Rev. Elev. Méd. vét. Pays trop., 1985, **38** (2) : 185-194.

Effects of *Cissus quadrangularis* on goats and sheep in Sudan

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RÉSUMÉ

BARAKAT (S. E. M.), ADAM (S. E. I.), MAGLAD (M. A.) et WASFI (I. A.). — Effets de *Cissus quadrangularis* sur des chèvres et des moutons au Soudan. *Rev. Elev. Méd. vet. Pays trop.*, 1985, **38** (2) : 185-194.

Sept moutons du désert et dix chèvres de Nubie ont reçu de 0,25 à 10 g/kg/j de tiges désséchées de *Cissus quadrangularis* et sont morts ou ont été sacrifiés à différents moments.

Les principaux symptômes de l'intoxication par cette plante étaient les suivants : inappétence, abattement, inaction, démarche ébrieuse, dyspnée, accès de diarrhée, perte de condition et voussure du dos. Les modifications pathologiques dominantes étaient représentées par des hémorragies dans les reins, les poumons, le cœur et l'intestin, de l'entérite catarrhale localisée, l'atrophie séreuse de la graisse cardiaque, de l'hydropéritoine et de l'hydropéricarde. Un accroissement de l'activité des G.O.T., une augmentation des concentrations de créatinine et d'ammoniaque ainsi qu'une baisse des protéines totales ont été décelés dans le sérum. Une leucocytose et une anémie étaient les seules modifications hématologiques.

Mots clés : Chèvre - Mouton - *Cissus quadrangularis* - Plante médicinale - Intoxication - Soudan.

SUMMARY

BARAKAT (S. E. M.), ADAM (S. E. I.), MAGLAD (M. A.), WASFI (I. A.). — Effects of *Cissus quadrangularis* on goats and sheep. *Rev. Elev. Méd. vét. Pays trop.*, 1985, **38** (2) : 185-194.

Seven Desert sheep and ten Nubian goats were given daily oral doses ranging from 0.25 to 10 g per kg per day of the dried stems of *Cissus quadrangularis* and died or were killed at various times after dosing. Inappetence dullness, stagging, dyspnoea, bouts of diarrhoea, loss of condition and arching of the back were the important signs of *C. quadrangularis* poisoning in goats and sheep. The outstanding pathological changes were haemorrhages in the kidneys, lungs, heart and intestine, focal catarrhal enteritis, serous atrophy of the cardiac fat, hydroperitoneum, and hydropericardium. An increase in the activity of G.O.T. and in the concentrations of creatinine and ammonia and a decrease in total protein were detected in the serum. Haematological changes were leucocytosis and anaemia.

Key words : Goat - Sheep - *Cissus quadrangularis* - Medicinal plant - Poisoning - Sudan.

INTRODUCTION

Cissus quadrangularis, a member of the family *Vitaceae* is recognized as a medicinal plant in African countries (14). The plant is suspected to cause significant livestock losses in the Sudan, but this has not been confirmed experimentally.

The present study was planned to examine the clinical, biochemical and pathological effects of dosing goats and sheep with the stems of *C. quadrangularis.*

MATERIALS AND METHODS

Animals. Twelve 3 to 6 month-old Nubian goats and nine 6 to 9 month-old Desert sheep were used. The animals were kept in pens at the Department of Veterinary Medicine, Toxico-

logy and Pharmacology, University of Khartoum and fed on lucerne and water *ad libitum*.

Dosing. The plant collected from El Guesi, Blue Nile Province was dried in the sun and finely ground in a mortar and given to the experimental animals as a suspension in water by stomach tube each day until death or slaughter.

Goats. The goats were divided into six groups. The powdered stems of *C. quadrangularis* were given at the dose rates of 10 g/kg/day to two goats (group I), 5 g/kg/day to two goats (group II), 1 g/kg/day to two goats (group III), 0.5 g/kg/day to two goats (group IV) and 0.25 g/kg/day to two goats (group V). The two goats in group VI were kept as undosed controls. The total amount of stems received by each animal is given in table n° I.

Sheep. The sheep were divided into four groups. The powdered stems of the plant were suspended in water and given by a stomach tube at the dose rates of 10 g/kg/day to two sheep (group I), 5 g/kg/day to three sheep (group II) and 1 g/kg/day to two sheep (group III). The two sheep in group IV were kept as untreated controls. The total amount of stems received by each sheep is given in table n° II.

Sampling. The animals were bled from the jugular vein before the experiment and at appropriate intervals afterwards for chemical investigations on serum and the cellular elements of the blood.

