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The effects of *Indigofera hochstetteri* on goats

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Influence d'*Indigofera hochstetteri* sur des chèvres au Soudan

Sur 12 chèvres nubiennes, 10 ont reçu une ration journalière de 1 à 10 g/kg de pousses desséchées d'*Indigofera hochstetteri*. Elles sont mortes ou ont été sacrifiées ensuite à différents intervalles. Les principaux symptômes d'intoxication étaient les suivants : gonflement, abattement, dyspnée, diarrhée, incoordination des mouvements, postures anormales et anémie. Une augmentation de l'activité de la transaminase glutamique oxaloacétique, des concentrations d'ammoniaque et d'urée et une diminution des protéines totales ont été décelées dans le sérum. La pathologie se manifestait par des hémorragies du cœur, des poumons et des reins, de l'entérite catarrhale et de l'abomasite, de la nécrose hépato-cellulaire et une modification graisseuse, de l'œdème pulmonaire et de l'emphysème.

L'activité de la 5-nucléotidase, de l'adénosine-triphosphatase, de la réductase tétrazolium succinique et du glucose-6 phosphate était réduite dans les cellules hépatiques et les cellules des tubules rénaux.

Mots clés : Intoxication — *Indigofera hochstetteri* — Chèvres — Soudan.

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Summary. — Out of twelve nubian goats, ten were given daily oral doses ranging from 1 to 10 g/kg/day of the dry shoots of *Indigofera hochstetteri* and died or were killed in extremis at various times after dosing. The main signs of poisoning were bloat, depression, dyspnoea, diarrhoea, incoordination of movement, abnormal postures and anaemia. An increase in the activity of GOT, in the concentrations of ammonia and urea and a decrease in total protein were detected in the serum. The main pathological changes were haemorrhages in the heart, lungs and kidneys, catarrhal enteritis and abomasitis, hepato-cellular necrosis and fatty change and pulmonary oedema and emphysema. The activity of 5-nucleotidase, adenosine triphosphatase, succinic tetrazolium reductase and glucose-6-phosphatase was reduced in the hepatocytes and the cells of the renal tubules.

Key words : Intoxication — *Indigofera hochstetteri* — Goats — Sudan.

INTRODUCTION

Several species in the genus *Indigofera (Papilionaceae)* have been the subject of investigations in the United States of America, Great Britain and Australia. For example, feeding of *Indigofera endecaphylla* to sheep and calves has caused hepato-renal injury.

Indigofera hochstetteri locally known as « Sharaya » is common in Central and Northern Sudan and is believed by livestock owners to cause poisoning in animals.

Reports of the toxic effects of *I. hochstetteri* on domesticated animals are lacking. The present study was planned to examine the effects on Nubian goats of the dry shoots of

TABLE N°I-Body weights (in kg) and survival times of goats fed with *Indigofera hochstetteri*

Group	Goat N°	Sex	Age (months)	Daily amount of *Indigofera* given (g/kg)	Total of *Indigofera* given (kg)	Weights of goats (in kg) at days after *Indigofera* given				Death at days
						Before *Indigofera* given	10	20	30	
I	11	M	9	10	0.798	11.40	-	-	-	7
II	12	F	10	10	1.906	14.50	11.40	-	-	14 (killed in extremis)
III	13	M	9	10	1.474	13.60	11.40	-	-	19 (killed in extremis)
	14	M	9	10	2.123	13.60	10.90	-	-	17
II	15	F	11	5	1.047	14.10	11.40	-	-	15 (killedin extremis)
	16	M	7	5	0.163	8.13	-	-	-	4
	17	M	13	5	0.616	15.40	-	-	-	8
III	18	F	10	1	0.413	13.60	12.30	10.90	9.00	35 (killed in extremis)
	19	M	10	1	0.370	12.30	11.40	10.00	8.20	33
	20	M	12	1	0.090	15.00	-	-	-	6

I. hochstetteri on the changes induced in the activities of serum GOT (Glutamic oxalacetic transaminase) and GPT (Glutamic pyruvic transaminase) and in the concentrations of total protein, total bilirubin, ammonia and urea as well as the cellular elements of the blood.

