

## Full Length Research Paper

# Effects of organophosphate insecticide, malathion on the liver and kidney of male Wistar albino rats by $\alpha$ -tocopherol and selenium

Abdulaziz M. Al-Othman<sup>1</sup>, Khaled S. Al-Numair<sup>1</sup>, Gaber E. El-Desoky<sup>2,3</sup>, Kareem Yusuf<sup>2</sup>, Zeid A. Al Othman<sup>2</sup>, Mourad A. M. Aboul-Soud<sup>3,4\*</sup> and John P. Giesy<sup>5,6,7,8</sup>

<sup>1</sup>Department of Community Health Sciences, College of Applied Medical Science, King Saud University, Kingdom of Saudi Arabia.

<sup>2</sup>Department of Chemistry, College of Science, King Saud University, Kingdom of Saudi Arabia.

<sup>3</sup>Biochemistry Department, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt.

<sup>4</sup>College of Science, King Saud University, P. O. BOX 2245, Riyadh 11451, Kingdom of Saudi Arabia.

<sup>5</sup>Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

<sup>6</sup>Department of Zoology, and Center for Integrative Toxicology, Michigan State University, East Lansing, MI, USA.

<sup>7</sup>School of Biological Sciences, University of Hong Kong, Hong Kong, SAR, China.

<sup>8</sup>Zoology Department, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia.

Accepted 22 September, 2020

Protection from effects of the organophosphate insecticide, malathion on the liver and kidney of male Wistar albino rats by  $\alpha$ -tocopherol and selenium was investigated. Significantly greater ( $P<0.01$ ) mean concentrations of malondialdehyde (MDA) and lesser concentrations ( $P<0.01$ ) of reduced glutathione (GSH) and tissues total proteins were observed in liver and kidney of rats exposed to malathion. Activities of the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were less in livers and kidneys of rats exposed to malathion. These effects caused by exposure to malathion were reversed when rats were subsequently treated orally with 100 mg/kg b.w. tocopherol and/or 0.1 mg selenium/kg b.w. Both tocopherol and selenium (Se) reduced the concentration of MDA, and increased concentration of total proteins and reduced glutathione and mean activities of SOD, CAT and GPx, to levels that were not significantly different from unexposed, control rats, previous to exposure to malathion. Selenium was significantly ( $P<0.05$ ) more effective at recovering activities of SOD and GPx in liver and kidney of malathion treated rats than was - tocopherol, whereas,  $\alpha$ -tocopherol was significantly ( $P<0.05$ ) more effective at recovering activities of CAT in liver and kidney than was Se. Both  $\alpha$ -tocopherol and Se were effective in alleviating oxidative damage in liver and kidney of rats caused by malathion.

**Key words:** Oxidative stress, antioxidant enzymes, lipid peroxidation, vitamin E, selenium.

## INTRODUCTION

Organophosphorous (OP) insecticides are used throughout the world for control of agricultural and domestic insect pests. OP insecticides are employed in medicine and industry, because of their relatively low persistence due to biodegradability (Uzun et al., 2009). OP insecticides were introduced as a replacement of the

more persistent organochlorine pesticides in the 1970s (Galloway and Handy, 2003). OP pesticides have been detected in the soil, water, bodies, vegetables, grains and other food products (IARC, 1983; Poet et al., 2004). Due to the general availability of OP compounds, poisoning are common (Garcia et al., 2003). OP insecticides are known to inhibit acetylcholinesterase and pseudocholinesterase activity in target tissues (John et al., 2001; Kalender et al., 2006). Other systems that can be affected by OPs are the immune system (Handy et al., 2002), reproductive system (Farag et al., 2000), nervous

\*Corresponding author. E-mail: [maboulsoud@ksu.edu.sa](mailto:maboulsoud@ksu.edu.sa). Tel: +966533203236. Fax: +96614678514.

system (Desi et al., 1998), pancreas (Gokalp et al., 2005) and liver (Kalender et al., 2006).

Malathion [O,O -dimethyl-S- (1,2-dicarbethoxyethyl) phosphorodithioate] is one of the most widely used OP insecticides for agriculture and public health programs (Maroni et al., 2000). Malathion causes toxicity through hyperexcitation of the nervous system through its bioactivated analog, malaoxon (Hazarika et al., 2003). Malathion is soluble in lipids and is stored in liver and other lipophilic tissues (Garcia-Repetto et al., 1995). Malathion has been found to exhibit rapid but asymmetrical transmembrane uptake by the liver. Therefore, the liver which is the most important organ in glucose and lipid homeostasis and production of related enzymes can be a target for malathion toxicity (Yang et al., 2000).

More recently, it has been postulated that OPs produce oxidative stress in different tissues through formation of reactive oxygen species (ROS) (Banerjee et al., 1999; Ahmed et al., 2000; Akhgari et al., 2003; Abdollahi et al., 2004). ROS such as hydrogen peroxide, super oxide anion and hydroxyl radical are produced in a number of cellular reactions by enzymes such as lipooxygenases, peroxidases and dehydrogenases (Halliwell and Gutteridge, 1999; Dal-Pizzol et al., 2001). Oxidative stress is also related to xenobiotic exposure and different levels of environmental contamination (Halliwell and Gutteridge, 1999). Peroxidation of membrane lipids seems to be an unavoidable process in tissue injury, which can impair antioxidant defenses, those results in oxidative damage by changing the balance between oxidants and antioxidants (Banerjee et al., 1988; Halliwell and Gutteridge, 1999; Torres et al., 2004).

The basis of OP toxicity in production of oxidative stress might be due to either: (a) their "redox -cycling" activity, where they readily accept an electron to form free radicals and then transfer them to oxygen to generate superoxide anions and hence hydrogen peroxide through dismutation reactions, or (b) to ROS generation via changes in normal antioxidant homeostasis that results in depletion of antioxidants (Banerjee et al., 1999; Kovacic, 2003; Vidyasagar et al., 2004). Regardless of OPs type and primary mode of toxic action, oxidative stress seems to be attenuated by non-enzymatic nutritional antioxidants such as  $\alpha$ -tocopherol and C (Ahmed et al., 2000). OP insecticides containing the P=S bond (called "thion") are converted to P=O (called "oxon") by microsomal enzymes called mixed-function oxidase (MFO), among which the enzymes cytochrome P<sub>450</sub> (CYP<sub>450</sub>) plays a major role (Giri et al., 2002). The oxons account for the cytotoxic effects of OPs.