TABLE N°I - Dosing schedule and time of death of *Cissus* poisoned goats

Group	Goat N°	Age (months)	Sex	Daily amount of *Cissus* fed* (g/kg)	Total amount of *Cissus* fed (g)	Onset of Clinical Signs (days)	Death (days)
I	83	5	M	10	1 080	6	12
	84	6	M	10	1 520	10	16
II	85	6	M	5	810	10	18
	86	5	M	5	800	14	20
III	87	4	F	1	225	21	30
	88	6	M	1	1 467	25	163 (killed)
IV	89	3	F	0.5	126	10	36
	90	4	M	0.5	204	18	51
V	91	4	M	0.25	61.9	21	33
	92	5	F	0.25	68	21	34

* Given by stomach tube.

TABLE N°II - Dosing schedule and time of death of *Cissus* poisoned Sheep

Group	Sheep N°	Age (months)	Sex	Daily amount of *Cissus* fed* (g/kg)	Total amount of *Cissus* fed (g)	Onset of Clinical Signs (days)	Death (days)
I	48	8	F	10	1 513.6	1	9
	49	9	M	10	2 481.8	1	13
II	50	7	M	5	2 990.9	6	28 (killed)
	51	6	F	5	1 431.8	4	14
	52	9	M	5	2 304.5	7	26
III	53	8	F	1	386.8	5	23
	54	7	M	1	290.9	7	20 (killed)

* Given by stomach tube.

Histological methods. Blocks of liver, intestines, spleen, kidney, lungs, heart, spinal cord and brain were fixed in 10 p. 100 formol-saline and paraffin sections 6 µm thick were stained with haematoxylin and eosin (H & E). Other methods used on selected sections were Masson's trichrome, Gordon and Sweet's method, periodic acid-Schiff (P.A.S.) method with and without prior incubation with diastase, Perl's prussian blue reaction for haemosiderin and Lillie's method for lipofuchsin.

Chemical methods. Blood samples, collected from goats and sheep before and during the experimental period, were allowed to clot and the sera were analysed for the activity of aspartate amino-transferase (G.O.T.) and alanine amino-transferase (G.P.T.) by the method of REITMAN and FRANKEL (11), total protein concentration by the biuret method, total bilirubin by the method of DANGERFIELD and FINLAYSON (7), ammonia by the method of VERLEY (13) and uric acid by the method of WHITE and FRANKEL (15). Creatinine concentrations were determined by the method of WHITE and FRANKEL (15).

Haematological methods. Blood samples were collected into dry clean bottles containing E.D.T.A. Haemoglobin concentration (Hb) was determined by the cyanmethaemoglobin technique with a haemoglobin meter (Evans Electroselenium Limited, England). Packed cell volume (P.C.V.) was measured in a micro-haematocrit centrifuge (Hawksley and Sons Limited, England). Red and white blood corpuscles (R.B.C. and W.B.C.) were counted in improved Neubauer haemocytometers. Mean corpuscular volume (M.C.V.) and mean corpuscular haemoglobin concentration (M.C.H.C.) were calculated from P.C.V., R.B.C. and Hb values.

RESULTS

The dosing schedule and the time of death of the *Cissus*-poisoned goats and sheep are given in tables I and II.

Nine of the *Cissus*-poisoned goats 83, 84 (group I), 85, 86 (group II) 87 (group III), 89, 90 (group IV), 91 and 92 (group V) died and one goat 88 (group III) was slaughtered. Five sheep 48, 49 (group I), 51, 52 (group II) and 53 (group III) died and two sheep 50 (group II) and 54 (Group III) were slaughtered.

Clinical findings

Goats. Goats 83, 84 (group I), 85 and 86 (group II) showed inappetence, dullness, staggering, diarrhoea, dyspnoea and recumbency between days 6 and 14. They died on days 12, 16, 18 and 20 respectively. Dyspnoea, inappetence, staggering, arching of the back and loss of condition were the main signs in goats 87 (group III), 89, 90 (group IV), 91 and 92 (group V) which died on days 30, 36, 51, 33 and 34 respectively. Goat 88 (group III) showed signs of toxicity and was slaughtered on day 163. The control goats 93 and 94 showed no clinical changes and were slaughtered on day 163.

Sheep. Sheep 48, 49 (group I), 50, 51, 52 (group II), 53 and 54 (group III) showed profound depression, inappetence, diarrhoea, dyspnoea, arching of the back, loss of condition and recumbency. They died or were slaughtered on days 9, 13, 28, 14, 26, 23 and 20 respectively. The control sheep 55 and 56 showed no clinical changes and were slaughtered on day 28.

