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Methyl parathion induced nephrotoxicity in male rats and protective role of vitamins C and E

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Abstract

Methyl parathion is an organophosphate insecticide that has been used in agriculture and domestic for several years. Vitamin C (200 mg/kg bw per day) + vitamin E (200 mg/kg bw per day), methyl parathion (0.28 mg/kg bw per day) and vitamin C (200 mg/kg bw per day) + vitamin E (200 mg/kg bw per day) + methyl parathion (0.28 mg/kg bw per day) combination were given to rats orally via gavage for 7 weeks. Body and kidney weights, malondialdehyde (MDA) levels and histopathological changes were investigated at the end of 4th and 7th weeks comparatively with control group. When methyl parathion-treated group and vitamins C and E + methyl parathion-treated group were compared to control group body and kidney weights decreased significantly at the end of 4th and 7th weeks. MDA levels increased in kidney tissues of the methyl parathion- and vitamins C and E + methyl parathion-treated groups compared to control group. MDA levels decreased significantly in vitamins C and E + methyl parathion treated group compared with methyl parathion treated group at the end of 4th and 7th weeks. In our light microscopic investigations, after 4 weeks of methyl parathion exposure, glomerular atrophy and vascular dilatation, and after 7 weeks, necrosis and edema were observed in the kidney tissues. After 4 weeks of vitamins C and E + methyl parathion exposure, mononuclear cell infiltrations, and after 7 weeks, calcification were detected in the kidney tissues.

Keywords: Organophosphate insecticide; Methyl parathion; Vitamin E; Vitamin C; Nephrotoxicity; Malondialdehyde; Histopathology

1. Introduction

Organophosphorus compounds are widely used in agriculture as insecticides and acaricides and also in medicine and industry. Residual amounts of organophosphate (OP) pesticides have been detected in the soil, water bodies, vegetables, grains and other foods products [1–3]. Due to the wide availability of organophosphorus compounds, poisonings are common [4]. OP pesticides are known to cause inhibition of acetylcholinesterase and pseudocholinesterase activity in the target tissues [2,5]. Other systems that could be affected by OP intoxicant are immune system [6,7], urinary system [8], pancreas [9], liver [10] and biochemical changes [11]. Some of the OP pesticides also have been reported to affect the reproductive system. It is embryotoxic and causes fetal anomalies in experimental animals [12].

Methyl parathion ($C_8H_{10}NO_5PS$), (*O*, *O*-dimethyl *O*-4-nitrophenyl phosphorothioate) is an OP insecticide with a broad range of activity which inhibits acetylcholinesterase activity [13]. It has been widely and effectively used throughout the world with applications in agriculture and horticulture for controlling insects and in crops, cotton, corn, cabbage, potatoes, wheat and soybean [14]. It appears as a white crystalline substance (pure) or as a technical grade liquid chemical with a light to dark tan color, and

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contains 80% active ingredient, 16.7% xylene and 3.3% inert ingredients [15]. Methyl parathion exposure has been linked to substantial adverse health effects on several organ system, the reproductive system and the nervous system [15]. Methyl parathion has been reported to cause DNA damage in rats [16].

Some studies have shown that both vitamin C and vitamin E are used in pesticide toxicity in the experimental studies [17,18]. Many insecticides are hydrophobic molecules, which bind extensively to biological membranes, especially to the phospholipids bilayers [19]. Vitamin E (α-tocopherol) is a family of lipid-soluble vitamins and acts as an antioxidant in cells, interrupting the propagation of lipid peroxidation in the plasma membrane and thus preserving membrane integrity [20,21]. Studies carried out with antioxidant such as α-tocopherol, have shown that they inhibit free radical formation [22,23] and may effectively minimize lipid peroxidation in biological systems [24,25]. Vitamin C is hydrophilic and a most important free radical scavenger in extracellular fluids, trapping radicals in the aqueous phase, and protecting biomembranes from peroxidative damage [18,26].

The aim of this study was to measure MDA levels and investigate the pathological changes of the kidney at the end of 4th week (subacute exposure) and 7th week (subchronic exposure) after methyl parathion administration to male rats and protective effect of vitamin C and vitamin E.

2. Material and methods

2.1. Animals

Forty-eight male Wistar-rats (90 days old) weighing between 320 and 340 g were obtained from the Refik Saydam Central Hygiene Institute, Ankara, Turkey were used. The animals were housed in plastic cages, fed a standard laboratory diet and water *ad libitum*. Rats were exposed to a 12 h light/dark cycle, at a room temperature of 18–22 °C. Animals were quarantined for 10 days before beginning of the experiments.

