Protective Effect of Vitamins C and E on Malathion-Induced Nephrotoxicity in Male Rats

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ABSTRACT

The male Wistar rats were given malathion and/or vitamin C+vitamin E daily *via* gavage for 4 weeks. When malathion-, vitamins C+E plus malathion-treated groups were compared to control, body and kidney weights decreased; serum urea, uric acid and creatinine levels increased significantly. Vitamins C+E plus malathion-treated group was compared to malathion-treated group there were statistically decrease in the serum urea level, while no significant changes were observed in other parameters. Light microscopic investigations revealed that both the malathion- and vitamins C+E plus malathion-treated groups exhibited histopathological changes in kidney. It appears that vitamins C and E didn't ameliorate malathion-induce nephrotoxicity.

1. INTRODUCTION

Organophosphate (OP) pesticides are among the most widely used synthetic chemicals for controlling a wide variety of pests. The main target of OP pesticides is acethylcholinesterase (AChE) which hydrolyses acetylcholine in cholinergic synapses and neuromuscular junctions [1]. Exposure these pesticides may cause alterations many systems [2, 3, 4].

Malathion [O,O-dimethyl-S-(1,2-dicarbethoxyethyl) phosphorodithioate] is one of the most widely used organophosphate pesticides for agriculture crops against several pests and public health programs, this insecticide is also considered as a low toxic for domestic use [5]. Toxic effects of malathion have been studied extensively in experimental animals and in exposed workers [6]. Malathion is known to inhibit acetylcholinesterase activity in target tissues [6, 7, 8] and has been linked to the dysfunction of several organ systems, including the liver [9], testis [10], pancreas [11], brain [12] and erythrocytes [13]. In recent years, studies have indicated that OP compounds, espacially malathion, are able to induce oxidative stress by changing the status of oxidant-antioxidant balance of body [14]. Some authors can demonstrate that malathion exposure causes toxic effects in animals and humans [2, 15].

The lipophilicity of organophosphate insecticides favors their incorporation in membranes. Therefore, insecticides may result from physicochemical changes at the level of membrane lipid structure and organization [16, 17]. Since vitamins C and E are known to be antioxidants, many studies have been performed to determine whether they can ameliorate the toxic effects of pesticides [18, 19, 20]. In the previous studies authors reported that, treatment with antioxidants can decrease oxidative stress and lipid peroxidation releated to OP-induced toxicity [4]. Vitamin E is the most important lipophilic antioxidant and exists mainly in the cell membranes, thus helping to maintain membrane stability [21]. Previous studies have shown that α-tocopherole inhibits free radical formation [22] and may effectively limit lipid peroxidation in biological systems [23]. Vitamin C is thought to be an important water soluble antioxidant which is reported to

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neutralize reactive oxygen species (ROS) and reduce the oxidative stress [1, 24].

The aim of this study was to determine the effect of subacute malathion exposure on kidney tissues of male rats and assess whether these effects can be ameliorated by co-treatment with vitamins C and E. To achieve this aim, rats were given malathion and/or vitamins C and E by gavage for 4 weeks.

2. MATERIAL AND METHODS

2.1. Animals

Sexually mature male Wistar rats (weighing approximately 300-320 g) obtained from the Gazi University Laboratory Animals Growing and Experimental Research Center (*GUDAM*) were used. The animals were housed in plastic cages, fed a standard laboratory diet and water *ad libitum*, exposed to a 12 h light/dark cycle, and maintained at a laboratory temperature of $20 \pm 2^{o}C$. The animals were quarantined for 10 days before beginning the experiments. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

2.2. Chemicals

Malathion was obtained from the 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

The rats were divided into two groups, the control (n=6) and experiment groups (n=18). The rats in the experiment group were divided into three groups, namely, the vitamins C and E-treated group (vitamin-treated group) (n=6), the malathion-treated group (n=6), and the vitamin plus malathion-treated group (n=6). The substances were administered in the morning (between 09:00-10:00 h) to non-fasted rats. The first day the animals were treated was considered as experimental day 0. At the end of the 4th week (28 days) of treatment, the six rats in each group were sacrificed and dissected. Blood and tissue samples were taken for the biochemical and light microscope investigations.

2.3.1. Control Group

Corn oil at a dose of 0,2 ml per animal was administered *via* gavage, once a day.