MATERIALS AND METHODS

Animals

Twelve 9 to 13 month-old Nubian goats of both sexes were used in the experiment, two as control animals. They were kept in pens at the Department of Veterinary Clinical Studies, University of Khartoum and fed on lucerne and water *ad libitum*.

Dosing

The plant collected from Wad Hussuna in the Butana area of the Sudan was dried in the sun, ground in a mortar and given to the experimental animals as a suspension in water by drench each day until death or slaughter.

The goats were divided into four groups and the dry shoots of *I. hochstetteri* were given by drench at the dose rates of 10 g/kg/day to four goats (group I), 5 g/kg/day to three goats (group II) and 1 g/kg/day to three goats (group III). The two goats in group IV were kept as undosed controls. The total amount of *Indigofera* shoots received by each animal is given in Table I.

Blood sampling

All goats were bled from the jugular vein before and at appropriate intervals after the commencement of *Indigofera* dosing for chemical investigations on serum and the cellular elements of the blood.

Chemical methods

Blood samples were allowed to clot, serum was separated by centrifugation at 3 000 r.p.m. for 10 minutes and stored at − 20 °C until analysed for the activities of aspartate amino-transferase (E.C.2.6.1.1 ; GOT) and alanine amino-transferase (E.C.2.6.1.2 : GPT) (10). The concentrations of ammonia (12), urea (14), total protein (13), total bilirubin (4), and total lipids (5) were determined by methods described elsewhere.

Histological and enzyme histochemical methods

Tissues fixed in 10 p. 100 formal-saline and paraffin sections 5 µm thick were stained with haematoxylin and eosin (H & E), Masson's trichrome, Gordon and Sweet's methods, Perl's Prussian blue reaction and periodic acid Schiff (PAS) method with and without prior incubation with diastase. For enzyme histochemistry, small blocks of liver and kidneys were immediately frozen in liquid nitrogen and transferred for sectioning to a cryostatic microtome (Slee, London) maintained at − 18 °C, 8 µm thick sections were stained for the activities of succinic tetrazolium reductase (succinic dehydrogenase) glucose-6-phosphatase, adenosine triphosphatase and 5-nucleotidase (9).

Haematological methods

Blood samples were collected into clean bottles containing EDTA as anticoagulant. Haemoglobin was determined by the cyanmethaemoglobin technique with a haemoglobin meter (Evans Electroselenium Ltd., England). Packed cell volume (PCV) was measured in a microhaematocrit centrifuge (Hawksley and Sons Ltd., England). Red and white blood corpuscles (RBC and WBC) were counted in an improved Neubauer haemocytometer. Red cell indices, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated from PCV, RBC and Hb values. A differential leucocyte count was made by the battlement method (11).

RESULTS

Information on goats, dose of *Indigofera* and time of death or slaughter are given in Table I.

Clinical findings

Goats 11, 12, 13, 14 (group I), 15, 16, 17 (group II) and 20 (group III) showed reduced

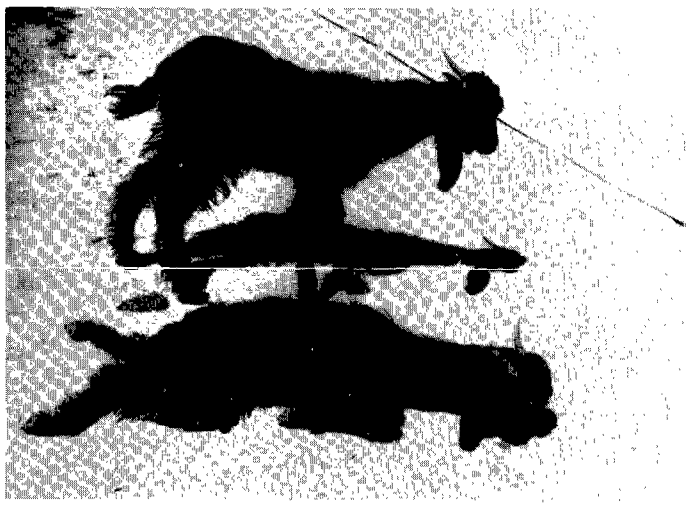

Figure 1. — Ataxia and abnormal postures in two goats fed with dry *Indigofera* shoots.

appetite depression, bloat, diarrhoea, weakness of the hind limbs, incoordination of movement, dyspnoea, abnormal postures, anaemia and recumbency (Fig. 1). They died or were slaughtered in a moribund condition on days 7, 14, 10, 17, 15, 4, 8 and 6 respectively. Goat 18 (group III) showed similar signs of poisoning and was slaughtered on day 35. Goat 19 (group III) died on day 33. There were no clinical changes in the control goats 21 and 22 (group IV).