2.2. Chemicals

Methyl parathion, purity 98%, was obtained from Agricultural Struggle Center, Ankara, Turkey. Vitamin E (DL-α-tocopherol acetate) was supplied by Merck (Germany). Vitamin C (L-ascorbic acid) was supplied by Carlo Erba (Milano, Italy).

2.3. Animal treatment schedule

Rats were divided into two groups, control ($n = 12$) and experiment groups ($n = 36$). Rats in experiment group were divided into three groups, vitamin C + vitamin E-treatment group (vitamins-treatment group) ($n = 12$), methyl parathion-treatment group ($n = 12$) and vitamins + methyl parathion-treatment group ($n = 12$). At the end of 4th and 7th weeks six rats were dissected from each group, tissue samples were taken for measurement of MDA levels and light microscope investigations.

The substances were administered in the morning (between 09:00 and 10:00 h) to non-fasted rats. First exposure day to methyl parathion was accepted experimental day 0.

2.3.1. Control group

Corn oil at a dose of 1 ml/kg body weight (bw) per day was given orally through gavage to rats once a day.

2.3.2. Vitamin C + vitamin E-treated group (vitamins-treated group)

Vitamin C (200 mg/kg bw per day) was administered orally through gavage to rats once a day. After vitamin E (200 mg/kg bw per day) was administered orally through gavage to rats once a day. Vitamin C and vitamin E were dissolved in water (1 ml/kg bw) and in corn oil (1 ml/kg bw), respectively.

2.3.3. Methyl parathion-treated group

Methyl parathion at a dose of 0.28 mg/kg bw (1/50 LD_{50} dose orally) per day in corn oil was given orally through gavage to rats once a day.

2.3.4. Vitamins + methyl parathion-treated group

Vitamin C (200 mg/kg bw per day, once a day in water) and vitamin E (200 mg/kg bw per day, once a day in corn oil) were administered orally through gavage 30 min before orally administration of methyl parathion (0.28 mg/kg bw per day, once a day in corn oil) through gavage.

2.4. Measurement of body and organ weights

Body and kidney weights of control and treated rats were measured at the end of the 4th and 7th weeks by automatic balance (AND GX-600, Japan). Kidneys were removed from adipose tissue and surrenal glands before weighing. Rats were anesthetized with diethyl ether after measurement of body and kidney weights were removed, and their weights were measured.

2.5. Measurement of malondialdehyde (MDA)

MDA occurs in lipid peroxidation and was measured in kidney tissues after incubation at 95 °C with thiobarbituric acid in aerobic conditions (pH 3.4). The pink colour produced by these reactions was measured spectrophotometrically at 532 nm to measure MDA levels [27]. Specific activity was defined as nmol per mg protein.

2.6. Histopathology

For histopathological examination, the kidney tissues were dissected and the tissue samples were fixed in Bouin's solution for 14–18 h, processed in a series of graded ethanol

and embedded in paraffin. Paraffin sections were cut with at 5 μm thickness and stained with hematoxylin and eosin for light microscopy examination. The sections were viewed and photographed on a Olympus light microscope (Olympus BX51, Tokyo, Japan) with attachment photograph machine (Olympus C-5050, Olympus Optical Co. Ltd., Japan). Ten slides were prepared from each kidney. All sections were evaluated for the degree of tubular and glomerular injury, inflammatory cell infiltration, necrosis, edema and calcification. Each kidney slides were examined and assigned for severity of changes using scores on a scale of none (−), mild (+), moderate (++) and severe (+++) damage.

2.7. Statistical analysis

Data were analyzed using SPSS 11.0 for windows. The significance was calculated using one-way analysis of variance (ANOVA) and followed by Tukey multiple comparison procedure to calculate the significance. $P < 0.05$ value was taken as statistically significant.

3. Results

3.1. Evaluation of body and organ weights

Death was not observed in any of the experimental groups during experimental period. But especially food intake of methyl parathion- and vitamins + methyl parathion-treated groups reduced during experiment. Body weight, absolute kidney weight and relative kidney weight did not show any significant changes between vitamins-treated group and the control group during experimental period.

In the methyl parathion- and vitamins + methyl parathion-treated groups, 4th and 7th weeks after, body weight, absolute kidney weight and relative kidney weight significantly decreased compared to control group ($P < 0.05$). When vitamins + methyl parathion-treated group was compared to methyl parathion-treated group no statistically significant changes were observed in the body weight, absolute kidney weight and relative kidney weight at the end of the 4th and 7th weeks (Table 1).

