along roads and on trampled veld. Kraalbos is a woody, perennial sub-shrub, growing *c.* 1 m high, and has small green leaves which turn yellow with age. Inflorescences are borne at the end of twigs and are 30–100 mm long, with many small yellow flowers (Vahrmeijer, 1981) (Fig. 1 & 2).

The toxic principle of kraalbos is not known. Under field conditions the plant has been associated with liver damage and severe ascites, referred to as 'waterpens' or water belly, in sheep and goats (De Kock, 1928; Kellerman, Coetzer & Naudé, 1988). Apart from severe abdominal distension, affected animals show mass loss, become apathetic and recumbent, and die. Cases of waterpens occur only in a small percentage of the flock. Animals in poor condition during severe droughts, when forced to browse the plant, are more prone to develop clinical disease (Kellerman *et al.*, 1988).

The pathology of *G. africana* poisoning has not been reported in detail. The marked liver lesions in sheep and occasionally in goats led to the belief that the plant is primarily hepatotoxic (Vahrmeijer, 1981). In a retrospective study of field cases of waterpens, however, Newsholme & Coetzer (1984) mentioned that the hepatic lesions were compatible with cyanotic induration and may have resulted from congestive right heart failure. In most of the cases where myocardium was available for examination, multifocal lesions, which ranged from acute to chronic, were in evidence.

This report describes lesions in some field cases of waterpens and the results of a dosing trial with *G. africana* in sheep, preliminary results of which have been reported elsewhere (Kellerman *et al.*, 1988). One of the objectives of the study was to determine whether the liver lesions were brought about by primary congestive heart failure.

FIG. 1 *Galenia africana* is a woody sub-shrub growing *c.* 1 m high

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FIG. 2 Inflorescences are borne on twigs with many small yellow flowers

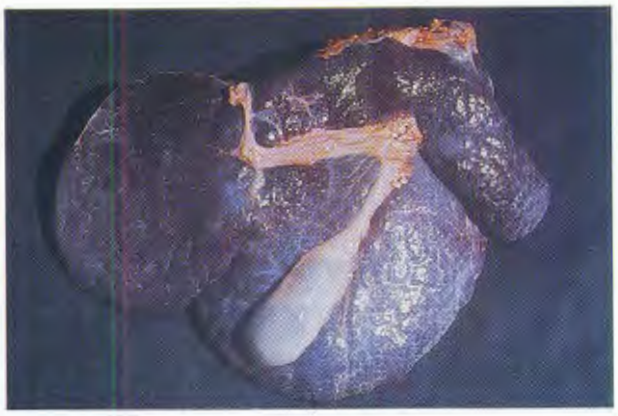

FIG. 4 The liver of Case 1 was swollen, greyish-blue and the surface irregular

MATERIALS AND METHODS

Field cases

Tissues fixed in buffered 10 % formalin from one goat (Case 1) and 3 sheep (Cases 2–4) were studied. Case 1 was an adult boer goat from the district of Willowmore in the Karoo. Due to severe drought and overgrazing, kraalbos was almost the only pasture plant available on the farm. At the time of examination the animal was unable to rise due to severe abdominal distension, but maintained a good appetite and habitus (Fig. 3). At necropsy, the abdominal cavity contained *c.* 15 ℓ transparent yellow fluid. The liver was mildly enlarged, greyish-blue, and the surface was irregular (Fig. 4). Cases 2–4 were adult sheep from farms near Calvinia, northwestern Cape Province and for each animal the clinical history was compatible with waterpens and there was known access to *G. africana.*

The entire hearts were received from the sheep and samples of interventricular septum and left ventricular wall from the goat, as well as samples of liver from each animal. In the sheep the ventricular myocardium was cut into 3 equal thickness transverse slices. Blocks of left and right ventricular wall and ventricular septum were taken from the distal surface of each slice. Blocks were also collected from each atrium. All tissues were embedded in paraffin wax, cut at 4–5 μm and stained with haematoxylin and eosin (HE). Sections of liver from selected paraffin blocks were also stained by a modified Masson's trichrome (MT) method (Luna, 1968), Gomori's reticulin impregnation (GRI) (Pearse, 1961) and Oil Red O (ORO) (Pearse, 1961).

FIG. 3 Case 1. The goat was unable to rise due to severe ascites

Experimental cases

Plant material

Two batches of *G. africana* plants were collected in July and August, 1985. The first batch was obtained from a locality near the office of the State Veterinarian, Calvinia and the second was collected in the Willowmore district. The plants were dried in the shade and the smaller branches bearing the leaves, which were separated from the thicker, wooded parts, were ground to a coarse powder in a hammer-mill and stored at −10 °C.

Experimental animals and dosing procedure

Plant material was administered per rumen fistula to 7 Merino sheep (Cases 5–11) which had not been exposed to the plant (Table 1). Cases 5–9 were dosed with plant material collected in Willowmore and Cases 10 and 11 with material from Calvinia. During the trials the animals were fed pellets (Onderstepoort formulated ration) and milled lucerne hay (*Medicago sativa*) *ad libitum.* Drinking water was available at all times.

Clinical pathology

Haematology

Blood haemoglobin (B-Hb) was determined by the cyanmethaemoglobin method (Merck, 1974); haematocrit (B-Ht) by using capillary tubes in a Damon IEC micro-haematocrit centrifuge; and erythrocyte sedimentation rate (B-ESR) in Wintrobe tubes for 1 h at 20 ± 3 °C.

