EFFECT OF MALATHION AND METHYL - PARATHION ON RAT LIVER ENZYMES

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Abstract

The effect of sub-lethal doses of Malathion and Methyl-Parathion was studied on the rat liver enzymes. Intravenous administration of both insecticides at weekly interval for four weeks resulted in increase in weight of various tissues, i.e., heart and spleen. Short term (24 hr) and long term (4 weeks) treatment with insecticides resulted in increased specific activities of liver enzymes, Acid phosphatase, Alkaline phosphatase, Glutamate dehydrogenase, Glutamate oxaloacetate transaminase and Glutamate pyruvate transaminase. The increase in enzyme activity was not as profound when the insecticide administration was spread over 4 weeks. Malathion had greater effect than Methyl-parathion on the biochemical parameters studied (JPMA 40 85, 1990).

INTRODUCTION

Pesticides are of benefit to mankind as they protect crops and increase the yields. They can also create serious problems when used, handled and stored improperly. In addition to their ability to destroy the environment, humans can also be poisoned by pesticides, if proper precautionary measures are not adopted. In Pakistan pesticide usage first started in 1954 with the import pf254m tons of formulated products which in 1987 increased to 4388m tons active ingredients[1]. Of the organophosphates, Malathion and Methyl-parathion are among the most widely used insecticides. Degree of toxicity of organophosphate insecticides varies widely. These compounds are absorbed in humans through all the routes including skin, lungs and gastrointestinal tract[2]. Long term exposure to residues of insecticides in food or water causes liver damage and responses of vein thrombosis, carcinomas, sarcomas and depressed growth[3,4]. Marked effects on nervous system and other body tissues of consumers have been reported[5]. In the present investigation the short term and long term effects of Malathion and Methyl-parathion on the general development of the animals and specifically on the activities of the liver enzymes were studied.

MATERIALS AND METHODS

Animals

Mature male albino rats, 8-10 weeks old were obtained from Department of Biological Sciences, Quaid-i-Azam University, Islamabad. The rats were maintained in controlled environment. Food and water were available ad jib.

CHEMICALS

The chemicals used in this study were of analytical grade. The Kits used for the assays of Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transminase (GPT) enzymes were supplied by the Wakopure Chemical Industries Ltd. Japan. Insecticides, Malathion (96% active ingredient) and Methyl-parathion (99% active ingredient) were obtained from National Institute of Health, Islamabad.

EXPERIMENTAL DESIGN

Short term treatment

The animals were divided into three groups, one serving as control carrier solution only, to the other group Malathion 0.96 mg/kg body weight, dissolved in corn oil was injected intravenously while the third group received Methyl-parathion 0.04 mg/kg body weight. The animals were killed after 24 hours by cervical dislocation and dissected. The liver removed from each animal was immediately weighed and processed further after washing with ice cold saline solution.

Long term treatment

The procedure was the same as that for short term except that the dose of insecticides was divided in 4 equal parts and each part injected at weekly interval. The animals were killed after 4 weeks and tissue further processed for analysis.

Preparation of liver tissue for enzyme assays

Liver tissues were minced with fine pair of scissors and homogenized in (1:10 v/v) ice cold 50mM phosphate buffer (pH 7) using a mechanized homogenizer. An aliquot of this homogenate was used for the estimation of total protein. Another aliquot of the homogenate was centrifuged, at 10,000 rpm for 20 minutes at 4°C, in Kokusan model H 251 centrifuge. The supernatant was decanted and analysed.

In vitro treatment

The supernatant from untreated rat liver preparations was assayed for enzyme activities with and without Malathion and Methyl- parathion dissolved in corn oil. The concentrations of insecticides were equivalent to those used for in vivo experiments.

ANALYTICAL PROCEDURES

(i) Protein concentrations

Protein in liver samples homogenates and supernatants was determined according to the method of Lowery[6], using Folin Phenol reagent. Protein concentration was calculated from calibration curve based on Bovine Serum Albumin as standard.

