

## COMBINED TOXICITY OF COUMAPHOS AND DIAZINON ON NUBIAN GOATS

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### ABSTRACT:

Two groups of Nubian goats were given 0.5 mg/kg/day coumaphos or diazinon. Another group of goats was given a mixture of coumaphos (0.25 mg/kg) and diazinon (0.25 mg/kg).

The clinical and pathological investigations indicated that coumaphos/diazinon mixture had synergistic toxic effect on Nubian goats and death occurred within a period between 8-15 days. Animals in the first two groups showed mild findings and they were slaughtered on the ninety-first day.

### المخلص

أربعة مجموعات من الماعز النوبي استعملت في هذه الدراسة والتي استمرت مدة ٩١ يوماً. المجموعة الأولى والثانية تناولت مبيد الكومافوس والديازينون يومياً وبالكم بمقدار 0.5 ملغ/كجم لكل منهم ، المجموعة الرابعة استعملت كمسيطر. المجموعة الأولى والثانية ذبحت في نهاية التجربة وكانت الأعراض الاكلينيكية والمرضية خفيفة. نتائج الدراسة الاكلينيكية المرضية اثبتت أن مخلوط المبيدين سام وقتل خلال ٨-١٥ يوم.

### INTRODUCTION:

One of the greatest challenges facing mankind today is to satisfy the nutritional needs of the growing world population, while at the same time preserving resources such as land, water, air and biodiversity. Livestock is a crucial element in this balancing process and a cornerstone of most rural population worldwide through their

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multiple functions. Growing much food on a small land by use of organic fertilizers, pesticides and appropriate agricultural technology is the target goal of Sudan government. Research in pesticides hazards and interaction, in public health livestock, agriculture and environment is another goal.

It is well known that extensive usage of chemical pesticides causes many problems such as occurrence of insect's resistance phenomenon. Pesticides combination is one of solutions suggested by scientists in order to control more than one pest at the same time, delaying occurrence of resistant insect strains, increasing knockdown effect and saving money and time (Dubois, 1961).

Diazinon (Schurman, 1987; Subramaniam and Varghese, 1991) and Coumaphos (Shaw and Baker, 1966; Mohammed, 1970; Drummond, 1981) are examples of the most commonly used organophosphorous compound in the Sudan in areas of the public health, agriculture and livestock pests' control.

Sahar *et al* (1997 and 1998) studied toxic effects of coumaphos and diazinon in Nubian goats at the dose rate of 0.5, 2.5 and 5 mg/kg/day and found out that animals tolerated dose of 0.5 mg/kg/ day without clinical signs for both insecticides, and that animals were slaughtered on the 91st day in healthy condition.

This study was performed to obtain scientific information on the clinico-pathological effect of coumaphos/ diazinon mixture on Nubian goats.

## MATERIALS AND METHODS

### ANIMALS AND DOSING :

Twelve healthy 5-9 months old male Nubian goats were housed in pens at the Central Veterinary Research Laboratory, Soba, fed on forage Sorghum (*Sorghum vulgare*) *ad libitum* and provided with free access to water.

Animals were randomly divided into 4 groups of three each. Goats No.1, 2 and 3 (group 1) and goats No. 4,5 and 6 (group 2) were each drenched coumaphos [0,0-diethyl-0-3-chloro-4-methyl-2-oxo-2H-1 benzopyran-7-yl-phosphorothioate, 50% (W.P.) Bayer, Leverkusen,

Germany) or diazinon (0,0-diethyl-0-2-isopropyl-6- methyl-4-pyrimidiny1)-thiophosphate, 60%, EC, Ciba Geigy Ltd., Basle, Switzerland ] at a dose rate of 0.5 mg/kg, respectively. Goats No. 7,8 and 9 (group3) were each drenched a mixture consisting of 0.25 mg/kg coumaphos and 0.25 mg/kg, diazinon forming a total of 0.5 mg/kg. Drenching was continued daily until animals were dead or slaughtered. Goats No .10,11 and 12 (group 4) were used as undosed control group.

## INVESTIGATION

### BLOOD

Blood samples were collected postdosing at 1 and then 7 days intervals thereafter, throughout the experiment from the jugular vein puncture. Blood sample from each goat was collected in two separate dry test tubes, one contained anticoagulant ethylene diamine tetra acetic acid (EDTA), and used for haematological investigations which include [haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell (RBC) count, mean corpuscular haemoglobin concentration (MCHC) and white blood cell (WBC) count] and were determined by the method suggested by Schalm *et al* (1975). The other sample was left to clot, centrifuged and collected sera were analyzed for the activities of serum aspartate aminotransferase (AST), alanine aminotrasferase (ALT) and also for concentration of total protein, albumin, urea and creatinine using methods described by Reitman and Frankel (1957), Weichselbaum (1946), Kertman(1971), Evans (1968) and White and Frankel (1965), respectively. Globulin was obtained by subtracting the albumin from the total protein.