Post-mortem findings

Goats. The post-mortem findings in the goats dosed with various levels of *C. quadrangularis* are summarized in table n° III. There were haemorrhages in the kidneys, spleen, lungs, heart and the wall of the small intestine, catarrhal enteritis and fatty change and congestion of the liver of *Cissus*-poisoned goats. Focal haemorrhages in the lungs, intestine and kidneys, serous atrophy of the epicardial fat, hydroperitoneum, hydropericardium and hydrothorax were seen in goats in groups III, IV and V. There were no changes in the control goats 93 and 94.

Sheep. In sheep 48, 49 (group I), 50, 51, 52 (group II), 53 and 54 (group III), the lesions were similar to those seen in *Cissus*-poisoned goats, but gelatinization of the epicardial fat, hydroperitoneum, and hydrothorax were less marked (Table IV).

TABLE N°III - Assessment of the post-mortem findings in goats fed with *Cissus quadrangularis*

Site	Findings	Group				
		I	II	III	IV	V
Abomasum and small intestine	Focal catarrhal abomasitis and enteritis	+++	+++	++	++	++
Liver	Fatty change and congestion	+++	+++	++	++	++
Kidney	Haemorrhage and fatty change	+++	+++	++	++	++
Spleen	Haemorrhage	++	++	+	+	+
Heart	Haemorrhage	++	++	+	+	+
	Gelatinization of fat	(-)	(-)	++	+	+
Lungs	Congestion and haemorrhage	+++	+++	+	++	+
	Pulmonary oedema	(-)	(-)	+	+	+
Serous cavities	Hydroperitoneum, hydrothorax and hydropericardium	(-)	(-)	+	+	+

+, ++, +++, increasing severity of lesions ; (-), absence of lesions.

TABLE N°IV - Assessment of the post-mortem findings in sheep fed with *Cissus quadrangularis*

Site	Findings	Group		
		I	II	III
Abomasum and small intestine	Focal catarrhal abomasitis, enteritis and erosions	+++	++	++
Liver	Congestion and fatty change	+++	+++	+++
Spleen	Haemorrhage	++	++	++
Kidney	Haemorrhage and fatty change	++	++	+
Heart	Haemorrhage and gelatinization of fat	++	++	++
Lungs	Congestion and haemorrhage	+++	+++	++
	Pulmonary oedeme	(-)	+	+
Serous cavities	Hydroperitoneum, hydrothorax and hydropericardium	(-)	+	+

+, ++, +++, increasing severity of lesions ; (-), absence of lesions.

Histological findings

Goats. There was cytoplasmic fatty vacuolation of the centrilobular hepatocytes, congestion and infiltration of fibroblasts in the hepatic portal areas, focal catarrhal abomasitis and enteritis, congestion and haemorrhage in the renal cortex and medulla, the splenic red pulp and pulmonary alveoli. Degeneration and/or necrosis of the renal convoluted tubules (Fig. 1), hepatic fatty change, haemorrhage and congestion of the intestinal *lamina propria* and degeneration of scattered cardiac muscle fibres were more severe in goats in group I and II. Pulmonary oedema and splenic haemosiderosis were seen especially in goats in groups IV and V.

Sheep. There was catarrhal enteritis and focal loss of the intestinal epithelium (Fig. 2), cytoplasmic fatty vacuolation of the centrilobular hepatocytes and accumulation of fibroblasts in the hepatic portal tract, necrosis and/or degeneration of the cells of the renal tubules in *Cissus*-poisoned sheep. The affected renal tubules contained acidophilic homogenous material and haemorrhage was seen in the cardiac muscle fibres, pulmonary alveoli and splenic red pulp.

Changes in serum constituents

Goats. There were no changes in the concentrations of bilirubin and uric acid and in the activity of G.P.T. in the serum of any of the

Fig. 1. — Kidney of goat 86, orally dosed with 5 g/kg/day of dry *Cissus* stems for 20 days. Degeneration of the cells of the convoluted tubules. H & E × 150.

Fig. 2. — Intestine of sheep 49, orally dosed with 10 g/kg/day of dry *Cissus* stems for 13 days. Catarrhal enteritis and focal loss of the intestinal epithelium. H & E × 150.

goats. Goats in groups I and II were exemplified by goat 86 which died on day 20. The concentration of G.O.T. and ammonia had risen on the 5th or the 7th day, reached peaks on the 8th or the 10th day and remained high at the time of death (Fig. 3). Creatinine sho-

