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The hematological effects of methyl parathion in rats

Ismail Celik [a,*], Huseyin Suzek [b]

[a] *Department of Biology, Arts and Sciences Faculty, Yüzüncü Yil University, 65080 Van, Turkey*
[b] *Mugla University, Health High School, Department of Biochemistry, Mugla, Turkey*

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Abstract

The effects of methyl parathion (MP) at sublethal concentration on hematological constituent [red blood corpuscles (RBC), white blood corpuscles (WBC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) counts, hemoglobin (Hb) and hematocrite (HCT) levels] and serum damage marker enzymes [aspartate aminotransferase (AST), alanin aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH)] of rats were investigated under laboratory conditions. 5 and 10 ppm dosages of MP were administered orally to six female rats *ad libitum* during the tests for 4 weeks consecutively. MP treatments caused different effects on the hematological constituents and the serum marker enzymes of the treatment groups as compared to the controls. According to the results, MP treatments increased significantly the levels of serum marker enzyme activities except for ALT with both dosages and LDH with 5 ppm dosage. Also, the hematological constituents were affected by MP. For example, WBC significantly increased in rats treated with both dosages of MP whereas the other hematologic constituents did not change at 5 and 10 ppm of MP treatments. The observations presented led us to conclude that the administration of subacute MP elevates tissue damage serum marker enzymes, and increases the number of WBC in rats. These data, along with the determined changes suggest that MP produce substantial systemic organ toxicity in rats during the period of a 28-day subacute exposure.

Keywords: Rat; Hematological constituents; Serum marker enzymes; Methyl parathion

1. 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. First, pesticides are toxicants, capable of affecting all taxonomic groups of biota, including non-target organism, which to varying degrees depend 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].

Methyl parathion (MP) 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 [4]. In literature, it is reported that OPIs are neurotoxic in nature by acting as inhibitors of neuronal cholinesterase (ChE) activity [5] and serum cholinesterase (SChE) [6]. Some studies have reported that OPIs cause lipid peroxidation [7–9] in vertebrates. Mutagenic effects of MP have been studied to determine the chemical's ability to cause a change in the DNA sequence of a gene. It was determined that MP, when administered to Wistar rats for a 6-week period of five treatment days per week at doses of 1/100, 1/75 and 1/50 of the LD50, displayed no significance effect in mutagenicity [10]. The cytogenetic and

* Corresponding author. Tel.: +90 432 2251707 2278; fax: +90 432 2251188.
E-mail addresses: icelik65@yahoo.com, icelik@yyu.edu.tr (I. Celik).

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cytotoxic effects of OPIs and organochlorine compounds following a single dose administration were studied. It was found that the frequency of chromosomal aberrations and micronuclei in bone marrow cells and an assay of the liver expressed the genotoxic capabilities of these chemicals [11]. MP was reported to cause DNA damage in rats [12]. Further, it was found that MP, as a result a single-exposure, was the most hazardous tested organophosphate showing definite pathology in the livers of treated rats. It was also concluded from genotoxicity studies of organophosphorus pesticides that methyl parathion had some genotoxic effects [13]. MP did not show acute testicular toxicity; instead it caused subacute and subchronic testicular toxicity [14]. A significant increase in the percentage of abnormal sperm was observed in mice treated orally with MP [15]. MP caused a significant decrease in sperm count of rats [16].

MP is one of the most widely used OPIs in agriculture and public health programmes. MP is also one of the most used OPIs in the region of Van, Turkey. The aim of this study was to investigate the effects of subacute exposure of MP at sublethal dosages on serum marker enzyme activities and the change of hematological constituents after methyl parathion administration to mature female rats. To this end, the treatment of MP was done orally because of the effect of chemicals, which represent a well characterized *in vivo* toxicity model system.

2. Materials and methods

2.1. Materials

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

2.2. Animals

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 three groups, each group containing six 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 $20 \pm 2\,^{\circ}\mathrm{C}$ daily in light/dark cycle.

2.3. Treatment of chemical

This investigation was performed on female rats. 5 and 10 ppm dosages of MP were used. Rats were exposed MP *ad libitum* during the tests for 4 weeks. Control rats were given only distillated water. Daily water consumption of rats was approximately 28 ± 2 ml during the tests.

At the end of the treatment, rats were anesthetized by inhalation of diethyl ether, and they were then sacrificed. Blood samples were obtained from the cardiac puncture by using syringe for the determination of hematological and biochemical constituent. Blood samples were drawn immediately into ice-chilled siliconized disposable glass tubes. The serum samples were obtained by centrifuging blood samples at 3000 rpm for 15 min at $4\,^{\circ}\mathrm{C}$, and serum marker enzyme activities were measured in these serum samples. For hematological constituents, the blood samples were drawn using EDTA as an anticoagulant.