2.3.2. Vitamins C and E-treated group (Vitamin-treated group)

Vitamin C and vitamin E were dissolved in water and corn oil, respectively. Once a day, the rats were treated, with first vitamin C (200 mg/kg bw per day) and then vitamin E (200 mg/kg bw per day), *via* gavage.

2.3.3. Malathion-treated group

Once a day, the rats were given malathion at a dose of 27 mg/kg bw (1/50 of the LD_{50} for an oral dose) in corn oil *via* gavage.

2.3.4. Vitamin plus malathion-treated group

Once a day, the rats were treated vitamin C dissolved in water (200 mg/kg bw per day) and vitamin E dissolved in corn oil (200 mg/kg bw per day), and thirty minutes later, malathion dissolved in corn oil (27 mg/kg bw per day) was administered *via* gavage.

2.4. Measurement of Body/organ Weights and Food Consumption

Body and kidney weights of control and treated rats were measured at the end of the 4th week by automatic balance (AND GX-600, Japan). Kidneys were removed from adipose tissue before weighing. Rats were anesthetized before measurement of body and kidney weights were removed. Food intakes/consumptions were recorded daily through the experiment by automatic balance (AND GX-600, Japan).

2.5. Biochemical Evaluation

At the end of the 4th week, blood samples of the rats were taken from the heart and collected into sterile tubes. Blood samples were centrifuged at 3500 rpm for 20 min, and serum was separated.

Urea, uric acid and creatinine were assessed in serum using a commercially available spectrophotometric-enzymatic kit (Thermo Trace-BECGMAN, Germany) and analyzed by an autoanalyzer (Bayer ope-RA).

2.6. Histopathology

For histopathological examination, the kidney tissues were dissected and the tissue samples were fixed in Zenker solution for 24 h, processed by using a graded ethanol series, and embedded in paraffin. The paraffin sections were cut into 5 μm-thick slices and stained with hematoxylin and eosin for light microscopic examination. The sections were viewed and photographed by using an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached photograph machine (Olympus E-330, Olympus Optical Co. Ltd., Japan). Ten slides were prepared from each kidney.

2.7. Statistical Analysis

The data were analyzed by using SPSS 11.0 for Windows. The significance of differences was calculated by using one-way analysis of variance (ANOVA) followed by Tukey's procedure for multiple comparisons. $P<0.05$ was considered statistically significant.

3. RESULTS

3.1. Evaluation of Body/organ Weights and Food Consumption

Death was not observed in any of the experimental groups during experimental period. 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 (Table 1).

By the end of the 4th week, in the malathion- and vitamin plus malathion-treated groups compared to control group, body weight, absolute kidney weight and

relative kidney weight significantly decreased (Table 1) (P<0.05). When vitamin plus malathion-treated group was compared to malathion-treated group, no statistically significant changes were observed in the body weight, absolute kidney weight and relative kidney weight at the and of the 4th week (Table 1).

No statistically significant differences in food consumption were observed when the vitamin-treated group was compared with the control group at the end of the 4th week. During the experiment food intakes/consumptions were recorded daily through the experiment and there were significantly decreased in malathion and vitamin plus malathion treated group compared to control group (Figure 1). There weren't significant changes between malathion and vitamin plus malathion treated rats rats (Figure 1).

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)		
Control	318,88±5,31	328,16±2,48	2,25±0,053	0,68±0,009
Vitamin C and E	319,33±3,20	327,83±1,94	2,22±0,056	0,67±0,005
Malathion	320,50±2,88	294,50±1,87 [a,b]	1,88±0,022 [a,b]	0,63±0,005 [a,b]
Vitamin C and E + Malathion	320,16±1,60	298,50±1,64 [a,b]	1,94±0,029 [a,b]	0,64±0,003 [a,b]

