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Influence of Malathion (0, 0'-dimethyl Dithiophosphate of Diethyl Mercaptosuccinate) on Body Enzymes in Dermal Subacute Toxicity Studies in *Bubalus bubalis* Species

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Among organophosphorus group of insecticides, malathion is routinely and selectively employed as an effective ectoparasiticide in the form of dermal spray in veterinary practice. The gross toxicological changes with dermal toxicity trials of malathion have been conducted by RADELEFF, et al. (1955) and RADELEFF and WOODARD (1957) where they observed that 1.0 per cent dermal application resulted into 75 per cent of mortality in baby calves. However, LEPELLY (1955) reported that adult cattle could withstand a concentration as high as 1.25 per cent of malathion spray without any untoward effect.

The inhibition of cholinesterase enzyme is usually considered as the sole mechanism responsible for organophosphate toxicity. However, very little information is available concerning the effects of insecticides especially organophosphate group on various biochemical parameters associated with enzymic activities in various mammalian species. Such information is absolutely lacking in Bubalus bubalis species. In the present communication the effect of malathion spray in different concentrations on various body enzymes in Bubalus bubalis species have been attempted. The information obtained from these investigations would go a long way in filling the gaps in the existing knowledge to understand the mechanism of toxicity more explicitly and also in establishing the safety for malathion as ectoparasiticide.

MATERIALS AND METHODS

Nine buffalo calves weighing 95 to 100 kg aged about one year and divided into three groups with three animals in each were used in this study. Malathion 50 E.C. in concentrations of 0.5, 1.0 and 5.0 per cent was sprayed daily for

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28 days on the clipped animals of various groups. Blood samples were drawn by jugular venipuncture and serum was separated at room temperature for studying the amino-transferases and phosphatases activity. The activity of various enzymes were assayed before, during (3, 7, 14 and 28 days) and after post-treatment (3 and 14 days).

Assay of enzymes

Cholinesterase (ChE): The activity of RBC ChE (E.C. 3.1.1.7) and plasma ChE (E.C. 3.1.1.8) was assayed according to the electrometric method of MICHEL (1949). The activity was calculated as Delta pH/hr and expressed in terms of per cent activity.

Aminotransferases: The activity of aspartate amino-transferase (E.C. 2.6.1.1.) and alanine aminotransferase (E.C. 2.6.1.2) was assayed in serum by the colorimetric procedure of REITMAN and FRANKEL (1957) as described by WOOTTON (1964). The activities were expressed as μ mol pyruvate/min/L of serum.

Phosphatases: The activity of alkaline phosphatase (E.C. 3.1.3.1) and acid phosphatase (E.C. 3.1.3.2) expressed as King Armstrong Unit (KAU) was assayed in serum by the colorimetric procedure of WOOTTON (1964).

The assay of all enzymes followed linear kinetics with respect to period of incubation and enzyme concentration.

Evaluation of the data: The data were analysed by the student's 't' test.

RESULTS

Cholinesterase (ChE): The effect of different concentrations of malathion spray on RBC ChE (RChE) and plasma ChE (PChE) represented as per cent of control (100 per cent) is shown in Fig. 1. With 0.5 per cent the appreciable inhibition in RChE and PChE became evident on 7th day (94.87 $\pm$ 2.96 and 95.24 $\pm$ 4.76 per cent, respectively) and was maximum on 28th day (67.66 $\pm$ 1.69 and 60.82 $\pm$ 4.08 per cent, respectively). With 1.0 per cent spray inhibition in RChE became evident on 3rd day (91.44 $\pm$ 2.13 per cent) and increased to 66.81 $\pm$ 2.12 per cent on 7th day. The maximum inhibition was recorded on 28th day (50.59 $\pm$ 4.53 per cent). With this concentration the inhibition in PChE on 7th day was of the order of 96.38 $\pm$ 3.62 per cent and the maximum inhibition was recorded on 28th day (46.14 $\pm$ 3.11 per cent). With 5.0