Determination on plasma and serum

Total plasma protein (P-TPP) was determined by the Biuret method (Merck, 1974), the albumin/globulin ratio with a Beckman serum protein electro-

TABLE 1 Dosing regimen, clinical pathological findings and cardiac function tests in sheep dosed with *G. africana* plant material

Sheep		Dosing regimen			Clinical pathology*	Clinical signs	Cardiac function (ECG and CPFI)	Fate
No.	Initial body mass (kg)	Dose (g/kg × n)	Days	Total dose (g/kg)				
5	19	5 × 3 7,5 × 3 10 × 1 7,5 × 13	0–2 3–5 6 9–21	145	Increased GGT: 59–85 U/ℓ (D7–D21)	Reduced ruminal movements (D7–D8)	No abnormalities	Euthanized on Day 23
6	29	5 × 3 7,5 × 3 10 × 1 7,5 × 21	0–2 3–5 6 9–29	205	Increased GGT: 70–86 U/ℓ (D7–D28)	Reduced ruminal movements (D7–D8)	No abnormalities	Euthanized on Day 30
7	25	5 × 3 7,5 × 3 10 × 1 7,5 × 22 10 × 20	0–2 3–5 6 9–30 31–50	412	Increased GGT: 55–76 U/ℓ (D7–D49)	Reduced ruminal movements (D7–D8)	No abnormalities	Euthanized on Day 52
8	21	5 × 3 7,5 × 3 10 × 1 7,5 × 22 10 × 34 12,5 × 11 15 × 5	0–2 3–5 6 9–30 31–64 66–75 76–80	765	Increased GGT: 57 U/ℓ (D66–D80)	Reduced ruminal movements (D7–D8); tachycardia (D75–death)	No abnormalities	Euthanized on Day 81
9	32	5 × 3 7,5 × 3 10 × 1 7,5 × 22 10 × 34 12,5 × 11 15 × 5	0–2 3–5 6 9–30 31–64 65–75 76–80	765	Increased GGT: 76–79 U/ℓ (D7–D90)	Reduced ruminal movements (D7–D8); mild cardiac arrhythmia and tachycardia (D48–death)	No abnormalities	Euthanized on Day 81
10	45	10 × 50	0–37 41–52	500	Increased GGT: 56–230 U/ℓ (D9–D56); AST (D51–death); LD (D56); B-Ht and B-Hb (D37–death)	Tachycardia (D38–death); transient inappetence and apathy (D40–death); ruminal stasis (D51–death)	CPFI increased from 7–11,5 (D21–D36 onwards)	Euthanized on Day 56
11	58	5 × 7 10 × 7 15 × 38	0–6 7–13 14–51	675	Increased GGT: 53–150 U/ℓ (D21–D58); AST (D58); B-Ht and B-Hb (D52–death)	Tachycardia (D23–death); ruminal stasis, inappetence, apathy, grinding on teeth (D52–death)	Elevated CPFI: 10 (D58)	Died on Day 61

* normal activity of GGT is < 40 U/ℓ

phoresis kit on agarose gel, plasma glucose using the GOD-Perid method (Boehringer Mannheim) and serum urea (SU) by the Berthelot method (Merck, 1974). The activities of the following enzymes were determined in the serum using Boehringer Mannheim test kits: lactate dehydrogenase (LD, EC 1.1.1.27), aspartate transaminase (AST, EC 2.6.1.1), creatine kinase (CK, EC 2.7.3.2) and gamma-glutamyltransferase (GGT, EC 2.3.2.2). In addition, isoenzymes of LD (LD_{1-5}) and CK (MM, MB and BB) were separated on agarose using the Beckman Paragon isoenzyme electrophoresis kit. All enzyme activity was measured at 25 °C. Isoenzymes and albumin/globulin ratios were quantified using a Model CDS-200 Beckman Densitometer. All above-mentioned determinations were done at Day −4, Day 0 and then twice a week.

Cardiac function

The electrical activity and function of the heart was monitored using Lead II of the electrocardiogram (Schultz, Pretorius & Terblanche, 1972) and by means of a cardiopulmonary flow index (CPFI) (Van der Walt & Van Rooyen, 1977; Van der Walt, Van Rooyen, Cilliers, Van Ryssen & Van Aarde, 1981). These tests were done once a week except in Case 11 where they were performed on Day 58 only. In addition, a Swan-Ganz catheter was positioned in the pulmonary artery after percutaneous jugular vein catheterisation in this animal. The following parameters were determined on Day 58: right atrial and ventricular pressure, mean pulmonary arterial pressure, pulmonary capillary wedge pressure, cardiac output (thermodilution) and stroke volume (Van Rooyen & Van der Walt, 1989).

Pathology

With the exception of Case 11, which died naturally, the other animals were killed by exsanguination after an intravenous overdose of pentobarbitone sodium. The sheep were euthanased on different days after commencement of dosing in an attempt to study the pathogenesis of the toxicosis (Table 1). Necropsies were performed on each animal and specimens of myocardium were collected for light microscopy as for the field cases. In addition, samples of liver, gall-bladder, skeletal muscles, lung, spleen, kidneys, pancreas, adrenal glands, rumen, abomasum, small and large intestines, lymph nodes, brain and spinal cord were taken from each animal. Tissues were embedded in paraffin wax, sectioned and stained as for the field cases.