Values are mean±SD of six rats in each group. Significance at *P* < 0.05.
[a]Comparison of control and other groups.
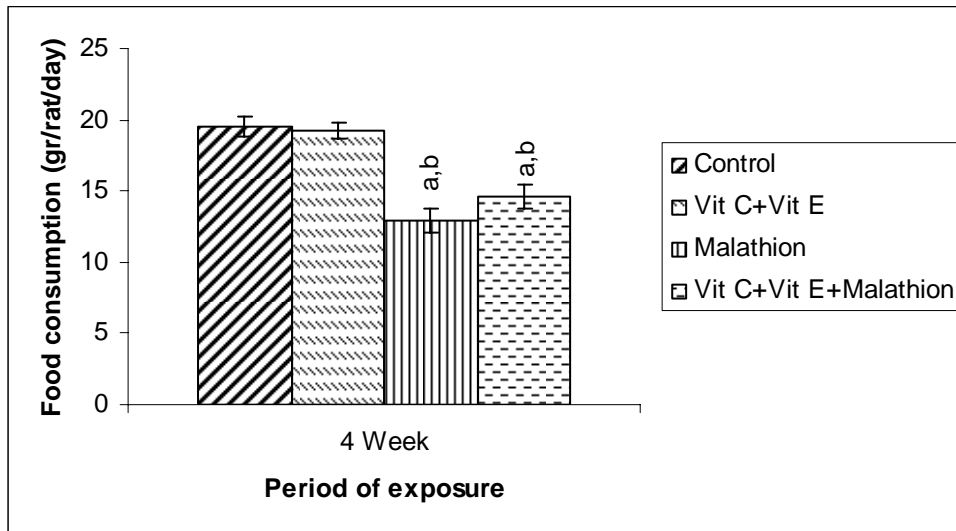[b]Comparison of vitamin-treated group with malathion- and vitamin plus malathion-treated groups.

Figure 1. Food consumption of control and experimental rats
Values are mean±SD of six rats in each group. Significance at *P* < 0.05.
[a]Comparison of control and other groups.
[b]Comparison of vitamin-treated group with malathion- and vitamin plus malathion-treated groups.

3.2. Evaluation of Biochemical Results

The control group was compared with all other groups at the end of the 4th week. In addition to this, the malathion-treated group was compared to the vitamin plus malathion treated group. No statistically significant differences were observed when the vitamin-treated group was compared with the control group at the end of the 4th week (Figure 2-4).

At the end of the 4th week, when the malathion- and vitamin plus malathion-treated groups were compared with the control group, there was a significant increase in the serum urea, uric acid and creatinine levels in both groups of treated rats (P<0.05) (Figure 2-4). In the vitamin plus malathion treated group there were statistically decrease in the serum urea level, while no statistically significant changes were observed in serum uric acid and creatinine levels compared to the malathion-treated group (Figure 2-4).
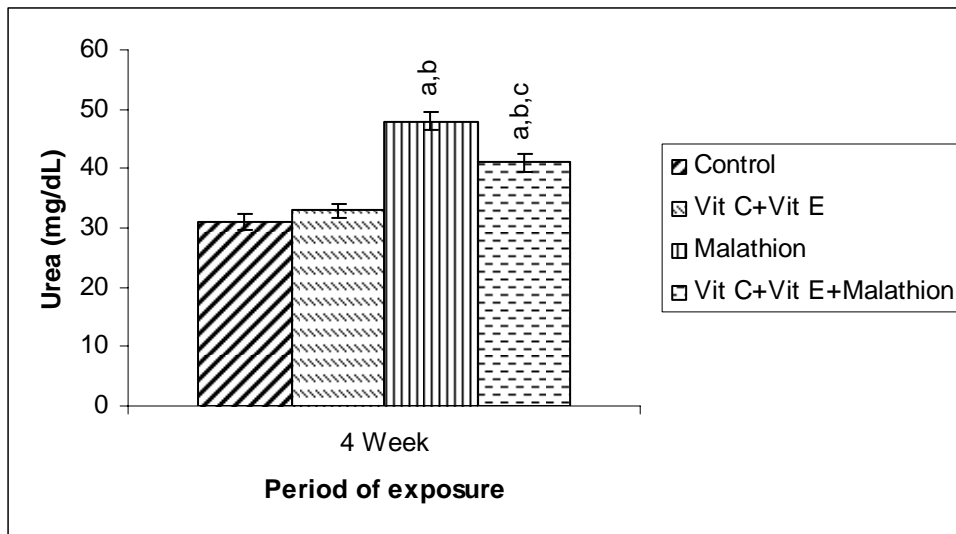
Figure 2. Serum urea levels in control and experimental rats
Values are mean±SD of six rats in each group. Significance at $P < 0.05$.
[a]Comparison of control and other groups.
[b]Comparison of vitamin-treated group with malathion- and vitamin plus malathion-treated groups.
[c] Comparison of malathion-treated group with vitamin plus malathion-treated group.
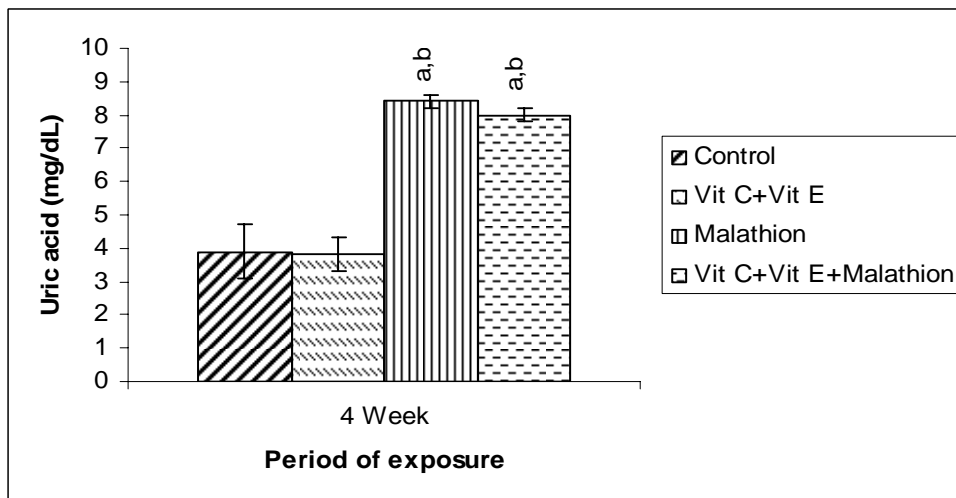