Post-mortem findings

There were ecchymotic haemorrhages in the heart, lungs and kidneys, catarrhal enteritis and abomasitis, pulmonary emphysema and fatty change and congestion in the liver of goats 11, 12, 13, 14 (group I), 15, 16, 17 (group II) and 20 (group III). Serous atrophy of the cardiac fat and fatty change in the liver and kidneys were particularly marked in goats 18 and 19 (group III). There were no changes in the control goats 21 and 22 (group IV).

Histological findings

There was haemorrhage and congestion in the renal cortex and medulla, the red pulp of the spleen and the submucosae of the small intestine and abomasum in *Indigofera*-poisoned goats. Catarrhal enteritis and abomasitis, degeneration and/or necrosis of the epithelial cells of the renal tubules and cytoplasmic fatty vacuolation of the centrilobular hepatocytes were marked in goats in groups II and III. The nuclei of the periportal liver cells were hyperchromic and some renal tubules contained acidophilic material. Congestion of the pulmonary alveolar capillaries, haemorrhages in renal glomeruli and pulmonary alveoli were seen especially in goats in groups I and II. Also, pulmonary oedema, perineuronal vacuolation in the grey matter of the spinal cord, hepatic portal fibroplasia and dilatation of the sinusoids and aggregates of mononuclear cells in the intestinal *Lamina propria,* renal glomeruli and pulmonary alveoli were seen.

Enzyme histochemical findings

The distribution of the activities of adenosine triphosphatase, 5-nucleotidase, succinic dehydrogenase and glucose-6-phosphatase in the liver and kidneys of control goats was similar to that described by BILAL (3).

Liver

In the liver of control goats, the activities of glucose-6-phosphatase (Fig. 2) and succinic dehydrogenase were greater in the periportal hepatocytes than in the cells of the inner 2/3 of the liver lobule. In goats in group I, the activities of these two enzymes were reduced in the necrotic foci. In goats of groups II and III, the activities of succinic dehydrogenase and

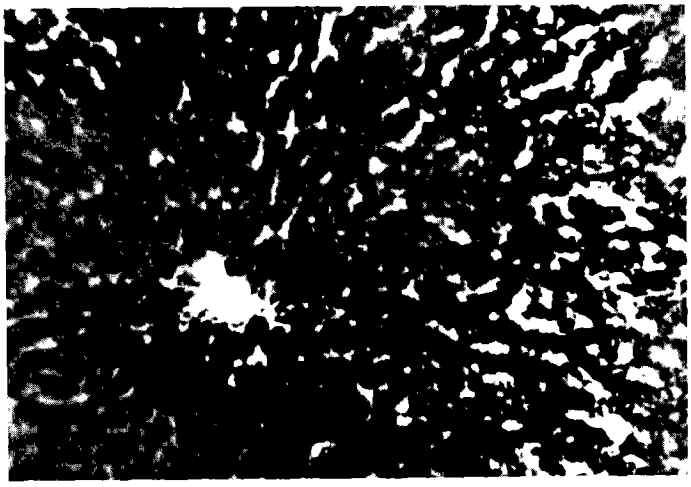

Figure 2. — Liver of control goat showing high activity of glucose-6-phosphatase in the periportal hepatocytes. Lead nitrate method × 100.