RESULTS

Field cases

Liver: In Case 1 the most striking feature was loss of hepatocytes in the centrilobular zones in the majority of lobules which resulted in collapse of the reticulin stroma and, in most of these lobules, centrilobular fibrosis (Fig. 5 & 6). The connective tissue sometimes bridged neighbouring centrilobular zones and formed connective tissue bands surrounding single portal tracts, giving rise to reversed lobulation. The majority of central veins were dilated. Areas of coagulative necrosis and lysis or degeneration of hepatocytes were seen adjacent to the fibrotic lesions. The necrosis was accompanied by pronounced haemorrhage and congestion and often extended into the midlobular zones, bridging adjacent lobules (Fig. 6). Degenerated cells had a finely granular and vacuolated cytoplasm or showed ballooning degeneration (Fig. 5). The latter cells were swollen 2 to 3 times the normal size, showed poorly defined outlines and appeared empty. The nuclei were pycnotic or lysed. These cells did not contain fat in sections stained with ORO. Large spaces, lined by a network of reticular tissue and sinusoidal lining cells remained where hepatocytes had disappeared. These spaces sometimes contained necrotic hepatocytes, cellular debris or serum. Small amounts of haemosiderin were evident in several Kupffer cells.

Hepatic cell cords at the periphery of the lobules were generally disrupted and the hepatocytes were variably swollen, showed marked anisokaryosis and binucleation and occasionally contained up to 5 nuclei. Lesions in the portal triads included mild to moderate fibrosis, mild dilation of lymphatics and mild bile ductular proliferation.

Lesions in Cases 2–4 were more chronic in nature and were characterized by extensive centrilobular and midlobular fibrosis, joining adjacent lobules (Fig. 7). Central veins were markedly congested, while some centrilobular areas contained dilated vascular channels. Several portal tracts were linked to adjacent portal zones by bands of connective tissue. Single or groups of hepatocytes became isolated by the connective tissue. Hepatocytes throughout the lobules were atrophic. The capsule of Glisson was diffusely thickened due to the deposition of collagen.

Heart: In Case 1 several variably sized areas of necrosis were evident in the inner two-thirds of the myocardium. Necrotic myocytes were shrunken, disrupted and fragmented and possessed strongly eosinophilic, hyalinized or granular cytoplasm with loss of striations (Fig. 8). Nuclei of necrotic fibres were often pycnotic. Fibres in the necrotic

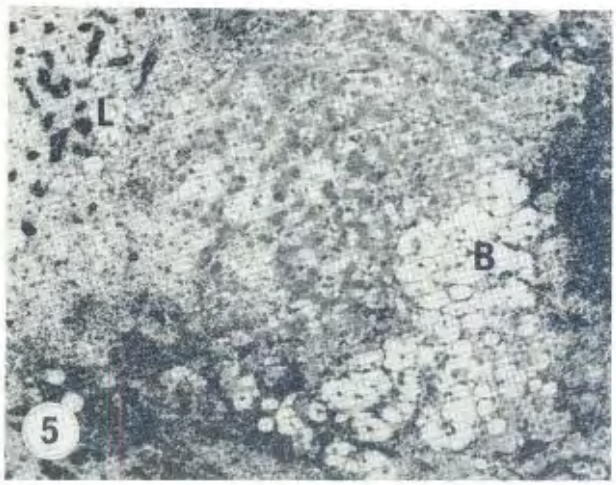

FIG. 5 Case 1: Liver. Ballooning degeneration (B) and loss (L) of hepatocytes. HE × 400

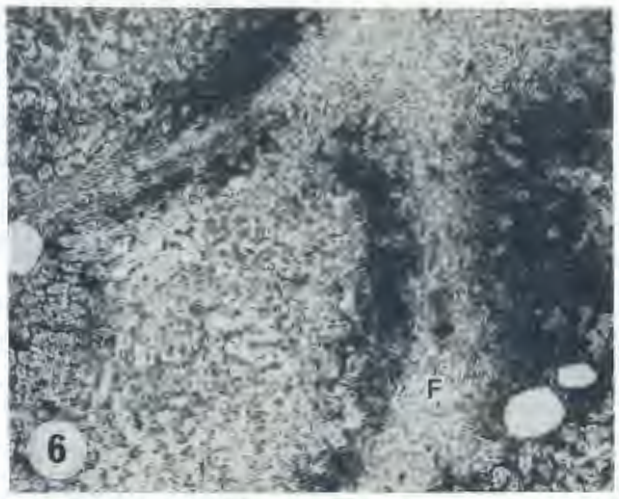

FIG. 6 Case 1: Liver. Centrilobular fibrosis with bridging between adjacent lobules (F). Areas of fibrosis are bordered by coagulative necrosis of hepatocytes, haemorrhage and congestion. HE × 200

FIG. 7 Case 3: Liver. Prominent centrizonal (C), portal (P) and intralobular fibrosis. HE × 200

FIG. 8 Case 1: Heart. Areas of necrosis and fibrosis in the ventricular myocardium. HE × 50

FIG. 9 Case 1: Heart. Necrotic myocytes are shrunken and fragmented (arrows) and are replaced by fibrous tissue. Adjacent fibres are hypertrophic and contain large nuclei (arrowhead). HE × 600

FIG. 10 Case 2: Heart. Diffuse atrophy of myocytes and foci of interstitial fibrosis in the ventricular wall. HE × 80

FIG. 11 Case 2: Heart. Fibromuscular proliferation of an intramural artery. HE × 150

areas not affected by necrosis were markedly hypertrophic and had a more granular appearence than normal and contained oval or large elongated vesicular nuclei with prominent nucleoli (Fig. 9). Necrosis followed by loss of myocytes resulted in collapse of the stroma and replacement by proliferating fibrous tissue; plump fusiform cells with weakly basophilic cytoplasm and large, ovoid vesicular nuclei, which were interpreted as immature fibroblasts, were observed amongst the necrotic myofibres and in the stromal tissue where myofibre loss had occurred. Remaining cardiac muscle cells appeared mildly thickened and had prominent nuclei. Occasionally groups of Purkinje fibres became surrounded by fibrous tissue.