3.2. Malondialdehyde (MDA) levels

Control group was compared with all groups at the end of 4th and 7th weeks after vitamins C and E, methyl parathion and vitamins + methyl parathion were given to rats. In addition to this, methyl parathion treated group was compared to vitamins + methyl parathion treated group. Changes in MDA levels were shown in Fig. 1.

No statistically significant changes were observed when control group was compared with vitamins-treated group at the end of 4th and 7th weeks.

A significant increase was observed in MDA levels at the end of 4th and 7th weeks in methyl parathion- and vitamins + methyl parathion-treated groups were compared with control group ($P < 0.05$), (Fig. 1). MDA levels decreased significantly in vitamins + methyl parathion-treated group compared with methyl parathion-treated group at the end of the 4th and 7th weeks ($P < 0.05$), (Fig. 1).

3.3. Histological changes in the kidney

The kidney in the control animals showed normal glomeruli, proximal and distal tubules (Fig. 2A). After 4 weeks of methyl parathion exposure, vascular dilatation was observed in kidney tissues. Most of glomeruli showed dilatation of Bowman's space with glomerular atrophy (Fig. 2B). 4 weeks after vitamins + methyl parathion was given to rats, interstitial inflammatory cell infiltrations were detected and some of the renal tubules were dilated (Fig. 2C). 7 weeks after methyl parathion-treatments to rats, edema, necrosis and mononuclear cell infiltrations were observed in kidney tissues (Fig. 2D). In addition to this, tubular dilatation and glomerular atrophy were detected in kidney tissues. 7 weeks after vitamins + methyl parathion-treatments to rats, calcification, tubular degeneration

Table 1
Body weight, kidney weight and relative kidney weight of control and experimental rats

Groups	Body weight			Absolute kidney weight (g)	Relative kidney weight (g/100 g body weight)
	Initial (g)	Final (g)	% Change		
4th week					
Control	326.78 ± 1.72	347.57 ± 4.59	6.36 ± 0.85	2.19 ± 0.02	0.63 ± 0.004
Vitamins C and E	328.18 ± 2.17	352.82 ± 5.00	7.50 ± 0.86	2.22 ± 0.03	0.62 ± 0.004
Methyl parathion	324.13 ± 2.71	304.78 ± 4.09[a,b]	−5.97 ± 0.49[a,b]	1.83 ± 0.03[a,b]	0.59 ± 0.004[a,b]
Vitamins C and E + methyl parathion	326.43 ± 3.11	310.80 ± 1.62[a,b]	−5.26 ± 0.47[a,b]	1.87 ± 0.03[a,b]	0.60 ± 0.008[a,b]
7th week					
Control	327.30 ± 3.28	363.03 ± 3.91	10.91 ± 0.14	2.28 ± 0.02	0.62 ± 0.004
Vitamins C and E	330.15 ± 1.97	366.70 ± 4.76	11.06 ± 0.80	2.29 ± 0.02	0.62 ± 0.005
Methyl parathion	326.13 ± 2.47	280.77 ± 3.44[a,b]	−13.90 ± 0.45[a,b]	1.69 ± 0.02[a,b]	0.60 ± 0.004[a,b]
Vitamins C and E + methyl parathion	329.57 ± 1.68	286.92 ± 3.71[a,b]	−13.04 ± 0.88[a,b]	1.72 ± 0.02[a,b]	0.59 ± 0.004[a,b]

Values are means ± SD for six rats in each groups. Significance at $P < 0.05$.
[a] Comparison of control and other groups.
[b] Comparison of vitamins-treated group with methyl parathion- and vitamins + methyl parathion-treated groups.

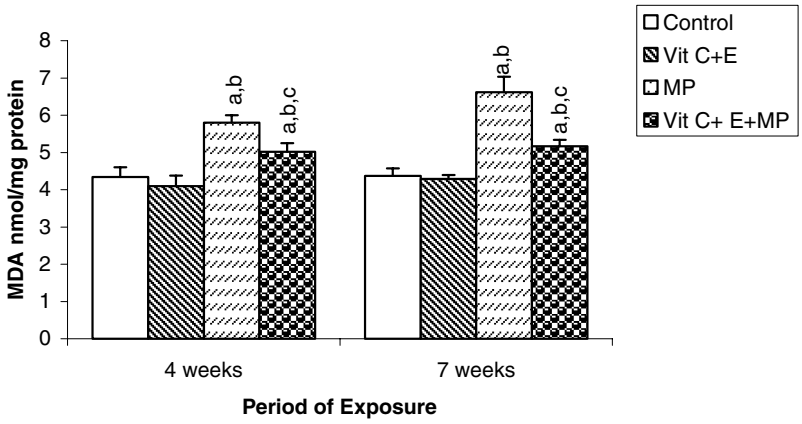

Fig. 1. Effects of vitamins, methyl parathion and vitamins + methyl parathion-treatments on malondialdehyde (MDA) levels in rat kidney tissue. [a]Comparison of control and other groups ($P < 0.05$). [b]Comparison of vitamins-treated group with methyl parathion- and vitamins + methyl parathion-treated groups ($P < 0.05$). [c]Comparison of methyl parathion-treated group with vitamins + methyl parathion-treated group ($P < 0.05$). Values are means ± SD of six animals in each group.