(ii) Acid Phosphatase (E.C. No. 3.1.3.2.) and Alkaline Phosphatase (E.C. No.3.13.1)

Acid phosphatase and alkaline phosphatase activity was determined according to the method described by Walter and Schmitt[7], using para-nitrophenol phosphate (p-NPP) as substrate.

(iii) Sorbitol Dehydrogenase (E.C. No. 1.1.1.14)

The sorbitol dehydroçenase activity was measured by the method of Bergmeyer[8] using fructose as substrate.

(iv) Glutamate Dehydrogenase (E.C. No.1.4.13).

Glutamate dehydrogenase activity was assayed by the method of Carlson et al[9].

(v) Glutamate Oxaloacetate Transminase (E.C. No. 2.6.1.1) and Glutamate Pyruvate Transaminase (E.C. No. 2.6.1.2).

Glutamate oxaloacetate transaminase and glutamate pyruvate transaminâse were assayed by the methods of Frankel and Reitman[10].

(vi) Statistical analysis

The results were analysed statistically by complete block design. Two way analysis of variance and Duncan's Multiple Range Test were applied to test the significance at F = 0.05.

RESULTS

The treated groups of rats receiving Malathion and Methyl- parathion for 4 weeks continued to grow normally with almost equal body weight as in the control rats and showed no visible toxic symptoms. The weight of various organs was recorded after 4 weeks treatment with insecticides (Table I).

TABLE I. Effect of Malathion and Methyl-parathion treatment (Long term) on various body organs of the rats.

	Control	Malathion Treatment	Methyl-parathion Treatment
Average body Weight (gm)	122.50	119.00	120.50
Organ	Average weight/body weight ($\times 10^{-3}$)		
Liver	45.70	47.00	47.50
Heart	3.27	4.20	4.15
Lungs	5.52	5.46	5.02
Kidneys	4.90	5.00	4.98
Spleen	2.85	3.36	3.30

No. of animals in each group = 3

The ratio of organ weight to body weight indicated that the weight of heart and spleen increased in the treated animals. Whereas there was no difference in the weights of liver, kidney and lungs of the control and the treated animals.

Effect on total protein in liver

Total protein concentration was estimated both in control and treated animals. After long term treatment with insecticides a decline in the level of protein content in the liver of animals was observed, the corresponding values for Malathion and Methyl- parathion were 29.60 and 37.5 mg/kg respectively. It was observed that decrease in protein contents was significantly greater in liver extracts of animals treated with Malathion than those treated with Methyl-parathion. Treatment with Malathion resulted in a decrease of 33.64% whereas a decrease of 16.8% was observed in Methyl-parathion treated animals as compared to control (Table II).

TABLE II. Protein concentration (mg/g wet weight) of liver extract after treatment with Malathion and Methyl-parathion.

Parameter	Exp. Conway.	Control	Malathion Treatment	Methyl-parathion Treatment
Protein	4 weeks	45.8±2.77 A	43.2±1.24 C	42.4±1.21 F
	24 hours	44.6±1.29 B	29.6±1.20 E	37.5±2.70 D
	in vitro	44.4±1.73 B	30.8±0.86 E	40.1±1.32 D

No. of animals in each group = 3; Results are Mean ± SD.
Difference in figures followed by the same letter are statistically non significant.

Short term treatment with Malathion and Methyl-parathion also resulted in a decrease of protein

concentration of liver. In in vitro experiment, decline in the level of protein was less but non-significant than for in vivo experiment for both insecticides.

Effect of insecticides on liver enzyme activities

The activities of various liver enzymes as affected by insecticides are presented in Table III.

TABLE III. Enzyme activities /min/mg protein in liver extract after treatment with Malathion and Methyl-parathion.

