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Effects of Subchronic Treatment of Parathion on Immune Potential Marker Enzymes of Rats

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The effects of methyl parathion in sublethal concentration on immune potential enzymes [adenosine deaminase and myeloperoxidase] of rats was investigated under laboratory conditions. Methyl parathion (5 and 10 ppm) was administered orally to 6 female rats *ad libitum* during the tests for 4 weeks consecutively. Various tissues adenosine deaminase and myeloperoxidase activities of rat were determined after treatment. The results showed that methyl parathion resulted different effects on the enzymes activities compared with control rats. Methyl parathion treatments increased significantly adenosine deaminase and myeloperoxidase activity except for liver myeloperoxidase activity with both two dosages treatment. The observations presented led us to conclude that methyl parathion produced substantial systemic organ toxicity in rats during the period of a 28 d subchronic exposure.

Key Words: Rat, Immune potential enzymes, Parathion.

INTRODUCTION

Environmental pollution by pesticide residues is a major environmental concern due to their extensive use in agriculture and in public health programs[1]. The environmental impact of pesticide use is related to several fundamental properties essential to their effectiveness as pesticides. Firstly, pesticides are toxicants, capable of affecting all taxonomic groups of biota, including non-target organism, to varying degrees depends on physiological and ecological factors. Secondly, many pesticides need to be resistant to environmental degradation so that they persist in treated areas and thus their effectiveness is enhanced. This property also promotes long-term effects in natural ecosystem[2]. Since pesticides are offered for plant protection, there has been improvement in the control of pest population and spread of infection born disease vectors. Public health programs in many developing countries including Turkey also utilize these studies as pesticides of choice to control disease-transmitting organism[3]. There is abundant evidence that many pesticides produce their acute toxic action by activating or inhibiting enzymes.

In addition, chemicals *via* food chain have harmed physiological mechanisms in man. On the other hand, many chemical even at relatively low dosages disturb the metabolism of biota by altering normal enzyme activity[3-6]. A considerable literature exists describing the effects of pesticides on populations and communities of organisms under field conditions. Major effects of pesticides on animal and insect populations result primarily in significant changes in species abundance and associated shifts in dynamics, thus they have been resulted in an imbalance in the natural system[7].

Methyl parathion is one of the most widely used organophosphate insecticides in agriculture. Organophosphorus insecticides (OPIs) are some of the most useful and diverse classes of insecticides in use for almost five decades. However, the uncontrolled use of these insecticides in agriculture and public health operation has increased the scope of ecological imbalance and thus many non-target organisms have become victims[8]. In the literature, it is reported that OPIs are neurotoxic in nature by acting as inhibitors of neuronal cholinesterase (ChE) activity[9]. However, some studies reported that OPIs caused lipid peroxidation[10-12] in vertebrates.

Methyl parathion is one of the most widely used OPIs in agriculture and public health programmes. Methyl parathion is also one of the most used OPIs in the region of Van, Turkey. The aim of the current study is to investigate the effects of subchronic administration of methyl parathion on immune potential enzymes activitiy changes in rats. For this aim, the treatment of methyl parathion was done by orally because of the effect of chemicals represent a well characterised *in vivo* toxicity model system.

EXPERIMENTAL

The commercial parathion (O,O-diethyl-*p*-nitrophenyl-phosphorothioate), Bayer, 500 g/L) was used in present studies. This stock solution was appropriately diluted with the test water to achieve the desired concentrations of methyl parathion.

Rats (Sprague-Dawley albino) weighing 150-200 g were provided by the animal house of the Sciences Faculty of Yuzuncu Yil University and were housed in 5 groups, each group containing 6 rats. All animals were fed a group wheat-soybean-meal-based diet and water *ad libitum* in stainless cages and received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institutes of Health. The animals were housed at 20 ± 2 °C in daily light/dark cycle.

Treatment of chemical: This investigation was performed on female rats. 5 and 10 ppm dosages of methyl parathion were used. Rats were exposed methyl parathion *ad libitum* during the tests for 4 weeks. Control rats were given only distilled water. Daily water consumption of rats was *ca.* 28 ± 2 mL during the tests.