Malathion is genotoxic in mice, and able to induce a variety of chromosomal aberrations in bone marrow cells (Rupa et al., 1991). The mutagenic activity of malathion might be due to the existence of electrophilic sites in the parent molecule or its metabolic intermediates, which are

capable of binding to nucleophilic sites of numerous biomolecules. In the malathion molecule, there are two potential electrophilic sites, alkyl group(s) and a phosphoryl groups, these might explain, at least in part, the occurrence of oxidative damage in rat liver sub-chronically exposed to malathion, because the liver is a primary site of oxidative metabolism and with CYP<sub>450</sub> activity related to biotransformation of xenobiotics (Halliwell and Guttridge, 1999).

The antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are the main enzymes that act as defenses and act in concert with non-enzymatic reduced glutathion (GSH) antioxidants and other antioxidants, such as  $\alpha$ -tocopherol and selenium (Halliwell and Gutteridge, 1999) protect against the adverse effects of ROS.  $\alpha$ -tocopherol (Vitamin E) is the major lipid soluble antioxidant and is known to protect cellular membranes and lipoproteins from peroxidation (Yavuz et al., 2004).  $\alpha$ -tocopherol inhibits free radical formation (Kalender et al., 2004) and can effectively minimize lipid peroxidation (Traber and Atkinson, 2007). To date, several studies have examined the protective effects of  $\alpha$ -tocopherol on pesticide induced oxidative stress (Aldana et al., 2001; John et al., 2001; El-Demerdash et al., 2004; Kalender et al., 2005; Durak et al., 2008; Sodhi et al., 2008). Selenium is a constituent of the cytosolic enzyme glutathione peroxidase and facilitates reduction peroxy radicals by - tocopherol (Kaneko, 1989). Absorption of  $\alpha$ -tocopherol is impaired by severe selenium deficiency and selenium alleviates  $\alpha$ -tocopherol deficiencies by permitting more - tocopherol to be absorbed (Machlin, 1991). The present study was conducted to: (1) Evaluate the effect of malathion on lipid peroxidation on production of MDA, and depletion of GSH and activities of the antioxidant enzymes such as SOD, CAT, and GPx, as well as evaluate its effect on total proteins in liver and kidney, and (2) determine the potency of the antioxidants, - tocopherol and selenium to modulate hepatic damage induced by malathion.

## MATERIALS AND METHODS

### Chemicals

A commercial formulation of malathion (agrothion 57% EC 500 g L<sup>-1</sup>) was purchased from a local market in Saudi Arabia and was used in this study. All chemicals were of analytical reagent grade and chemicals required for all biochemical assays were obtained from Sigma-Aldrich Chemicals Co. (St.louis, Mo, USA) and Merck (Darmstadt, Germany).

### Animals

Male Wister albino rats, weighing 200 to 250 g, were obtained from the animal rearing facility of the College of Medicine at King Saud University. Rats were housed throughout the experiment in polypropylene cages, with 10 animals per cage, and allowed to

acclimatize to the laboratory environment for 10 days. Animals were maintained under controlled conditions of temperature at  $25\pm 2^\circ\text{C}$ , relative humidity of  $50\pm 15\%$  and normal photoperiod (12 to 12 h light-dark cycle). The animals were allowed free access to standard dry pellet diet and water *ad libitum*.

Experiments reported here complied with current laws and regulations of Saudi Arabia on the care and handling of experimental animals and the animal ethical committee of King Saud University, College of Medicine, Saudi Arabia.

### Experimental design

Rats were divided into five groups: control (Group I) ( $n=10$ ), malathion-treatment (Group II) ( $n=10$ ), malathion plus  $\alpha$ -tocopherol and selenium treatment (Group III) ( $n=10$ ), malathion plus -tocopherol treatment (Group IV) ( $n=10$ ), malation plus selenium treatment (Group V) ( $n=10$ ). The substances were administrated via gavage in the morning (between 09:00AM and 10:00AM) to non-fasting animals.

#### Normal control group (Group I)

Corn oil at a dose of 0.5 ml per animal was given via gavage, once a day for the treatment period of 45 days.

#### Malathion-treated group (group II)

Once a day, the rats were given malathion at a dose of 27 mg/kg ( $1/50 \text{ LD}_{50}$ ) body weight per day in 0.5 ml corn oil *via* gavage.

#### Malathion plus $\alpha$ -tocopherol and selenium-treated group (Group III)

Selenium and  $\alpha$ -tocopherol were dissolved in water and corn oil, respectively. Once a day, the rats were treated, *via* gavage, first with selenium (0.1 mg Se/kg b.w. per day) and then 100 mg -tocopherol/kg b.w. per day, and 30 min later, malathion dissolved in corn oil (10 mg/kg b.w. per day) was administrated *via* gavage.

#### Malation plus $\alpha$ -tocopherol treated group (Group IV)

Once a day, rats were given  $\alpha$ -tocopherol dissolved in corn oil (100 mg/kg b.w. per day) by gavage and 30 min later, malathion dissolved in corn oil (10 mg/kg b.w. per day) was administrated *via* gavage.

#### Malathion plus selenium treated group (Group V)

Once a day, rats were given selenium as sodium selenite ( $\text{Na}_2\text{SeO}_4$ ) dissolved in water (0.1 mg/kg b.w. per day) by gavage and 30 min later, malathion dissolved in corn oil (10 mg/kg b.w. per day) was administrated *via* gavage.

The selected dose of malathion was based on previous studies in which  $1/50 \text{ LD}_{50}$  (27 mg/kg b.w.) of malathion induced biochemical alteration in rat without morbidity (Uzun et al., 2009; Aboul-Soud et al., 2011), while that of  $\alpha$ -tocopherol and selenium was according to previous reports (Aksoy et al., 2005; Sodhi et al., 2008). At the end of the exposure period, rats were killed by cervical dislocation and their liver and kidney were removed, weighed and treated for further measurements.