### PATHOLOGY:

At death or slaughter of an animal, post-mortem findings were recorded. Specimens of brain, spinal cord, peripheral nerves, trachea, lungs, heart, liver, spleen, kidneys, abomasum and intestine were immediatley fixed in 10% formal saline prepared in histological sections, stained with haematoxylin and eosin(H&E).

Data were analyzed using Student's t-test according to the method of Mendenhall (1971).

## RESULTS:

Details of goats dosed with coumaphos, diazinon and their mixture are illustrated in Table 1.

**TABLE 1: DETAILS OF GOATS DOSED WITH COUMAPHOS, DIAZINON AND THEIR MIXTURE**

Group	Goat No.	Daily dose (mg/kg)	Clinical Results	
			Day onset of clinical signs	Fate and time of death (days)
1	1	0.5 coumaphos	No signs appeared	91 (slaughtered)
	2			91 (slaughtered)
	3			91 (slaughtered)
2	4	0.5 diazinon	No signs appeared	91 (slaughtered)
	5			91 (slaughtered)
	6			91 (slaughtered)
3	7	0.25 coumaphos + 0.25 diazinon	8	8 (died)
	8			15 (slaughtered)
	9			15 (died)
4	10	-	No signs appeared	91 (slaughtered)
	11			91 (slaughtered)
	12			91 (slaughtered)

**CLINICAL SIGNS:** Goats of group 1 and 2 which received 0.5 mg/kg coumaphos or diazinon respectively, showed no clinical signs and the animals looked healthy until slaughtered on the 91<sup>st</sup> day. Group 3 goats which received the mixture of coumaphos/diazinon (50:50) exhibited massive salivation, lachrymation, staggering, ataxia, neck torticoiling, muscle tremors, and abnormal posture. Then the animals suffered respiratory distress, profuse diarrhoea, inappetance, dullness, sunken eyes, recumbency and eventually death occurred within 8-15 days. These signs started on the 8<sup>th</sup> day, thirty minutes post dosing, and severity of signs increased as dosing continued. The control group showed no clinical signs.

**PATHOLOGY:**

Table 2 summarizes the post-mortem findings in experimental animals. Varying degrees of congestion and haemorrhages were observed in vital organs of dosed animals. The pulmonary and digestive tract lesions were most prominent in goats of group 3 and consisted of oedema, emphysema of the lungs and froth in respiratory passages, abomasitis, enteritis and abomasal ulcers. Fatty changes and /or necrosis in liver and kidneys were more clear in diazinon-dosed goats (group2). No clinical signs were seen in the control group (group 4).

Table 2: POST-MORTEM FINDINGS IN GOATS DOSED WITH COUMAPHOS, DIAZINON AND THEIR MIXTURE

Organs and lesions	Group1	Group2	Group3	Group 4
Heart				
Congestion and haemorrhage	+	+	+	-
Flappiness	-	+	+	-
Hydropericardium	-	++	++	-
Lungs				
Congestion and haemorrhage	+	+	++	-
Odema	+	+	++	-
Emphysema	+	+	++	-
Trachea				
Froth	-	-	++	-
Liver				
Congestion and haemorrhage	+	+	+	-
Fatty change and/ or necrosis	+	++	++	-
Kidneys				
Congestion and haemorrhage	+	++	+	-
Fatty change and/or necrosis	+	+	++	-
Abomasum				
Congestion and haemorrhage	+	+	+	-
Ulceration	-	-	++	-
Abomasitis	-	-	+	-
Intestines				
Congestion and haemorrhage	-	+	+	-
Enteritis	-	-	+	-

→ ++ increasing severity. (-) absence of lesions

**HISTOPATHOLOGY:**

Severe to moderate haemorrhage and, congestion of hepatic blood vessels and foamy cytoplasm were noticed in all dosed animals. Goats of group 3 showed bile ductules hyperplasia, sinusoidal dilatation and hepatocytic megalocytosis. Kidneys were slightly haemorrhagic and medullary rays were congested in addition to slight renal tubular epithelial cells degeneration in all dosed animals. Goats of group 3 showed cystically dilated proximal tubules. Some renal collecting ducts and renal tubules contained acidophilic homogenous material. Rupture of alveoli and exudates were the most prominent features seen in lungs of dosed animals, but severe in goats of group 3. RBC collections were noticed between the cardiac muscle bundles. Mucosal and submucosal lymphocytic infiltration were apparent in goats of group 3. Goats of group 1, 2 and 3 showed diffused congestion and perivascular cuffing in cerebral cortex and no demyelination of the nerve fibres in white and grey matter of any of the experimental animals. No significant lesions were observed in control group.