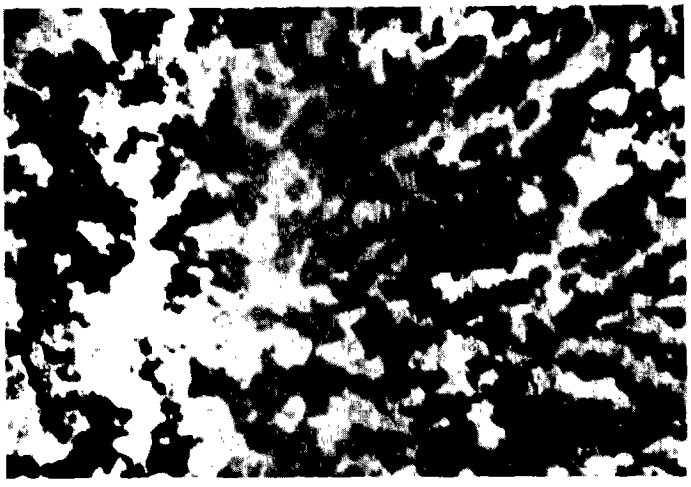

Figure 3. — Liver of goat 19 in group III, orally dosed with 1 g/kg/day of dry *Indigofera* shoots for 33 days. Loss of glucose-6-phosphatase activity in necrotic hepatocytes. Lead nitrate method × 100.

Figure 4. — Kidney of control goat showing succinic tetrazolium reductase activity in the cytoplasm of the cells of the renal tubules. Nitro-BT. × 100.

glucose-6-phosphatase were lost from necrotic hepatocytes (Fig. 3). In the normal liver of goats, the activity of ATP-ase was seen in the bile canaliculi, the walls of the central veins, the blood vessels of the portal areas and the adventitia of bile ducts and walls of sinusoids. 5-nucleotidase activity in the liver of normal goats was high in the sinusoids and the bile canaliculi. In *Indigofera*-poisoned goats, the reaction of ATP-ase and 5-nucleotidase was lost from the canaliculi and sinusoids in the necrotic area.

Kidneys

In the kidney of control goats, the activities of glucose-6-phosphatase and succinic dehydrogenase were confined to the cytoplasm of the renal tubules (Fig. 4). In *Indigofera*-poisoned goats, the reaction of glucose-6-phosphatase and succinic dehydrogenase was completely lost from the necrotic renal tubules (Fig. 5). In the kidney of control goats, the activities of 5-nucleotidase and ATP-ase were intense in the glomeruli, blood vessels and basement membrane and cytoplasm of the renal tubules. In *Indigofera*-intoxicated goats, there was a patchy reduction in the activities of ATP-ase and 5-nucleotidase in the affected tubules but the blood vessels still showed strong reaction.

Changes in serum constituents

There were no significant changes in the concentration of total bilirubin or in the activity of GPT in the serum of any animal.

Animals in group I were exemplified by goat 13 which was slaughtered on day 10. There were increases in the concentrations of urea and ammonia and in the activity of GOT and a decrease in the concentration of total protein at the time of slaughter (Fig. 6). In goat 15 (group II), there were terminal increases in the activity of GOT and in the concentrations of ammonia and urea and a decrease in the

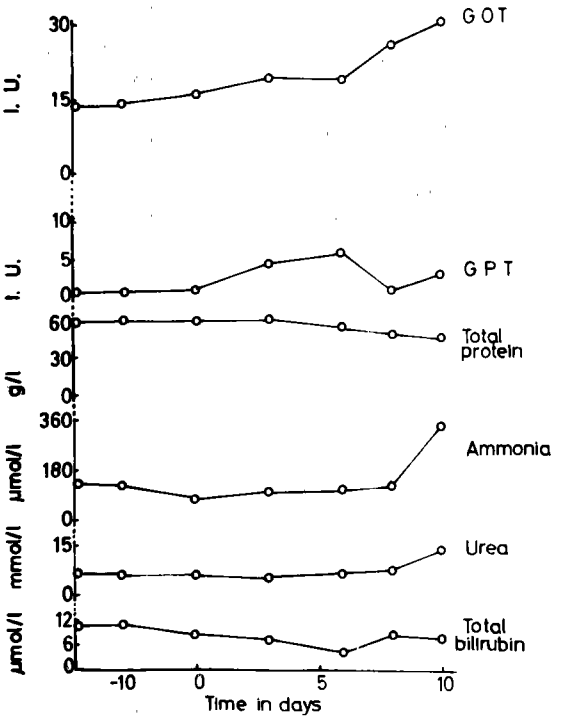

Figure 6. — Serum changes in goat 13 in group I, orally dosed with 10 g/kg/day of dry *Indigofera* shoots for 10 days.

