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BIOCHEMICAL EFFECTS OF SOME ORGANOPHOSPHORUS PESTICIDES ON THE OVARIES OF ALBINO RATS

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Abstract : An evaluation of the toxic effects of three organophosphates; monocrotophos, dimethoate and methyl parathion on female reproduction was made by biochemical estimations of cytoplasmic and membrance bound proteins, lipids, phospholipids and cholesterol in the rat ovaries after treatment with their low residual level doses (LD_{50} 1/8–1/5) to three groups of six rats each for 90 days. All the three pesticides caused degenerative changes in the ovaries as evidenced by a significant decrease in the concentration of cytoplasmic as well as membrane bound proteins, total lipids, phospholipids and cholesterol. The observations are thus indicative of the reproductive toxicity caused by organophosphates at cellular and molecular level in the ovaries of rats.

Key words : organophosphates rats ovaries proteins lipids phospholipids cholesterol

INTRODUCTION

Organophosphates are commonly used as insecticides in agriculture in India and are potent toxicants (1). A serious problem with some organophosphorus compounds has been their high acute toxicity to man and non-target organisms (2). Many environmental pollutants are known to cause reproductive toxicity and have resulted in a decrease in the fertility level of human population (3), but a few reproductive toxicants have been clearly identified and the mechanisms of chemical toxicity on reproductive organs are not well defined (4). Therefore, in the present studies, it was planned to investigate the biochemical effects of three organophosphates; dimethoate, methyl parathion and monocrotophos, after their long-term treatment in low residual level doses on ovaries of albino rats.

METHODS

Animals

Adult (1½–2 months age; 150–200 g body weight) female rats of Wistar strain were housed in groups of two per cage, maintained under controlled conditions of temperature (22 ± 2°C) and light (12:12 L : D) and provided with food and water ad

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libitum. Cyclicity of rats was confirmed by examining their vaginal smears daily and the ones showing two or more regular 4–5 days estrous cycles were selected for the experiment.

Treatment with organophosphates

Groups of six rats each were given daily oral doses of dimethoate (1/5 LD_{50}; 76 mg/kg bw), methyl parathion (1/5 LD_{50}; 4.8 mg/kg bw) and monocrotophos (1/8 LD_{50}; 2.9 mg/kg bw) for 90 days. An equal number of animals were vehicle (water) treated and kept as control for each pesticide.

Biochemical estimations

A day after the last dose, the animals were sacrificed by decapitation, ovaries and liver extirpated, put in normal saline and freed from fat and adhering connective tissues. The cleared ovaries and liver were soaked on a piece of filter paper, weighed, minced and homogenized in known volume of ice-cold phosphate - buffer saline (PBS, pH 7.4) and the ovarian homogenate was centrifuged.

Determination of proteins

The soluble (cytoplasmic) proteins were estimated in the supernatant and the residue (pellet) was washed with PBS by centrifugation at 1000 rpm for 5 minutes. The supernatant was separated and pellet dissolved in PBS and shaken with known

volume of 1% solution of the detergent deoxycholate (DOC) for 1 hr at 37°C. The solution was ccntrifuged at 10,000 rpm for 30 minutes, pellet discarded and supernatant used for estimation of membrane bound proteins and lipids. In the liver homogenate and both the components of ovaries, total proteins were estimated by the method of Lowry et al (5) and expressed as mg/g fresh liver/ovary.

Determination of total lipids, phospholipids and cholesterol

The liver homogenate and both the ovarian supernatants left after the estimation of proteins were used for the estimation of total lipids by the method of Folch et al (6). Total lipids expressed as mg/g fresh liver or ovary were further analysed for phospholipids and cholesterol by using standard techniques of Ames (7) and Zlatkis and Zak (8) respectively and expressed as mg/g of fresh liver/ovary as well as percent of total lipids. The data were subjected to statistical analysis applying student's t-test.

RESULTS

Effects of OPs on ovarian proteins, lipids, phospholipids and cholesterol

The concentration of total cytoplasmic as well as membrane bound proteins, lipids, phospholipids and cholesterol significantly decreased in the ovaries of rats treated with each of the organophosphate (Table I and II). The decrease in concentrations of these biochemical components was statistically significant ($P<0.05–0.1$) when compared with their respective control levels. (Table I and II). The concentration of phospholipids and cholesterol, when expressed in terms of percentage of total lipid content of the

TABLE I: Effect of some organophosphates on concentration of cytoplasmic proteins, total lipid, phospholipids and cholesterol in the ovaries of albino rats (Mean ± SD).

