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Effect of repeated oral administration of quinalphos to male goat (*Capra hircus*)

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Abstract. Quinalphos given in daily oral doses of 0.5 mg/kg for 110 days induced severe signs of organophosphorus poisoning in male goats. The inhibition of acetylcholinesterase activity in erythrocyte was highly significant. The activity of liver glutamic; oxaloacetic transaminase, glutamic; pyruvic.transaminase, alkaline phosphatase and protein indicated marked alteration. The haematological changes were however, relatively less significant with the exception of a very low count of red blood cells and white blood cells in the treated animals. Among the vital organs, only liver suggested mild cellular changes due to quinalphos intoxication. There was no significant pathological change in other organs of the treated animals.

In animals observed after 15 and 30 days rest, the activity of acetylcholinesterase in red blood cells and haematological picture showed a fairly good recovery. This study suggests that although quinalphos in low concentrations did not produce discernible cellular changes, it induced highly significant enzymatic and haematological changes in the goat.

Keywords. Organophosphorous; acetylcholinesterase; erythrocyte; quinalphos.

Introduction

Hazards and environmental contamination through abuse of a variety of pesticides has attracted global attention. In recent years emphasis has been given to organophosphorous pesticides instead of organochlorine compounds to overcome the problem of residue. Quinalphos (O, O-diethyl-O-quinoxalin-2-yl phosphorothioate) due to its acaricidal and insecticidal properties is in large scale use in this country. From an annual consumption of 300 metric tons during 1977, the use has risen to 1000 metric tons (Anon 1978, 1979). Quinalphos is presently used in different formulations such as emulsion concentrates, granules and dust.

Single and also multiple feeding of quinalphos produced highly significant toxicological effects in rats (Dikshith T. S. S., Datta, K. K. and Raizada, R. B. unpublished). It also induced significant inhibition· in the activity of acetylholinesterase in red blood cells (RBC) and brain of guinea-pigs (Dikshith *et al.,* 1980a). Rats pretreated with carbon tetrachloride were more susceptible to quinalphos intoxication (Dikshith *et al.,* 1980b). Response and toxic effects of

Abbreviations used: RBC, red blood cells; WBC, white blood cells.

pesticides have been associated with species differences. The mechanism of disposing of lipid-soluble toxic chemicals such as pesticides by different species of animals has already been reported (Murthy and DuBios, 1957; Hayes, 1959; Brodie and Maickel, 1962; Durham, 1967; Fouts, 1970 and Cantilema *et al.,* 1979). Since domestic goat is associated with the food chain of man and also represents the livestock animals the present study could help in identifying cases of organophosphorous poisoning among livestock animals and also in occupational workers handling these potentially toxic insecticides.

Materials and methods

Animals

Four domestic male goats (average body weight 16 kg) were procured from the breeders. The animals were under observation for one month before the commencement of medication with quinalphos. They were maintained on green leaves, grass and peeled husks of grains and were allowed to move freely in the field during the day unless otherwise mentioned.

Preparation of the compound and treatment

Technical quinalphos obtained from Regional Research Laboratory, Jorhat, was used in this study. The compound dissolved in peanut oil was orally administered daily to two male goats (0.5 mg/kg) for a period of 110 days. Two goats of the control group were administered equal volume of peanut oil in a similar manner. Blood was sampled periodically at 60, 90 and 110 days during treatment and 15 and 30 days during post-treatment rest periods in oxalated tubes (2 mg of 6 parts amonium oxalate and 4 parts potassium oxalate/ml blood) for acetylcholinesterase and haematoiogical assays. Experimental and control groups of animals were killed after 140 days of treatment.

Period of exposure and sampling

Earlier we have observed that low dose of quinalphos given to guinea-pigs for 30 days could not elicit significant toxicological effects (Dikshith *et al.,* 1980a). In the present study we therefore exposed the animals for much longer periods and also sampled after 60, 90 and 110 days after treatment. Since the treated goats became very weak after 110 days of exposure, further administration of quinalphos was stopped. The animals were under close observation till the experiment was terminated. Blood was also sampled after 15 and 30 days of post-treatment rest for the estimation of acetylcholinesterase activity.