## Biochemical analysis

### Lipid peroxidation (malondialdehyde MDA) and reduced glutathione

The method described by Ohkawa et al. (1979) was used to quantify concentrations of MDA in tissue. In brief, liver or kidney tissues (200 mg) were homogenized in aqueous 0.15 M KCl solution to give 10% homogenate. One milliliter of homogenate was mixed with 1 ml of 10% trichloroacetic acid (TAC) and centrifuged at 3,000 rpm for 15 min. Then, 1 ml supernatant was suspended in 1 ml of 0.67% 2-thiobarbituric acid. Samples were then placed in a boiling water bath for 15 min. Samples were allowed to cool to room temperature and then centrifuged at 3000 rpm for 15 min. Optical density of the clear pink supernatant was measured at 532 nm on a spectrophotometer (APEL, PD-303 uv, Japan). Glutathione (GSH) in tissues was measured according to the method described by Sedláč and Lindsay (1968). A cross section of liver or kidney tissues (200 mg) was dissected and homogenized in ice-cold 0.02 M ethylenediaminetetraacetic acid (EDTA). Aliquots of 0.5 ml of tissues homogenates were mixed with 0.2 M Tris buffer, pH 8.2 and 0.1 ml of Ellman's reagent, [5,5 -dithio-bis-(2-nitro- benzoic acid)] (DTNB). Sample tubes were centrifuged at 3000 rpm at room temperature for 15 min. Absorbance of the clear supernatant was recorded using a spectrophotometer (APEL, PD-303 uv, Japan) at 412 nm in one centimeter quartz cell. The quantification of reduced glutathione was done by using a standard curve.

### Total proteins

Total concentrations proteins in liver and kidney were measured by use of a modified Lowry method of Schacter and Pollack (1973). Bovine plasma albumin was used as standard.

### Antioxidant enzymes activity

**Superoxide dismutase (SOD) activity:** Superoxide dismutase (SOD) activity in liver and kidney was measured spectrophotometrically (560 nm) by the method described by Kakkar et al. (1984) by use of nitroblue tetrazolium as the indicator reagent. Briefly, the kidney tissues (200 mg) were homogenized with 10 times (w/v) 0.1 M sodium phosphate buffer (pH 7.4). The reagents: sodium pyrophosphate buffer 1.2 ml (0.052 M) pH 8.3, 0.1 ml phenazine metho-sulphate (186  $\mu\text{M}$ ), 0.3 ml nitro blue tetrazolium (300  $\mu\text{M}$ ) and 0.2 ml NADH (780  $\mu\text{M}$ ) were added to 0.1 ml of processed tissue sample. The mixture was then incubated for 90 min at  $30^\circ\text{C}$ . Four milliliters of n-butanol and 1 ml of acetic acid were then added. The mixture was shaken vigorously. Following centrifugation at 4000 rpm for 10 min, the organic layer was withdrawn and absorbance was measured at 560 nm using a spectrophotometer (LKB-pharmacia, Mark II, Ireland).

**Catalase (CAT) activity:** Catalase (CAT) activity in liver and kidney was measured by the method of Aebi (1983) using hydrogen peroxide as the substrate. The disappearance of  $\text{H}_2\text{O}_2$  was followed at 240 nm. The activity was expressed as  $\mu\text{mole}^{-1} \text{min}^{-1} \text{mg}^{-1}$  protein using the extension coefficient of  $0.0436 \text{ mM}^{-1} \text{mg}^{-1}$ . CAT exerts a dual-function decomposition of  $\text{H}_2\text{O}_2$  to give  $\text{H}_2\text{O}$  and  $\text{O}_2$  and oxidation of H donors. In the ultraviolet range,  $\text{H}_2\text{O}_2$  shows a continual increase in absorption with decreasing wavelength. The decomposition of  $\text{H}_2\text{O}_2$  can be followed directly by measuring the decrease in absorbance at 240 nm. The difference in absorbance per unit time is a measure of CAT activity. Liver or kidney tissues (200 mg) were homogenized in 8 ml of 0.05 M phosphate buffer at pH 7.0. The tissue homogenates were centrifuged at  $4^\circ\text{C}$  for 15 min

**Table 1.** Effect of  $\alpha$ -tocopherol and selenium on MDA, GSH and proteins content in liver and kidney of malathion- treated rats.

| Treatment | Liver                            |                             |                                | Kidney                           |                             |                                |
|-----------|----------------------------------|-----------------------------|--------------------------------|----------------------------------|-----------------------------|--------------------------------|
|           | GSH<br>(nmole/100 mg wet tissue) | MDA<br>(nmole/g wet tissue) | Proteins<br>(mg/100 mg tissue) | GSH<br>(nmole/100 mg wet tissue) | MDA<br>(nmole/g wet tissue) | Proteins<br>(mg/100 mg tissue) |
| Group I   | 381.64±10.6                      | 238.60±5.9                  | 17.15±0.4                      | 95.13±4.1                        | 268.20±14.8                 | 17.24±0.6                      |
| Group II  | 238.76±36.0**                    | 384.92±7.2**                | 10.44±0.6**                    | 25.19±6.1**                      | 377.12±14.0**               | 11.48±0.8**                    |
| Group III | 378.91±4.3                       | 234.02±2.9                  | 16.75±0.9                      | 92.98±2.4                        | 274.54±7.5                  | 16.78±1.0                      |
| Group IV  | 367.90±19.5                      | 256.44±25.7                 | 14.85±2.6                      | 91.07±0.9                        | 245.51±28.9                 | 14.99±2.5                      |
| Group V   | 367.78±21.9                      | 251.98±23.3                 | 15.41±2.9                      | 91.67±1.3                        | 249.05±33.9                 | 15.83±1.5                      |

Values are expressed as mean  $\pm$  S.D; \*\*  $P < 0.01$  relative to Group I. Group I: Control; Group II: Malathion; Group III: Malathion+  $\alpha$ -tocopherol + Selenium; Group IV: Malathion+  $\alpha$ -tocopherol; Group V - Malathion+ Selenium.

at 1500 g. The supernatants were removed into separate test tubes and kept on ice until the enzymes assay. Sample was measured against a blank containing 2.8 ml (1:500 v/v) phosphate buffer instead of  $H_2O_2$  (30 mM hydrogen peroxide) and 0.2 ml enzyme solution. The reaction was started by addition of  $H_2O_2$ . The initial absorbance should be  $A=0.500$  followed by the decrease in absorbance for about 30 s.