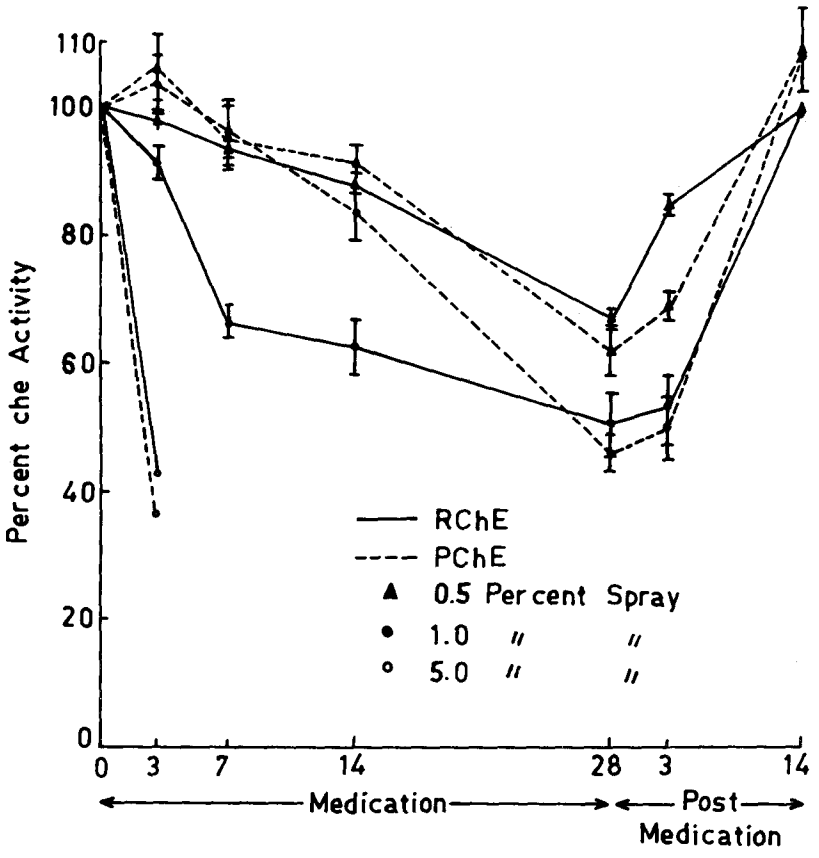

FIGURE 1 : Effect of continuous dermal spray of malathion on RBC cholinesterase (RChE) and plasma cholinesterase (PChE) in buffalo calves. The control activity was taken as 100 per cent.

per cent inhibition in RChE and PChE were of the order of 42.68 and 36.77 per cent, respectively on 3rd day when all the animals died.

Aminotransferases and phosphatases (Table 1 and 2): No significant ($P > 0.05$) change in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and acid phosphatase was observed with 0.5 per cent spray. With 1.0 per cent, significant ($P < 0.05$) increase in aspartate-and alanine aminotransferases

TABLE 1

Effect of continuous dermal spray of malathion on aminotransferases in buffalo calves.

Parameter	Treat-ment (%)	Period in days						
		Medication					Post-medication	
		0	3	7	14	28	3	14
Aspart ate aminotransferase (μ mol pyruvate/ min/L) (Mean ± S.E.)	0.5	54.72 ±2.23	55.18 ±0.99	55.48 ±0.89	56.21 ±1.48	57.17 ±1.79	56.30 ±1.36	54.80 ±1.20
	1.0	56.32 ±0.92	57.10 ±1.05	62.31* ±0.67	67.13* ±0.53	68.11* ±1.40	62.53* ±1.95	56.56 ±1.97
	5.0	55.69 ±0.64	82.95**					
Alanine aminotransferases (μ mol pyruvate/ min/L) (Mean ± S.E.)	0.5	46.18 ±1.85	46.17 ±1.67	46.53 ±1.54	46.97 ±2.02	46.74 ±1.80	46.68 ±1.86	46.33 ±1.83
	1.0	48.64 ±0.06	49.64 ±0.44	55.17* ±1.97	57.13* ±0.98	64.13* ±2.17	56.34* ±2.49	49.43 ±0.44
	5.0	44.33 ±1.85	59.10**					

* P/0.05 ** Single observation

(62.31 ± 0.67 and 55.17 ± 1.97 μ mol pyruvate/min/L, respectively) occurred on 7th day and maximum increase was observed on 28th day (68.11 ± 1.40 and 64.13 ± 2.17 μ mol pyruvate/min/L, respectively). The significant ($P<0.05$) increase in alkaline phosphatase (1067 ± 0.17 KAU) and acid phosphatase (0.87 ± 0.0[illegible] KAU) with this concentration occurred on 14th and 7th day respectively and maximum increase was observed on 28th day (12.17 ± 0.13 and 1.37 ± 0.09 KAU, respectively).

The values of various enzymes activities returned to normal after two weeks of post-medication.