Histological studies

Sections of the liver, kidney, adrenal, brain and testis were fixed in normal-saline solution (100 ml formalin + 900 ml distilled water + 8.5 g sodium chloride). After routine processing, paraffin sections were cut at 6 μm thickness and stained with haematoxylin-eosin (McManus and Mowry, 1960).

Biochemical studies

The liver was washed free of extraneous material using chilled saline solution. The

liver was homogenized in 0.25 M ice-cold sucrose solution (10% w/v) in a Potter-Elvehjem type homogenizer. The homogenate was centrifuged at 700 *g* for 10 min and the supernatant was collected. Serum was separated. The supernatant and serum were used for estimation of enzyme activities. The activity of glutamic-oxaloacetic transaminase (E.C.2.6.1.1) glutamic-pyruvic acid transaminase (E.C.2.6.1.2) and alkaline phosphatase (E.C.3.1.3.1, orthophosphoric monoester phosphohydrolase) were determined by the method of Wootton (1964). Serum bilirubin was estimated by the procedure of Mallary and Evelyh, modified by Ducci and Watson (1945). The method of Lowry *et al.* (1951) was followed for the determination of protein while the Nelson-Somogyi (1965) method was adopted for blood sugar.

Acetylcholinesterase determination

Brain was quickly removed, washed free from extraneous material and homogenized in 0.25 M ice-cold sucrose solution 10% w/v in a Potter-Elvehjem type homogenizer. RBC was separated by centrifuging the blood at 2500 *g* for 10 min. Acetylcholinesterase (E.C.3.1.1.7) activity was assayed by the method of Hestrin (1949).

Haematological studies

Total RBC and total white blood cell (WBC) were determined by the method of Wintrobe and Landsberg (1935) and haemoglobin was estimated according to the method of Kolmer *et al.* (1951).

Results

Clinical signs

Male goats which received a daily dose of quinalphos (0.5 mg/kg) for a period of 110 days indicated body tremor, profuse salivation, weakness, diarrhoea and reduced food consumption. The animals became weak after 30 days of continuous exposure to quinalphos but there was no paralysis or death.

Microscopic examination

Liver showed fatty degenerative changes in the parenchyma. Hepatocytes of the centrolobular area appeared vacuolated and carried granular cytoplasm and the nuclei were pushed to a corner of the cell. However there was no necrosis of the liver (figure 1, 2). Other organs did not suggest any kind of morphological change due to quinalphos.

Biochemical studies

The activity of liver and serum glutamic-oxaloacetic, glutamic pyruvic transminases, alkaline phosphatase activities, protein content and serum bilirubin is shown in table 1. The activity of the transaminases, protein content as well as serum protein in the quinalphos treated animals showed a highly significant reduction ($p<0.001$ to $p<0.01$) in comparison to the value in the control animals. However the activity of alkaline phosphatase both in liver and serum was increased ($p<0.01$). The level of serum bilirubin was very high ($p<0.001$) in exposed animals (table 1), while that of blood sugar was comparable with the control.