**Estimation of glutathione peroxidase (GPx) activity in tissues:** Glutathione peroxidase (GPx) activity was measured by use of the method developed by Paglia and Valentine (1967). The reaction mixture contained 2.6 ml of 100 mmol/L phosphate buffer (pH 7.0) with 3 mmol/L EDTA, 0.05 ml of 10 mg/ml GSH solution, 0.1 ml GR (10 mg/ml), 0.05 (10 mg/ml) NADPH-Na salt, 0.1 ml 90 mmol/L hydrogen peroxide solution and 0.1 ml of tissue supernatant. The GPx activity was monitored by the decrease in absorbance due to the consumption of NADPH, which absorbs at 340 nm on spectrophotometer (LKB-pharmacia, Mark II, Irland).

#### Statistical analyses

All data are expressed as mean  $\pm$  standard deviation (SD); data were analyzed by ANOVA test using SPSS version 17.0 for Windows» statistical program and the individual comparison of groups were obtained by using Scheffe's multiple comparison procedure. Pearson correlation test was used to determine the significant correlation between

variable.  $P$  values of less than 0.05 and 0.01 were selected as the levels of significance.

## RESULTS

### Effects of $\alpha$ -tocopherol and selenium on, MDA, GSH, and total protein

The concentration of MDA was significantly ( $p < 0.01$ ) greater in liver and kidney of Group II rats (malathion treatment) than those in I (control) (Table 1). However, the mean MDA concentrations in liver and kidney of Group III rats (malathion plus  $\alpha$ -tocopherol and selenium treatment), Group IV rats (malathion plus - tocopherol treatment) Group V rats (malathion plus selenium treatment) were not significantly different from those of the controls (Table 1).

The concentrations of GSH and total protein liver and kidney of Group II rats were significantly ( $p < 0.01$ ) less than those of the controls. Treatment with  $\alpha$ -tocopherol and selenium in combination, or with  $\alpha$ -tocopherol or selenium individually when rats were also exposed to malathion, Groups III, IV and V, respectively resulted in concentrations of GSH and

total protein that were not significantly different from those in the controls (Table 1).

### Effects of $\alpha$ -tocopherol and selenium on antioxidant enzymes

Both  $\alpha$ -tocopherol and selenium significantly mitigated the effects of malathion on activities of antioxidant enzymes. Significantly ( $p < 0.01$ ) lesser activities of CAT, SOD and GPx were observed in the liver and kidney of rats exposed to malathion alone (Group II), relative to the controls (Group I) (Table 2). When rats were exposed to - tocopherol and selenium in combination, or - tocopherol or selenium individually (Groups III, IV and V), there were no significant differences in activities of enzymes than in the controls (Group I). Activities of SOD and GPx in liver and kidney of rats treated with Se (Group V) were significantly ( $p < 0.05$ ) greater than those of rats treated with - tocopherol (Group IV) in the presence of malathion. However, the effect of  $\alpha$ -tocopherol on the activity of CAT in liver and kidney of malathion treated rats (Group V) were significantly ( $p < 0.05$ ) greater than that of selenium (Group V) (Table 2).

When the results of all of the treatments

**Table 2.** Effect of  $\alpha$ -tocopherol and selenium on SOD, CAT and GPx activities in liver and kidney of malathion-treated rats.

| Treatment | Liver                    |                                    |                                    | Kidney                   |                                    |                                    |
|-----------|--------------------------|------------------------------------|------------------------------------|--------------------------|------------------------------------|------------------------------------|
|           | SOD<br>(U/mg of protein) | CAT<br>( $\mu$ mole/mg of protein) | GPx<br>( $\mu$ mole/mg of protein) | SOD<br>(U/mg of protein) | CAT<br>( $\mu$ mole/mg of protein) | GPx<br>( $\mu$ mole/mg of protein) |
| Group I   | 6.07 $\pm$ 0.6           | 595.58 $\pm$ 16.8                  | 183.09 $\pm$ 15.0                  | 5.54 $\pm$ 0.4           | 598.30 $\pm$ 50.9                  | 239.13 $\pm$ 19.3                  |
| Group II  | 4.39 $\pm$ 0.3**         | 447.04 $\pm$ 24.4**                | 135.56 $\pm$ 2.6**                 | 3.41 $\pm$ 0.1**         | 411.96 $\pm$ 5.6**                 | 128.99 $\pm$ 9.8**                 |
| Group III | 5.61 $\pm$ 0.2           | 597.03 $\pm$ 19.6                  | 190.25 $\pm$ 3.8                   | 5.92 $\pm$ 0.3           | 583.27 $\pm$ 36.2                  | 262.88 $\pm$ 18.5                  |
| Group IV  | 5.62 $\pm$ 0.1           | 594.89 $\pm$ 11.3*                 | 166.76 $\pm$ 16.8                  | 5.43 $\pm$ 0.2           | 578.282 $\pm$ 0.1*                 | 228.85 $\pm$ 11.7                  |
| Group V   | 5.87 $\pm$ 0.1*          | 570.43 $\pm$ 20.3                  | 191.22 $\pm$ 6.2*                  | 5.85 $\pm$ 0.2*          | 548.99 $\pm$ 8.8                   | 261.93 $\pm$ 27.4*                 |

Values are expressed as mean  $\pm$  S.D; \*\* P < 0.01 relative to Group I. Group I: Control; Group II: Malathion; Group III: Malathion+  $\alpha$ -tocopherol + Selenium; Group IV: Malathion+  $\alpha$ -tocopherol; Group V - Malathion+ Selenium.