2.4. Measurement of enzyme levels

Serum enzyme activities [aspartate aminotransferase (AST) (EC 2.6.1.1), alanin aminotransferase (ALT) (EC 2.6.1.2), lactate dehydrogenase (LDH) (EC 1.1.1.27) and alkaline phosphatase (ALP) (EC 3.1.3.1)] were measured by an auto analyzer (BM/HITACHI-911), using the kits (DPC; Diagnostic Products Corporation, USA).

2.5. Hematological study

Freshly collected blood samples were analyzed for hematological assay using an automatic hematological assay analyzer (Swelab 24D). Different tested hematological parameters were as follows: red blood corpuscles (RBC), white blood corpuscles (WBC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) counts, hemoglobin (Hb) and hematocrite (HCT) levels.

2.6. Analysis of data

All data were expressed as mean ± standard deviation (S.D.). The statistical analyses were made using the minitab 13 for windows packet program. Means and standard deviations were calculated according to the standard methods for all parameters. One way ANOVA statistical test was used to determine the differences between means of the treatments and the control group accepting the significance level at $p \leq 0.05$.

3. Results

Following the exposure to 5 and 10 ppm dosages of MP, the body weight of rats were found to have been slightly reduced. The effects of MP administration on tissue damages index were evaluated as marker enzymes in serum samples from control and treated rats. The results showed that MP caused an increase in AST and ALP activities exposed with both dosages and also LDH with 10 ppm exposure (Table 1). With regard to the haematotoxicity of MP subacute exposure, no alteration in the hematological values was observed in rats treated with both dosages of MP except for WBC number (Table 2).

4. Discussion

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 the effect of these pollutants on human beings, plants and animals were initiated. MP

Table 1
Effect of MP on serum marker enzyme activities and body weight of rats (mean ± S.D.)

Parameters	Control, mean ± S.D.	5 ppm (∼0.019 mM) MP, mean ± S.D.	10 ppm (∼0.038 mM) MP, mean ± S.D.
Body weight (g)	Beginning: 162 ± 13; finally: 171 ± 11	Beginning: 174 ± 16; finally: 165 ± 12	Beginning: 185 ± 20; finally: 179 ± 19
AST (U/L)	137.3 ± 11.8	182.1 ± 32.9*	235.2 ± 25.8**
ALT (U/L)	29.5 ± 7.5	32.8 ± 5.8	39.1 ± 10.1
LDH (U/L)	2308.1 ± 123.2	2853.7 ± 714.1	3297.3 ± 505.9**
ALP (U/L)	97.5 ± 23.1	138.3 ± 19.3***	141.3 ± 24.5*

Each value represents the mean ± S.D. Significantly different from control rats at (*) 0.01, (**) 0.001 and (***) 0.008 (one way ANOVA).

is widely used throughout the world as a wide-spectrum insecticide for numerous harmful agricultural crops. Also, it has widely and effectively been used throughout the world with applications in agriculture and horticulture for controlling insects in crops, cotton, corn, cabbage, potatoes, wheat and soybean. Although the usage against to pest control of MP on plants is well-known, little is known as regards to which extend the hematotoxic and hepatic effect the animal.