At the end of the treatments, the rats were anesthetized by inhalation of diethyl ether and were sacrificed for the tissues. The tissues were dissected and put in petri dishes. After washing the tissues with physiological saline (0.9 % NaCl), samples taken and kept at -78 °C until analysis. The tissues were homogenized for 5 min in 100 mM ice-cold phosphate solution pH 7.8) (1:10 w/v) using a glass-porcelain homogenizer (20 KHz frequency ultrasonic, Jencons Scientific Co.) and then centrifuged at 7000x g for 15 min. All processes were carried out at 4 °C. Supernatants were used to determine immune potential enzymes.

Biochemical analysis: Adenosine deaminase was assayed by the method described by Giusti[13]. Adenosine deaminase (EC 3.4.5.5) assay is based on indirectly measuring the formation of NH_3 produced when adenosine deaminase acts in excess of adenosine. The release of ammonia was determined colorimetrically at 630 nm after the development of an intense blue colour with hypochlorite and phenol in an alkaline solution. myeloperoxidase (EC 1.11.1.7) was assayed by the method described by Bradley *et al.*[14].

Analysis of data: All data were expressed as mean ± standard deviation (SD). For statistical analysis, the SPSS/PC+ package (SPSS/PC+, Chicago, IL, USA) was applied. For all parameters, means and SD were calculated according to the standard methods. Mann Whitney U-Test for differences between means of the treatments and the control rats was employed. The significance level was accepted at $p = 0.05$ for all tests.

RESULTS AND DISCUSSION

The data provided were all from one time-point of the experiment. To find out the significance of adenosine deaminase and myeloperoxidase activity changes in rats exposed to methyl parathion for 28 d, the data have been subjected to Man Whitney-U test. According to the result, the tissues adenosine deaminase and myeloperoxidase activities of rats feeding with 5 and 10 ppm presence of methyl parathion were found to be changed. The effects of methyl parathion administrations on tissue damages index were evaluated as marker enzymes in tissues samples from control and treated rats. The results showed that methyl parathion caused an increase in myeloperoxidase and adenosine deaminase, but did not change adenosine deaminase activity in the liver exposed with 5 and 10 ppm dosages (Tables 1 and 2).

The effects of pollutants on nature became a field of interest for scientists from the beginning of the second half of 20th century and subsequently investigation on effect of these pollutants on human beings, plants and animals were initiated. Methyl parathion is widely used throughout the world as a wide-spectrum insecticide for numerous agricultural crops.

TABLE-1
EFFECTS OF 5 AND 10 ppm DOSAGES OF METHYL PARATHION (MP) ON ADENOSINE DEAMINASE (ADA) ACTIVITY IN VARIOUS TISSUES OF RATS

Dose	Parameters (U/g)	Control (X ± SD)	MP (X ± SD)
5 ppm	Liver	4.43 ± 0.65	16.7 ± 3.70*
	Lung	4.7 ± 0.72	16.7 ± 3.60*
	Spleen	4.19 ± 0.50	16.34 ± 3.50*
	Hearth	4.1 ± 1.10	12.6 ± 4.20*
	Kidney	3.6 ± 0.62	15.14 ± 2.70*
	Brain	4.6 ± 0.70	16.7 ± 3.60*
10 ppm	Liver	4.7 ± 0.72	16.1 ± 2.50*
	Lung	3.94 ± 0.60	16.1 ± 2.03*
	Spleen	4.19 ± 0.50	15.9 ± 1.80*
	Hearth	4.1 ± 1.10	16.1 ± 2.60*
	Kidney	3.6 ± 0.62	15.9 ± 1.90*
	Brain	4.6 ± 0.70	16.1 ± 1.03*

Each value represents the Mean ± SD.

*Significantly different from control rats at $p \leq 0.05$ (Mann-Whitney U-test).