**Table 3.** Correlation coefficient (r) among oxidative stress and the antioxidant enzymes activities in kidney of malathion treated rats.

| Antioxidant enzymes |                     | SOD     | GPx     | CAT     |
|---------------------|---------------------|---------|---------|---------|
| MDA                 | Pearson correlation | -0.735- | -0.426- | -0.728- |
|                     | Sig. (2-tailed)     | 0.096   | 0.400   | 0.101   |
|                     | N                   | 6       | 6       | 6       |
| GSH                 | Pearson correlation | 0.929** | 0.713   | 0.934** |
|                     | Sig. (2-tailed)     | 0.007   | 0.112   | 0.006   |
|                     | N                   | 6       | 6       | 6       |
| P                   | Pearson correlation | 0.919** | 0.727   | 0.939** |
|                     | Sig. (2-tailed)     | 0.010   | 0.102   | 0.005   |
|                     | N                   | 6       | 6       | 6       |

\*, Correlation is significant at the 0.05 level (2-tailed); \*\*, correlation is significant at the 0.01 level (2-tailed).

were considered together, there was a statistically significant correlation between the concentration of MDA and level of antioxidant enzymes of liver and kidney injury. These associations indicated that lipid peroxidation was involved in toxicity of malathion to both liver and kidney. Indicators of toxicity in both liver and kidney were inversely correlated with activities of antioxidant enzymes, which is consistent with the importance of

antioxidants in protecting against the effects of ROS (Tables 3 and 4).

## DISCUSSION

Malathion causes both acute and chronic toxicity to non-target animals. Malathion not only has toxic effects on mammals, it also has toxic effects on

fish, chicks, and non-target invertebrates (Senger et al., 2005; Sodhi et al., 2008). The oral LD<sub>50</sub> of malathion for male rats is 1350 mg/kg (John et al., 2001). Several case-control studies have associated parenteral exposure to insecticides or insecticide use in the home with childhood brain tumors, leukemia, lymphomas, testicular cancer and other types of cancer (Eskenazi et al., 1999). Birth defects reproductive abnormalities and

**Table 4.** Correlation coefficient (r) among oxidative stress and the antioxidant enzymes activities in liver of malathion treated rats.

| Antioxidant enzymes |                     | SOD       | GPx       | CAT       |
|---------------------|---------------------|-----------|-----------|-----------|
| <b>MDA</b>          | Pearson correlation | -0.945-** | -0.938-** | -0.974-** |
|                     | Sig. (2-tailed)     | 0.004     | 0.006     | 0.001     |
|                     | N                   | 6         | 6         | 6         |
| <b>GSH</b>          | Pearson correlation | 0.954**   | 0.907*    | 0.984**   |
|                     | Sig. (2-tailed)     | 0.003     | 0.013     | 0.000     |
|                     | N                   | 6         | 6         | 6         |
| <b>P</b>            | Pearson correlation | 0.931**   | 0.935**   | 0.951**   |
|                     | Sig. (2-tailed)     | 0.007     | 0.006     | 0.004     |
|                     | N                   | 6         | 6         | 6         |

\*, Correlation is significant at the 0.05 level (2-tailed); \*\*, Correlation is significant at the 0.01 level (2-tailed).

genetic damage have also been linked to exposure of humans and laboratory animals to malathion. Disorders of vision, behavioral changes, impaired learning and sensitization of skin have also been associated with exposures to malathion (Brenner, 1992).

The objective of the study upon which is reported here, was to evaluate the effects of malathion on lipid peroxidation, antioxidant defense mechanisms and histological changes in rat liver and kidney. Alleviation of the effects of malathion by  $\alpha$ -tocopherol and selenium were investigated. Lipid peroxidation, which is caused by reactive oxygen species, has been implicated in the pathogenesis of various liver and kidney injuries. The most widely used marker of lipid peroxidation is formation of MDA, which is assessed by measuring the thiobarbituric acid assay. The significantly ( $p < 0.01$ ) greater concentration of MDA in liver and kidney of rats exposed to malathion was consistent with lipid peroxidation and the results of previous studies that have demonstrated oxidative damage in erythrocyte membranes following oral exposure to 0.13 mg malathion/kg, bw for 6 weeks (John et al., 2001). Lipid peroxidation was also observed in erythrocytes and liver of rats exposed orally to 5, 25 or 75 mg/kg, bw daily for 4 weeks. Exposure to another OP insecticide, 125 mg acephate/kg, bw caused injury to kidney tubules, which was accompanied by increased of  $H_2O_2$  production and elevated lipid peroxidation (Poovala et al., 1998). It is hypothesized that in the present study, radicals generated during metabolism of malathion by cytochrome P<sub>450</sub>-oxidase induced lipid peroxidation. Simultaneous treatment with  $\alpha$ -tocopherol and selenium in combination resulted in higher decrease in the content of MDA in liver (39.20%) and kidney (27.20%) when compared with malathion treated group. Also, pretreatment with  $\alpha$ -tocopherol alone or selenium alone resulted in higher decrease in the content of MDA in liver and kidney

when compared to the malathion treated rats. However, the significant decrease in the hepatic and renal MDA content confirms that pretreatment with  $\alpha$ -tocopherol and/or selenium could effectively protect against the hepatic and renal lipid peroxidation induced by malathion.

Supporting the present findings, malathion also significantly ( $p < 0.01$ ) decreased total protein and reduced glutathione (GSH) content in liver and kidney tissues compared with the control group. Glutathione dependent defense against xenobiotic toxicity is a multifaceted phenomenon that has been well characterized in animals. GSH plays a key role in the detoxification of the reactive toxic metabolites and liver necrosis is known to be initiated when reserves of GSH are depleted (Ozden et al., 2009). In the present study, the decrease of renal GSH level (73.52%) was more than the decrease of hepatic GSH level (37.43%) confirming that liver has more antioxidant potential than kidney. The mechanism of GSH depletion involves diminishing of the overall antioxidant potential of liver and kidneys as a result of increased lipid peroxidation following malathion treatment. However, pretreatment with  $\alpha$ -tocopherol and selenium combination significantly prevented the malathion induced depletion of GSH in liver and kidney indicating the antioxidant effect of  $\alpha$ -tocopherol and selenium.