In the sheep (Cases 2–4) most cardiac muscle cells in the left and right ventricular walls and to a lesser extent in the interventricular septum were moderately atrophic, while scattered groups of myocytes appeared hypertrophic (Fig. 10). Atrophy was most pronounced in the inner two thirds of the myocardium. Atrophic fibres were thin and sometimes arranged in a wavy pattern and possessed mildly hypertrophic nuclei. Foci of muscle fibre loss with stromal condensation and interstitial fibrosis were scattered throughout the left ventricular wall and interventricular septum in the 3 sheep. In addition, fibromuscular proliferation associated with medial and adventitial thickening affected some medium-sized intramural arteries in the ventricular walls and interventricular septum in the sheep (Fig. 11).

Experimental cases

Clinical signs, clinical pathology and cardiac function

The findings are summarized in Tables 1 and 2 and Fig. 12. Increases in the activity of GGT, indicative of liver involvement, was the most prominent clinical pathological change noticed in all the sheep. In 5 animals abnormally high values initially occurred as early as 9 days after commencement of dosing, and in all the sheep the values gradually increased throughout the dosing period. The activities of LD and AST remained within normal limits and increased only terminally in Cases 10 and 11. The activities of CK and the isoenzymes did not follow any particular pattern. Neither were significant changes detectable in the haematological parameters except for increases in B-Hb and B-Ht in the terminal phase of intoxication, coinciding with clinical signs of inappetence and ruminal atony.

Tachycardia occurred in Cases 8–11. Cardiac involvement reflected by elevated CPFI values were recorded in Cases 10 and 11. In Case 11 the CPFI was 10 and the heart rate 130 on Day 58. Other parameters including cardiac output, stroke volume and pulmonary arterial pressure were within normal limits and the right atrial pressure was lower than normal on that day (Table 2).

TABLE 2 Cardiac parameters in Case 11 (Day 58) and control sheep

Parameter †	Case 11	Control sheep* (n=6)	Control sheep** (n=16)
		Mean (SD)	Mean (SD)
CPFI	10	7,1 (0,8)	6,9 (1,2)
HR (beats/min)	130	85 (11)	94 (14)
CO (ℓ/min)	4,5	3,7 (1,0)	3,6 (0,7)
SV (mℓ)	31 (HR = 145)	44,3 (14,4)	39,3 (9,0)
RAP (mmHg)	1,25	3,94 (1,23)	3,9 (2,5)
PAP (mmHg)	16,25	16,0 (1,8)	17,6 (4,1)

† CPFI = cardiopulmonary flow index; HR = heart rate; CO = cardiac output; SV = stroke volume; RAP = right atrial pressure; PAP = pulmonary arterial pressure; SD = standard deviation
* Van Rooyen, 1987;
** Van Rooyen & Van der Walt, 1989

Macroscopical pathology

No noteworthy lesions were seen in Cases 5 and 6. In Cases 7–9 the livers were mildly swollen and the lobulation slightly accentuated with light red centrilobular areas surrounded by pale almost yellowish peripheral zones.

The liver in Case 10 was mildly swollen. Several yellow, ill-defined, slightly raised patches were noticed subcapsularly, while the remaining parenchyma was greyish-blue. The lobulation was slightly

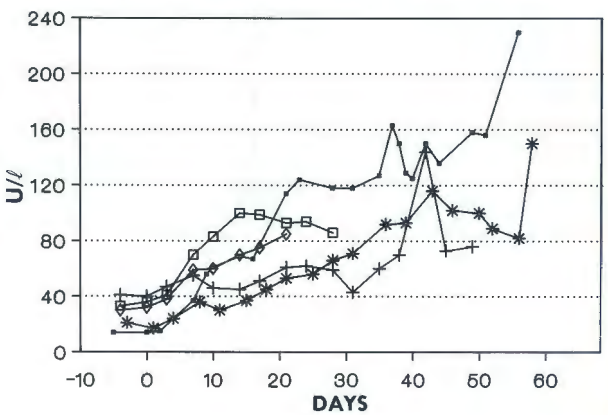

FIG. 12 Serum activities of GGT (U/ℓ) of Cases 5 (—÷—), 6 (—□—), 7 (—┼—), 10 (—✱—) and 11 (—✱—). Normal activity is < 40 U/ℓ.