Parameter	Exp. Cond.	Control	Malathion treated	Methyl-parathion treated
Acid phos-	4 weeks	25.8±1.71 E	35.4±0.82 D	34.9±1.60 D
phatase	24 hours	24.0±1.36 EF	59.5±0.84 A	48.1±0.82 B
	in vitro	24.2±1.20 EF	40.4±1.20 C	35.5±1.20 D
Alkaline	4 weeks	28.8±1.42 E	43.3±2.83 B	36.5±0.41 CD
Phos-	24 hours	24.8±1.03 F	81.3±0.73 A	47.3±0.80 B
phatase	in vitro	24.1±1.85 F	46.9±0.99 B	35.1±0.82 C
Glutamate	4 weeks	65.6±1.00 E	72.3±0.93 CD	69.1±0.13 D
dehydro-	24 hours	63.8±1.67 F	93.9±0.52 A	83.2±0.40 B
genase	in vitro	63.6±1.23 F	80.3±3.80 B	78.3±2.35 C
Sorbitol	4 weeks	68.5±0.41 FG	70.8±1.08 DF	71.4±2.27 DE
dehyd-	24 hours	65.9±1.36 G	103.5±1.50 A	98.3±1.36 B
rogenase	in vitro	65.9±2.12 G	75.9±2.40 C	74.1±0.85 C
Glutamate	4 weeks	37.4±2.00 D	48.0±1.48 B	41.7±0.68 C
Oxaloace-	24 hours	36.3±0.57DE	65.6±1.19 A	41.5±0.44 C
tate trans-	in vitro	34.8±1.04 E	47.6±1.90 B	39.6±1.67 CD
aminase				
Glutamate				
Pyruvate	4weeks	32.3±0.52 DE	34.4±1.32 D	34.0±1.35 D
Transa-	24 hours	30.1±1.50 EF	56.1±1.64 A	38.6±2.20 C
minase	in vitro	28.6±0.27 F	46.5±1.20 B	34.5±1.24 D

No. of animals in each group = 3; Results are Mean ± SD.
Difference in figures followed by the same letter for each enzyme is statistically non significant.

(1) Acid Phosphatase

Specific activity of acid phosphatase in liver extract of animals treated with Malathion and Methyl-parathion showed a significant increase as compared to the control under all the experimental conditions. The increase in specific activity after short term treatment was significantly higher than

after long term treatment. However, the administration of Malathion resulted in a greater enzyme activity than Methyl-parathion after short term treatment but the effect of the long term treatment with both insecticides for four weeks did not differ from each other.

(2) Alkaline Phosphatase

The administration of Malathion and Methyl-parathion to animals for short term in vivo and in vitro and for long term in vivo resulted in significant increase in the specific activity of this enzyme. In short term treatment with Malathion and Methyl-parathion, the specific activity of the enzyme increased by about 3 and 2 folds respectively than controls. After long term treatment the increase was 1.5 and 1.2 times respectively compared to corresponding contrOls. Malathion had a greater effect on specific activity of this enzyme as compared to Methyl-parathion.

(3) Glutamate Dehydrogenase

The rate of increase in the activity of GDH varied under different conditions. The maximum increase (47.3%) in specific activity was observed in liver of animals treated with Malathion for 24 hours. While in animals treated with Methyl-parathion it was 30.5% higher than controls. The treatment of liver extracts with insecticides in vitro had the same effect on specific activity but it was significantly less than in vivo conditions. In in vitro conditions the increase in specific activity was 26.3% and 22.6% with Malathion and Methyl-parathion respectively. The long term treatment with insecticides had almost the same effect on the specific activity of GLDH but the activity was significantly higher as compared to control.

(4) Sorbitol Dehydrogenase

The short term treatment with Malathion in vivo had more prominent effect on the specific activity of SDH than in in vitro and long term treatment. The specific activity of SDH increased from 65.91 moles/min/mg protein in the control to 103.5 moles/min/mg protein with Malathion treatment. Short term treatment in vivo and in vitro with Methyl-parathion also significantly increased the specific activity of SDH. Its specific activity increased by 48.7% in in vivo and 8.13% in vitro, as compared to the control. Administration of Methyl-parathion in multiple doses had little effect on the activity of SDH.

(5) Glutamate Oxaloacetate Transaminase.

In short term treatment with Malathion in vivo 80.6% and in vitro 36.7% increase in specific activity of GOT was observed while there was only 28.2% increase in specific activity of GOT in liver of animals receiving multiple dose of Malathion over a longer period. Short and long term effects of Methyl-parathion on the specific activity of GOT revealed no statistical difference. The increase in specific activity was 14.29% as compared to control after short term administration of insecticide in vivo, whereas after long term treatment, this increase was only 11.4%.

