

Experimental metolachlor toxicosis in Nubian goats in the Sudan

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MOHAMED (O.S.A.), AHMED (Khaldia E.), ADAM (S.E.I.), IDRIS (O.F.). Intoxication expérimentale par le métolachlor de chèvres de race Nubienne au Soudan. *Revue Elev. Méd. vét. Pays trop.*, 1994, 47 (3) : 315-318

Dans un lot de quinze chèvres de race Nubienne, six ont reçu oralement en une seule fois des doses de métolachlor (Dual 720 EC[®]) de 2 000 ou 500 mg/kg de poids vif. Elles sont mortes dans un délai de 1 h après l'administration du produit. Six autres ont reçu quotidiennement des doses orales de 200 ou 25 mg/kg de poids vif. Elles sont mortes ou ont été abattues entre le 8^e et le 25^e jour. Chez les sujets ayant reçu une dose unique du produit, les signes d'empoisonnement ont consisté en des épisodes convulsifs, de l'incoordination motrice, des tremblements, des spasmes musculaires sévères, de la raideur, une salivation intense, de la détresse respiratoire, des attitudes anormales et du décubitus. Chez les chèvres ayant reçu le métolachlor aux doses quotidiennes, les signes cliniques ont été similaires mais se sont développés lentement. L'augmentation d'activité des AST et GGT sériques, celle de la concentration en urée et la diminution de la concentration des protéines totales ont évolué en corrélation avec les changements cliniques et l'apparition des lésions.

Mots clés : Caprin - Chèvre Nubienne - Intoxication - Herbicide - Protéine - Sérum sanguin - Urée - Soudan.

INTRODUCTION

In the Sudan, the commercial use of herbicides has increased tremendously over the last few years as a result of the establishment of new agricultural schemes. In these projects, many crops have been cultivated and livestock has been introduced in the agricultural round with improvement of productivity/feddian crop. Metolachlor is a new selective herbicide which contains an acetanilide as an active ingredient and possesses an excellent action against annual grass weeds in soybeans, groundnuts, corn, maize, sunflowers, sugarcane and sugarbeet when applied at pre-emergence stage. It is taken up by the plants mainly through the shoots when present in the upper-most soil layer (4, 5, 7). Information on the toxicity of metolachlor in livestock is scarce. The present study was planned to investigate the toxic effects of metolachlor in Nubian goats, because this herbicide is frequently used in this country and cases of poisoning have been observed.

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Reçu le 22.11.1993, accepté le 30.9.1994.

MATERIALS AND METHODS

Animals and dosing

Fifteen 4 to 7 month-old male Nubian goat kids were used. The goats were clinically healthy, kept within the premises of Central Veterinary Research Laboratory, Soba and fed on forage Sorghum bicolor (ABU 70) and water *ad libitum*. Goat kids 1, 2 and 3 (group 1), 4, 5 and 6 (group 2) were each given single oral doses of 2,000 and 500 mg/kg of metolachlor (Dual 720 EC[®]) [2-ethyl-6-methyl-N-(2-methoxy-1-methyl-ethyl) - x chloroacetanilide, 72 % technical, Ciba Geigy Ltd., Basle Switzerland] respectively. Goat kids 7,8 and 9 (group 3), 10, 11 and 12 (group 4) were each given daily oral metolachlor doses of 200 mg/kg for 8-12 days and 25 mg/kg for 25 days respectively. Goat kids 13, 14 and 15 (group 5) were undosed controls.

Serum chemistry

Goats kids were bled from the jugular vein before and after dosing and blood samples were collected, centrifuges at 3,500 rpm for 10 min. Sera were analysed for the activities of AST, GGT and LDH and concentrations of total protein, total cholesterol, creatinine and urea by commercial kits (Cromatest Laboratories, Knickerbocker, SAE, Barcelona, Spain). Serum ALP activity and sodium, potassium and inorganic phosphate concentrations were determined by standard methods (10, 11). The concentrations of magnesium (8) and calcium (9) were estimated.

Histological methods and statistical analysis

All goat kids were examined immediately after death or slaughter for gross lesions. Specimens of brain, spinal cord, peripheral nerves, lungs, trachea, heart, liver, spleen, kidneys, abomasum and intestines were fixed in 10 % neutral buffered formalin, embedded in paraffin wax, sectioned with 5 µm and stained with hematoxylin and eosin (H&E). Statistical significance was assessed by Student's test (6).