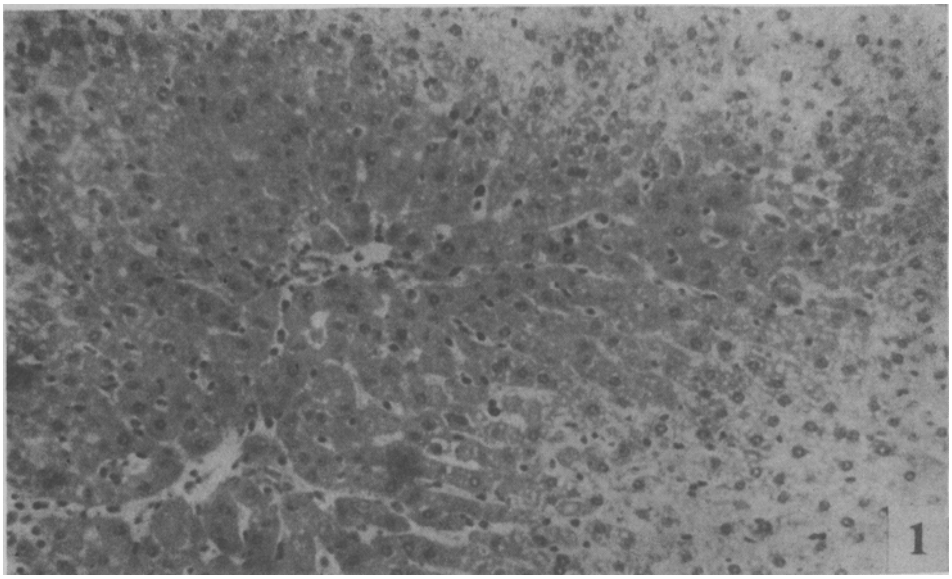

Figure 1. Section of liver of control goat (H and E × 164).

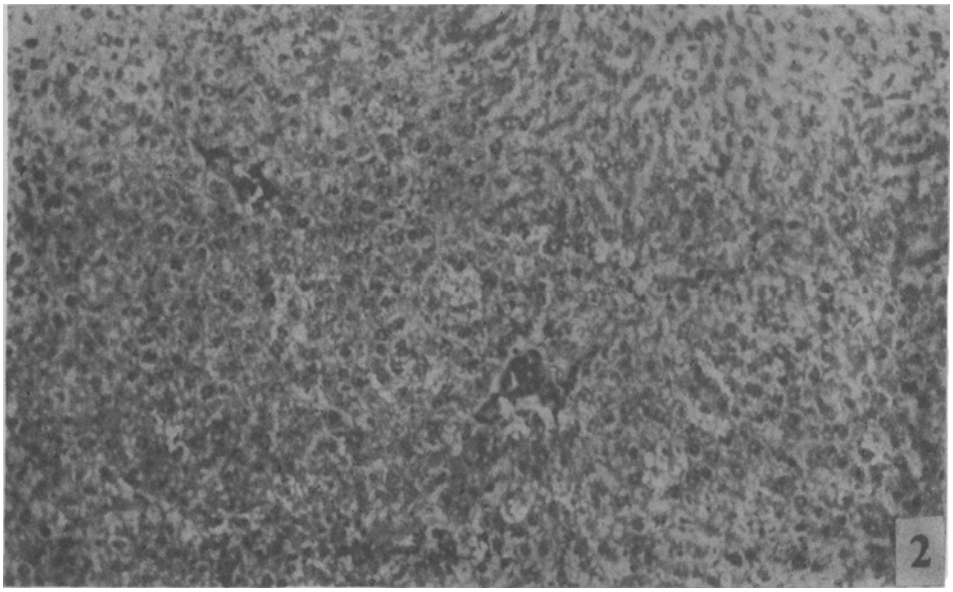

Figure 2. Section of liver of goat exposed to quinalphos. (H and E × 164).

Table 1. Biochemical changes in liver, serum and blood of male goats exposed to quinalphos.

Treatment	Control (peanut oil 0.5 ml/day)	Quinalphos (0.5 mg/kg/day)
Liver		
GOT	7.52±0.32	4.29[a]±0.10
GPT	6.05±0.19	3.46[b]±0.35
Alkaline phosphatase	0.26±0.17	1.912[b]±0.19
Total protein	31.12±0.13	14.25[a]±0.13
Serum		
GOT	0.04±0.003	0.30±0.003
GPT	0.013±0.002	0.019±0.001
Alkaline phosphatase	0.076±0.001	0.27[b]±0.003
Total protein	23.42±0.23	14.6[a]±0.17
Bilirubin	0.34±0.02	0.70[a]±0.01

GOT—glutamic-oxaloacetic transaminase; GPT — glutamic-pyruvate transaminase; alkaline phosphatase (µmol/ g or ml/min)

Total protein (mg/g or ml); Bilirubin— (mg/ 100 ml serum)

a = $p<0.001$; *b* = $p<0.01$

Table 2. Activity of RBC acetylcholinesterase in male goats exposed to quinalphos.