and a weak mononuclear cell infiltrations were occurred (Fig. 2E). No necrosis was detected in the kidney tissue. The histopathological changes were graded and summarized in Table 2.

4. Discussion

Acute and chronic toxicity studies indicate that methyl parathion is very highly toxic to mammals. Mammals are expected to be adversely affected by methyl parathion through oral, dermal and inhalation exposure pathways [4]. Methyl parathion not only has toxic effects on the mammals it also has toxic effects on fishes, birds and non-target invertebrates [28–30]. Oral LD_{50} of methyl parathion is 14 and 24 mg/kg for male and female rats, respectively [31]. Institoris et al. [32] gave 0.218 mg/kg and 0.872 mg/kg methyl parathion to rats orally and observed neurotoxicologic and immunotoxicologic changes. In the present study, even though methyl parathion was given at 1/50 LD_{50} dose orally, we observed pathological findings in rat kidneys. But no death was observed during experiments.

OP insecticides cause reduction of body and organ weights in experimental animals [33–35]. In the present study, decreases of body, absolute kidney and relative kidney weights were observed 4th and 7th weeks after methyl parathion and vitamins + methyl parathion treatment. We thought that this decrease in rats occurred according to food intake. Because, when methyl parathion- and vitamins + methyl parathion-treated groups rats were compared to control group rats, it was observed that food consumption was lesser than control group. According to present study, it was detected that methyl parathion reduced appetite of rats. We can say that vitamin C and vitamin E did not show protective effect on body, absolute kidney and relative kidney weights.

MDA, the end product of lipid peroxidation, has also been measured to indicate the presence of free radicals and lipid peroxidation induced nephrotoxicity. Organophosphorus compounds cause increase of lipid peroxidation level [22,26]. Lipid peroxidizing effect of anilofos following acute exposure has been reported in rats [36]. Diazinon caused increase of lipid peroxidation level of rat erythrocytes and pancreas [9,37]. In the present study, methyl parathion caused increase of MDA level, the end product of lipid peroxidation. The increase in the MDA level was observed at the end of the 4th and 7th weeks. The lipid peroxidation is an autocatalytic process which is caused by free radicals. The increase of MDA level in this study is an indicator of free radical formation caused by methyl parathion in kidney tissue of rats.

Pesticides cause various histopathological and cytopathological changes in the male urinary system [26,38,39]. Methidathion, an OP insecticide, caused histopathological changes in the rat kidney [26]. Mohssen [39] reported that thimet, an OP insecticide, caused pathological changes in rat kidney. In the present study, methyl parathion caused pathological changes in the kidney after 4 and 7 weeks exposure. Especially 7 weeks after, it caused necrosis and edema in the rat kidney tissue.

Some studies have reported that a combination of vitamin C and vitamin E reduce lipid peroxidation caused by toxic substances [17,18,40]. In many studies, vitamin E neutralizes lipid peroxidation and unsatured membrane lipids because of its oxygen scavenging effect [23,24]. While vitamin E was showing protective effect on some biochemical indices and fine structure of cells in different periods of the organochlorine insecticide endosulfan and organophosphate insecticide diazinon toxicities, it did not show protective effect on some parameters [10,22,41]. Vitamin E is a potential antioxidant, and a lipid soluble vitamin present in biological membranes. Vitamin C would serve to scavenge free radicals within the extracellular space whereas lipid soluble vitamin E would effectively scavenge free radicals within the cells where reactive metabolites are produced. In addition, vitamin C may remove free radicals that are bound to vitamin E, thus