RESULTS

The dosing protocol and survival times of the goat kids are given in table I.

Clinical findings

Within two min post dosing, goat kids receiving single oral doses of metolachlor at 2,000 mg/kg (group 1) and 500 mg/kg (group 2) developed tremors, staggering, severe muscular spasm of the fore and hind limbs, neck and back (photo 1), profuse salivation, licking of the lips, dilatation of the pupils, dyspnoea, ruminal tympany, uneasiness, prostration and recumbency. Death took place within 60 min. Goat kids given daily doses of metolachlor at 200 mg/kg (group 3) and 25 mg/kg (group 4) showed moaning, salivation, grinding of the teeth, urination, defecation, uneasiness, slight tremors and weakness of the hind limbs. In these groups, signs developed within 5 min post dosing and disappeared after 4 h following daily dosing. The goats died or were slaughtered between days 8 and 25. The control goat kids (group 5) showed no clinical signs and were slaughtered on day 25.

Post mortem changes

There was congestion and/or hemorrhage in the brain, trachea, lungs, liver, kidneys, heart, spleen and small intestines in all metolachlor-dosed goats in groups 1, 2, 3 and 4. Varying degrees of pulmonary emphysema, oedema and froth at the trachea were observed while fatty change in the kidneys in group 2, 3 and 4 was more severe than in group 1. Slight catarrhal abomasitis or enteritis was seen in groups 2, 3 and 4. Control goat kids (group 5) showed no lesions.

Histopathologic changes

Liver

In goat No 4 in group 2, the liver showed cytoplasmic fatty vacuolation or necrosis of the centrilobular hepatocytes. In groups 3 and 4, the hepatic lesions extended to the peripheral hepatocytes and the portal tracts were infiltrated with lymphocytes and a few fibroblasts. The blood vessels and sinusoids were congested and bile ductule hyperplasia was observed.

Kidneys

The epithelial cells of some proximal convoluted tubules were degenerated, some glomerular tufts were shrunken or necrotic, and congestion hemorrhage was seen in both cortex and medulla.

Lungs

There was congestion of the alveolar capillaries, alveolar hemorrhage, oedema and emphysema with peribronchiolar lymphocytic infiltration, especially in groups 1, 2 and 3.



Photo 1 : Severe muscular spasm of the limbs, neck and back in kid No. 4 (group 2) given a single oral dose of 500 mg/kg of metolachlor.

TABLE I Details of male goat kids given single or repeated doses of metolachlor by drench.

Group No.	Goat Kid No.	Age (m)	Dose (mg/kg)	Time of death	
1	1	4	2 000	10 min	
	2	4	2 000	10 min	
	3	4	2 000	10 min	
	2	4	5	500	60 min
		5	5	500	30 min
		6	6	500	30 min
3	7	7	200	18 d	
	8	7	200	8 d	
	9	7	200	12 d	
	4	10	7	25	25 d (slaughtered)
		11	7	25	25 d (slaughtered)
		12	7	25	25 d (slaughtered)

Abomasum and intestines

The lamina propria of the intestinal and abomasal mucosae was congested and infiltrated with lymphocytes. Catarrhal abomasitis and/or enteritis was detected and hemosiderin deposits were seen in the red pulp of the spleen in groups 2, 3 and 4.

Changes in serum constituents (table II)

The activities of serum AST, GGT, ALP and LDH and the concentration of total protein, total cholesterol, urea, creatinine, sodium, potassium, inorganic phosphate, calcium and magnesium were not assayed in groups 1 and 2 because the test animals died within 1 h post dosing. In

TABLE II Changes in serum constituents of metolachlor poisoned goat kids.

Group	AST (i.u)	GGT (i.u)	Total protein (g/100 ml)	Urea (mg/100 ml)	Inorganic phosphate (mg/100 ml)
5 (controls)	11.74 ± 8.16	14.74 ± 4.55	7.47 ± 0.65	17.8 ± 2.25	6.1 ± 1.1
3 (200 mg/kg/d)	51.92 ± 12.8***	47.01 ± 16.15***	6.5 ± 0.6**	31.23 ± 4.68**	4.8 ± 1.4*
4 (25 mg/kg/d)	42.2 ± 7.04***	47.12 ± 17.25***	6.55 ± 0.75**	45.1 ± 17.04***	5.3 ± 1.5 ^{NS}

NS = Not Significant ; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

groups 3 and 4, there were significant increases ($p < 0.001$) in the activities of AST and GGT and decreases ($p < 0.01$) in the concentration of total protein. The concentrations of urea were higher ($p < 0.01-0.001$) in the test groups than in the control group. The concentration of inorganic phosphate decreased in group 3 ($p < 0.05$). No significant differences in the activities of LDH and ALP or in the concentrations of total cholesterol, creatinine, sodium, potassium, calcium and magnesium were observed between the test goat kids in groups 3 and 4 and the control animals in group 5.