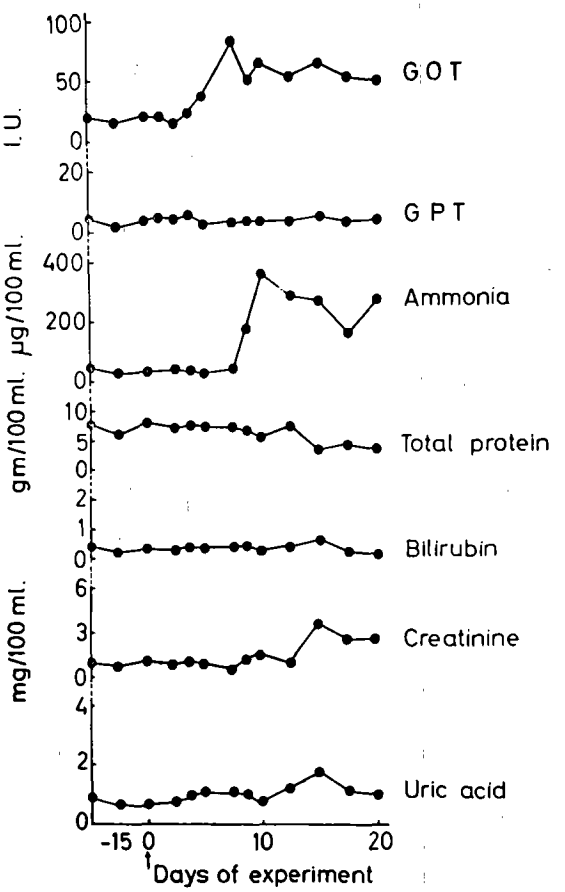

Fig. 3. — Changes in the concentrations in serum of G.O.T., G.P.T., ammonia, total protein, total bilirubin, creatinine and uric acid in goat 86, orally dosed with 5 g/kg/day of dry *Cissus* stems for 20 days. The arrow on the time axis indicates the commencement of dosing.

wed terminal raised concentration. The fall in total protein occurred between days 15 and 20. Animals of group III were exemplified by goat 88 which was slaughtered on day 163. The activity of G.O.T. commenced to rise on the 52nd day, reached a peak on the 85th day and fluctuated above normal until the time of death (Fig. 4). The increase in the concentration of ammonia and the fall in total protein occurred between days 90 and 163.

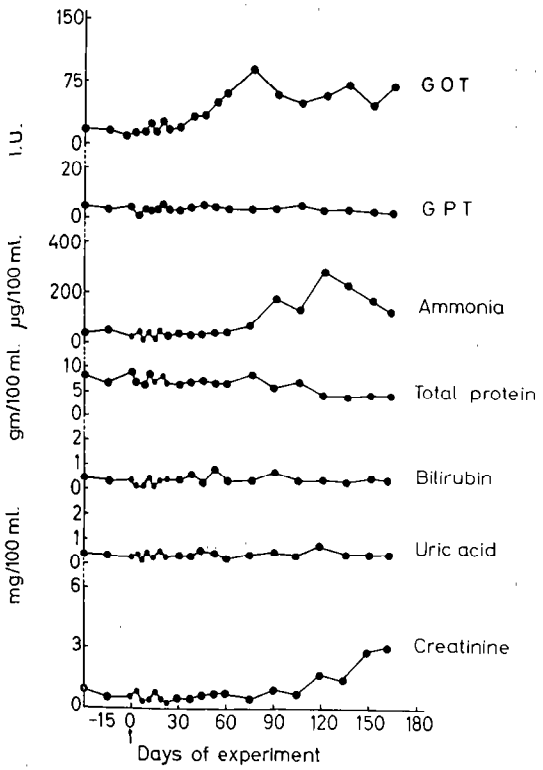

Fig. 4. — Changes in the concentrations in serum of G.O.T., G.P.T., ammonia, total protein, total bilirubin, creatinine, and uric acid in goat 88, orally dosed with 1 g/kg/day of dry *Cissus* stems for 163 days.

Creatinine showed raised concentrations terminally. Animals in group IV were exemplified by goat 90 which died on day 51. The activity of G.O.T. had risen on the 15th day, reached a peak on the 53rd day and remained high at the time of death (Fig. 5). Ammonia and creatinine showed raised concentrations terminally. The concentrations of total protein fell between days 35 and 51. In goat 92 (group V), the activity of G.O.T. and the concentration of ammonia had risen on the 12th day, reached peaks on the 15th or the 25th day and remained high at the time of death (Fig. 6). The