Treatment	*Proteins (mg/g)*	*Total lipids (mg/g)*	*Phospholipids (mg/g)*	*Cholestrol (mg/g)*
Control	30.49±5.86	44.72±15.52	5.69±1.83	2.65±0.19
Dimethoate	23.61±1.50	14.03±16.46	2.28±0.74*	1.05±0.26*
Control	32.50±6.90	37.41±13.50	4.95±1.63	2.51±0.15
Methyl parathion	19.19±5.32*	21.05±4.76	1.50±0.35*	0.16±0.009**
Control	28.03±3.77	40.15±6.49	4.93±0.31	2.55±0.04
Monocrotophos	14.87±1.44*	9.68±1.44**	1.90±1.0*	0.44±0.02**

*P<0.05; **P<0.1

TABLE II: Effect of some organophosphates on membrane bound (DOC extracted) proteins, total lipid, phospholipids and cholesterol in the ovaries of albino rats (Mean ± SD).

Treatment	*Proteins (mg/g)*	*Total lipids (mg/g)*	*Phospholipids (mg/g)*	*Cholestrol (mg/g)*
Control	11.79±1.67	102.07±36.45	10.46±2.18	2.83±0.96
Dimethoate	5.14±1.04**	79.42±6.19**	3.95±0.63**	1.10±0.49**
Control	2.47±2.86	110.73±16.48	9.06±1.37	2.19±0.16
Methyl parathion	6.05±0.90**	77.00±4.18**	7.77±1.26	0.93±0.06**
Control	10.31±3.27	96.00±11.79	8.56±11.79	2.61±0.11
Monocrotophos	3.08±0.3**	35.96±3.18**	2.87±0.64**	0.95±0.12**

*P<0.1

TABLE III: Effect of some organophosphates on the percentage of cholesterol and phospholipids in total lipids in ovaries of albino rats.

Treatment	*% in cytoplasmic total lipids*		*% in DOC extracted membrane lipids*	
	Cholesterol (%)	*Phospholipids (%)*	*Cholesterol (%)*	*Phospho (%) lipids*
Control	5.93	1.27	2.77	10.25
Dimethoate	7.48	16.25	1.39	4.97
Control	6.71	13.23	1.98	8.18
Methyl parathion	0.76	7.13	1.21	10.09
Control	6.35	12.28	2.72	8.92
Monocrotophos	4.56	19.63	2.64	7.98

ovaries, was also found to be decreased after treatment with all the OPs (Table III). The concentration of proteins and lipids also decreased in the liver of OP treated rats as compared to that in their control rats (Table IV).

TABLE IV: Effect of some organophosphates on protein and lipid content in liver of female albino rats (mean ± SD).

Treatment	*Protein (mg/g)*	*Lipid (mg/g)*
Control	7.04±0.13	29.16±4.77
Dimethoate	5.97±0.35*	20.83±4.21*
Control	6.47±0.26	28.90±2.50
Methyl parathion	4.73±0.07*	19.66±4.21*
Control	6.56±0.86	33.33±4.21
Monocrotophos	4.95±0.18*	25.00±0*

*P<0.05

DISCUSSION

The decrease in the proteins, lipids, phospholipids and cholesterol (cytoplasmic and membrane bound) in the ovaries of rats after long term treatment with low doses of dimethoate, methyl parathion and monocrotophos indicates degenerative changes. A decrease in the total lipid content in schiatic nerve, kidney, skeletal muscle, brain and spinal cord was also observed after treatment of chicks with two organophosphates; disyston and folithion (9). Phospholipids and the ratio of phospholipids to total cholesterol also decreased in the nervous tissue of the chicks with these two pesticides, which are indicative of degenerative changes (9). Similar lipid changes have also been reported with other organophosphates like lebaycid and metasystox-R (10). The toxicity of organophosphates varies with structures (WHO, 1986). All organophosphorus pesticides (OP) are lipophilic and these environmental xenobiotics are known to have a strong affinity for interaction with membrane phospholipids (11) and the phospholipid component of the biomembrane is believed to be the site of action of OP insecticides (12). There are also evidences that oxygen free radical formation can be a factor in the toxicity of organophosphates (13). One of the targets of creative oxygen-induced injury is lipid peroxidation, and peroxidation of membrane phospholipids not only alters lipid milieu and structural and functional integrity of cell membrane but also affects the activities of various membrane bound enzymes including total ATPase, Na^+-K^+-ATPase and Mg-ATPase (14–16). Thus in addition to acctylcholinestrase being the principal mode of action of OP pesticides (17) increased lipid peroxidation (12–13; 18–20) and altered ATPase (21–26) have also been implicated in mediating OP toxicity in animals. The inhibitory alterations in the membrane bound lipid composition of the OP treated rat ovaries in the present observations might be result of their this activity and present studies are thus indicative of such degenerative changes induced by dimethoate, methyl parathion and monocrotophos at the cellular and molecular level in the ovaries and liver of rats after their long term exposure even in very low residual level doses.

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