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Full Length Research Paper

Potential hepatoprotective effects of vitamin E and selenium on hepatotoxicity induced by malathion in rats

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The aim of this present study was to analyze the hepatotoxic effect of malathion in adult male rats and evaluate the possible hepatoprotective effect of vitamin E and/or selenium. Oral administration of malathion for 45 days significantly induced marked hepatic injury as revealed by increased activity of the plasma enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and gamma-glutamyl transferase GGT). Oral administration of vitamin E and selenium in combination with malathion exhibited a significant protective effect by lowering the elevated plasma levels of the previous enzymes. Light microscopic investigation revealed that malathion exposure was associated with necrosis of hepatocytes, marked changes of liver tissues in the form of dilated veins, hemorrhagic spots and some degenerative signs of hepatocytes. Co-administration of vitamin E and selenium with malathion to rats showed mild histopathological changes. Thus, it appears that vitamin E and/or selenium ameliorate malathion hepatotoxicity but are not completely protective.

Key words: Malathion, rats enzymes, selenium, vitamin E.

INTRODUCTION

Widespread use of pesticides in public health and agricultural programs has resulted in the pollution of water, air and food that has lead to severe acute and chronic cases of human poisoning (Moghadamnia and Abdollahi, 2002). The World Health Organization (WHO, 1997) estimating that the incidence of pesticide poisoning in developing countries has doubled in between 1987 and 1997, and one million serious accidental and two million suicidal poisonings with insecticides occur, worldwide, from which, approximately 220,000 die (WHO, 1997). International or accidental acute organophosphates pesticides (OP) poisoning is unfortunately very common and many fatal cases are reported. However, the use of OP has increased considerably due to their low potency (Moghadamnia and Abdollahi, 2002; Jalali et al., 2003). OP compounds are primarily recognized for their ability to induce toxicity in mammals through the inhibition of acetyl cholinesterase (AChE) in target tissues (Rezg et al., 2008a; Rezg et al., 2008b) and have been found to affect the mammalian reproductive system (Bustos-Obregon and Gonzales-Hormazabal, 2003).

Other organs that could be affected by OPs intoxication include liver, pancreas and kidney (Possamai et al., 2006;

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Pournourmohammadi et al., 2007; Kalender et al., 2010). As studied in hen, mouse, rat, cow and men, Malathion is highly lipid soluble and it stored in liver and other lipophilic tissues (Garcia-Repetto et al., 1995). Additionally, malathion was found to have a 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). The toxicity of OP agents may be due to, at least in part, the formation of reactive oxygen species (ROS), lipid peroxidation (LPO), which is generally assessed by an increase in the levels of thiobarbituric acid reactive substances (TBARS) (Verma and Srivastava, 2001; Hazarika et al., 2003; Cemek et al., 2010; Kalender et al., 2010; Olorunnisola et al., 2011). In addition, Malathion is a lipophilic substance; it may enhance LPO by directly interacting with the cellular plasma membrane (Hazarika et al., 2003; Yan-Bin et al., 2011) so it may damage the membranes by inducing LPO. It is evident that LPO is accompanied by an alteration (inhibition or activation) in the antioxidant defense system in different organs following acute, subchronic and chronic exposure to OP compound (Hazarika et al., 2003; Fortunato et al., 2006; Jingtao et al., 2011). Vitamin E (α-tocopherol) is the major lipid-soluble antioxidant and is known to protect cellular membranes and lipoproteins from peroxidation (Yavuz et al., 2004). Moreover, several studies have shown that α-tocopherol inhibits free radical formation (Kalender et al., 2004) and may effectively minimize lipid peroxidation in biological system (Traber and Atkinson, 2007).

Also, selenium is known to be antioxidant. It is a constituent of cytosolic enzyme glutathion peroxidase and facilitates the action of vitamin E in reducing peroxy radicals (Kaneko, 1989). In chicken, absorption of vitamin E is impaired by severe selenium deficiency and selenium alleviates vitamin E deficiencies by permitting higher level of vitamin E to be absorbed (Machlin, 1991).

The current study was undertaken to evaluate the hepatoprotective potential of vitamin E and selenium against malathion-induced hepatotoxicity.

MATERIALS AND METHODS

Chemicals

A commercial formulation of malathion (agrothion 57% EC) was purchased from local market of 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 Company (St. Louis, Mo, USA) and Merck (Darmstadt, Germany). Kits were obtained from Human GmbH, Germany.