Figure 3. Serum uric acid levels in control and experimental rats
Values are mean±SD of six rats in each group. Significance at $P < 0.05$.
[a]Comparison of control and other groups.
[b]Comparison of vitamin-treated group with malathion- and vitamin plus malathion-treated groups.

Figure 4. Serum creatinine levels in control and experimental rats
Values are mean±SD of six rats in each group. Significance at $P < 0.05$.
[a]Comparison of control and other groups.
[b]Comparison of vitamin-treated group with malathion- and vitamin plus malathion-treated groups.

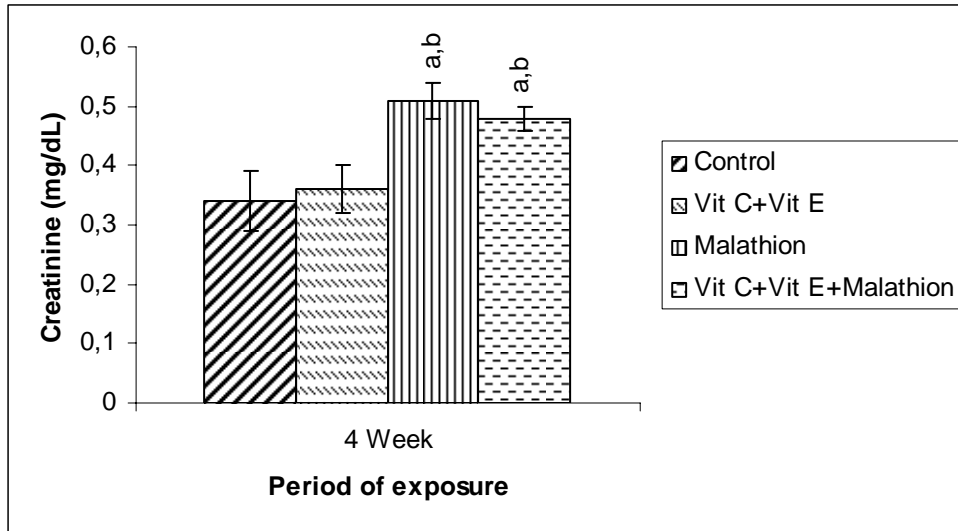### 3.3. Histopathological Changes in the Kidney

