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Modification of Fenitrothion-Induced Circulating Enzymatic Alterations in *Bubalus bubalis* by 2,3-Butanedione Monoxime

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Modern agricultural practices have increased health hazard to farm animals of exposure to organophosphate (OP) insecticides. Apart from other sources, ruminants including buffaloes may be exposed to OP insecticidal agents through consumption of contaminated forages (Singh 1981; Hatch 1988). These chemicals are responsible for many cases of poisoning in farm animals due to their anticholinesterase activity (Singh 1981; Smith and Lewis 1988; Pritchard 1989). The OP insecticide fenitrothion is being extensively used to control insect pests on different crops including forages (Jha 1987). This insecticide is more toxic to buffaloes than to rodents (Vadlamudi 1974; Gupta et al. 1981).

The acetylcholinesterase (AChE)-reactivating oximes control acute OP intoxication in man and animals (Hatch 1988; Taylor 1991). The choice of an oxime may vary as certain oximes may be more efficacious than others. A widely accepted oxime, pralidoxime was found to be ineffective in combating acute malathion intoxication in the buffalo (Gupta 1984). Besides inhibiting AChE, OP insecticides are known to cause an increase in the blood levels of aminotransferases and phosphatases in animals (Gupta et al. 1981; Sandhu and Malik 1988). Little has been reported on the effect of oximes on OP-mediated elevation of these enzymes. The purpose of this study was to determine whether 2,3-butanedione monoxime has the potential to reverse fenitrothion-induced circulating enzymatic alterations in buffalo calves.

MATERIALS AND METHODS

The experiments were performed on clinically healthy male buffalo calves weighing between 74 and 90 kg.

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These animals were purchased from the local market and brought into the experimental house for acclimatisation two weeks before the studies started. Calves were fed green fodder, wheat straw and water was available ad libitum. All the calves were fasted for 20 hr and were given fenitrothion [0,0-dimethyl 0-(4-nitro-m-tolyl) phosphorothioate; Folithion 50 EC; Bayer (India) Ltd., Bombay] in a single oral (p.o.) dose of 217.5 mg/kg body wt. The fenitrothion-exposed animals were assigned randomly to 2 groups each comprised of 3 calves. Group 1 was left untreated whereas animals of group 2 were given a single intravenous dose (30 mg/kg body wt) of 2,3-butanedione monoxime (Aldrich Chemical Co., Milwaukee, WI) within 52-70 min of fenitrothion exposure followed 12 hr later by the intramuscular dose (15 mg/kg body wt). All calves were observed closely for adverse reactions during the study.

Blood samples were drawn from the jugular vein prior to and at 0.25, 0.5, 1, 2, 4, 6, 12, 24, 36, 48 and 72 hr and 7, 14, 21 and 28 d after insecticide administration and initiation of antidotal therapy. Many data points that offered no additional information have been omitted in Tables 1 and 2. Blood samples collected in nonheparinized tubes were allowed to clot and the serum was harvested after centrifugation to determine the activities of aspartate aminotransferase, alanine aminotransferase, acid phosphatase, alkaline phosphatase and lactate dehydrogenase according to the procedures described by Wootton (1964). The level of cholinesterase (ChE) activity was determined in the heparinized whole blood according to the method described previously (Awal et al. 1988). Student's t-test was employed to compare significant difference between the baseline and later values of various parameters and the significance was assessed at $P < 0.05$ and $P < 0.01$ levels.

RESULTS AND DISCUSSION

Changes in the levels of various blood enzymes in fenitrothion-exposed untreated calves are presented in Table 1. Fenitrothion strongly depressed whole blood ChE activity within 1 hr and the maximal inactivation (79%) was noticed at 24 hr. Significant ($P < 0.01$) increase in the serum levels of aspartate aminotransferase, alanine aminotransferase, acid phosphatase, alkaline phosphatase and lactate dehydrogenase was seen with peak effect (87, 377, 208, 59 and 53%, respectively) occurring at 24 hr. Elevation of these enzymes in serum following exposure to fenitrothion and other related OP insecticides is possibly as a result of increased plasma membrane

Table 1. Effect of fenitrothion (217.5 mg/kg, p.o.) on blood enzymes in buffalo calves

Before fenitrothion	Time after administration of fenitrothion (hr)					
	1	2	6	12	24	48
	Whole blood cholinesterase (nmol acetylthiocholine hydrolyzed/min/ml)					
770 ± 20	470 ± 30[a]	405 ± 15[a]	278 ± 30[a]	213 ± 37[a]	165 ± 38[a]	260[b]
	Serum aspartate aminotransferase (nmol pyruvate formed/min/ml)					
75 ± 2.3	89 ± 3.5[c]	93 ± 1.7[a]	119 ± 4.6[a]	130 ± 9.9[a]	140 ± 14[a]	126[b]
	Serum alanine aminotransferase (nmol pyruvate formed/min/ml)					
47 ± 7.2	83 ± 13	95 ± 15[c]	149 ± 15[a]	189 ± 22[a]	224 ± 25[a]	214[b]
	Serum acid phosphatase (nmol phenol liberated/min/ml)					
3.9 ± 0.34	6.9 ± 0.97[c]	8.1 ± 0.97[c]	11 ± 0.66[a]	12 ± 0.79[a]	12 ± 0.68[a]	10[b]
	Serum alkaline phosphatase (nmol phenol liberated/min/ml)					
75 ± 2.1	97 ± 9.2	105 ± 9.9[c]	111 ± 9.0[c]	116 ± 9.4[c]	119 ± 9.5[c]	114[b]
	Serum lactate dehydrogenase (nmol pyruvate reduced/min/ml)					
253 ± 11	315 ± 11[c]	333 ± 11[a]	361 ± 14[a]	379 ± 28[c]	386 ± 26[a]	370[b]