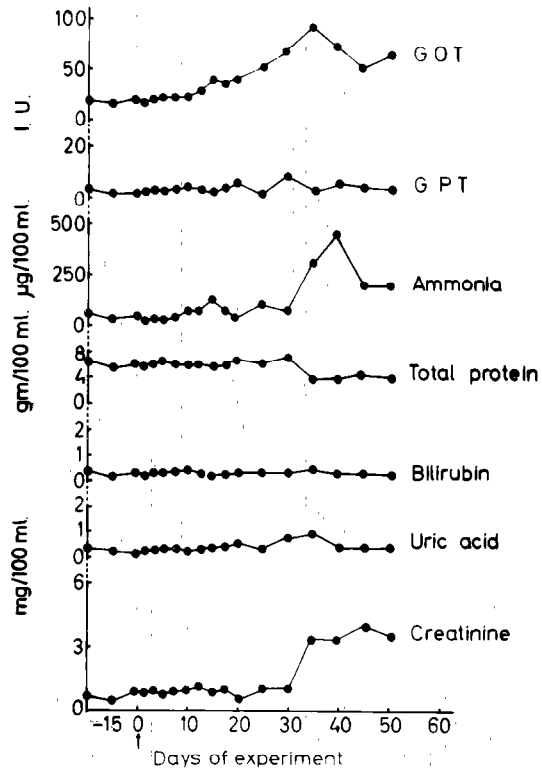

Fig. 5. — Changes in the concentrations in serum of G.O.T., G.P.T., ammonia, total protein, total bilirubin, creatinine and uric acid in goat 90, orally dosed with 0.5 g/kg/day of dry *Cissus* stems for 57 days.

concentrations of total protein fell and that of creatinine increased between days 25 and 34. The concentrations of total protein, total bilirubin, ammonia, creatinine and uric acid and the activity of serum enzymes in control goats did not change.

Sheep. There were no changes in the activity of G.P.T. or in the concentrations of bilirubin and uric acid in the serum of any sheep. Animals of group I were exemplified by sheep 49 which died on day 13. The activity of G.O.T. commenced to rise on day 6, reached a peak on day 10 and remained high at the time of death (Fig. 7). Ammonia and creatinine showed raised concentrations terminally. The fall in total protein occurred between days 11 and 13. Animals in group II were exemplified by sheep 52 which died on day 26. The activity of G.O.T. and the concentration of ammonia had risen on the 12th day, reached peaks on the 14th or the 21st day and remained high at the time of death (Fig. 8). The concentration of creatinine had risen on the 18th day and reached a peak on the 25th day. Total protein

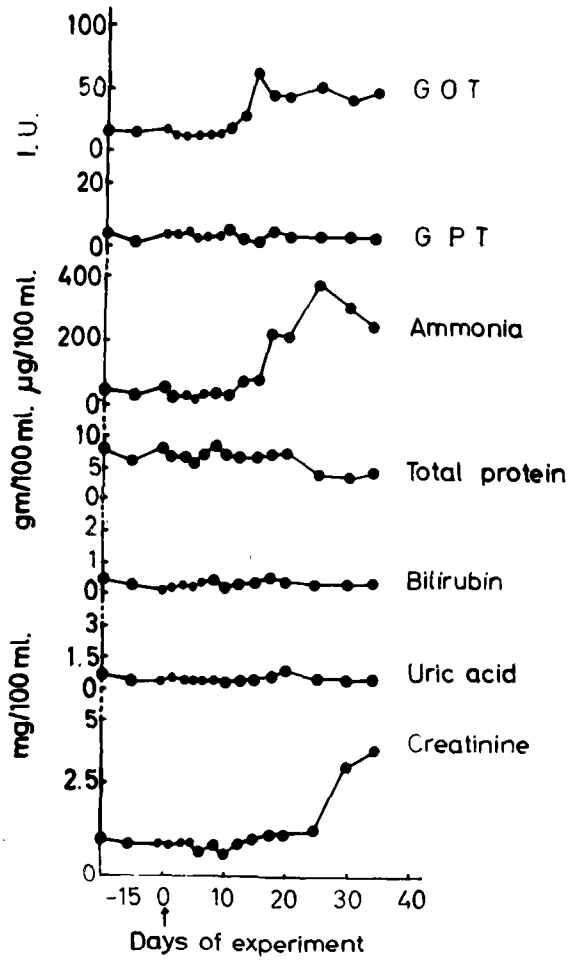

Fig. 6. — Changes in the concentrations in serum of G.O.T., G.P.T., ammonia, total protein, total bilirubin, creatinine and uric acid in goat 92, orally dosed with 0.25 g/kg/day of dry *Cissus* stems for 34 days.