Animals

Male Wister albino rats, weighting 200 to 250 g were obtained from animal house, College of Medicine, King Saud University. The animals were housed throughout the experiment in polypropylene cages (with each cage housing ten animals) and allowed to acclimatize to the laboratory environment for 10 days. Animals were maintained under controlled conditions of temperature at 25±2ºC, relative humidity of 50±15% and normal photoperiod (12-12 h light-dark cycle). The animals were allowed free access to standard dry pellet diet and water *ad libitum*. The 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

Fifty adult male Wister albino rats were divided into 5 groups (n = 10). Group I (Normal Control): Corn oil (0.5 ml/animal). Group II: Malathion (27 mg/kg b.wt.). Group III: Malathion (27 mg/kg b.wt.) + Vitamin E (100 mg/kg b.wt.) + Selenium (0.1 mg/kg b.wt.). Group IV: Malathion (27 mg/kg b.wt.) + Vitamin E (100 mg/kg b.wt.). Group V: Malathion (27 mg/kg b.wt.) + Selenium (0.1 mg/kg b.wt.). All medications were given orally by gastric intubation in the morning (between 09:00 AM and 10:00 AM) to non fasting animals for 45 days.

Blood sampling

On the 45th day blood samples were collected from all rats (under ether anesthesia) into heparinized tubes by puncturing their retro-orbital venous plexus with fine sterilized glass capillary tubes. Blood samples were left for 20 min at room temperature, then centrifuged at 3000 rpm for 10 min using Hereaeus Labofuge 400R, Kendro Laboratory Products GmbH, Germany, to separate the plasma. The obtained plasma samples were kept in a deep freezer (-20°C) until analyzed within 1 week for the biochemical estimations.

Biochemical analysis

The plasma activity of marker enzymes such as AST and ALT was assayed following the method of Reitman and Frankel (1957), while the activities of alkaline phosphatase enzyme (ALP) was estimated by the method of Douglas et al. (1979). The activities of LDH and GGT were estimated in plasma according to Tietz (1995) and Szasz (1969), respectively.

Effect on body weight gain and relative organ weight

Body weight of all rats was recorded at the beginning and the end of the experiment. After blood collection, the rats were sacrificed by cervical dislocation; their livers were separated and weighted individually. Then, the relative liver weight was calculated.

Histopathological examination

Liver samples were dissected and fixed in 10% neutral formalin, dehydrated in ascending grades of alcohol and imbedded in paraffin wax. Paraffin sections (5 µm thick) were stained with haematoxylin and eosin (H&E) as a routine stain. Tissue slides were examined under light microscope and scored according to Kerem et al. (2007) as follows: 0 = normal appearance, 1 = mild cellular disruption in less than 25% of field area, 2 = moderate cellular disruption of 25 to <50% of field area and 3 = extensive cell disruption in greater than 50% of field area.

Table 1. Effect of α-tocopherol and selenium on AST, ALT, ALP, GGT and LDH activities in plasma of malathion-treated rats.

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)
Group I	31.27±1.8	42.03±2.2	101.76±15.6	10.15±1.5	153.83±18.0
Group II	41.92±1.4**	52.98±0.9**	58.89±7.0**	15.43±0.9**	214.36±6.5**
Group III	32.38±1.6	41.89±1.3	102.79±19.1	11.20±0.7	155.63±14.9
Group IV	33.11±1.6	42.54±5.2	100.99±15.5	11.56±1.6	164.32±27.8
Group V	36.39±5.2	46.5±4.4	80.79±15.9	12.73±2.2	185.47±27.2

Values are expressed as Mean±S.D. **P < 0.01 relative to group I, group I – Control, Group II – Malathion Group III - Malathion+ Vit.E+ Selenium, Group IV - Malathion+ Vit.E, group V - Malathion+ Selenium.

Table 2. Body and relative organs weights of experimental rats.

Treatment	Group I	Group II	Group III	Group IV	Group V
Initial body weight (g)	243.2±1.9	245.2±2.8**	245.0±2.5	245.2±3.1	244.8±3.4
Final body weight (g)	274.0±12.2	208.0±18.3**	283.6±21.0	249.0±16.8	231.2±8.3
Body weight gain (in 45 day)	30.8±10.3	-37.2±15.5**	38.6±18.5	3.8±0.8	-13.6±4.9
Liver weight (g)	4.3±0.2	2.7±0.1**	3.9±0.1	4.1±0.2	3.2±0.1
Liver-to-body weight ratio	0.016±0.002	0.013±0.002**	0.016±0.001	0.15±0.002	0.014±0.001

Values are expressed as mean ± S.D. **P < 0.01 relative to Group I. Group I – Control. Group II – malathion Group III - malathion+ Vitamin E+ Selenium. Group IV - malathion+ Vitamin E. Group V - malathion+ Selenium.