DISCUSSION

The results of this study indicate that malathion dermal spray in recommended concentrations of 0.5 and 1.0 per cent was not free from biochemical defects and 5.0 per cent resulted in death of all the animals. Both the recommended concentrations produced inhibition in PChE and RChE which became evident between 3rd to 7th day depending upon the concentration and the intensity was concentration dependent. The lower concentration of 0.5 per cent, however, did not produce any effect on other enzymes studied. The inhibition in PChE was more marked than RChE in all the concentrations. Similar findings have been reported by VADLAMUDI (1974) in his dermal subacute toxicity study with fenitrothion in Bubalus bubalis species. GUPTA and PAUL (1977) also observed more inhibition in PChE than RChE in their subacute oral toxicity studies, with malathion. These observations are also in agreement to the findings of FRAWLEY et al. (1952) who have reported greater inhibition of PChE than RChE in rats intoxicated with malathion, EPN and parathion.

In addition to cholinesterase inhibition, the higher concentrations of 1.0 and 5.0 per cent also produced significant ($P<0.05$) elevation in activities of aminotransferases and phosphatases, which is in agreement with the findings of VADLAMUDI (1974), MALIK (1975) and GUPTA and PAUL (1977) in their acute and subacute toxicity trials with various organophosphorus insecticides in Bubalus bubalis species. WRIGHT et al. (1966) and YOUNGER and WRIGHT (1971) have also reported elevation of aminotransferases in calves intoxicated with single oral doses of caumaphos (37.5 and 100 mg/kg). However, POLAN et al. (1969) did not observe any change in aminotransferases in calves medicated with Supracide, another organophosphate compound in the doses of 0.5, 1.0 and 2.0 mg/kg for 10 weeks. The possible mechanism involved in the elevation of aminotransferases may be due to tissue damage, particularly in liver, kidney and heart (ROUILLER, 1964) or due to increased synthesis or decreased catabolism of aminotransferases (DENMANN et al. 1963).

TABLE 2

Effect of continuous dermal spray of malathion on phosphatases in buffalo calves.

Parameter	Treat-ment (%)	Period in days						
		Medication					Post-medication	
		0	3	7	14	28	3	14
Alkaline Phosphatase (KAU) (Mean ± S.E.)	0.5	9.33 ±0.08	9.42 ±0.08	9.61 ±0.11	9.93 ±0.22	9.92 ±0.08	9.83 ±0.17	9.58 ±0.08
	1.0	9.70 ±0.30	10.28 ±0.08	10.48 ±0.16	10.67* ±0.17	12.17* ±0.13	11.39* ±0.24	9.83 ±0.17
	5.0	6.67 ±0.11	12.75**					
Acid Phosphatase (KAU) (Mean ± S.E.)	0.5	0.80 ±0.11	0.80 ±0.11	0.83 ±0.09	0.87 ±0.49	0.93 ±0.07	0.93 ±0.07	0.83 ±0.09
	1.0	0.77 ±0.03	0.82 ±0.03	0.87* ±0.08	1.03* ±0.02	1.37* ±0.09	1.25* ±0.07	0.77 ±0.07
	5.0	0.66 ±0.07	1.75**					

* $P < 0.05$ ** Single observation.

The elevation of alkaline phosphatase activity in this study is in accordance with the findings of MURPHY (1966) in rats intoxicated with several organophosphate compounds. The increase in alkaline phosphatase may possibly be attributed to leakage of this enzyme to the circulation from hepatocytes (ROUILLER, 1964). The obstruction of bile duct as a result of liver damage (ABDERHALDEN, 1961) and renal damage (ABRAHAM and ROBBSMITH, 1970) may be the other factor responsible for the observed elevation in alkaline phosphatase. Apart from the organophosphate toxicity, the elevation of acid phosphatase has also been observed in silicotic rats (GUPTA et al. 1972) and in Asbestotic guinea pigs (VISWANATHAN, 1973). Although the sole mechanisms involved in increase of acid phosphatase is beyond the scope of this study but the possibility of these compounds having cytotoxic action on lysosomes, thereby releasing these enzymes by disrupting the membranes cannot be ruled out as postulated by VISWANATHAN et al. (1973), NADLER and GOLDFISCHER (1970) for other chemicals viz. Asbestos, Manganese and Silica.

These observations tend to suggest that malathion spray for ectoparasiticidal purposes should not be employed for more than three days even in recommended concentration (0.5 and 1.0 per cent). The concentration as high as 5.0 per cent is lethal.

SUMMARY

The effect of daily dermal spray of malathion for four weeks in recommended (0.5 and 1.0 per cent) and higher (5.0 per cent) concentration on various enzymes in Bubalus bubalis species were studied. The higher concentration of 5.0 per cent showed lethal effect after 2 to 3 exposures. The cholinesterase activity in both RBC (RChE) and plasma (PChE) were inhibited with all the concentrations. There was also significant ($P<0.05$) elevation in the activities of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase with 1.0 and 5.0 per cent spray and enzyme activities remained altered even during post-medication. The extent of various biochemical changes were dose and time dependent.

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