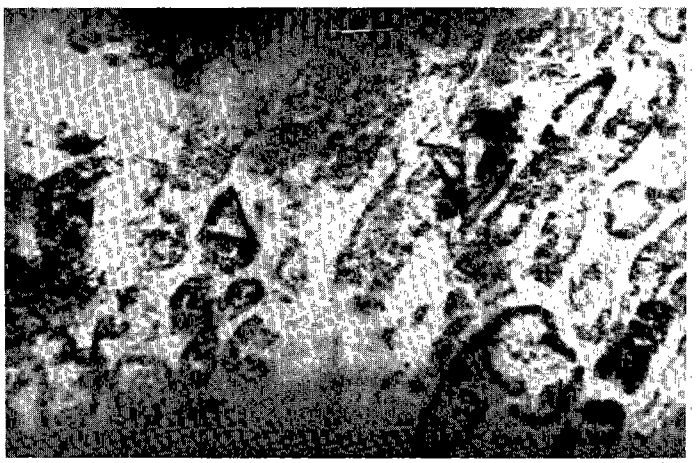

Figure 5. — Kidney of goat 17 in group II, orally dosed with 5 g/kg/day of dry *Indigofera* shoots for 8 days. Loss of succinic tetrazolium reductase activity in necrotic renal cells. Nitro-BT. × 100.

concentration of total protein (Fig. 7). In goat 19 (group III) which died on day 33, serum protein showed slight falls towards terminal stages of the disease (Fig. 8). Ammonia and urea showed terminal increases and the activity of GOT was elevated between days 19 and 33.

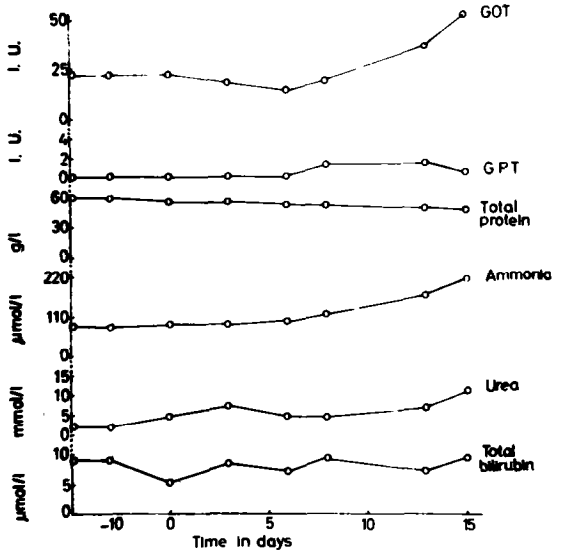

Figure 7. — Serum changes in goat 15 in group II, orally dosed with 5 g/kg/day of dry *Indigofera* shoots for 15 days.

Haematological findings

Again, animals in group I were exemplified by goat 13. There was a decrease in the values of Hb, PCV, RBC and MCHC between days 3 and 10 (Fig. 9). Leucocytosis was detected during the same period. In goat 15 (group II), Hb, PCV, RBC and MCHC fell below predosing values between days 6 and 15. There was an increase in leucocyte number on day 6 and a progressive decrease afterwards. In goat 19 (group III), leucocytosis occurred between days 14 and 22 and was due to an increase in the number of neutrophils. Hb, PCV and MCV decreased towards terminal stages of the disease.

Tissue lipids

The average concentrations of total lipids in the liver, heart and kidneys of the control animals and of the *Indigofera*-intoxicated goats are given in Table II. Raised values of total lipids were found in the liver, heart and kidneys of Indigofera-poisoned goats.