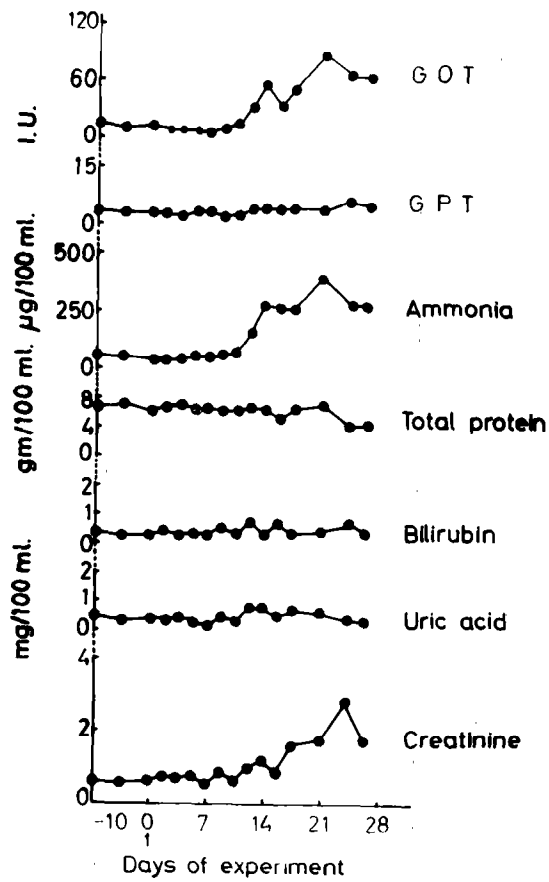

Fig. 8. — Changes in the concentrations in serum of G.O.T., G.P.T., ammonia, creatinine, total protein, total bilirubin and uric acid in sheep 52, orally dosed with 5 g/kg/day of dry *Cissus* stems for 26 days.

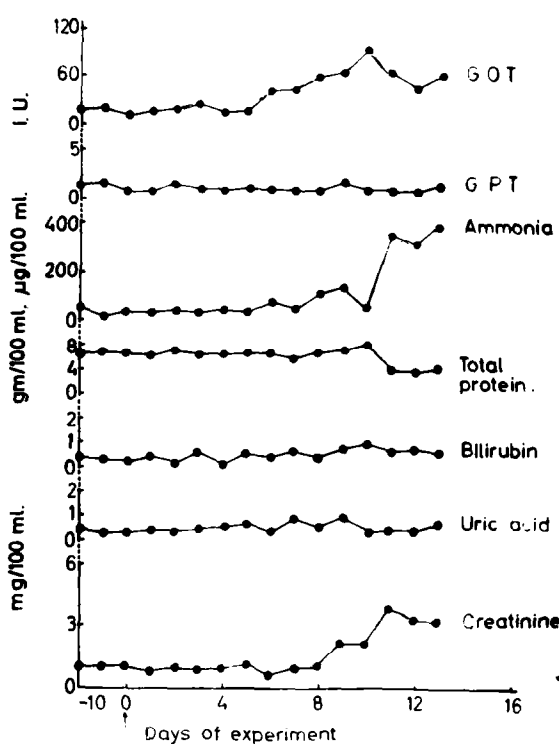

Fig. 7. — Changes in the concentrations in serum of G.O.T., G.P.T., ammonia, creatinine, total protein, total bilirubin and uric acid in sheep 49, orally dosed with 10 g/kg/day of dry *Cissus* stems for 13 days.

concentration was reduced at the time of death. In sheep 53 (group III), the activity of G.O.T. commenced to rise on the 10th day, reached a peak on the 14th day and remained high at the time of death (Fig. 9). Ammonia and creatinine showed raised concentrations terminally. The concentration of total protein was reduced between days 16 and 23. The activity of serum enzymes and concentration of bilirubin, total protein, ammonia, creatinine and uric acid in control sheep did not change.

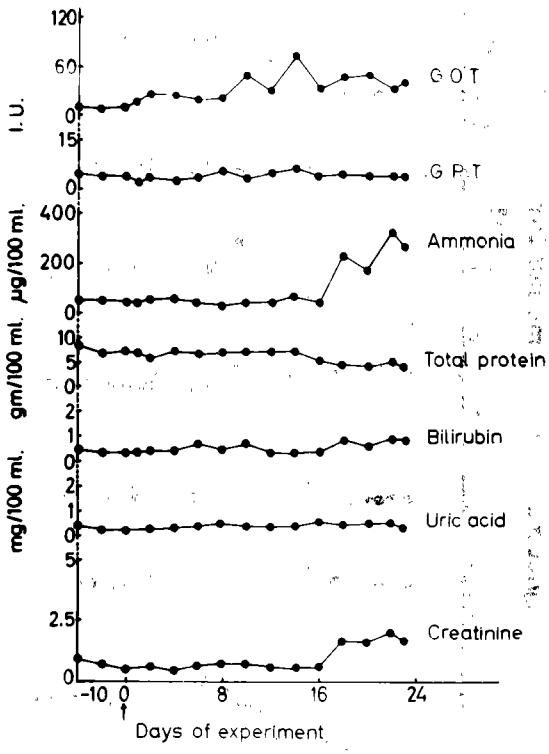

Fig. 9. — Changes in the concentrations in serum of G.O.T., G.P.T., total protein, total bilirubin, ammonia, creatinine and uric acid in sheep 53, orally dosed with 1 g/kg/day of dry *Cissus* stems for 23 days.