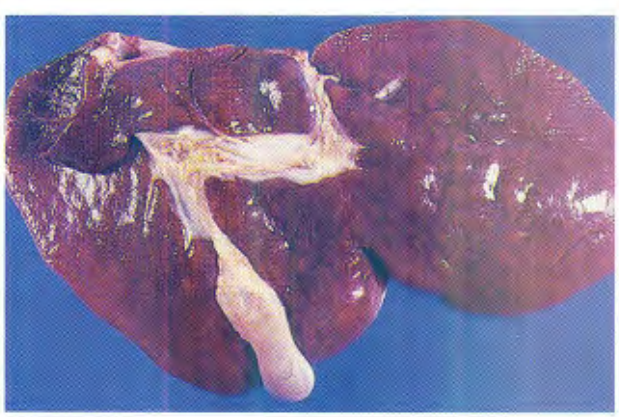

FIG. 13 The liver of Case 11 is yellowish-brown and has a uneven surface

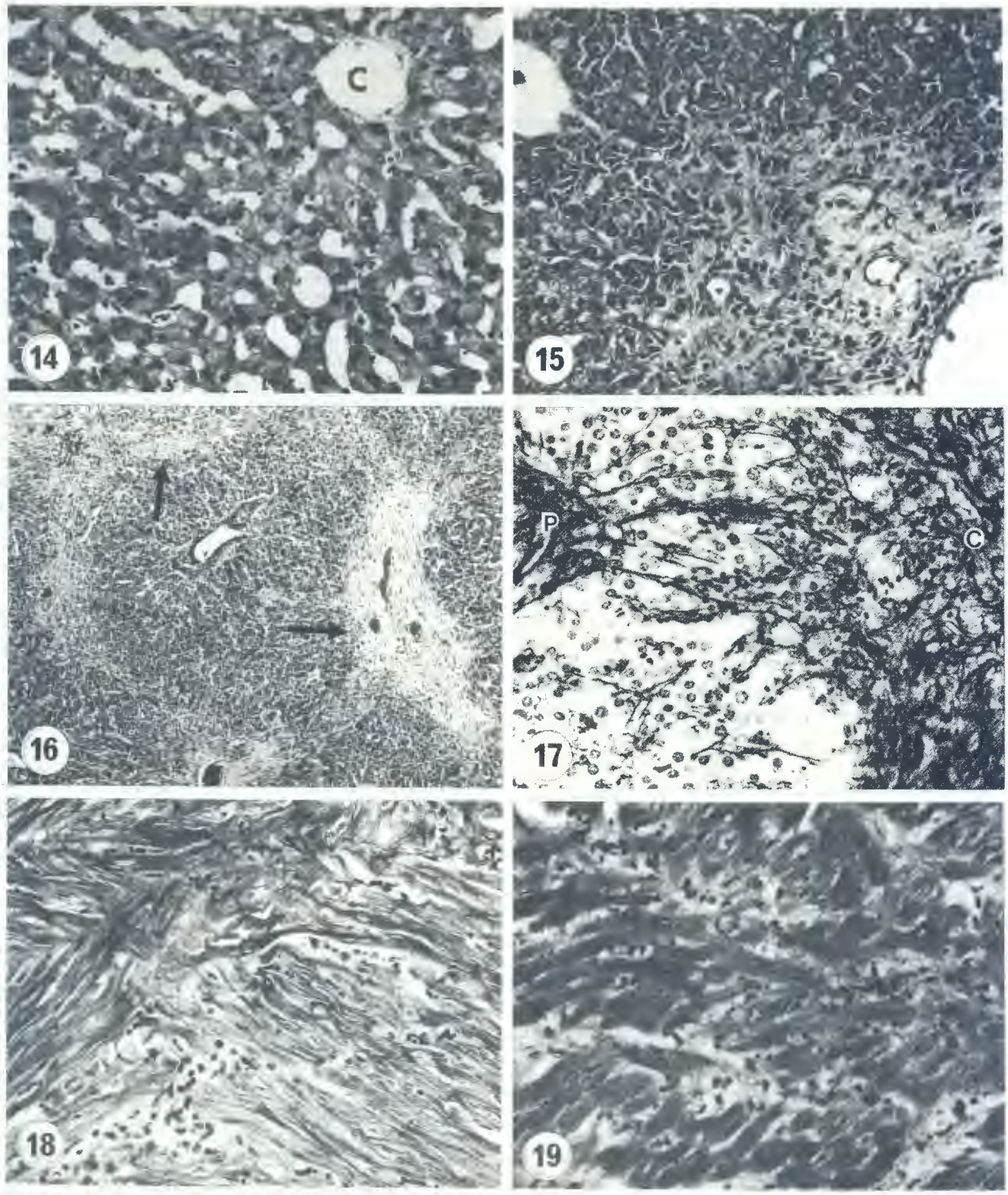

FIG. 14 Case 6: Liver. Dilation of central vein (C) and centrilobular sinusoids. The hepatocytes are mildly atrophic. HE × 600

FIG. 15 Case 9: Liver. Two central veins are linked by proliferating connective tissue which is infiltrated by lymphocytes and macrophages. HE × 500

FIG. 16 Case 11: Liver. Centrizonal fibrosis joining adjacent central veins (arrows) giving rise to a reversed lobular pattern. HE × 200

FIG. 17 Case 11: Liver. Collapse and condensation of the reticulin network in the centrilobular (C) and portal areas (P) and linking of these areas by collapsed stroma. GRI × 500

FIG. 18 Case 9: Heart. Cardiac muscle cells in the interventricular septum are disorganized with crossover patterns. HE × 200

FIG. 19 Case 10: Heart. Hypertrophy and mild interstitial fibrosis in the interventricular septum. Note disorganization of the myocytes. HE × 600

more distinct. In addition, several petechial haemorrhages were scattered throughout the parenchyma.

In Case 11 the abdominal cavity contained 30 mℓ of slightly turbid, dark yellow fluid. Oedema of the mediastinum and mild congestion of the kidneys and spleen was present. The moderately enlarged liver was yellowish-brown and the surface uneven (Fig. 13). In addition, several congested, poorly circumscribed patches, 5 to 20 mm in diameter were distributed under the capsule. The hepatic lobulation was moderately accentuated, each lobule having a pale tan-coloured slightly sunken centre surrounded by a brownish peripheral zone. The gallbladder contained watery, dark green bile.

Microscopical pathology

Liver: Changes in Cases 5, 6 and 8 were minimal to mild and comprised dilation of central veins as well as sinusoids for variable distances towards the portal areas (Fig. 14). Centrilobular hepatocytes were mildly atrophic and pale-staining and the cytoplasm had a finely granular and vacuolar appearance. Hepatocytes in the midzonal and peripheral areas evinced increased cytoplasmic granularity.