Values given are mean ± SE of the results obtained from 3 animals unless otherwise stated

[a] Significantly different from respective control values ($P < 0.01$)

[b] Values are mean of 2 animals

[c] Significantly different from respective control values ($P < 0.05$)

Table 2. Effect of 2,3-butanedione monoxime on blood enzymes in fenitrothion-poisoned buffalo calves[a]

Before fenitrothion	Time after administration of 2,3-butanedione monoxime (hr)						
	0	1	2	6	12	24	48
	Whole blood cholinesterase (nmol acetylthiocholine hydrolyzed/min/ml)						
796±20	368±35[b]	496±26[b]	535±7.6[b]	622±43[c]	728±17	705±26	744±20
	Serum aspartate aminotransferase (nmol pyruvate formed/min/ml)						
71±1.1	91±3.7[b]	81±5.6	76±5.0	75±2.9	75±1.1	70±0.60	72±2.1
	Serum alanine aminotransferase (nmol pyruvate formed/min/ml)						
60±2.4	98±5.3[b]	82±4.5[c]	74±4.5	66±8.2	63±5.9	54±3.1	53±1.2
	Serum acid phosphatase (nmol phenol liberated/min/ml)						
4.4±0.12	9.7±0.37[b]	7.6±0.45[b]	6.8±0.31[b]	5.1±0.47	4.5±0.22	4.3±0.41	4.0±0.11
	Serum alkaline phosphatase (nmol phenol liberated/min/ml)						
66±0.79	88±1.4[b]	72±3.7	69±2.8	63±1.6	65±2.2	64±1.1	67±2.1
	Serum lactate dehydrogenase (nmol pyruvate reduced/min/ml)						
293±8.2	352±5.3[b]	340±3.1[b]	335±1.5[b]	321±6.2	316±5.3	315±5.3	309±8.2

Values given are mean ± SE of the results obtained from 3 animals

[a]Details of dosages and routes of administration of fenitrothion and 2,3-butanedione monoxime are given in Materials and Methods

[b]Significantly different from respective control values ($P < 0.01$)

[c]Significantly different from respective control values ($P < 0.05$)

permeability and/or cellular injury (Antunes-Madeira and Madeira 1979; Malik et al. 1980; Gupta et al. 1981; Gupta 1984). Alterations in the levels of circulating ChE and other enzymes monitored may be of notable value in the diagnosis of acute fenitrothion intoxication. All animals given fenitrothion displayed toxic signs characteristic of anticholinesterase poisoning (Vadlamudi 1974; Singh 1981; Gupta 1984; Srivastava 1984; Sandhu and Malik 1988). One calf died after 24 hr and a second one on the 15th d after fenitrothion administration. The dose (217.5 mg/kg body wt) employed has been reported to be the median lethal dose of fenitrothion in male buffalo calves (Vadlamudi 1974).

Treatment with 2,3-butanedione monoxime eliminated the clinical toxic symptoms within 18-30 min and completely protected animals against fenitrothion- induced lethality. In fenitrothion-exposed oxime- treated calves, whole blood ChE activity recovered to control values within 12 hr after the initiation of therapy (Table 2).

Administration of 2,3-butanedione monoxime completely reversed the rise in the serum levels of aminotransferases, phosphatases and lactate dehydrogenase. The activities of these enzymes were not significantly ($P > 0.05$) different from baseline values within 6 hr after the first administration of oxime (Table 2). Gupta (1984) also demonstrated that pralidoxime prevented excess and sustained leakage of aminotransferases into serum of malathion-poisoned calves. However, the exact mechanism of oximes involved in reducing enzymatic release still remains to be elucidated.

Unlike pralidoxime, 2,3-butanedione monoxime readily crosses blood-brain barrier and reactivates AChE enzyme in the central nervous system (Taylor 1991). Comparative studies on the disposition of 2,3-butanedione monoxime and pralidoxime in the buffalo have also revealed considerably higher concentrations of the former drug in different parts of brain and spinal cord (Srivastava 1984). In addition to its ability to accelerate dephosphorylation of inhibited AChE, 2,3-butanedione monoxime may react directly with OP to form a relatively nontoxic complex that can be eliminated from the body (Cohen and Wiersinga 1960). From the present findings, however, it could not be ascertained whether all of these factors and/or some other mechanism are involved in the observed prominent beneficial effects of 2,3-butanedione monoxime.

It has been well documented that oximes are not effective in restoring aged phosphorylated AChE enzyme

(Hatch 1988; Taylor 1991). Although dimethoxy-phosphoryl-AChE formed by fenitrothion is less susceptible to aging as compared to the phosphorylated enzyme derived by OP compounds containing tertiary alkoxy groups (Taylor 1991), an early treatment with 2,3-butanedione monoxime would be highly desirable.

The results of this study demonstrated that deleterious effects of fenitrothion on circulating ChE, aminotransferases, phosphatases and lactate dehydrogenase in the buffalo may be rapidly and completely antagonized by 2,3-butanedione monoxime.

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