Antioxidant enzymes, mainly SOD, CAT and GPx are the first line of defense against free radical induced oxidative stress. SOD is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to hydrogen peroxide (McCord and Fridovich, 1969). CAT is responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water (Aebi, 1984). GPx, responsible for enzymatic defense against hydrogen peroxide, is strictly linked with the concentration of GSH because it catalyses the reaction between glutathione and hydrogen peroxide,

resulting in the formation of glutathione disulphide (Paglia and Valentine, 1967). In the present study, malathion caused a significant ( $p < 0.01$ ) decrease in activities of SOD, CAT and GPx in rat liver and kidney confirming the increased production of free radicals caused by administration of malathion.

These findings are in accordance with those of Gultekin et al. (2001) who reported that MDA values increased with increasing organophosphorous compounds concentration and incubation period, but SOD and CAT activities decreased with increasing its concentration and incubation period. Also Rezg et al. (2008) reported that subchronic treatment with malathion, gradually decreased the activities of SOD and CAT in rat liver. Sodhi et al. (2008) found that the mean activity of GPx decreased progressively in chicks after receiving malathion (10 mg/kg b.w.) as compared to control. Our data show that depletion of SOD, CAT and GPx which was directly mediated by malathion, proved the role of enzymes as antioxidant, and they accelerated the conversion of superoxide radicals to hydrogen peroxide, then decomposition of hydrogen peroxide to molecular oxygen and water. In rats pretreated with  $\alpha$ -tocopherol and selenium, the activities of these antioxidant enzymes were significantly higher compared to the rats treated with malathion, and were very similar to the values noted in normal control rats. In the same respect, the effect of selenium alone on the activity of SOD and GPx in malathion treated rats [Group V] was significantly higher than the effect of  $\alpha$ -tocopherol alone (Group IV), whereas  $\alpha$ -tocopherol was highly effective on the activity of CAT than selenium. This may be attributed to the role of selenium in the active sites of SOD and GPx. As clearly demonstrated in the present study, pretreatment with  $\alpha$ -tocopherol and selenium combination significantly decreased the level lipid peroxidation, attenuated the levels of GSH and the activities of antioxidant enzymes in liver and kidney. The protective effect of  $\alpha$ -tocopherol and selenium on liver and kidney resulted from antioxidant activity including stabilization in the intracellular defense systems and reduction in the lipid peroxidation products and the production of reactive oxygen species. Thus, it may be suggested that  $\alpha$ -tocopherol and selenium, the parent antioxidants can partially quench the deleterious effects of chronic toxicity of malathion by scavenging the free radicals and ROS, as alleviation of hepatic damage by  $\alpha$ -tocopherol and selenium also tended to alleviate induced increase in the activity of serum transaminases and hypoproteinemia in a previous study (Sodhi et al., 2001).

Previously, it had been reported that  $\alpha$ -tocopherol is protective against pesticide-induced toxicity (Giray et al., 2001; John et al., 2001; Kalender et al., 2004; Atessahin et al., 2005; Sutcu et al., 2006; yousef et al., 2006; Guney et al., 2007). In addition, studies have reported that supplementation with  $\alpha$ -tocopherol and selenium can play an important role in the development and maintenance of

the defense system (Raza et al., 1997). Most studies concerning such supplementation have focused on the preventive and curative properties in diseases (Tengerdy and Nockels, 1975; Bollengier et al., 1998). Moreover, selenium is an essential element, plays an important antioxidant role, binding active site of glutathione peroxidase and thioredoxin reductase. The most important metabolic role of Se in mammalian cell occurs due to its function in the active site of selenoenzyme GPx. It also facilitates the action of  $\alpha$ -tocopherol in reducing peroxy radicals through permitting higher levels of  $\alpha$ -tocopherol to be absorbed (Machlin, 1991). Ozardali et al. (2004) found that the necrotic, fibrotic and cirrhotic changes in the liver sections were reduced after being treated with Se and they suggested that Se can diminish liver injury fibrosis and cirrhosis induced by pesticides.

In conclusion, the biochemical evaluation demonstrated that exposure to malathion results in the induction of lipid peroxidation (MDA), depletion in tissue protein and reduced glutathione (GSH) and significant reduction in antioxidant enzymes (SOD, CAT and GPx) lead to changes in antioxidant system in living cells. Moreover, results of the present study showed that pretreatment with  $\alpha$ -tocopherol and selenium may attenuate malathion-induced oxidative damage by decreasing lipid peroxidation and altering antioxidant defense system in rat liver and kidney. Thus, more attention is needed to limit the use of OP insecticides, particularly malathion, which is contained in most agricultural pesticides used in the developing countries as much as possible and reduce their entrance into the human food cycle via environmental pollution.

## ACKNOWLEDGEMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No RGP-VPP-130

## REFERENCES

- Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A (2004) Pesticides and oxidative stress: A review. *Med. Sci. Monit.*, 10: RA141-RA148.
- Aboul-Soud MAM, Al-Othman AM, El-Desoky GE, Al-Othman ZA, Yusuf K, Ahmad J, Al-Khedhairi AA (2011). Hepatoprotective Effects of Vitamin E/Selenium against Malathion-Induced Injuries on the antioxidant Status and Apoptosis-Related Gene Expression in Rats. *J. Toxicol. Sci.*, 36: 285-296.
- Aebi H (1983). Catalase. In: *Methods of Enzymatic Analysis*, Ed: Bergmeyer, H.U. Weinheim and Academic Press, 227-282
- Aebi H (1984). Catalase *in vitro*. *Methods Enzymol.*, 105: 121-126.
- Ahmed RS, Seth V, Pasha ST, Banerjee BD (2000). Influence of dietary Ginger (*Zingiber officinale* Rosc) on oxidative stress induced by malathion in rats. *Food Chem. Toxicol.*, 38: 443-450.
- Akhgari M, Abdollahi M, Kebryaezadeh A, Hosseini R, Sabzevari O (2003). Biochemical evidence for free radical-induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. *Hum. Exp. Toxicol.*, 22: 205-211.
- Aldana L, Tsutsumi V, Craigmill A, Silveira MI, Gonzalez de Mejia E