In Cases 7 and 9 several lobules showed mild centrilobular fibrosis, occasionally joining adjacent lobules (Fig. 15). The connective tissue had a more cellular appearance than normal due to the infiltration of small numbers of inflammatory cells, especially lymphocytes and to a lesser extent macrophages and neutrophils. The macrophages contained granular, yellowish-brown intracytoplasmic pigment. In the centrizonal and midzonal areas the liver cells were either degenerated or atrophic and possessed pale-staining, finely granular cytoplasm. Hepatocytes in the remainder of the lobules had more acidophilic and granular cytoplasms containing small, irregular, often elongated clumps of eosinophilic material. Sinusoids throughout the liver were variably dilated. Portal tracts were mildly fibrotic and were infiltrated by focal aggregates of mononuclear cells.

Liver lesions in Cases 10 and 11 were similar to those described in Case 1, but were less severe. There was centrizonal fibrosis and proliferating connective tissue joining central veins in some lobules, resulting in a reversed lobular pattern (Fig. 16 & 17). Dilation and occasional duplication of central veins were noticeable. The fibrotic areas were infiltrated by small numbers of plasma cells, lymphocytes as well as pigment-laden macrophages. Parenchymal cells adjacent to fibrotic lesions often showed ballooning degeneration or necrosis accompanied by haemorrhage and congestion. Hepatocytes at the periphery of the lobules were variably degenerated with marked anisonucleosis, and in Case 11, frequently contained several bright acidophilic, homogenous and well-delineated hayline globules of different sizes. In addition, there was pronounced dilation of the spaces of Disse in the peripheral zones in this animal. Changes in the portal tracts in the 2 sheep were characterized by dilation of lymphatics, mild fibrosis and bile ductular proliferation and infiltrations of small numbers of mononuclear cells with a predominance of lymphocytes and plasma cells.

Heart: In Cases 5–7 the interventricular septum and ventricular walls contained scattered foci of necrosis and loss of myocytes with interstitial fibrosis sometimes accompanied by infiltrations of small numbers of lymphocytes.

Lesions in Cases 8 and 9 resembled those described in Cases 2–4, but were less severe. Myocytes in the interventricular septum and ventricular walls were mildly atrophic. Foci of myocyte necrosis and interstitial fibrosis were infiltrated by small numbers of mononuclear cells predominantly macrophages and lymphocytes. In Case 9 myocardial fibres neighbouring these foci were occasionally disorganized with cross-over patterns, and showed apparent myofibrillar disruption and disarray (Fig. 18).

In Case 10 there were several areas of hypertrophy of muscle cells in the inner two thirds of the left ventricular wall and interventricular septum, and to a lesser extent in the right ventricular wall. These muscle cells contained large oval or elongated nuclei, while some fibres possessed a slightly more acidophilic cytoplasm and pycnotic nuclei. Affected areas showed short runs of hypertrophic fibres interrupted by mild fibrosis (Fig. 19). Fibres in the ventricular walls and interventricular septum not affected by hypertrophy appeared mildly atrophic, sometimes had a wavy pattern and possessed mildly hypertrophic nuclei, particularly in the endocardial and epicardial zones. The myocardium contained a few scattered foci of mononuclear cell infiltrates.

Lesions in Case 11 were similar to those described in Case 1, but were more severe. Extensive areas of necrosis and loss of cardiac muscle cells with replacement fibrosis were noticeable throughout the interventricular septum and ventricular walls (Fig. 20). Individual fibres neighbouring the areas of necrosis appeared mildly thickened and had a more eosinophilic and slightly vacuolated sarcoplasm and pycnotic nuclei.

FIG. 20 Case 11: Heart. Large area of necrosis and loss of muscle fibres with replacement fibrosis in the left ventricle. HE × 600

Other organs: In Cases 9–11 the alveolar walls were mildly to moderately thickened by mononuclear cells, fibrosis and congestion. A small number of alveolar macrophages were present as well as focal emphysema.

Discussion

In 5 of the experimental sheep liver involvement at a very early stage of the intoxication was reflected by increases in the activities of GGT in the serum within 9 days after commencement of dosing. Apart from the kidney, GGT occurs in significant amounts in the liver and pancreas with only minor quantities in the intestines, heart and other tissues (Boyd, 1988). In the liver the enzyme is membrane-bound in hepatocytes and bile duct epithelium and is most commonly associated with cholestasis and bile duct damage (Boyd, 1988; Malherbe, Kellerman, Kriek & Haupt, 1977). The initial, prompt rises in the experimental animals can probably be attributed to microsomal induction when morphological alterations in the hepatocytes were minimal (Malherbe *et al.,* 1977). Gradual increases in the activities of GGT subsequent to the initial rises may result from a direct effect of the toxin(s) of *G. africana* on the liver.

Except for marked terminal elevations in 2 sheep, the activities of AST were normal. This enzyme is found in substantial quantities in the liver, cardiac and skeletal muscles of sheep (Boyd, 1988) and is commonly used at the Onderstepoort Veterinary Institute to monitor acute hepatotoxic damage in ruminants (Malherbe *et al.,* 1977; Van der Lugt, Nel & Kitching, 1991; Van der Lugt, Nel & Kitching, 1992). It also proved to be the most reliable indicator of cardiac damage in gousiekte in sheep (Fourie, Schultz, Prozesky, Kellerman & Labuschagne, 1989). The lack of change in AST activity indicates that acute hepatocellular and myocardial damage was absent during the major part of the intoxication.

The activities of LD followed the same pattern, confirming that the heart was only terminally involved. Rises in the activities of this enzyme do not indicate hepatic damage since liver tissue contains less LD than the myocardium. Since the profile of activity of the various isoenzymes in cardiac and hepatic tissue and serum in sheep are similar with LD_1 predominating (Beatty, 1983), this pattern is maintained when serum LD activity is elevated (Fourie *et al.,* 1989). Cardiac damage therefore cannot be distinguished from liver damage on the basis of disparities in the activities of the various isoenzymes in the serum.