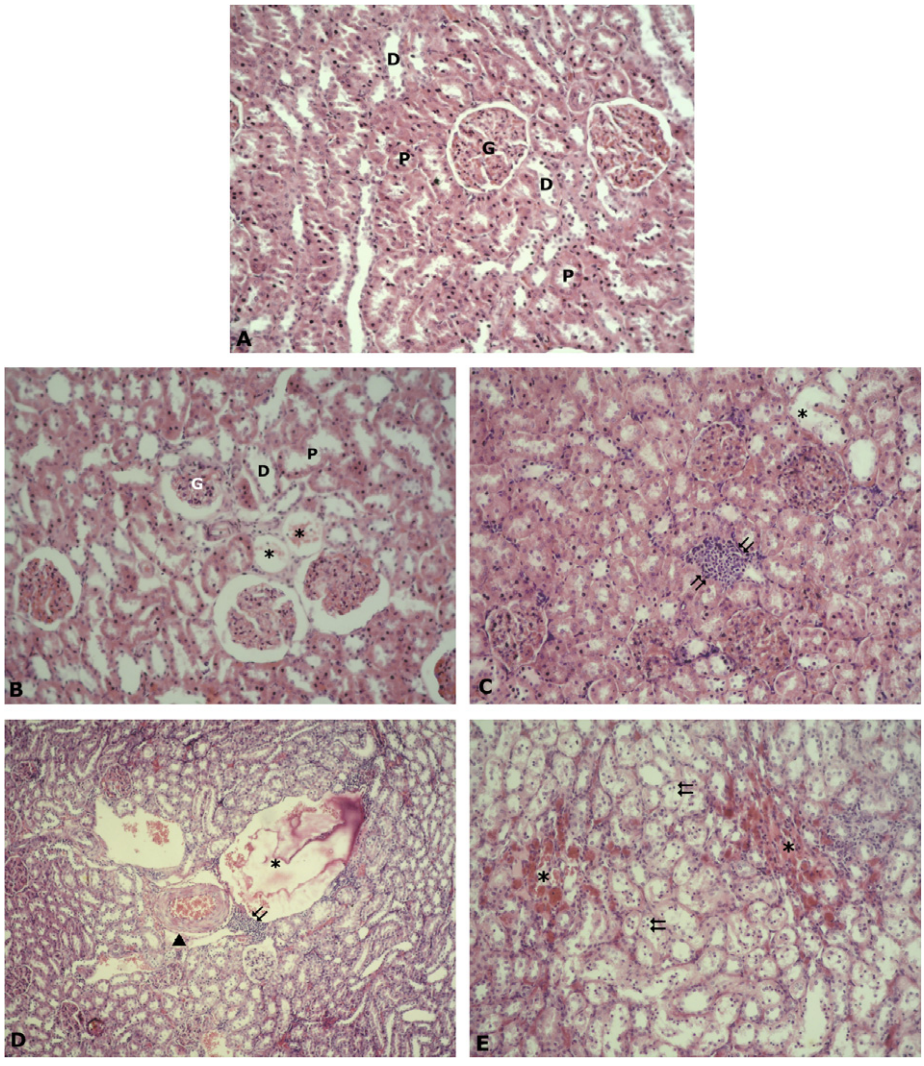

Fig. 2. (A) Kidney section of control rats, ×200. (B) Glomerular atrophy (G) and vascular dilatation (✱) in kidney tissue 4 weeks after methyl parathion treatment to rats, ×200. (C) Interstitial cell infiltration (arrows) in kidney 4 weeks after vitamins + methyl parathion treatment to rats, ×160. (D) Necrosis (✱), edema (▲) and interstitial cell infiltration (arrows) in kidney 7 weeks after methyl parathion treatment to rats, ×140. (E) Calcification (✱) and tubular degeneration (arrows) in kidney 7 weeks after vitamins + methyl parathion treatment to rats, ×160. P, proximal tubules; D, distal tubules; G, Glomerulus.

Table 2
Grading of the histopathological changes in the kidney sections

Group	Tubular degeneration		Infiltration		Glomerular atrophy		Calcification		Edema		Necrosis	
	4 w	7 w	4 w	7 w	4 w	7 w	4 w	7 w	4 w	7 w	4 w	7 w
Control	–	–	–	–	–	–	–	–	–	–	–	–
Vitamins C and E	–	–	–	–	–	–	–	–	–	–	–	–
Methyl parathion	++	+++	++	+++	+++	+++	–	++	+	++	+	+++
Vitamins C and E +methyl parathion	+	++	++	+++	+	++	–	++	+	+	+	+

Scoring was done as follows: none (–), mild (+), moderate (++) and severe (+++), w: weeks.

serving to regenerate vitamin E [42]. In the present study, when vitamins + methyl parathion-treated group was compared with methyl parathion-treated group, MDA decreased at the end of 4th and 7th weeks. Light microscopic findings support this.

As a result, in the present study methyl parathion caused subacute and subchronic nephrotoxicity. Vitamin C and vitamin E decreased subacute and subchronic nephrotoxicity.

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