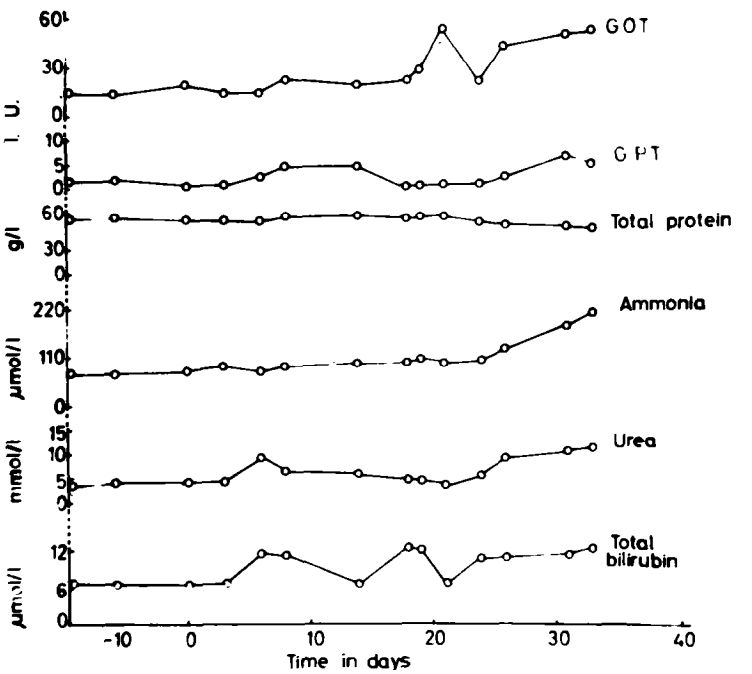

Figure 8. — Serum changes in goat 19 in group III, orally dosed with 1 g/kg/day of dry *Indigofera* shoots for 33 days.

TABLE N°II-Concentrations of total lipids in tissues of goats fed with *Indigofera hochstetteri*

Group	Average concentrations of total lipids in tissues (mg/g)		
	Liver	Kidneys	Heart
I	58.6	28.4	21.5
II	68.4	33.3	26.2
III	74.5	35.2	23.7
IV (controls)	30.5	23.5	16.2

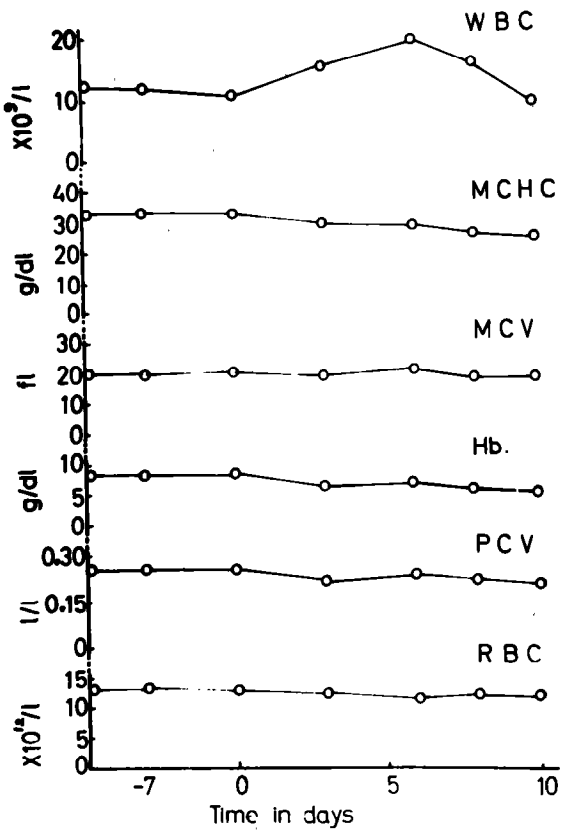

Figure 9. — Changes in the red cell parameters and in leucocyte number in goat 13 in group I, orally dosed with 10 g/kg/day of dry *Indigofera* shoots for 10 days.