In the last stage of the intoxication the CPFI increased to values of 11,5 (heart rate 135) and 10 (heart rate 130) in Cases 10 and 11, respectively. Values between 8,5 and 14 in man and sheep are associated with decreased cardiac function, while values greater than 14 are indicative of congestive heart failure (J. J. van der Walt & J. M. van Rooyen, personal communication, 1991). In Case 11 however, cardiac output and stroke volume were within normal limits and the right atrial pressure was lower than normal (Table 2). These findings, especially the low right atrial pressure, suggest that towards the end of the dosing period in the above 2 animals there were indications of impaired cardiac function but no congestive heart failure. It is worthy of mention that the CPFI may increase at heart rates higher than 130 beats/min as a result of a decreased filling time of the ventricles (Van der Walt *et al.*, 1981).

The liver lesions in the experimental and field cases could be attributed to a primary hepatotoxic effect. Coagulative necrosis and lysis and in particular ballooning degeneration are changes suggestive of toxin-induced injury. Ballooning degeneration is associated with viral hepatitis in man (Ishak, 1976), though it may be found in other conditions such as drug-induced toxicity (Zimmerman & Ishak, 1979). This type of degeneration was also described recently in cattle poisoned by the plant *Cestrum laevigatum* (Van der Lugt *et al.,* 1991). Collapse and proliferation of the reticulin network with subsequent fibrosis in the centrilobular areas may follow on toxin-induced necrosis, while passive venous congestion may have exacerbated these lesions. Dilation of central veins, portal lymphatics and spaces of Disse as well as a reversed lobular pattern, noticed in some of the animals, is associated with passive venous congestion (Kelly, 1985). Cases 5–9 were drenched with a different batch of plant material which proved to be less toxic. Hepatic lesions in these animals were minimal to mild and comprised dilation of central veins and adjacent sinusoids as well as atrophy of centrizonal hepatocytes, and centrilobular fibrosis in the slightly more advanced cases. In the light of the clinical pathological findings and heart function tests the pathogenesis of these lesions cannot be explained in terms of congestive heart failure, although lesions similar in nature have been associated with chronic passive venous congestion in man (Dunn, Hayes, Breen & Schenker, 1973; Sherlock, 1951) and animals (Kelly, 1985).

Gross cardiac lesions were not observed in the present study but microscopical changes varying in nature and severity were present in the myocardium of all the animals. Variations in the nature of these lesions could probably be related in part to the toxicity of the plant material and chronicity of the toxicosis. In Cases 5–7 myocardial lesions were minimal and characterized by multifocal fibre loss and interstitial fibrosis. Muscle fibres in the ventricles and interventricular septum in Cases 1 and 11 were diffusely hypertrophic with areas of necrosis and replacement fibrosis. In Case 10 there were areas of hypertrophy and disorganization of myofibres, while fibres in the remainder of the ventricles and septum appeared atrophic. In cases of longer duration (Cases 2–4, 8 and 9) lesions were characterized by diffuse atrophy of cardiac muscle cells accompanied by foci of interstitial fibrosis. No distinct pattern was noticeable in the distribution of the myocardial lesions in the experimental cases and the lesions were distributed throughout the ventricular walls and interventricular septum. In 3 of the field cases (Cases 2–4), however, microscopical changes were more pronounced in the inner third of the myocardium.

Although only a limited number of cases were studied, it appears that the initial myocardial lesions

in *G. africana* poisoning is hypertrophy of myocytes which may be followed by degeneration and necrosis of hypertrophic fibres with resultant replacement fibrosis. In cases of longer duration the most prominent change was atrophy of myocytes. Myocyte degeneration occurs commonly in hypertrophic human hearts and is thought to represent a final common pathway of cellular damage, leading to fibrosis (Ferrans, 1978). Meerson (1969) developed the concept that cardiac muscle evolves through three different functional stages during the time course of hypertrophy. Hypertrophy begins during the first stage, in which there is an increase in energy production and protein synthesis. A stable state of cardiac hyperfunction exists during the second stage. The third stage is characterized by gradual exhaustion of protein synthesis associated by myofibrillar damage and myocyte atrophy. Based on this concept it is speculated that in longstanding cases of *G. africana* poisoning, hypertrophic fibres, instead of undergoing degeneration, become atrophic.

Hyperplasia of the wall of intramyocardial arteries was noticeable in three field cases (Cases 2–4). Similar vascular lesions have been reported in cardiomyopathies in humans, dogs and cats (Van Vleet & Ferrans, 1986) and in sheep with gousiekte (Prozesky, Fourie, Neser & Nel, 1988). These changes may contribute to focal myocardial ischemic injury with subsequent necrosis and fibrosis in hearts with cardiomyopathy (Van Vleet, Ferrans & Weirich, 1981). The importance of this finding is not known, but it seems unlikely that the vascular lesions play a role in the pathogenesis of *G. africana* poisoning.

This study emphasises the difficulty in experimentally reproducing toxicoses as they are encountered in field situations. This was especially highlighted by our inability to induce the marked ascites which is the important diagnostic feature of the naturally occurring disease from which the name "waterpens" (waterbelly) is derived. The available clinical pathological and pathological findings in this report suggest that *G. africana* is primarily hepatotoxic and that the heart is affected in the later stages of the intoxication. Further experiments are needed to elucidate the pathogenesis of this plant poisoning.

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REFERENCES

BEATTY, ELSPETH H., 1983. A study of selected isoenzymes in sheep. D. Phil. thesis, University of Edinburgh.

BOYD, J. W., 1988. Serum enzymes in the diagnosis of diseases in man and animals. *Journal of Comparitive Pathology,* 98, 381–404.