Statistical analysis

The data were analyzed by using PASW (SPSS version 18.0) for Windows and expressed as means±SD. Paired samples t-test was used to compare between the data of the control and those of treatments significance levels were considered for p<0.01.

RESULTS

Body weight and relative liver weights

No mortality occurred during the experimental period. Data of final body weights and relative liver weights of male rats subjected to different treatments are shown in (Table 1) it was observed that malathion-treated rats achieved significant decreases (P<0.01) in body weights and relative liver weights compared to control and other treatments. Co-treatment of malathion exposed rats with vitamin E + selenium combination or vitamin E or selenium alone showed body weights and relative liver weights of no significant differences than those of control group (Table 2).

Liver dysfunction

In order to determine whether the malathion dosing (27 mg/kg b.w/day) produced toxicity to animals, our results (Table 1 and Figure 1) showed that AST, ALT, LDH and GGT activities in plasma of malathion induced rats were significantly (P<0.01) increased by 34.06, 26.05, 39.34 and 52.02%, respectively, whereas malathion dosing produced significant (P<0.01) reduction in ALP activity by 42.13% relative to the control. This evidence suggests that this malathion dose is able to induce some degree of liver toxicity. Long-term vitamin E and selenium combination treatment to Malathion induced rats significantly (P<0.01) reduced the activities of AST, ALT, LDH and GGT enzymes but significantly (P<0.01) increased the activity of ALP compared with those of Malathion treated rats. Also these enzymes activities were arrange near the control values, on the other hand, long term treatment with selenium alone to malathion induced rats (group V), was not able to reverse malathion-induced increase in plasma AST, ALT, LDH and GGT activities or decreases in ALP activity.

Moreover, selenium produced a slight reduction in AST, ALT, LDH and GGT activities and a slight elevation in ALP activity in malathion induced rats, but it were marginally significant compared to those of control (*P*= 0.07, 0.08, 0.06, 0.06 and 0.06, respectively) and significantly differences compared to those of malathion treated rats. In the same respect, long-term treatment with vitamin E to malathion induced rats (group IV) was able to reverse malathion induced increase in AST, ALT, LDH and GGT activities or decrease in ALP activity and produced significant reduction in AST, ALT, LDH and GGT activities and significant elevation in ALP activity compared to those of malathion induced rats and arranged near to those of the control activities (Table 1

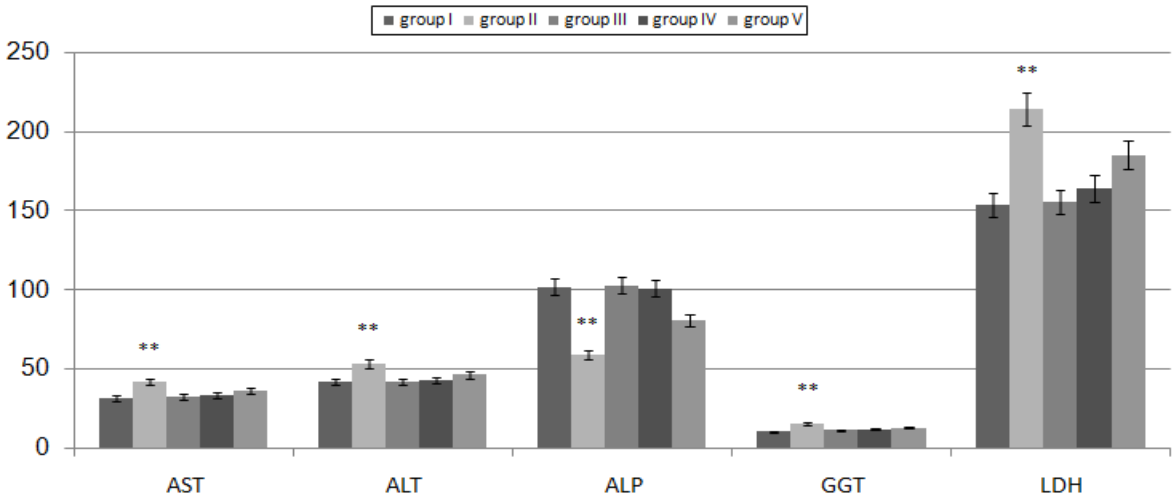

Figure 1. The effect of vitamin E and selenium on AST, ALT, ALP, GGT and LDH activities (U/L) in malathion-treated rat plasma. Values are expressed as mean ±SD. ** significantly differ from control group [group I] (p<0.01) (one way ANOVA).