Haematological findings

Goats. Goats 83, 84 (group I), 85, 86, (group II), 87, 88 (group III), 89, 90 (group IV), 91 and 92 (group V) showed similar haematological changes and were exemplified by goat 92 (group V) which died on day 34. There was a decrease in the values of Hb., P.C.V. and R.B.C. between days 19 and 34 (Fig. 10). No significant changes were noticed in M.C.H.C. value. The M.C.V. had decreased between days 19 and 26. Leucocytosis was noticed between days 5 and 19 and was due to an increase in neutrophils.

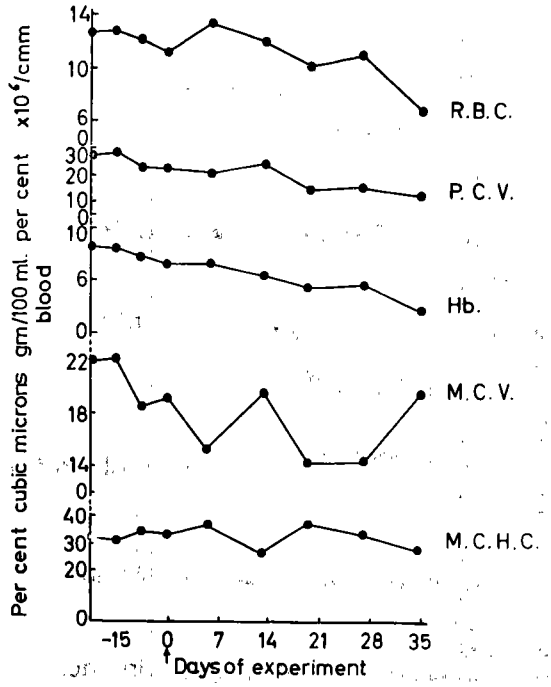

Fig. 10. — Changes in the values of Hb., P.C.V., R.B.C., M.C.V. and M.C.H.C. in goat 92, orally dosed with 0.25 g/kg/day of dry *Cissus* stems for 34 days.

Sheep. Sheep 48, 49 (group I), 50, 51, 52 (group II), 53 and 54 (group III) showed similar haematological changes and were exemplified by sheep 50 which died on day 28. There was a decrease in the values of Hb., P.C.V. and R.B.C. between days 17 and 28. There were no significant changes in the M.C.V. and M.C.H.C. Leucocytosis was due to an increase in neutrophils.

DISCUSSION

Cissus poisoning in varying degrees of severity was produced in ten goats and seven sheep. Four goats 83, 84, 85 and 86 were severely affected ; there was ample evidence of tissue damage and they died within 20 days. Five goats 87, 89, 90, 91 and 92 were moderately affected and died within 51 days. One goat 88 was slaughtered on day 163. The seven sheep were severely affected and died or were killed within 28 days. Desert sheep are more susceptible to the effect of *C. quadrangularis* than are Nubian goats. This is based on the rapid onset of morbidity and the short course

of the toxic effects. The difference between calves, sheep and goats in their response to ingestion of poisonous plants e.g. *Jatropha curcas* and *Capparis tomentosa* has been observed previously (4, 5) and is further exemplified in this study.

The toxic constituents of the stems of *C. quadrangularis* appeared to cause marked damage to the liver, kidney, intestine, and lungs. The clinical features of anorexia, paresis of the hind limbs, arching of the back and dyspnoea were correlated with the damage to these vital organs. The lesions in the liver consisted of centrilobular fatty change, loss of glycogen and congestion and/or haemorrhage. The depletion of hepatic glycogen in *Cissus*-poisoned sheep and goats indicates interference with carbohydrate metabolism. Effect on hepatic parenchyma is further supported by the high levels of G.O.T. and ammonia in serum and the associated fall in protein concentration. However, the lack of increase in the activity of G.P.T. and in the concentration of bilirubin in the serum of young ruminants fed with hepatotoxic plants has been described and fully explained by FORD, RITCHIE and THORPE (9), GOPINATH and FORD (10), ADAM (1), and ADAM and MAGZOUB (2).

