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Comparative Toxicity of the Crabamate Insecticides Bendiocarb and Propoxur in Nubian Goats

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Abstract: This work examined the sequential development of some clinical, biochemical, haematological and pathological lesions in young Nubian goats given single oral doses of two carbamate insecticides, bendiocarb and propoxur, alone and in combination. The influence of pretreatment with the drug metabolising enzyme inducer phenobarbitone was also investigated. Signs of poisoning with both insecticides were dose-related, similar in severity and included restlessness, excessive salivation, arching of the back and recumbency. All the animals died or were killed 21 to 30 days post treatment. The toxicity caused significant decrease in haemoglobin concentration, haematocrit and erythrocyte numbers. Biochemically, there were significant inhibition of cholinesterase and a rise in aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) indicative of tissue damage. Increases in creatinine and urea were suggestive of renal damage. Histopathologically, there were degeneration and/or necrosis of hepatocytes and of cells of the renal convoluted tubules, congestion in the heart, lungs, kidneys and intestines. Phenobarbitone pretreatment increased the signs of toxicity of both insecticides, indicating that the toxicity may be caused by metabolites of these agents.

Key words: Bendiocarb, goats, propoxur, toxicity

Introduction

In many countries, especially in the developing world, the expansion in agricultural schemes is often accompanied with indiscriminate and ever-increasing use of pesticides. One of these insecticides is the group termed crabamates, which are N-substituted esters of carbamic acid (Ashton and Craft, 1973). In the Sudan, the carbamate insecticides have been used for the last 25 years or so. It is established that the use of these insecticides is associated with toxicity in mammals (Vandekar *et al*., 1971; Tyrkiel and Bojanowska, 1978; Weisbroth *et al*., 1983). Nonetheless, we are not aware of any reports in the literature about their toxicity in Nubian goats or other domestic animals. Therefore in the present work we have attempted to document and compare the clinical, biochemical, haematological and pathological effects of administration of two carbamate insecticides, bendiocarb ($C_{11}H_{15}NO_3$) and propoxur ($C_{11}H_{13}NO_4$) in young Nubian goats when given singly and in combination. The effect of the drug metabolising enzyme inducer phenobarbitone on the insecticide toxicity has also been undertaken in order to see if the toxicity of these insecticides is caused by the parent compounds, their metabolites or both.

Materials and Methods

Animals: Clinically healthy male young Nubian goats (weighing about 9-12 Kg and aged 6 months old) were bought from a local market in Khartoum North. They were housed in groups in a well-ventilated shaded barn in the clinic of the Veterinary College and were fed *ad libitum* on Lucerne (*Medicago sativa*), sorghum grains and water. They were kept for a week to acclimatise before the start of the experiment, during which time the freedom of the kids from internal and external parasites was checked by standard methods and insured.

Experimental design: Forty-four goats were divided randomly into eleven equal groups (designated group 1-11) and treated orally as follows:

Group 1, 2 and 3: Treated with bendiocarb at a dose of 100, 250 and 1000 mg kg^{-1}, respectively

Group 4 and 5: Treated with propoxur at a dose of 100 and 250 mg kg^{-1}, respectively

Group 6: Treated with bendiocarb (100 mg kg^{-1}) plus propoxur (100 mg kg^{-1})

Group 7: Treated with bendiocarb (250 mg kg^{-1}) plus propoxur (250 mg kg^{-1})

Group 8 and 9: Treated with phenobarbitone Na at a dose of 20 mg kg^{-1} for 14 consecutive days, and then received either a dose of 100 mg kg^{-1} of bendiocarb (group 8) or propoxur (group 9)

Group 10: Treated with phenobarbitone only as above

Group 11: Treated with normal saline and kept as control

The body weights of all kids were measured at the beginning, during and at the end of the experiment. Heart and respiratory rates were taken before and after dosing. The same veterinarian (who was unaware of the treatments) closely observed the animals several hours post dosing.

Blood (10 ml) was collected from the jugular vein on a weekly basis. Part (2 ml) was used immediately for haematological measurements, and the rest was left in a refrigerator for 2 h, following which it was centrifuged for 15 min at 900 g to obtain serum. The harvested serum was stored at -20°C to await analysis.

Haematological measurements: The packed cell volume (haematocrit, PCV), haemoglobin (Hb) concentration, erythrocyte and leukocyte concentration, mean corpuscular

volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were determined by standard methods (Schalm, 1965).

