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Effect of Chronic Sublethal Daily Dosing of Monocrotophos on Some Aspects of Protein Metabolism in Rat Brain

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Monocrotophos (O,O-dimethyl-2(methyl carboxy)-1-methyl vinyl phosphate) is an effective organophosphorous (OP) insecticide which is also toxic to mammals (Stickel 1975). The chemical which is registered for use on crops, is extensively employed in agricultural operations in India for the control of pests. However, inadvertant and indiscriminate use of monocrotophos poses health hazards to domestic animals either by ingestion of fodder or inhalation from the contaminated environment (Shlosberg *et al* 1980). It is well known that the primary target of organophosphates is the cholinesterase (Arnal *et al* 1990). Consequently organophosphates and their residues cause inhibition of esterases to varying degrees in animal systems, leading to behavioral lesions characteristic of cholinergic impairment. In addition to their cholinergic effects organophosphorous compounds can also modify the general metabolic state of the animals by influencing different metabolic segments. Organophosphates cause changes in protein metabolism (Sandhu and Malik 1988a; Sandhu *et al* 1991). Monocrotophos has significant effect on serum proteins and phosphatases of buffaloes (Sandhu and Malik 1988b) and brain proteins and phosphatases of fish (Joshi and Desai 1983). Since peripheral metabolic events are well known to affect brain functions, it is important to assess the metabolic toxicity of monocrotophos in brain areas. Hence, in the present study the changes in soluble and total proteins, free amino acids, proteases and phosphatases were investigated in the cerebral cortex, cerebellum, hippocampus and medulla in albino rats under subacute monocrotophos toxicity.

MATERIALS AND METHODS

Male albino rats (Wistar strain 130±20g) maintained in animal house at 25±2° C, with a normal photoperiod of 12h light and 12h darkness, were used in the present investigation. They were fed with standard pellet diet and water *ad libilitum*. Technical grade monocrotophos (O,O-dimethyl-2(methyl carboxy)-1-1 methyl vinyl phosphate) of 98% purity, obtained from NOCIL, Bombay was used in the present study. Monocrotophos was administered by oral intubation. Distilled water was used as the vehicle, since monocrotophos

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is highly soluble in water. The animals were dosed daily with 9 mg/kg body weight on the first day followed by 6 mg/kg body weight on remaining days. This dose was arrived at, after determining the LD_{50} and then testing a number of sublethal doses. Control animals were given distilled water. The animals were sacrificed at the same time of the day (11 A.M - 1 P.M.) to avoid circadian variations. After sacrificcing the animals by cervical dislocation, the brain was quickly isolated on ice immediately and the brain areas were dissected on a chilled plate following the standard anatomical marks (Glowinski and Iversen 1966). The biochemical estimations were done at 1,3,7,11 and 16 days of daily treatments. Total and soluble proteins were estimated by the method of Lowry *et al* (1951). Free aminoacid content was estimated by the method of Moore and Stein (1954). The activity of proteases was estimated by the method of Davis and Smith (1955). Acid and alkaline phosphatases were estimated by the method of Bodanski (1933) and the liberated inorganic phosphates were estimated by the method of Fiske and Subba Row (1954). Statistical significance of the data was assessed through student's "t" test.

RESULTS AND DISCUSSION

Toxic signs and symptoms were noticed as the daily dosing of monocrotophos was continued which were mainly cholinergic. The severity of signs and symptoms was in between 4-9 days and majority of the signs and symptoms were conspicuous by their absence after 10-11 days. The signs of toxicity were salivation, sweating, tremors, urination, defacation and occasional convulsions. The results presented in Table 1 show a significant decrease of total and soluble proteins and an increase in the content of free aminoacids (FAA) in all brain areas. The decrease in proteins and elevation in free aminoacids were progressive till the dosing was terminated after 16 days. Maximum decrease of total and soluble proteins and maximum elevation of FAA was observed in cerebral cortex followed by other regions.