It is well known that the determination of urea nitrogen and creatinine is a useful measure of renal function. The increase in the concentration of both urea and creatinine in the serum of *Cissus*-poisoned sheep and goats indicates severe renal damage. CORNELIUS and KANEKO (6) suggested that the rise in urea concentration in the blood may probably reflect a deficient blood flow to the kidney. There is evidence that the toxic constituents in *C. quadrangularis* have a direct effect on the renal glomeruli to produce necrosis and that the tubular cells, particularly those of the convoluted tubules, are damaged by the action of the plant poisons. The blood levels of creatinine and urea nitrogen showed marked changes in sheep given mercuric chloride (8, 12).

Finally, it would appear from the results of this experiment that *Cissus* toxicity in sheep and goats is of economic importance. Even at the low dose levels of the plant that were used, significantly marked tissue damage was recorded.

ACKNOWLEDGMENTS

This work was supported by a grant from the Graduate College, University of Khartoum, Sudan.

RESUMEN

BARAKAT (S. E. M.), ADAM (S. E. I.), MAGLAD (M. A.) y WASFI (I. A.). — Efectos de *Cissus quadrangularis* en cabras y carneros en Sudan. *Rev. Elev. Méd. vét. Pays trop.*, 1985, **38** (2) : 185-194.

Se alimentaron 7 carneros del desierto y 10 cabras de Nubia con 0,25 a 10 g/kg/día de tallos desecados de *Cissus quadrangularis*. Estos animales murieron o fueron matados a diferentes momentos. Los principales síntomas de la intoxicación por dicha planta eran los siguientes : inapetencia, abatimiento, vertigos, disnea, accesos de diarrea, pérdida de condición y encorvadura del lomo. Se representaban las modificaciones patológicas dominantes por hemorragias en los riñones, los pulmones, el corazón y el intestino, enteritis catarral localizada, atrofia serosa de la grasa cardiaca, hidroperitoneo e hidropericardio. Se observaban un aumento de la actividad de los G.O.T. y de las concentraciones de creatinina y de amoniaco así como una disminución de las proteinas totales en el suero. Una leucocitosis y una anemia eran las solas modificaciones hematológicas.

Palabras claves : Cabra - Carnero - *Cissus quadrangularis* - Planta medicina - Intoxicación - Sudan.

REFERENCES

1. ADAM (S. E. I.). Toxicity of indigenous plants and agricultural chemicals in farm animals. *Clin. Toxicol.*, 1978, **13** : 269-280.
2. ADAM (S. E. I.) and MAGZOUB (M.). Toxicity of *Jatropha curcas* for goats. *Toxicology*, 1975, **4** : 347-354.
3. AHMED (O. M. M.). The toxicity of *Capparis tomentosa* and of *Jatropha curcas* in calves, sheep and goats. M. V. Sci. thesis, University of Khartoum, Sudan, 1978.
4. AHMED (O. M. M.) and ADAM (S. E. I.). Toxicity of *Jatropha curcas* in sheep and goats. *Res. vet. Sci.*, 1979, **27** : 89-96.
5. AHMED (O. M. M.) and ADAM (S. E. I.). Effects of *Jatropha curcas* on calves. *Vet. Path.*, 1979, **16** : 476-482.

6. CORNELIUS (C. E.) and KANEKO (J. J.). Clinical biochemistry of domestic animals. New York, Academic Press, 1963.
7. DANGERFIELD (W. C.) and FINLAYSON (R.). Estimation of bilirubin in serum. *J. clin. Path.*, 1953, **6** : 173-177.
8. FORD (E. J. H.). Activity of gamma-glutamyl transferase and other enzymes in the serum of sheep with liver or kidney damage. *J. comp. Path.*, 1974, **84** : 231-243.
9. FORD (E. J. H.), RITCHIE (H. E.) and THORPE (E.). Serum changes following the feeding of ragwort (*Senecio jacobaea*) to calves. *J. comp. Path.*, 1968, **78** : 207-218.
10. GOPINATH (C.) and FORD (E. J. H.). Location of liver injury and extent of bilirubinaemia in experimental liver lesions. *Vet. Path.*, 1972, **9** : 99-108.
11. REITMAN (S.) and FRANKEL (S.). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. clin. Path.*, 1957, **28** : 56-63.
12. ROBINSON (M.) and HESKETH (A.). Effect of mercuric chloride on the structure and function of the kidney of sheep. *J. comp. Path.*, 1976, **86** : 307-318.
13. VARLEY (H.). Practical clinical biochemistry. 4th ed., New York, William Heinemann Medical Books and Interscience, 1967.
14. WATT (J. M.) and BREYER-BRANDWIJK (N. G.). Medicinal and poisonous plants of Southern and Eastern Africa, 2nd ed., Edinburgh, Livingstone, 1962.
15. WHITE (W. L.) and FRANKEL (S.). Chemistry for medical technologists, 2nd ed., St. Louis, U.S.A., C. V. Mosby Company, 1965.