DISCUSSION

The clinical changes in Nubian goats orally dosed with *I. hochstetteri* were anorexia, loss of condition, diarrhoea, bloat, dyspnoea, nervous signs and anaemia. The presence of perineuronal vacuolation in the grey matter of the spinal cord is in accord with the clinical evidence of locomotary disturbances. Perineuronal vacuolation in the grey matter of the spinal cord was produced in goats, sheep and calves by *Capparis tomentosa* (1, 2). The severity of the nervous signs during the course of *Indigofera* poisoning in goats might also be increased by the hepato-renal damage. The lesions in the kidneys consisted of necrosis and/or degeneration of the cells of the convoluted tubules, loss of enzyme activity, haemorrhage and congestion in both renal cortex and medulla and disappearance of the glomerular tufts. This effect on the kidney probably contributed to the high level of serum urea and ammonia. The high concentrations of GOT and ammonia and the fall in total protein concentration in the serum indicate liver-cell damage. The distribution of the activities of adenosine triphosphatase, 5-nucleotidase, succinic dehydrogenase and glucose-6-phosphatase in the liver and kidneys of control goats was similar to that described by BILAL (3). The loss of succinic tretrazolium reductase and glucose-6-phosphatase activity from the hepatocytes together with irregular distribution of the activity of the canalicular ATP-ase and 5-nucleotidase add further evidence of liver-cell injury. It is known that in ruminants, the supply of glucose by the process of gluconeogenesis from propionate is very important. Clearly, absence or decreased activity of the enzyme glucose-6-phosphatase, as was shown in *Indigofera*-poisoned goats, probably impaired glucose production, since this is the final enzyme in the gluconeogenic pathway. In such conditions it is conceivable that other sources of energy will be resorted to, for example fat and protein, by the processes of lipolysis and proteolysis respectively. That our study has indirectly proved that was indicated by increased concentration of urea in the serum

and by increased hepatic lipids as a result of increased amino acid metabolism and by increased supply of fatty acids to the liver respectively. Decreased energy supply from the tricarboxylic acid cycle as a result of succinic dehydrogenase deficiency is also expected to be reflected on increased mobilization of fat and protein, similar to that of glucose-6-phosphatase deficiency. Whether the effect of *Indigofera* on these enzymes is cause or effect, however, remain to be determined. For this reason it would be of interest to directly measure the activity of such enzymes and/or to measure rates of gluconeogenesis, lipolysis or proteolysis in *Indigofera*-poisoned animals. Damage to such vital enzymes concerned with energy homeostasis in the body may prove to be an important mechanism of poisonous plants on their overall pathological, biochemical and clinical effects.

The development of anorexia and loss of condition and the changes in Hb, PCV and RBC counts during the terminal stages of the disease indicate that anaemia is present in *Indigofera*-poisoning. It seems that anaemia is hypochromic as indicated by the low MCHC values obtained shortly before death.

It seems reasonable, therefore, to conclude from the above findings that the dry shoots of *I. hochstetteri* are toxic to goats by causing structural and functional changes in vital organs of the body especially the kidneys, liver, intestine and central nervous system.

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SULIMAN (H. B.), WASFI (I. A.), TARTOUR (G.), ADAM (S. E. I.). Efectos de *Indigofera hochstetteri* en cabras en Sudan. *Rev. Elev. Méd. vét. Pays trop.*, 1983, **36** (4) : 393-402.

Resumen. — De 12 cabras de Nubia, 10 recibieron una dosis diaria de 1 à 10 g/kg de brotes de *Indigofera hochstetteri*. Murieron o fueron matadas después a varios intervalos. Eran los principales síntomas de intoxicación los siguientes : hinchazón, prostración, disnea, diarrea, incoordinación de los movimientos, posturas anormales y anemia. Se notaron un aumento de la actividad de la transaminase glutamica oxalo-acetica, de las concentraciones de amoniaco y de urea y una baja de las proteinas totales en el suero. La patologia se manifestaba por hemorragias del corazón, de los pulmones y de los riñones, enteritis catarral y abomasitis, necrosis hepatocellular y modificación de la grasa, edema pulmonar y enfisema. Se reducía la actividad de la 5-nucleotidase ; del adenosinatrifosfato, de la reductase tetrazolium succínica, y de la glucosa-6-fostato en las células hepáticas y los tubulos renales.

Palabras claves : Intoxicatión — *Indigofera hochstetteri* — Cabras — Sudan.

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