Kidneys of the control and vitamin-treated group showed a normal structure (Fig. 5A). In light microscopic examinations, histopathological changes were observed in the kidneys of vitamin plus malathion-treated group and malathion-treated group compared to control group (Fig. 5B-E). After 4 weeks of malathion exposure, mononuclear cell infiltration, break away from basal membrane, glomerular atrophy were observed in the kidney tissues (Fig. 5B, C). Also we observed mononuclear cell infiltration, break away in basal membrane, glomerular atrophy in the kidney tissues of vitamin plus malathion treated-group(Fig. 5D, E).
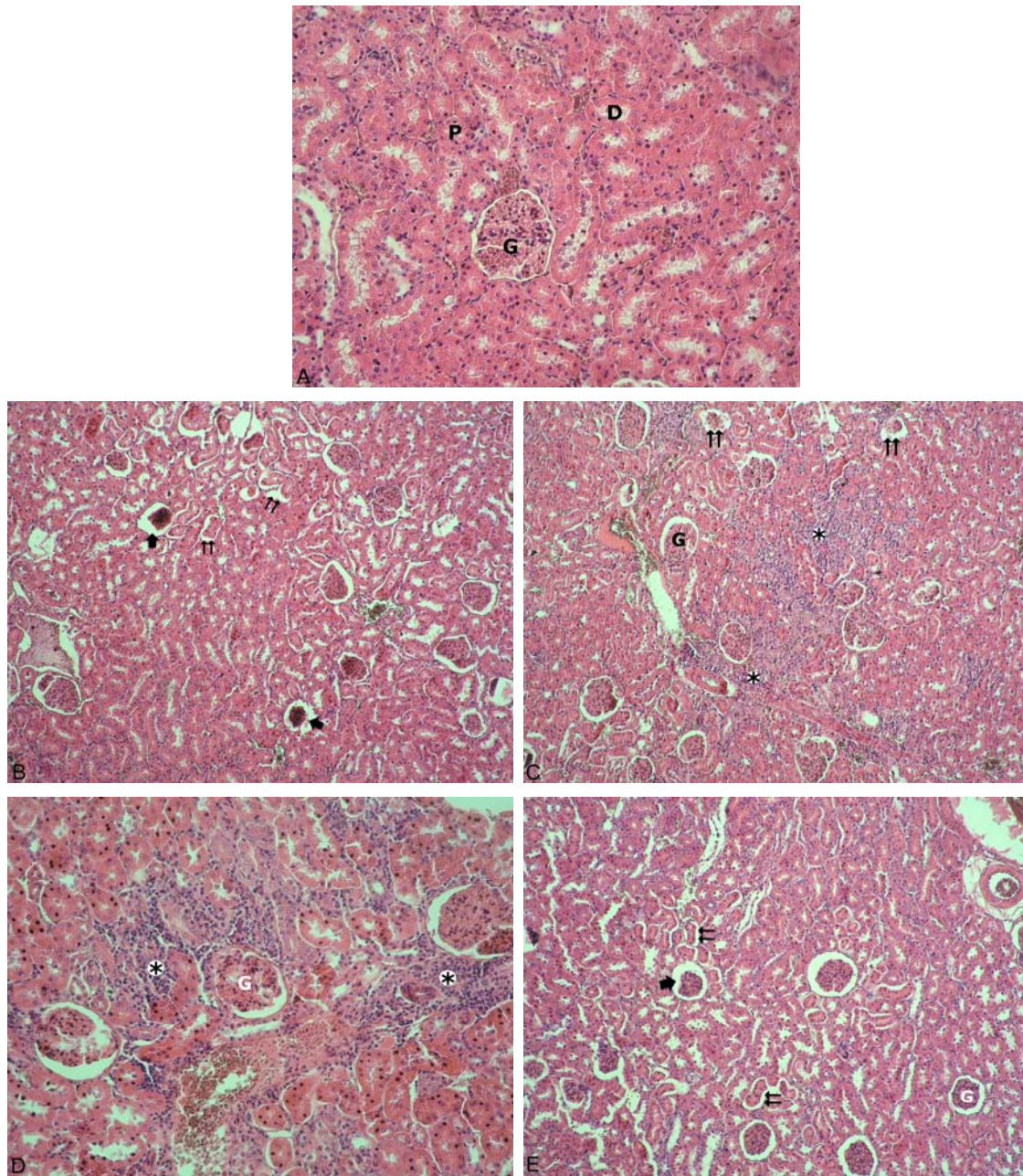
Figure 5. (A) Kidney section of control rats, proximal tubule (P), distal tubule (D) and glomerulus (G), x400. (B-C) Glomerular atrophy (➔), break away from basal membrane (⇉) and mononuclear cell infiltration (✶) in kidney 4 weeks after malathion treatment to rats, x200. (D-E) Kidney sections of vitamin plus malathion-treated rats Glomerular atrophy (➔), break away from basal membrane (⇉) and mononuclear cell infiltration (✶), (D) x400, (E) x200.

