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Biochemical Alterations after Single Oral Dose of Monocrotophos in *Bubalus bubalis*

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The wide spread use and in many cases their presence and/or concentration in food chain, organophosphorus insecticides constitute a major group of potential health hazards to man and livestock. Like other organophosphorus insecticides, the biological activity of monocrotophos is not limited to insects only but it is also toxic to mammals and birds (Stickel 1975, Mendelssohn 1977). The indiscriminate and inadvertent use of monocrotophos poses health hazards to domestic animals and birds exposed either by ingestion of insecticide sprayed fodder or inhalation from the contaminated environment (Shlosberg et al. 1980). The detailed information on the acute toxicity of monocrotophos is, however, absolutely lacking in buffalo species. Previous reports have indicated that susceptibility of buffaloes to organophosphorus insecticides differ markedly from other species of animals including rodents (Umetsu et al. 1977, Clarke et al. 1981). The present study was therefore undertaken to investigate the effects of single oral administration of monocrotophos on plasma cholinesterase (ChE), serum aminotransferases and total plasma proteins.

MATERIALS AND METHODS

Twelve healthy male buffalo calves (70-130 kg), purchased from local market, were maintained on green fodder (maize or barseem) and wheat straw. Water was provided ad libitum. The calves were randomly divided into three groups, four animals each. Based on preliminary trials, animals of these groups received single dose of monocrotophos (Nuvacron, 36 EC w/w, Hindustan Ciba-Geigy Ltd., Bombay) at the dose rate of 10, 20 and 40 mg/kg body weight. The requisite quantity

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monocrotophos was suspended in 100 ml of tap water and given by drenching. All the calves were fasted for 16 hours prior to administration of insecticide.

Monocrotophos treated animals were observed for the appearance of any toxic symptoms and extent of lethality. The surviving animals were kept under close observation over a period of 28 days after the ingestion of insecticide. Blood samples were collected by jugular venipuncture prior to and at different time intervals after monocrotophos administration. Plasma and serum were separated at room temperature. Plasma ChE (E.C. 3.1.1.8) was determined according to the methods of Voss (1970) as modified by Morai et al. (1976).The levels of serum aspartate aminotransferase (E.C. 2.6.1.1); serum alanine aminotransferase (E.C. 2.6.1.2) and plasma protein were measured by the procedures of Wotton (1964). The statistical significance between two means based on individual observations was assayed by student's t-test.

RESULTS AND DISCUSSION

Monocrotophos was administered in single doses of 10, 20 and 40 mg/kg body weight by oral route. At all doses it produced mild to moderate degree of toxic symptoms which were characteristics of typical anticholinesterase poisoning. The severity of toxic symptoms were dose dependent. The lowest dose (10 mg/kg) was not lethal whereas 20 and 40 mg/kg doses produced 50 and 100 per cent lethality within 106-116 and 14-32 h,respectively. The occurance of toxic symptoms following monocrotophos administration was considerably delayed. The delay in appearance of toxic symptoms may be due to slower absorption of monocrotophos from the gastrointestinal tract; as absorption of xenobiotics from the gastrointestinal tract is a complex process involving interaction of many factors i.e. lipophilicity, binding, pH, degree of ionization etc. (Hwang and Schanker 1974; Ahdaya et al. 1981). In buffalo species an other organophosphorus insecticide, dimethoate, has also shown delayed toxicity following acute poisoning (Singh 1981).

All the doses of monocrotophos produced marked inactivation of plasma cholinesterase. The extent of inhibition of plasma cholinesterase correlated well with the severity of toxicity in buffalo calves given 20 and 40 mg/kg doses of monocrotophos. Maximal inactivation of plasma cholinesterase was observed at 24 h and at this time animals showed severe toxic symptoms. However no relationship between recovery of apparent toxic symptoms and reactivation of plasma cholinesterase enzyme could be established in animals survived

Table 1. Effect of single oral administration of monocrotophos on plasma cholinesterase and total plasma proteins in buffalo calves