DE KOCK, G., 1928. Diseases of sheep in relation to the pasture under South African conditions. *Journal of the South African Veterinary Medical Association,* 1, 29–38.

DUNN, G. D., HAYES, P., BREEN, K. J. & SCHENKER, S., 1973. The liver in congestive heart failure. *American Journal of Medical Science,* 265, 174–189.

FERRANS, V. J., 1978. Myocardial ultrastructure in human cardiac hypertrophy. *In:* KALTENBACH, M., LOOGEN, F. & OLSON, E. G. J. (eds.). Cardiomyopathy and myocardial biopsy. Berlin, New York: Springer-Verlag.

FOURIE, N., SCHULTZ, R. ANITRA, PROZESKY, L., KELLERMAN, T. S. & LABUSCHAGNE, LEONIE, 1989. Clinical pathological changes in gousiekte, a plant-induced cardiotoxicosis of ruminants. *Onderstepoort Journal of Veterinary Research,* 56, 73–80.

ISHAK, K. G., 1976. Light microscopic morphology of viral hepatitis. *American Journal of Clinical Pathology,* 65, 787–827.

KELLERMAN, T. S., COETZER, J. A. W. & NAUDÉ, T. W., 1988. Plant poisonings and mycotoxicoses of livestock in southern Africa. Cape Town: Oxford University Press.

KELLY, W. R., 1985. The liver and biliary system. *In:* JUBB, K. V. F., KENNEDY, P. C. & PALMER, N. (eds.). Pathology of domestic animals. Vol. 2, 3rd edn. Orlando, San Diego, New York: Academic Press, Inc.

LUNA, L. G. (ed)., 1968. Manual of the histological staining methods of the Armed Forces Institute of Pathology. 3rd edn. New York: McGraw-Hill.

MALHERBE, W. D., KELLERMAN, T. S., KRIEK, N. P. J. & HAUPT, W. H., 1977. Gamma-glutamyl transpeptidase activity in sheep serum: normal values and an evaluation of its potential for detecting liver involvement in experimental lupinosis. *Onderstepoort Journal of Veterinary Research,* 44, 29–38.

MEERSON, F. Z., 1969. The myocardium in hyperfunction, hypertrophy and heart failure. *Circulation Research,* 25, Supplement 11, 1–169.

MERCK, E., 1974. Clinical laboratory. 11th edn. Darmstadt: E. Merck.

NEWSHOLME, S. J. & COETZER, J. A. W., 1984. Myocardial pathology of domestic ruminants in southern Africa. *Journal of the South African Veterinary Association,* 55, 89–96.

PEARSE, A. G. E., 1961. Histochemistry, theoretical and applied. 2nd edn. London: J. & A. Churchill.

PROZESKY, L., FOURIE, N., NESER, J. A. & NEL, P. W., 1988. A field outbreak in Ile-de-France sheep of a cardiotoxicosis caused by the plant *Pachystigma pygmaeum* (Schltr) Robyns (Rubiaceae). *Onderstepoort Journal of Veterinary Research,* 55, 193–196.

SCHULTZ, R. ANITRA, PRETORIUS, P. J. & TERBLANCHE, M., 1972. An electro-cardiographic study of normal sheep using a modified technique. *Onderstepoort Journal of Veterinary Research,* 39, 97–106.

SHERLOCK, S., 1951. The liver in heart failure. *British Heart Journal,* 13, 273–293.

VAHRMEIJER, J., 1981. Poisonous plants of southern Africa that causes stock losses. Cape Town: Tafelberg Publishers Limited.

VAN DER LUGT, J. J., NEL, P. W. & KITCHING, J. P., 1991. The pathology of *Cestrum laevigatum* (Schlechtd.) poisoning in cattle. *Onderstepoort Journal of Veterinary Research,* 58, 211–221.

VAN DER LUGT, J. J., NEL, P. W. & KITCHING, J. P., 1992. Experimentally-induced *Cestrum laevigatum* (Schlechtd.) poisoning in sheep. *Onderstepoort Journal of Veterinary Research,* 59, 135–144.

VAN DER WALT, J. J. & VAN ROOYEN, J. M., 1977. Use of technetium-99m to determine haemodynamic changes during the development of ventricular failure with gousiekte. *South African Medical Journal,* 52, 375.

VAN DER WALT, J. J., VAN ROOYEN, J. M., CILLIERS, G. D., VAN RYSSEN, J. C. J. & VAN AARDE, M. N., 1981. Ratio of cardiopulmonary blood volume to stroke volume as an index of cardiac function in animals and in man. *Cardiovascular Research,* 15, 580–587.

VAN ROOYEN, J. M., 1987. Die beheer van die kardiopulmonêre bloedvolume en slagvolume by normale en versakende harte van skape. DSc thesis, Potchefstroom University for Christian Higher Education.

VAN ROOYEN, J. M. & VAN DER WALT, J. J., 1989. The regulation of the ratio of the cardiopulmonary blood volume to stroke volume in sheep. *Cardiovascular Research,* 23, 453–459.

VAN VLEET, J. F. & FERRANS, V. J., 1986. Myocardial disease of animals. *American Journal of Pathology,*124, 98–178.

VAN VLEET, J. F., FERRANS, V. J. & WEIRICH, W. E., 1981. Pathological alterations in congestive cardiomyopathy of dogs. *American Journal of Veterinary Research,* 42, 416–424.

ZIMMERMAN, H. J. & ISAK, K. G., 1979. Hepatic injury due to drugs and toxins. *In:* MACSWEEN, R. N. M., ANTHONY, P. P. SCHEUER, P. J. & POPPER, H. (eds.). Pathology of the Liver. Edinburgh, London, New York: Churchill Livingstone.