**BLOOD****a) HAEMATOLOGY:**

No significant changes were observed in the tested goats for haematological values measured during the experiment.

**b) SERUM CONSTITUENTS:**

These are summarized in Table 3. Significant increases were observed in the activity of AST ( $P < 0.001$ ) in group 2 and 3. The activity of ALT was not affected in groups 1 and 2 but significant increases ( $P < 0.001$ ) was observed in goats of group 3. Serum urea concentration increased significantly ( $P < 0.05$  and  $0.001$ ) in goats of group 1 and 3. The total protein concentration was affected, but the globulin: albumin ratio was changed towards the globulin in goats of group 2 and 3. No significant changes were observed in serum creatinine concentration of experimental animals. No changes were recorded in serum parameters of control group.

## DISCUSSION

It is well known that a drug interaction is a pharmacological phenomenon, which occurs when effects of one drug are modified by the prior or concurrent administration of another. The coumaphos/diazinon mixture of 0.5mg/kg/day on Nubian goats was not reported before.

In the present study 0.5mg/kg of coumaphos or diazinon alone drenched to Nubian goats was tolerated for 91 days without apparent toxicity. When coumaphos and diazinon were each given in 0.25mg/kg in a combined dose of 0.5mg/kg, severe clinical signs were observed in this group and manifested organophosphorous compounds known toxicity, with death occurring within 8-15 days. In addition, the pathological lesions such as pulmonary oedema, hepatitis, nephritis, gastroenteritis and varying degrees of congestion and haemorrhage in different body tissues coupled with changes in blood such as increase in the activity of AST and in concentration of ureas are other marks for pesticide potentiating toxicity.

In spite of not measuring the cholinesterase activity in poisoned goats. The authors are of the opinion that both of the administered organophosphorous compounds are cholinesterase inhibitor (Clarke *et al.*, 1981). Pesticide toxicity potentiation was reported by Mohamed and Adam (1990a, 1990b) when they studied the combined effects of dursban with reldan (organophosphorus), temik (carbomate) with sumicidin (pyrethroid) and dieldrin with endosulfan (organochlorine) Abdelsalam *et al* (1982), on Nubian goats.

The present study concludes that coumaphos and diazinon when given in a mixture is toxic and fatal to Nubian goats.

Moreover, further studies should be undertaken to understand the dynamics of these compounds in potentiating the toxicity in small ruminants and other animal species.

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TABLE 3: SERUM CONSTITUENTS ANALYSIS IN EXPERIMENTED GOATS

Group	Daily dose (mg/kg)	ALT (iu/L)	AST (iu/L)	Total Protein (g/L)	Albumin (g/L)	(Globulin g/L)	Urea (mmol/L)	Creatinine ( $\mu$ mole/L)
1	0.5 coumaphos	11.62 $\pm$	26.09 <sub>1</sub>	60.06	29.61	30.45	4.45 $\pm$	42.42 $\pm$
		5.23 <sup>NS</sup>	17.23 <sup>NS</sup>	$\pm$ 9.27*	$\pm$ 4.62**	$\pm$ 7.46 <sup>NS</sup>	1.79 *	7.09 <sup>NS</sup>
2	0.5 diazinon	16.66 <sub>1</sub>	62.39 <sub>2</sub>	74.99	28.59	46.40	2.35 $\pm$	45.78 <sup>NS</sup>
		12.49 <sup>NS</sup>	21.14 <sup>***</sup>	$\pm$ 8.08 <sup>NS</sup>	$\pm$ 2.73 <sup>***</sup>	$\pm$ 7.11 <sup>***</sup>	0.74 <sup>NS</sup>	$\pm$ 4.90
3	0.25 coumaphos	40.00 <sub>1</sub>	59.00 <sub>2</sub>	70.88	26.00	44.88	7.18 $\pm$	46.38 <sub>1</sub>
		2.55 <sup>***</sup>	7.92 <sup>***</sup>	$\pm$ 10.3 <sup>NS</sup>	$\pm$ 2.53 <sup>***</sup>	$\pm$ 8.74 <sup>**</sup>	2.0 <sup>***</sup>	10.14 <sup>NS</sup>
4	untreated control	12.25	17.85	69.23	37.43	31.8	2.63	42.79
		$\pm$ 2.47	$\pm$ 2.63	$\pm$ 5.7	$\pm$ 7.88	$\pm$ 7.42	$\pm$ 1.9	$\pm$ 5.97

\* (P&lt; 0.05), \*\* (P&lt; 0.01), \*\*\* (P&lt; 0.001), NS: not significant

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