Biochemical methods: These were all measured spectrophotornetrically. Total proteins were determined sing the Biuret reagent (White and Rankel, 1965) and albumin by a commercial kit (Sigma, St. Louis, MO, USA). Urea was measured by the method of Coulombe and Favreau (1963). Total lipids were measured using commercial kits (Kit number 61402, Bio-Mirieux, France). The activity of aspartate amino transferase (AST) was measured according to Reitman and Frankel (1957). Lactate dehydrogenase (LD) was measured using a kit (Human Gesselschaft fur Biochemica and Diagogonstica, Germany).

Residue determination: An attempt was made to isolate and identify bendiocarb, propoxur and their metabolite(s) from viscera and urine of goats treated with a combination of the two insecticides at a dose of 250 mg kg^{-1}. The samples were made acidic at pH 2.0 and then extracted with chloroform. The extract was then evaporated in a steam bath. The residue was spotted in thin-layer chromatography (TLC) plates. These were immersed in a system of chloroform, methanol and ammonia (90:10.1). After development, the TLC plates were examined under ultraviolet (UV) light and sprayed with potassium dichromate. The rate of flaw (R_f) was then measured against that of authentic bendiocarb and propoxur.

Histopathology: Post mortem specimens were immediately fixed in 10% formalin, followed by Bouin's fluid, embedded in paraffin wax, sectioned at 6 mm and stained with hematoxylin and eosin (H and E).

Statistical Methods: Values are expressed as means ± SEM (number of observations). Differences between groups were determined by the Student's t-test. P higher than 0.05 was considered insignificant.

Results

Clinical findings: The goats either died or were killed in exrremis within 21-30 days post treatment. The weights of all the treated animals decreased by about 12-18%. The weight of untreated controls increased by 14-33% during the same period. The acute signs of toxicity appeared minutes after treatment with the insecticides. These include: uneasiness, back arching, abduction of the forelimbs, hindlimb paralysis, inappetance, salivation, nasal discharge, diarrhoea, red and oedematous eyes and lateral bending of the neck. In addition, temperature and respiratory, pulse and heart rates were elevated. These acute signs lasted for about 3 h, then subsided. Goats not treated with the insecticides did not show any of these signs.

Haematological findings: There were dose-dependent decreases in the RBC counts, Hb concentration and haematocrit (PCV) values. Goats given phenobarbitone only had a haemogram similar to that of saline-treated animals (Table 1).

Biochemical findings: These are summarised in Table 2. Most of the variables measured were significantly affected by the treatments given.

Post-mortem findings: The lesions seen were mostly dose-dependent and ranged from mild to severe. The most affected organs were hearts, lungs, livers and kidneys. The heart was flappy and congested. In the lungs there were oedema, congestion and emphysema. In the hepatic and renal tissues, congestion, patchy areas of ischeamia, fatty changes and necrosis were seen. In animals on high doses of the insecticides and in when the two insecticide were combined, the gall bladder was distended. The signs were severer in those goats receiving pretreatment with phenobarbitone. Control animals had none of the above signs (Fig. 1).

Histopathological findings: The histopathological findings in animals treated with the two insecticides were more or less similar, but were more severe in goats receiving the higher doses of the insecticides and in those animals that were given the combination of the two compounds. In the liver there was variable degrees of congestion of the central veins and sinusoids in all goats treated with the insecticides.

In the heart, focal areas of capillary congestion, vacoulations and degeneration of cardiac muscle cells and fibres were seen (Fig. 2). In the lugs there was congestion of the pulmonary alveolar capillaries, patchy areas of emphysema and increased cellularity of the alveoli. There was moderate thickening of the interalveolar septa as a result of mononuclear cellular infiltration (Fig. 3). The intestines showed acute catarrhal enteritis as reflected by congestion of blood vessels and capillaries, particularly in submucosa and heavy infiltration of mononuclear cells (mainly lymphocytes) on the mucosa. There were also excessive destruction of the mucosal glands and desquamation of the lining epithelium. In the urinary bladder there was substantial oedema in the submucosa and massive hydropic degeneration of the surface epithelium, with focal areas of erosion in certain parts. In goats treated with phenobarbitone or saline none of the above histopathological findings were observed (Fig. 4).

Tissue residues: The values of R_f for authentic bendiocarb and propoxur were 0.08 and 0.04, respectively. The urine showed three spots, two of which had the same value as that of the authentic compounds. The third R_f value was 0.3 and was suggestive of a metabolite.

Discussion

The present results have shown that bendiocarb and propoxur are toxic to goats at single doses of 100 mg kg^{-1} and above. The degree of toxicity of the two insecticides was similar and their combination resulted in an augmented (additive) effect.