Rapid loss of brain proteins during pesticide toxicity was reported (Richardson 1981). The decrease in total proteins and soluble proteins indicates their metabolic utilization. Monocrotophos reduced the protein content of fish brain, *Tilapia mossambica* (Joshi and Desai 1983). However, there was an increase in the levels of plasma proteins manifested by the oral dosing of monocrotophos in buffalo calves (Sandhu and Malik 1988b). In the present study elevation of FAA content was observed when decrease in protein levels was noticed. Presumably the degradation of proteins would have led to FAA accumulation. The free amino acid pool may form a possible source of energy to meet the energy requirement.

The results presented in Table 2 show elevation of acidic, neutral and alkaline proteases in all brain areas. The increase in neutral and alkaline proteases was greater than that in acidic proteases. The elevation of protease activity was progressive as the dosing was continued, and maximum elevation was observed on the 16th day. Maximum elevation of acidic protease activity was

Table 1. Changes in protein and free aminoacid contents in brain areas of male albino rats exposed to monocrotophos. Values in parentheses indicate per cent change from control.

Brain area	control	Period of treatment (days) 1	3	7	11	16
		Total Proteins (mg protein/g wet weight)				
Cc	130.93	122.38	115.08	112.49	110.91	108.93
	±1.14	±2.51	±3.12	±2.42	±4.30	±4.60
		(-6.53)	(-12.10)	(-14.08)	(-15.29)	(-16.8)
C	128.22	123.13	120.15	118.11	115.15	110.86
	±1.23	±4.93	±3.23	±2.44	±4.26	±4.62
		(-3.97)	(-6.20)	(-7.88)	(-10.19)	(-13.53)
H	125.52	118.13	115.49	111.36	109.36	108.32
	±2.15	±4.68	±3.12	±3.20	±3.36	±3.49
		(-5.89)	(-7.99)	(-11.28)	(-12.87)	(-13.70)
M	127.50	119.39	117.81	115.35	112.25	108.24
	±1.06	±5.19	±4.33	±2.50	±6.32	±3.24
		(-6.36)	(-7.60)	(-9.53)	(-11.96)	(-15.1)
		Soluble proteins (mg protein/g wet weight)				
Cc	74.73	66.29	59.17	58.26	51.31	49.43
	±1.59	±1.78	±2.67	±4.24	±3.23	±2.29
		(-11.29)	(-20.82)	(-22.04)	(-31.33)	(-33.85)
C	64.09	58.53	56.02	54.81	52.19	47.95
	±1.28	±4.95	±2.01	±1.57	±3.33	±1.49
		(-8.67)	(-12.59)	(-14.48)	(-18.57)	(-25.18)
H	64.87	59.30	56.60	53.21	51.25	47.51
	±1.15	±1.90	±1.48	±2.07	±1.68	±1.48
		(-8.59)	(-12.75)	(-17.97)	(-20.99)	(-26.76)
M	72.25	64.82	61.82	56.43	52.14	49.53
	±1.95	±1.75	±3.12	±2.30	±2.80	±2.60
		(-10.28)	(-14.14)	(-21.90)	(-27.83)	(-31.45)
		Free aminoacids (μ moles of tyrosine equivalents/g wet wt of tissue)				
Cc	87.30	96.21	101.83	104.63	106.04	110.10
	±2.54	±2.28	±2.06	±4.80	±3.90	±3.05
		(10.20)	(16.64)	(19.85)	(21.47)	(26.12)
Cb	85.46	89.66	96.56	97.66	99.62	102.69
	±2.93	±2.76	±2.64	±2.32	±3.05	±3.35
		(4.27)	(12.99)	(15.45)	(16.57)	(20.16)
H	89.39	89.98	100.81	104.57	106.90	108.45
	±2.57	±2.74	±3.39	±2.57	±4.77	±3.47
		(0.66)	(12.78)	(16.98)	(19.59)	(21.32)
M	92.03	101.38	104.57	106.14	107.78	110.20
	±1.99	±2.21	±2.57	±3.80	±3.90	±4.77
		(10.16)	(13.63)	(15.33)	(17.11)	(19.74)

Cc : Cortex, Cb : Cerebellum, H : Hippocampus, M : Medulla
Values are mean ±SD of six observations.
Values are significant at $P < 0.01$.

noticed in cerebellum, and the elevation of neutral and alkaline protease activities was maximum in cerebral cortex. The increase in the acitivity of proteases correlated with the decrease of soluble and total proteins. Lysosomal (acidic) proteases found to have a role in protein degradation (Marzella *et al* 1981). The decrease in proteins could be attributed to the enhanced activities of acidic and neutral proteases to some extent and to that of alkaline proteases. It was reported that impaired energy supply leads to the breakdown of tissue proteins making them susceptible to the action of

Table 2. Changes in the activity of acidic, neutral and alkaline proteases (μ moles of tyrosine equivalents/mg protein/h) in brain areas of male albino rats exposed to monocrotophos. Values in parentheses indicate per cent change from control.