DISCUSSION

The present study has shown that single oral doses of 500 mg/kg and above of metolachlor are fatal to goat kids within 10 min-1 h of dosing probably due to respiratory failure. The daily oral dosages of 200 or 25 mg/kg of metolachlor in goat kids caused toxic manifestations and death between days 8 and 25. GENTILE and CALABRESE (3) found a significant reduction in erythrocyte acetylcholinesterase activity in Dorset sheep dosed with metolachlor. In the present study, the signs of toxicosis in goat kids might have been due to cholinesterase inhibition or diffuse stimulation of the central nervous system or to both. None of the test animals showed demyelination of the peripheral nerves or the white matter of the spinal cord. The hepatorenal lesions were accompanied with increases in the activities of serum AST and GGT and in the concentration of urea and decreases in the concentrations of total protein. EL SADEK *et al.* (2) reported a decrease in the activity of serum AST and creatine phosphokinase (CPK) in rats given different oral doses of metolachlor for 15 days.

In this study, the presence of hemosiderosis in the spleen may be due to destruction of erythrocytes. CORNELIUS and KANEBO (1) have suggested that in human beings and rabbits, damage to renal tissue causes deficiency of the hormone, erythropoietin, and consequently results in failure in erythropoiesis. Experiments to test the carcinogenicity, teratogenicity and pharmacokinetics of this herbicide have not been undertaken.

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MOHAMED (O.S.A.), AHMED (Khalda E.), ADAM (S.E.I.), IDRIS (O.F.). Experimental metolachlor toxicosis in Nubian goats in the Sudan. *Revue Élev. Méd. vét. Pays trop.*, 1994, **47** (3): 315-318

Six out of 15 Nubian goats kids were given single oral doses of metolachlor (Dual 720 EC®) at 2,000 or 500 mg/kg liveweight and died within 1 h of the dosing. Other 6 goats were given daily oral doses at 200 or 25 mg/kg and died or were slaughtered between days 8 and 25. In goats receiving single doses, the signs of poisoning were convulsive episodes, incoordination of movement, tremors, severe muscular spasms, stiffness, profuse salivation, respiratory distress, abnormal posture and recumbency. In goats receiving metolachlor at daily doses, the signs were similar, but developed slowly. Increases in the activities of serum AST and GGT and in the concentration of urea, and decreases in total protein concentration were correlated with clinical changes and lesions.

Key words : Goat - Nubian goat - Poisoning - Herbicide - Protein - Blood serum - Urea - The Sudan.

MOHAMED (O.S.A.), AHMED (Khalda E.), ADAM (S.E.I.), IDRIS (O.F.). Toxicosis experimental por metolachlor, en cabras de raza Nubiana en Sudán. *Revue Élev. Méd. vét. Pays trop.*, 1994, **47** (3): 315-318

Se administraron dosis individuales de 2 000 o 500 mg/kg de metolachlor ((Dual 720 EC®) a seis de 15 cabritos de la raza Nubiana, los cuales murieron una hora después de la administración. Otras seis cabras recibieron diariamente dosis orales del compuesto de 200 o 25 mg/kg ; estos animales murieron o fueron matados entre el día 8 y el día 25. Las cabras que recibieron dosis individuales de Dual, presentaron signos de envenenamiento, con episodios convulsivos, movimientos de incoordinación, temores, espasmos musculares severos, contracturas, salivación profusa, disfunción respiratoria, posturas anormales y decúbito. En las cabras que recibieron el metolachlor en dosis de 200 o 25 mg/kg, los signos fueron similares, pero con un desarrollo más lento. Se correlacionaron aumentos en la actividad sérica de AST y GGT, en la concentración de úrea y en la disminución de la concentración de las proteínas totales con los cambios clínicos y las lesiones.

Palabras clave : Caprino - Cabra Nubiana - Intoxicación - Herbicida - Proteína - Suero sanguíneo - Urea - Sudán.