The rise in the activity of plasma enzymes (AST and LDH) and the clinical, post mortem and histopathological signs are evidence of the toxicity. The rise in plasma enzymes was probably due to tissue damage. The enzyme cholinesterase was dose-dependently inhibited. The administration of phenobarbitone 10 days prior to the administration of the insecticides caused increased toxicity, probably due to the drug metabolising-inducing action of the barbiturate. This suggests that the metabolites of these insecticides are toxic. The induction of liver microsomal

Table 1: Some haematological findings in goats orally dosed with bendiocarb (B) and/or propoxur (P)

Group	Treatment	RBS (10^6/mm^3)	HB (g/dl)	PCV (%)	MCHC	MCV
1.	B (100 mg kg^{-1})	12.9 ± 1.3	9.9 ± 0.2	27.1 ± 0.2	36.5 ± 0.6	20.9 ± 0.7
2.	B (250 mg kg^{-1})	11.1 ± 0.3	9.1 ± 0.3	26.2 ± 0.2	35.0 ± 0.5	32.4 ± 0.7
3.	B (1000 mg kg^{-1})	10.1 ± 0.4*	8.4 ± 0.6*	25.0 ± 0.5*	33.3 ± 0.7*	24.8 ± 0.9*
4.	P (100 mg kg^{-1})	11.5 ± 0.5	9.5 ± 0.9	26.2 ± 0.6	36.3 ± 0.7	22.6 ± 0.6
5.	P (250 mg kg^{-1})	10.9 ± 1.2	8.9 ± 0.3	25.2 ± 0.7	35.3 ± 0.8	23.1 ± 0.4
6.	B (100 mg kg^{-1}) + P (100 mg kg^{-1})	10.0 ± 0.9*	8.1 ± 0.2*	24.5 ± 0.8*	33.1 ± 1.3*	24.5 ± 0.6*
7.	B (250 mg kg^{-1}) + P (250 mg kg^{-1})	10.4 ± 1.0*	8.2 ± 0.5*	23.5 ± 2.1*	32.1 ± 2.0*	23.3 ± 1.4*
8.	Phenobarbitone (20 mg kg^{-1}) + El (100 mg kg^{-1})	9.5 ± 0.9*	8.8 ± 0.6*	24.2 ± 1.6*	31.3 ± 2.2*	22.7 ± 1.8*
9.	Phenobarbitone (20 mg kg^{-1}) + P (100 mg kg^{-1})	9.7 ± 0.8*	8.9 ± 0.5*	23.7 ± 1.5*	32.6 ± 2.2*	22.4 ± 2.0*
10.	Phenobarbitone (20 mg kg^{-1}) + saline	13.3 ± 0.1	10.1 ± 0.3	28.0 ± 0.6	36.1 ± 0.6	21.1 ± 1.5
11.	Saline (control)	13.5 ± 0.2	10.5 ± 0.4	28.1 ± 0.6	37.4 ± 0.8	20.8 ± 1.2

Values are means ± SEM (n = 4).
Asterisks denote significant difference from the saline-treated animals ($p < 0.05$)

Table 2: Changes in serum biochemical constituents in male goats dosed with bendiocarb (B) and/or propoxur (P) with or without treatment with phenobarbitone