TABLE-2
EFFECTS OF 5 AND 10 ppm DOSAGES OF METHYL PARATHION (MP) ON MYELOPEROXIDASE (MPO) ACTIVITY IN VARIOUS TISSUES OF RATS

Dose	Parameters (U/g)	Control (X ± SD)	MP (X ± SD)
5 ppm	Liver	186.5 ± 50.6	276.6 ± 78.4
	Lung	4082.8 ± 392.7	7728.4 ± 1259.8*
	Spleen	4423.9 ± 973.6	7741.2 ± 1959.8*
	Hearth	3021.6 ± 488.4	4796.9 ± 1354.9*
	Kidney	960.6 ± 289.2	1791.9 ± 499.7*
	Brain	604.1 ± 110.2	989.8 ± 298.7*
10 ppm	Liver	186.5 ± 50.6	324.4 ± 111.2
	Lung	4082.8 ± 392.7	6676.6 ± 1158.2*
	Spleen	4423.9 ± 973.6	7170.1 ± 1288.1*
	Hearth	3021.6 ± 488.4	4299.5 ± 784.1*
	Kidney	960.6 ± 289.2	1708.1 ± 385.9*
	Brain	604.1 ± 110.2	923.8 ± 174.3

Each value represents the Mean ± SD.

*Significantly different from control rats at $p \leq 0.05$ (Mann-Whitney U-test).

Although the usage against to pest control of methyl parathion on plants known clearly, but the little knowledge is known how methyl parathion contributes to the oxidative stress on animal.

In the present study, methyl parathion caused a significant increase in the myeloperoxidase and adenosine deaminase activities in rats treated with methyl parathion in comparison to those of controls (Tables 1 and 2). Although the reasons for such effect of methyl parathion are not understood at the present, it is desirable that methyl parathion might be interacting primarily with the tissues, resulting in enzymes activities by the way of increased reactive oxygen radicals and under inflammatory conditions, neutrophil enzyme, myeloperoxidase, is activated, releasing damaged hypochlorous acid. As know, adenosine deaminase is essential for the proper functioning of the vertebrates' body immune system. Because adenosine deaminase is the major enzyme responsible for the degradation of adenosine deaminase, the change of its activity should represent one of the best ways to increase accumulation of adenosine deaminase in tissues under chemicals stress conditions. On the other hand, the presence of abnormal levels of enzymes in tissues is used in clinical practice to indicate whether or not tissue damage which organ has been affected. In phagolysosomes, this enzyme works together with other oxidases and proteases to cause the destruction of ingested organisms[15]. Low myeloperoxidase levels are the most common neutrophilic lysosomal deficiency, but usually occur without a noticeable increase susceptibility to infection or reduced immune response[15,16]. Myeloperoxidase detection also has been used as a marker of neutrophile infiltration into tissues[17,18].

The results of present study indicate that methyl parathion possesses the various effects. This is evidenced from present observation that, upon methyl parathion treatment of rats *in vivo*, the levels of immune potential marker enzymes increased and decreased. So far, no study is available on the affect of these chemicals *in vivo* state in rats. Because of this, we couldn't have the chance to compare with the previous results. In addition, due to inconsistent factors like treatment time and manner, purity and species tissue differences *etc.*, it is difficult to compare data from different laboratories regarding the ranking of test chemicals for toxically effect.

The observations presented led us to conclude that administration of subacute methyl parathion affect immune defense cells. Thus any external stressor, such as methyl parathion, even at non-lethal concentration can have a toxic effect on organism. From the foregoing observations it is postulated that immune potential maker enzyme activities might offer a certain result of choice for monitoring biotoxicity of direct acting compounds such as methyl parathion. However, individual variations, in the biochemical characters of animal, as proven in the past, are important phenomena to consider when final conclusion is made. Such a test will also be of value in pollution studies and also be of interest to understand molecular basis of methyl parathion toxicity. On the other hand, it is imposible to forbid the

utilization of this kind of chemicals, which are used against harmful insects and giving rise losing product under these conditions today. However, the necessity of using regulators should be decreased by improving resistant plants species to diseases and unfavourable conditions. This kind of plant species can be developed by aid of biotechnological and plant improving procedure.

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