4. DISCUSSION

Malathion is used in public health, agriculture and household purposes [5, 6, 11]. Several studies show that malathion caused hepatotoxicity [9], testicular toxicity [10], hematotoxicity [13], genotoxicity [25]. In addition to this previous studies have shown that malathion can alter biochemical [8, 26] and hematological parameters in experimental animals [5]. And malathion also caused changes in antioxidant enzyme activities different tissues like kidney [27], brain [28], liver [29]. The oral LD_{50} of malathion for male rats is 1350 mg/kg [30]. In the present study even though malathion was given at $1/50$ of the oral LD_{50} and none of the rats died during the experimental period, histopathological changes were observed in rat kidney tissues.

OP insecticides cause decrease of body and organ weights [19, 31, 32]. In the present study body weights, absolute kidney and relative kidney weights of animals treated with malathion and vitamin plus malathion-treated groups were significantly less as compared to the control group. And also food intake was reduced malathion and vitamin plus malathion treated rats

compared to control rats. In the present study, the weight loss in these animals may be due to enhanced catabolism and reduction in food intake [33].

Many pesticides can cause some toxic and adverse effects on the kidney tissues [34]. Kidney is one of the targets organs of experimental animals attacked by OP compounds [32, 35]. Urea, uric acid and creatinine levels are kidney function parameters [32, 36]. Pesticides can alter plasma urea, uric acid and creatinine levels [32, 37, 38]. In this study, malathion and vitamin plus malathion exposure also increased in the urea, uric acid and creatinine levels in malathion and vitamin plus malathion-treated rats compared to control rats. This increase may be due to kidney damage caused by malathion.

Urea is the end product of protein catabolism. Increased blood urea is correlated with an increased protein catabolism in mammalian body and/or referred to kidney dysfunction [38, 46]. The levels of urea in the plasma of rats are tested as indicators for kidney functions [33]. Also, the high levels of blood urea results caused from increased breakdown of tissue or impaired excretion [38]. In this study, this increased urea level may be due to toxic effects of malathion.

Uric acid is the end product of purine catabolism and can reduce oxidative stress by scavenging various reactive oxygen species [39, 40]. Many chemicals can affect uric acid levels [39]. Serum uric acid is reported to increase in proportion to the decrease in creatinine clearance and correlate with the degree of renal tubulo-interstitial damage [41]. In this study uric acid increase may be related to either increase in protein degradation, which is involved in uric acid formation, or the toxic effect of malathion on the kidneys [42].

The creatinine excretion is dependent almost on the process of glomerular filtration. Previous study reported that significant rise in the serum creatinine level may due to the impairment of the glomerular function and tubular damage in the kidneys [32]. Creatinine level is a good risk marker for chronic renal insufficiency [37, 43]. Increased creatinine level shows that damage of the glomerular function and tubular damage in the kidneys [44, 45]. In the present study, it was shown that treatment with malathion and vitamin plus malathion to rats caused degeneration in kidney tissues and it was correlate with the creatinine levels in plasma.

Pesticides cause various histopathological changes in kidney tissues of experimental animals [19, 34, 44, 47]. We observed that malathion-treatment led to histopathological changes in kidney tissues. Moreover, we observed that the histopathological changes in the vitamin plus malathion-treated rats. In this study, alterations in biochemical parameters were well correlated with the histological results. These changes may result from the toxic effects of malathion.

Antioxidant vitamins have a various biological activities. Vitamins C and E are a well known antioxidant. Vitamin E was thought to be an important chain breaking antioxidant. Vitamin C is another important water soluble antioxidant [1]. Vitamin E is also the most important lipophilic antioxidant and is residing mainly in the cell membranes and helping to maintain membrane stability. Vitamin C is the most important free-radical scavenger in extracellular fluids and protecting biomembranes from peroxidative damage [47]. In our study, serum urea levels were at least partially normalized when vitamins C and E were given together with malathion. But other biochemical parameters which include uric acid and creatinine and also histopathological changes were not normalized when vitamins C and E were given together with malathion.

Thus, in summary these data show that a low dose of malathion caused subacute nephrotoxicity, and the antioxidants vitamin C and E didn't ameliorate this toxicity.

ACKNOWLEDGEMENTS

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