Group	Treatment	T. protein (g/l)	albumin (g/l)	urea (mg/dl)	T. lipids (g/l)	AST U/l	CE U/l	LDH U/l
1.	B (100 mg kg^{-1})	56.7 ± 2.5*	34.4 ± 2.3	45.9 ± 4.4*	60.0 ± 6.2*	24.3 ± 2*	189 ± 15*	240 ± 23
2.	B (250 mg kg^{-1})	52.3 ± 2.2*	22.2 ± 0.3*	51.6 ± 4.4**	62.0 ± 5.7*	28.5 ± 24*	149 ± 13*	288 ± 25*
3.	B (1000 mg kg^{-1})	49.6 ± 2.2*	20.9 ± 1.2*	60.7 ± 5.2**	63.0 ± 5.1*	30.1 ± 23*	124 + 23*	301 ± 27*
4.	P (100 mg kg^{-1})	55.6 + 1.6*	24.3 ± 0.25	46.7 ± 4.3*	61.0 + 5.5*	24.7 ± 23*	185 ± 17*	248 ± 26
5.	P (250 mg kg^{-1})	51.7 ± 2.1*	21.7 ± 0.5*	52.3 ± 4.6**	62.5 ± 6.2*	28.7 ± 23*	143 ± 12*	290 ± 25
6.	B (100 mg kg^{-1}) P (100 mg kg^{-1})	46.4 ± 4.2*	19.7 ± 2.0*	64.3 ± 5.5**	63.5 ± 6.1*	31.9 ± 31**	121 ± 12*	281 ± 1.21
7.	(250 mg kg^{-1}) 1P (250 mg kg^{-1})	45.9 ± 4.9*	18.9 ± 1.4*	68.7 ± 6.3**	64.3 ± 5.5*	33.3 ± 28**	119 ± 10*	309 ± 26*
8.	Phenobarbitone (20 mg kg^{-1}) + B(100 mg kg^{-1})	57.3 ± 5.4*	24.2 ± 2.2*	47.1 ± 4.3*	64.5 ± 6.0*	26.3 ± 24*	180 ± 11*	235 ± 21
9.	Phenobarbitone (20 mg kg^{-1}) + P (100 mg kg^{-1})	56.1 ± 5.5*	23.9 ± 1.9*	48.2 ± 4.2*	65.3 ± 6.5*	28.1 ± 27*	174 ± 13*	245 ± 22
10.	Phenobarbitone (20 mg kg^{-1}) + saline	59.2 ± 5.3	31.2 ± 2.2	39.1 ± 2.4	52.5 ± 5.3	13.2 ± 1.4	260 ± 24	220 ± 20
11.	Saline (control)	67.6 ± 5.4	34.4 ± 2.3	38.7 ± 3.6	51.0 ± 3.9	13.9 ± 1.2	266 ± 24	224 ± 21

Values are means ± SEM (n = 4). Asterisks denote significant difference from the control ($p < 0.05$)

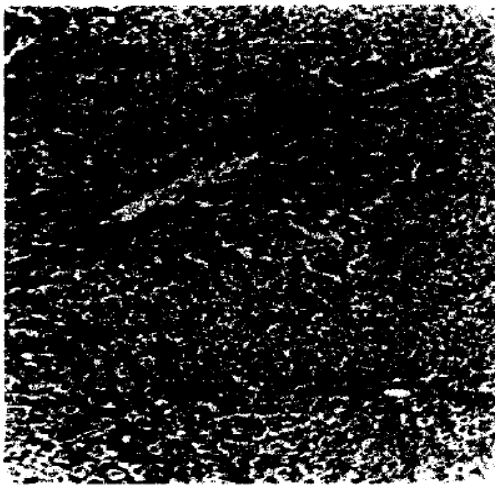

Fig. 1: Centrilobular necrosis of the hepatocytes with vcoulation changes in the peripheral area in a goat receiving propoxur (250 mg kg^{-1}) orally route

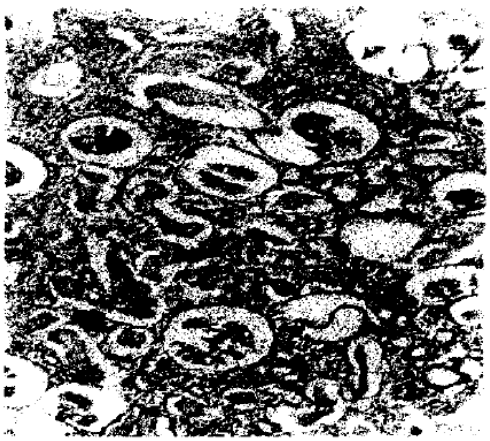

Fig. 2: Dilated renal tubules, shrunken or destroyed glomeruli in the kidney of a goat that received bendiocarb together with propoxur, each at an oral dose of 250 mg kg^{-1}

Fig. 3: Wide spread vacoulation and fatty change in the sarcoplasm of the cardiac muscle cell of a goat that was given bendiocoarb at an oral dose of 250 mg kg^{-1}

Fig. 4: Focal emphysema and thickening of alveoli in a goat that was given propoxur at an oral dose of 250 mg kg^{-1}

enzymes was found to increase or to increase the toxicity of mice to several organophosphorus compounds including malathion, coumaphos, parathion, demeton and cattonientNon (DuBois, 1961).

Although the chemical structure of carbamate insecticides seem to suggest that they may be potentially carcinogenic, a working group of the International Agency for research on cancer found no evidence of carcinogenicity with these insecticides.

In conclusion, the present work has provided evidence that the carbamates bendiocarb and propoxur are toxic to young goats at doses of 100 mg kg^{-1} and above and that their toxicity is more or less similar in this species.

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