- (2001). alpha-tocopherol modulates liver toxicity of the pyrethroid cypermethrin. *Toxicol. Lett.* 125: 107–116.
- Atessahin A, Yilmaz S, Karahan I, Pirincci I, Tasdemir B (2005). The effects of vitamin E and selenium on cypermethrin-induced oxidative stress in rats. *Turk. J. Vet. Anim. Sci.*, 29: 385–391.
- Banerjee BD, Pasha ST, Hussain QZ, Koner BC, Ray A (1988). A comparative evaluation of immunotoxicity of malathion after subchronic exposure in experimental animals. *Indian J. Exp. Biol.*, 36: 273–282.
- Banerjee BD, Seth V, Bhattacharya A, Pasha ST, Chakraborty AK (1999). Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. *Toxicol. Lett.*, 107: 33–47.
- Bollengier LS, Mitchell MA, Utomo DB, Williams PEV, Whitehead CC (1998). Influence of high dietary vitamin E supplementation on egg production and plasma characteristics in hens subjected to heat stress. *Brit. Poult. Sci.*, 39: 106–112.
- Brenner L (1992). Malathion. *J. Pest. Reform.*, 12: 29–37.
- Dal-Pizzol F, Klamt F, Dalmolin RJS, Bernard EA, Moreira JCF (2001). Mitogenic signaling mediated by oxidants in retinal treated Sertoli cells. *Free Radic. Res.*, 35: 749–755.
- Desi I, Nagymajteny L, Papp A, Schulz H (1998). Experimental model studies of pesticide exposure. *Neurotoxicology*, 19: 611–616.
- Durak D, Uzun FG, Kalender S, Ogutcu A, Uzunhisarcikli M, Kalender Y (2008). Malathion-induced oxidative stress in human erythrocytes and the protective effect of vitamins C and E *in vitro*. *Environ. Toxicol.*, 24: 235–242.
- El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH (2004). Role of alphatocopherol and beta-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters, and semen quality of male rats. *J. Environ. Sci. Health B.*, 39: 443–459.
- Eskenazi B, Bradman A, Castorina R (1999). Exposures of children to organophosphate pesticides and their potential adverse health effects. *Environ. Health Perspect.*, 107: 409–419.
- Farag AT, Ewediah MH, El-Okazy AM (2000). Reproductive toxicology of acephate in male mice. *Reprod. Toxicol.*, 14: 457–462.
- Galloway T, Handy R (2003). Immunotoxicity of organophosphorus pesticides. *Ecotoxicology*, 12: 345–363.
- Garcia SJ, Abu-Qare AW, Meeker-O'Connell WA, Borton AJ, Abou-Donia MB (2003). Methyl parathion: a review of health effects. *J. Toxicol. Environ. Health B Crit. Rev.*, 6: 185–210.
- Garcia-Repetto R, Martinez D, Repetto M (1995). Malathion and dichlorvos toxicokinetics after the oral administration of malathion and trichlorfon. *Vet. Hum. Toxicol.*, 37: 306–309.
- Giray B, Gurbay A, Hincal F (2001). Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E or allopurinol. *Toxicol. Lett.*, 118: 139–146.
- Giri S, Prasad SB, Giri A, Sharma GD (2002). Genotoxic effects of malathion: An organophosphorus insecticide, using three mammalian bioassays *in vivo*. *Mutat. Res.*, 514: 223–231.
- Gokalp O, Buyukvanli B, Cicek E, Ozer MK, Koyu A, Altuntas I, Koylu H (2005). The effects of diazinon on pancreatic damage and ameliorating role of vitamins E and C. *Pestic. Biochem. Phys.*, 81: 123–128.
- Güney M, Oral B, Demirin H, Take G, Giray SG, Altuntas I, Mungan T (2007). Fallopian damage induced by organophosphate insecticide methyl parathion, and protective effect of vitamins E and C on ultrastructural changes in rats. *Toxicol. Ind. Health*, 23: 429–438.
- Gultekin F, Delibas N, Yasar S, Kilinc I (2001). *In vivo* changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. *Arch. Toxicol.*, 75: 88–96.
- Handy RD, Abd-El Samei HA, Bayomy MF, Mahran AM, Abdeen AM, El-Elaimy EA (2002). Chronic diazinon exposure: pathologies of spleen, thymus, blood cells, and lymph nodes are modulated by dietary protein or lipid in the mouse. *Toxicology*, 172: 13–34.
- Hazarika A, Sarkar SN, Hajare S, Kataria M, Malik JK (2003). Influence of malathion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. *Toxicology*, 185: 1–8.
- IARC (1983). Monograph on the evaluation of carcinogenic risk of chemicals to man. Miscellaneous pesticides. International Agency for Research on Cancer, vol. 30. Lyon France.
- John S, Kale M, Rathore N, Bhatnagar D (2001). Protective effect of Vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. *J. Nutr. Biochem.*, 12: 500–504.
- Kakkar P, Das B, Viswanathan PN (1984). A modified spectrophotometric assay of superoxide dismutase. *Ind. J. Biochem. Biophys.*, 21: 130–132.
- Kalender S, Kalender Y, Ogutcu A, Uzunhisarcikli M, Durak D, Acikgoz F (2004). Endosulfan-induced cardiotoxicity and free radical metabolism in rats: the protective effect of vitamin E. *Toxicology*, 202: 227–235.
- Kalender S, Ogutcu A, Uzunhisarcikli M, Acikgoz F, Durak D, Ulusoy Y, Kalender Y (2005). Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. *Toxicology*, 211: 197–206.
- Kalender Y, Uzunhisarcikli M, Ogutcu A, Acikgoz F, Kalender S (2006). Effects of diazinon on pseudocholinesterase activity and haematological indices in rats: the protective role of vitamin E. *Environ. Toxicol. Pharmacol.*, 22: 46–51.
- Kaneko JJ (1989). *Clinical Biochemistry of Domestic Animals*. 4th ed., Academic Press, San Diego.
- Kovacic P (2003). Mechanism of organophosphates (nerve gases and pesticides) and antidotes: electron transfer and oxidative stress. *Curr. Med. Chem.*, 10: 2705–2709.
- Machlin LJ (1991). Vitamin E. In "Handbook of Vitamins," (L.J. Machlin, ed.), 2nd Ed., Marcel Dekker, Inc., New York.
- Maroni M, Colosio C, Ferioli A, Fait A (2000). Biological monitoring of pesticide exposure: a review. *Introduction. Toxicol.*, 7: 1–118.
- McCord JM, Fridovich I (1969). Superoxide dismutase. An enzymic function for erithrocuprein (hemocuprein). *J. Biol. Chem.*, 244: 6049–6055.
- Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, 8: 95–351.
- Ozardali I, Bitiren M, Karakilcik AZ, Zerir M, Aksoy N, Musa D (2004). Effects of selenium on histopathological and enzymatic changes in experimental liver injury of rats. *Exp. Toxicol. Pathol.*, 56: 59–64.
- Ozden S, Catalgol B, Gezginci-Oktayoglu S, Arda-Pirincci P, Bolkent S, Alpertunga B (2009). Methiocarb-induced oxidative damage following subacute exposure and the protective effects of vitamin E and taurine in rats. *Food Chem. Toxicol.*, 47: 1676–1684.
- Paglia DE, Valentine WN (1967). Studies on the qualitative and quantitative characterization of erythrocyte glutathione peroxidase. *Lab. Clin. Med.*, 70: 158–169.
- Poet TS, Kousba AA, Dennison SL, Timchalk C (2004). Physiologically based pharmacokinetic/pharmacodynamic model for the organophosphorus pesticide diazinon. *Neurotoxicology*, 25: 1013–1030.
- Poovala VS, Kanji VK, Tachikawa H, Salahudeen AK (1998). Role of oxidant stress and antioxidant protection in acephate-induced renal tubular cytotoxicity. *Toxicol. Sci.*, 46: 403–409.
- Raza H, Lakhani MS, Ahmed I, John A, Morgenstern R, Montague W (1997). Tissue specific expression of glutathione S-transferases, glutathione content and lipid peroxidation in camel tissues. *Comp. Biochem. Physiol. B.*, 118: 829–835.
- Rezg R, Mornagui B, El-Fazaa S, Gharbi N (2008). Biochemical evaluation of hepatic damage in subchronic exposure to malathion in rats: effect on superoxide dismutase and catalase activities using native PAGE. *C. R. Biol.*, 331: 655–662.
- Rupa DS, Reddy PP, Sreemannaranaya OS, Reddi OS (1991). Frequency of sister chromatid exchanges in peripheral lymphocytes of male pesticide applicators. *Environ. Mol. Mutagen.*, 18: 136–138.
- Schacter GR, Pollack RL (1973). Simplified method for quantitative assay of small amounts of protein in biologic material. *Anal. Biochem.*, 51: 654–655.
- Sedláč J, Lindsay RH (1968). Estimation of total protein-bound and non protein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.*, 25: 192–205.
- Senger R, Rico EP, Arizi MB, Rosemberg DB, Dias RD, Bogo MR, Bonan CD (2005). Carbofuran and malathion inhibit nucleotide hydrolysis in zebrafish (*Danio rerio*) brain membranes. *Toxicology*, 212: 107–115.
- Sodhi S, Sharma A, Brar APS, Brar RS (2008). Effect of a tocopherol