In this study, experimental group was exposed to two dosage of chemical substance. Liver enzymes and hematologic constituents were used as important biomarkers for detection of hepatotoxic and hematotoxic nature of this pesticide. Four serum hepatic marker enzymes (ALT, AST, LDH and ALP) were evaluated for hepatotoxsisiz. According to the results, MP caused a significant increase in the AST, LDH and ALP activities, and slightly increased the levels of ALT activity in rats treated with MP in comparison to those of controls. The reasons for such effect of MP are not understood at present certainly. But, our findings indicate that the liver damage may occur in the rats exposed to sublethal dosages of toxic substance for subacute period. As known, several of soluble enzymes of blood serum have been considered as indicators of the hepatic dysfunction and damage. Transaminases (AST and ALT) are important and critical enzymes in the biological processes. The increase in the activities of AST and ALT in serum of rats treated with MP is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream [17]. The activity of AST increases significantly in such cases and escapes into the plasma from the injured hepatic cells. In addition, ALT level is of value, which also indicates the existence of liver diseases, as this enzyme is present in large quantities in the liver. It increases in serum when cellular degeneration or destruction occurs in this organ [18]. These results are also supported by some other studies conducted on the same classes of chemicals, although the treatment and the setting of studies are different. For example, an increase in ALP confirms the presence of hepatic damage [19]. A significant rise in AST occurs after chronic exposure to pesticides [20]. It was found that the administration of fenthion (an organophosphate) results in *in vitro* and *in vivo* increase in serum enzymes including ALT, AST and ALP [21]. Also, the increase in the activity of these enzymes in the serum may result only consequent to impairment of the function of tissues with subsequent liberation of the enzymes into the circulation from the damaged tissue. Further, a statistically significant increase in levels of ALT, AST and ALP had been detected in exposed workers OPIs with respect to the control group [22]. On the other hand, phosphatases and dehydrogenases are important and are critical enzymes in biological processes. They are responsible for detoxification, metabolism and biosynthesis of energetic macromolecules for different essential functions. Any interference in these enzymes leads to biochemical impairment and lesions in the tissue and cellular function [23]. Yamaguchi et al. [24] and Yousef et al. [25] reported that the changes in the activities of these enzymes in $SnCl_2$-treated rats were regarded as the biochemical manifestation of the toxic action of inorganic tin. Also, Rahman et al. [26] suggested that the increase in the activities of ALP and ACP in plasma might be due to the increased permeability of plasma membrane or cellular necrosis, and this showed the stress condition of the treated animals. Also, the increase in plasma LDH activity may be due to the hepatocellular necrosis leading to leakage of the enzyme into the blood stream [27].

In addition to the increased level of serum marker enzymes, the results of the present study have also demonstrated that the

Table 2
Effect of MP on hematological constituents of rats (mean ± S.D.)

Hematological constituent	Control, mean ± S.D.	5 ppm (∼0.019 mM) MP, mean ± S.D.	10 ppm (∼0.038 mM) MP, mean ± S.D.
RBC ($10^6/mm^3$)	8.7 ± 0.4	8.6 ± 0.3	8.4 ± 0.2
WBC ($10^3/mm^3$)	5.3 ± 0.4	8.4 ± 1.1*	11.5 ± 1.8*
HGB (g/dl)	14.6 ± 1	15.3 ± 0.5	14.7 ± 0.3
HCT (%)	42.4 ± 2.8	44.2 ± 0.9	41.5 ± 1.4
MCV (μm^3)	50.2 ± 1.5	51.8 ± 0.8	49.9 ± 1.4
MCH (pg)	17.9 ± 0.5	18.1 ± 0.1	17.6 ± 0.5
MCHC (%)	34.5 ± 0.3	34.6 ± 0.4	35.1 ± 0.4
PLT ($10^3/mm^3$)	553.2 ± 71	567.0 ± 65.1	516.7 ± 49.7

Each value represents the mean ± S.D. Significantly different from control rats at (*) 0.001 (one way ANOVA).

applied dosages of MP could have affected the immune defense systems in rats. This is evidenced from our observation that, upon MP treatment *in vivo*, the numbers of WBC differ from those of controls. We observed that while MP caused a significant increase in the level of WBC, the levels of the other hematological constituents did not change. From the observed values of WBC, it is clear that an increase in the number of WBC is a normal reaction of rats to substances, which alter their normal physiological processes. The leucocytosis observed in present study indicates an immune system to protect the rats against infection that might have been caused by chemical and also secondary infections, which may be contracted after the weakening condition of the rats. Leucocytosis, which may be directly proportional to the severity of the causative stress condition may be attributed to an increase in leukocyte mobilization [28]. However, in contrast, Bhalli et al. [22] reported that individuals exposed to OPIs exhibited cytogenetic damage with increased frequencies of binucleated cells with micronuclei and total number of micronuclei in binucleated lymphocytes and have shown an increase in hematologic constituent such as RBC, MCV, MCH, MCHC, Hb and HCT levels. Thus, although the treatment, chemicals and the setting of studies are different, these results are in contradiction with our results.

Our observations led us to conclude that the administration of subacute MP promotes immune defense cells and elevates tissue damage serum marker enzymes and depletes body weight in the rats. Thus, any external stressor, such as MP, even at nonlethal concentration can have a toxic effect on the organism. From the foregoing observations it may also be postulated that hematological and biochemical constituents might offer a certain result of choice for monitoring biotoxicity of direct acting compounds such as MP. However, individual variations, in the biochemical characters of animal, as proven in the past, are very 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 refractoriness of MP toxicity. On the other hand, it is impossible to forbid the utilization this kind of chemicals, which are used against harmful insects and give rise to lose the product under these conditions today. However, the necessity of using regulators should be decreased by improving resistant plants species to diseases and unfavorable conditions. This kind of plant species can be developed by the aid of biotechnological and plant improving procedure.

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