Treatment (days)	Acetylcholinesterase (µmol/ml/10 min)
Control peanut oil (O)	5.85 ±0.12
Treatment	
60	2.84[a] ±0.12
90	1.89[a] ±0.09
110	0.82[a] ±0.14
Post-treatment rest	
15	1.96[b]* ±0.06
30	3.38[a]* ±0.20

RBC —Red blood cells

[a] $p<0.001$; [b]$p<0.01$

* Values significantly different in comparison to the value of 110 days exposure.

Acetylcholinesterase activity

Male goats exposed to quinalphos for 110 days showed significant reduction in acetylcholinesterase activity ($p<0.001$) in comparison with the control (0.82 compared to 5.85). Acetylcholinesterase after 15 days and 30 days of post-treatment rest period was 1.96 and 3.38 respectively indicating that a highly significant recovery in the acetylcholinesterase activity had occurred on stopping the treatment (compared to 0.82 of 110 days treatment) (table 2).

Haematological studies

Although goats treated with quinalphos for 110 days exhibited significant decrease ($p<0.001$) of RBC and WBC counts the values were within the normal range. There was a significant recovery of RBC and WBC counts after 15 and 30 days of post-treatment rest (table 3).

Table 3. Haematological changes in male goats exposed to quinalphos.

	Treatment (days)					
	Control (peanut oil)				Post-treatment rest	
	0	60	90	110	15	30
RBC	15.21±0.15	13.42±0.96	14.45±0.27	10.9[a]±0.21	14.92±0.19	20.52*±0.27
WBC	6800±170	7150±80	6000±260	3850[a]±110	3950[a]±192	6350±250
Hb	9.2±0.31	14.5±1.99	10.4±0.31	10.0±0.31	11.0±0.42	12.20*±0.31
N	39±0.22	4[a]±0.57	55[a]±0.57	40±0.57	35[a]±0.67	32[a]±0.57
L	58±1	44[a]±0.51	43[a]±0.57	59±0.57	58±0.67	67[a]±0.57
M	3±0.57	2±0.5	2±0.5	.1±0.29	4±0.5	2.9

[a] $p<0.001$; [b] $p<0.05$

RBC–Red blood cells (million/mm^3; WBC-white blood cells (1000/mm^3); Hb-haemoglobin (g/100 ml); N — neutrophils (%); L—lymphocytes (%); M—monocytes (%).

* Normal values for RBC 9-19; Hb 12.7-14.2 (Kolmer *et al.,* 1951).

Discussion

The present study has indicated the manner of organophosphorus poisoning in the domestic goat. Animals dosed with 0.5 mg/kg/day for 110 days showed severe signs of poisoning with highly significant inhibition of the acetylcholinesterase activity. It is of interest to observe here that guinea-pigs which were exposed to different doses of quinalphos did not show clinical signs of organic phosphorus poisoning up to 30 days. However the inhibition of acetylcholinesterase activity was highly significant both in guinea-pigs as well as in goats after quinalphos treatment (Dikshith *et al.,* 1980a). Similarly, while the guinea-pigs failed to show any morphological change in the vital organs, the liver of goats exposed to quinalphos for 110 days indicated mild cellular changes associated with biochemical alterations.

The activity of liver transaminases and protein showed a significant decrease in the quinalphos treated goats. In contrast to liver, the level of serum transaminases was unchanged. Absence of liver necrosis corelates with the unaltered levels of serum transaminases. However the activity of alkaline phosphatase in the serum increased significantly.

There was a highly significant decrease in RBC and WBC counts in the male goats after 110 days of treatment but were within the normal range of a healthy goat.

Experiments suggest that on stopping the quinalphos treatment followed by a period of rest, the decreased acetylcholinesterase activity as well as the lowered RBC and WBC counts returned to normal values. The pattern of recovery in these animals is similar to that observed on feeding the methyl demeton (Dikshith *et al,* 1980c).

This study is of relevance since it suggests that occupational workers handling organophosphorous compounds as spray and formulations need periodical surveillance and rest. Such periodical check and reentry may minimise the incidence of poisoning.

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