(6) Glutamate Pyruvate Tkansaminase

The short term effect of Malathion on specific activity of GPT resulted in 86.3% increase in in vivo and 27.9% increase in vitro, while the administration of Methyl-parathion in vivo and in vitro did not differ significantly. It was observed that there was statistically no change in the specific activity of the enzyme when both the insecticides were given to the animals for a longer period. To conclude, it was observed that if the insecticides were given in smaller doses for a longer period, the animals adjusted by probably detoxifying these toxicants. However, if the insecticides are given as a single dose and samples analysed 24 hours after administration all the enzymes studied showed enhanced specific activities.

DISCUSSION

The average body weight of the animals after insecticide treatment for 4 weeks showed no variation than the controls. However, the loss of weight has earlier been observed in rats trea$ed with

Malathion[11] and also in rabbits treated with Methyl-parathion[12]. The ratio of organ weight to body weight increased to some extent in all the organs studied, but it was more prominent in heart and spleen, after the administration of Malathion and Methyl-parathion. Similar results have been reported when rats were treated with organophosphates[11,13,14]. The post treatment analysis revealed loss in protein concentration after both short and long term exposure to Malathion and Methyl-parathion. Earlier reports have also shown the reduction in protein concentration in liver of rats and fish by the administration of Malathion and other organophosphates[14,15]. Reduction in protein maybe due to inhibition of amino-acid metabolizing enzymes with subsequent inhibition of translation. Organophosphates induce alteration in enzyme activities in liver tissues[16]. In the present investigation the activity of acid phosphatase was increased with the treatment of Malathion and Methyl-parathion. This agrees with the findings for fish ovary[17] and in brain tissues of hens treated with Parathion[18]. Although a decline was observed in the total protein, the increase in AcP may be the reflection that most of the decrease occurred in non enzymic protein, therefore, changing the ratio in the favour of enzymic protein. Alkaline phosphatase activity was also increased by the administration of the insecticides as compared to the control animals. Similar observations have been made in rat liver and fish liver after treatment with Parathion, EPN, Dichlorvos (DDVP) and Malathion[17,19]. The increase in the enzyme activity may be due to liver cell damage. Glutamate dehydrogenase activity was observed to be enhanced under all the experimental conditions. These results are in agreement with the earlier findings which show elevation in GLDH activity in various tissues of fish with the treatment of Methyl-parathion and other organophosphates[20]. Sorbitol dehydrogenase (SDH) activity was also observed to increase after the administration of Malathion and Methyl-parathion. The increase in the activity of SDH in liver, brain and muscle was also found after treating the Indian cat and fish with organophosphate insecticides[21]. Similarly increase in the activity of GOT was observed after administration of Malathion and Methyl-parathion. These findings are in agreement with the previous findings on the influence of Chiorpyrifos and Diazinon on the GOT activity in rat liver[22]. The activity of GPT was increased with the treatment of Malathion and Methyl-parathion. Similar kinds of results have been reported when the insecticides were administered to the rats[21]. Malathion and Methyl-parathion had more profound effect on the liver enzymes in vivo (short term) rather than in vitro treatment. This maybe attributed to the fact that Malathion and Methyl-parathion are converted to their metabolic derivatives in liver whose toxicity-is several times greater than that of the original compounds[23]. In a similar study Jabeen[22] has reported increase in various rat liver enzymes under the effect of organophosphate insecticides, Chiorpyrifos and Diazinon. Most of the enzymes tested in liver tissue showed increased specific activities suggesting intra and extra hepatocellular release by necrosis of the hepatocytes. Since both cytoplasmic (SGPT) and particular mitochondrial (SGOT) and lysosomal enzymes (Ac + Al P) are elevated. This suggests generalized toxic effects within the hepatocytes. Finally, it may be emphasized that the results of our study using sub-lethal doses may create some awareness among the users to the fact that even if these insecticides are taken in only small quantities they are dangerous to health hence proper precautionary measures should always be taken while handling them.

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