Brain area	control	Period of treatment (days) 1	3	7	11	16
			Acidic protease			
Cc	1.32 ±0.06	1.45 ±0.06 (9.84)	1.57 ±0.05 (18.94)	1.75 ±0.07 (32.58)	2.07 ±0.08 (56.82)	2.11 ±0.06 (59.85)
Cb	1.06 ±0.05	1.12 ±0.04 (5.66)	1.33 ±0.04 (25.47)	1.47 ±0.05 (38.68)	1.60 ±0.06 (50.94)	1.68 ±0.05 (58.49)
H	1.09 ±0.03	1.17 ±0.04 (7.34)	1.28 ±0.06 (17.43)	1.37 ±0.08 (25.69)	1.56 ±0.06 (43.12)	1.45 ±0.07 (51.38)
M	1.19 ±0.03	1.34 ±0.02 (12.60)	1.46 ±0.05 (22.69)	1.65 ±0.05 (38.66)	1.79 ±0.07 (50.42)	1.89 ±0.08 (58.82)
			Neutral protease			
Cc	1.48 ±0.02	1.55 ±0.03 (4.73)	1.73 ±0.05 (16.89)	2.04 ±0.07 (37.83)	2.09 ±0.04 (41.22)	2.19 ±0.18 (47.97)
Cb	1.20 ±0.02	1.33 ±0.03 (10.83)	1.42 ±0.05 (18.38)	1.54 ±0.06 (28.33)	1.62 ±0.04 (35.00)	1.79 ±0.05 (49.17)
H	1.22 ±0.03	1.32 ±0.06 (8.20)	1.55 ±0.12 (27.05)	1.70 ±0.04 (39.34)	1.82 ±0.06 (49.18)	1.94 ±0.09 (59.02)
M	1.21 ±0.02	1.41 ±0.11 (16.53)	1.58 ±0.12 (30.58)	1.85 ±0.10 (52.89)	1.89 ±0.08 (56.20)	1.94 ±0.11 (60.33)
			Alkaline protease			
Cc	1.43 ±0.02	1.79 ±0.05 (25.17)	1.98 ±0.09 (38.46)	2.11 ±0.05 (47.55)	2.23 ±0.03 (55.94)	2.34 ±0.12 (63.64)
Cb	1.19 ±0.03	1.34 ±0.05 (12.60)	1.55 ±0.04 (30.25)	1.63 ±0.08 (36.97)	1.79 ±0.08 (50.42)	1.89 ±0.05 (58.82)
H	1.20 ±0.02	1.37 ±0.04 (14.17)	1.54 ±0.08 (28.33)	1.66 ±0.03 (38.33)	1.75 ±0.07 (45.83)	1.83 ±0.08 (52.50)
M	1.39 ±0.03	1.69 ±0.01 (21.58)	1.84 ±0.12 (32.37)	1.99 ±0.05 (43.16)	2.10 ±0.05 (51.08)	2.18 ±0.16 (56.83)

Cc : Cortex, Cb : Cerebellum, H : Hippocampus, M : Medulla
Values are mean ±SD of six observations.
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tissue proteolytic enzymes and leading to their consequent degradation by proteases (Berger *et al* 1983). The involvement of phosphorylated proteins in these results, however, is not known.

The results presented in Table 3 show elevation in the activity of phosphatases. However, the elevation of acidic and alkaline phosphatases was rapid till 7 days of daily treatments and the elevation was less at 11 and 16 days after daily dosings. The activity of alkaline phosphatase was more to acidic phosphatase. Maximum effect was seen in cerebral cortex. The

Table 3 Changes in acid and alkaline phosphatase activity (μ moles of pi formed/mg protein/h) in brain areas of male albino rats exposed to monocrotophos. Values in parentheses indicate per cent change from control.