Dose (mg/kg)	Time after administration (h) 0	2	4	12	24	48	168	672
	Plasma cholinesterase (n mol acetylthiocholine hydrolysed/min/ml)							
10	175.2± 2.1	147.4±** 2.2	137.1±** 4.2	112.6±** 6.4	105.6±** 8.4	114.3±** 8.4	150.7±** 11.0	170.8± 3.3
20	176.8± 1.0	146.9±** 5.7	130.0±** 7.9	66.9±** 3.9	52.2±** 2.9	45.2±** 4.5	71.8[a]	162.1[a]
40	178.5± 5.0	133.8±** 5.7	102.3±** 9.2	32.1±** 2.9	32.6[b]			
	Total plasma proteins (g/100 ml)							
10	7.0± 0.0	7.5±* 0.1	7.6±** 0.1	8.1±** 0.1	8.4±** 0.1	8.4±** 0.2	7.2± 0.1	7.0± 0.0
20	7.2± 0.1	7.9±* 0.2	8.2±** 0.1	9.0±** 0.2	9.0±** 0.4	8.4±** 0.4	7.4[a]	7.0[a]
40	7.4± 0.1	8.9±* 0.2	9.6±** 0.1	10.6±** 0.2	11.0[b]			

Values given are mean ± SE of the results from 4 animals unless otherwise stated.
a - Value is mean of 2 animals
b - Value of 1 animal
* $P < 0.05$
** $P < 0.01$

Table 2. Effect of single oral administration of monocrotophos on serum aminotransferases in buffalo calves

Dose (mg/kg)	Time after administration (h) 0	2	4	12	24	48	168	672
	Serum aspartate aminotransferase (n mol pyruvate formed/min/ml)							
10	59.7± 1.0	64.8± 0.8	66.2±* 0.3	75.4±** 1.3	78.7±** 2.2	76.5±** 3.2	63.9± 2.7	58.6± 1.1
20	63.4± 0.5	72.3±** 0.5	75.9±** 0.8	90.2±** 2.0	94.4±** 2.1	90.5±** 2.4	65.3[a]	60.3[a]
40	65.3± 1.1	83.8±** 1.8	96.9±** 1.5	137.1±** 2.1	140.7[b]			
	Serum alanine aminotransferase (n mol pyruvate formed/min/ml)							
10	50.4± 1.1	60.4±* 1.9	62.6±** 1.9	82.5±** 5.7	83.7±** 3.4	77.6±** 2.9	56.5± 2.3	50.9± 1.6
20	46.6± 1.8	69.8±** 1.1	80.4±** 2.8	108.6±** 3.7	115.3±** 6.8	113.1±** 4.8	69.8[a]	44.3[a]
40	50.4± 1.9	107.5±** 1.4	119.7±** 2.7	174.0±** 4.9	192.9[b]			

Values given are mean ± SE of the results from 4 animals unless otherwise stated.
a - Value is mean of 2 animals.
b - Value of 1 animal
* $P \leq 0.05$
** $P \leq 0.01$

after monocrotophos administration as toxic symptoms in surviving animals were recovered within 24-31 h but during this time plasma cholinesterase activity remained markedly inhibited ($P < 0.01$). Similar results have been reported with various other organophosphorus insecticides in different species of animals (Jovic 1974, Malik et al. 1978).

There was significant elevation in the serum levels of aspartate and alanine aminotransferases (Table 2). At 24 h the levels of aspartate and alanine aminotransferases were increased to the extent of 32.4-115.4 and 65.9-282.4 per cent, respectively. The increase levels of serum aminotransferases have been used as indicator of cellular damage (Drotman and Lawhorn 1978), increased plasma membrane permeability (Ramazzotto and Carlin 1978) or altered metabolism of these enzymes (Dinman et al. 1963). The oral administration of organophosphorus esters is known to severely effect the liver, an organ primarily involved in their activation and/or detoxification (Murphy 1980). In addition, organophosphorus compounds increases the permeability of artificially prepared lipid membranes (Antunes-Madeira and Madeira 1979). From the present results, however, it could not be ascertained whether monocrotophos mediated elevation of these enzymes was a result of increased plasma membrane permeability or cellular damage or a combination of these factors.

The oral administration of monocrotophos produced marked elevation in the levels of plasma proteins (Table 1). It has been suggested that organophosphorus insecticides may stimulate the growth of cellular proteins and RNA in animals (Puga et al. 1974). Similar to our findings Mihai et al (1972) also observed increased levels of aminoacids in blood and liver of horses given trichlorfon.

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