- and selenium on antioxidant status, lipid peroxidation and hepatopathy induced by malathion in chicks. *Pestic. Biochem. Phys.*, 90: 82–86.
- Sodhi S, Sharma A, Chawla JS, Brar APS (2001). Supplementation of vitamin E and selenium in malathion toxicity in broiler chicks. *Indian J. Poult. Sci.*, 36: 100–102.
- Sutcu R, Altuntas I, Yildirim B, Karahan N, Demirin H, Delibas N (2006). The effects of subchronic methidathion toxicity on rat liver: role of antioxidant vitamins C and E. *Cell Biol. Toxicol.*, 22: 221–227.
- Tengerdy R, Nockels C (1973). The effect of vitamin E on egg production, hatchability, and humoral response of chickens. *Poult. Sci.*, 52: 778–783.
- Torres RL, Torres ILS, Gamaro GD, Fontella FU, Silveira PP, Moreira JSR, Lacerda M, Amoretti JR, Rech D, Dalmaz C, Bell'ó AA (2004). Lipid peroxidation and total radical-trapping potential of the lungs of rats submitted to chronic and sub-chronic stress. *Braz. J. Med. Biol. Res.*, 37: 185–192.
- Traber MG, Atkinson J (2007). Vitamin E, antioxidant and nothing more. *Free Radic. Biol. Med.*, 43: 4–15.
- Uzun FG, Kalender S, Durak D, Demir F, Kalender Y (2009). Malathion-induced testicular toxicity in male rats and the protective effect of vitamins C and E. *Food Chem. Toxicol.*, 47: 1903–1908.
- Vidyasagar J, Karunakar N, Reddy MS, Rajnarayana K, Surender T, Krishna DR (2004). Oxidative stress and antioxidant status in acute organophosphorous insecticide poisoning. *Indian J. Pharmacol.*, 36: 76–79.
- Yang MC, McLean AJ, Rivory LP, Le Couteur DG (2000). Hepatic disposition of neurotoxins and pesticides. *Pharmacol. Toxicol.*, 87: 286–291.
- Yavuz T, Delibas N, Yildirim B, Altuntas I, Candir O, Cora A, Karaman N, Ibrisim E, Kutsal A (2004). Vascular wall damage in rats induced by methidathion and ameliorating effect of vitamins E and C. *Arch. Toxicol.*, 78: 655–659.
- Yousef MI, Awad TI, Mohamed EH (2006). Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by vitamin E. *Toxicology*, 22: 240–247.