Brain area	control	Period of treatment (days) 1	3	7	11	16
			Acid phosphatase			
C	3.02 ±0.06	3.57 ±0.19 (18.21)	3.71 ±0.22 (22.81)	3.88 ±0.14 (28.48)	3.78 ±1.47 (25.17)	3.90 ±0.35 (29.14)
Cb	3.62 ±0.09	4.12 ±0.15 (13.81)	4.38 ±0.21 (20.99)	4.61 ±0.17 (27.35)	4.49 ±0.31 (24.03)	4.62 ±0.23 (26.62)
H	2.93 ±0.08	3.31 ±0.17 (12.91)	3.49 ±0.13 (19.11)	3.68 ±0.24 (25.60)	3.76 ±0.28 (28.33)	3.58 ±0.33 (22.18)
M	2.90 ±0.06	3.29 ±0.22 (13.45)	3.39 ±0.25 (16.90)	3.62 ±0.14 (24.83)	3.58 ±0.13 (23.45)	3.69 ±0.22 (27.24)
			Alkaline phosphatase			
Cc	3.81 ±0.14	4.23 ±0.21 (11.02)	4.86 ±0.32 (27.56)	4.97 ±0.27 (30.45)	5.07 ±0.28 (33.01)	5.19 ±0.30 (36.22)
Cb	3.72 ±0.10	4.09 ±0.19 (9.95)	4.17 ±0.23 (12.10)	4.58 ±0.20 (23.12)	4.72 ±0.19 (26.88)	4.78 ±0.24 (28.49)
H	3.68 ±0.18	4.19 ±0.16 (13.86)	4.43 ±0.19 (20.38)	4.64 ±0.32 (26.09)	4.73 ±0.21 (28.53)	4.80 ±0.15 (30.43)
M	3.23 ±0.05	3.69 ±0.17 (14.24)	3.84 ±0.08 (18.88)	4.11 ±0.22 (27.24)	4.17 ±0.24 (29.10)	4.28 ±0.32 (32.50)

Cc : Cortex, Cb : Cerebellum, H : Hippocampus, M : Medulla
Values are mean ± SD of six observations.
Values are significant at $P < 0.01$.

enhancement of phophatase activities indicate that monocrotophos has caused some damage to brain areas. Increased acid phosphatase activity of a tissue is an indication of tissue damage (Tietz 1970). Serum phosphatases were elevated following single and chronic oral doses of monocrotophos and greater effect was found on acidic phosphatase than on alkaline phosphatase, indicating an effect on lysosomes (Sandhu and Malik 1988b). Both phosphatases were affected to a similar extent in the present study. Lower effect of monocrotophos in terms of slower elevation of phosphatase activity after 7 days of treatment suggests that it has a lowered effect on long-term treatment.

Results of the present study clearly show disturbances in proteins and related enzymes of protein metabolism in brain areas on monocrotophos administration. The results differ from our results on acetylcholinesterase (AChE) activity (Swamy *et al* 1992). The AChE activity decreased progressively up to 7 days and then showed some reversal towards control on the 11th day. The activity eventually reached a plateau, still maintaining a lower level than control at 16 days in all the brain areas. Despite this the animals showed apparent behavioral normalcy (Swamy *et al* 1992). In the

present study each of the parameters studied under protein metabolism either progressively increased or decreased throughout the period of study (16 days). Behavioral normalcy was apparent at 16 days in the present study also. Thus, although differing from the trend from cholinesterase inhibition the changes in protein metabolism may have their own adaptive value under monocrotophos stress. The changes were predominant in the cerebral cortex followed by other regions. Similar findings were reported earlier (Sadovnikova *et al* 1981).

The present study also indicates that daily treatment of animals with a cholinesterase inhibitor leads to changes in the activities in tissue enzymes other than cholinesterases. The number of such enzymes/proteins involved are unknown. The extent of change probably depends upon the amount of OP administered and the affinity of the OP for these enzymes. The lack of correlation between the disappearance of toxic signs and symptoms and the changes in the metabolic parameters in the present study indicate little or no role for these parameters in behavioral adaptations under chronic monocrotophos treatment.

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