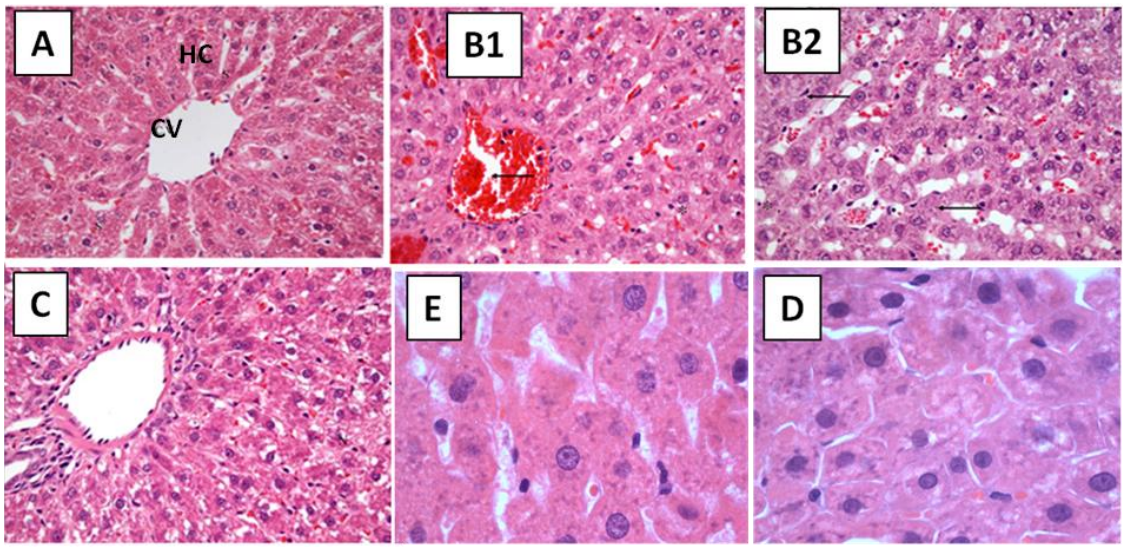

Figure 2. Paraffin sections stained by haematoxylin and eosin (H&E, X 400) for histopathological examination of hepatocytes: **A** Liver tissue of control showing normal structure, central vein (C.V.), normal arrangement of hepatic cords (H.C.), normal blood sinusoids (S) and hepatocytes; **(B1)** Liver tissue of malathion treated rats showing hemorrhage in the central vein (←), necrosis; **(B2)** Liver tissue of malathion treated rats showing dilation of blood sinusoids (←), and necrosis; and **(C)** Liver tissue of vitamin E + selenium-treated rats showing few necrosis (N). **(D)** Liver of malathion plus vitamin E treated rat showing mild necrotic changes; **E** Liver of malathion plus selenium treated rat showing moderate degenerative changes, congestion and hyperplasia.

and Figure 1).

Histopathological findings

In control rats (Group I) the histopathological examination of liver showed no pathological alterations. Liver tissues of rats showing normal structure, normal hepatocytes with normal nuclei and normal blood sinusoids appeared between the liver cords (Figure 2A). However, liver tissues of animals intoxicated with (27 mg/kg b.w) Malathion (Group II), showed vacuolization of hepatocytes, congested blood sinusoids in between the liver cords with intense mononuclear inflammatory cellular

Table 3. Histopathological changes in the liver of experimental rats, based on scoring severity of injury.

Treatment	Score average (range)	Severity
Control	0.0	Normal
Malathion	3.0	Sever
Malathion+ Vit.E+ selenium	0.0	Normal
Malathion+ Vit.E	1.0	Mild
Malathion+ selenium	2.15	Moderate

[a] Scores in terms of numerical values are mentioned in Section 2.3.3. Histopathological studies.

infiltration. Liver cells appeared swollen and areas of hemorrhage were noticed between the cells. Nuclei of the cells become flattened-shaped (Figure 2B).

The number of rats displaying histopathological changes in liver was decreased in (Group III and IV) which had received malathion plus vitamin E and selenium combination and malathion plus vitamin E alone as the rats of this group revealed only mild necrotic changes in the liver tissues (Figure 2C and D), but, in selenium plus malathion treated rats (Figure 2E), moderate degenerative changes was observed in the hepatocytes associated with congestions oedema and hyperplasia. Nuclei of the cells become flattened shaped.

Histopathologic examinations, based on scoring severity of injury in the livers of the rats are presented in Table 3. The livers of malathion-treated rats showed sever injury. In contrast, treatment with vitamin E and selenium combinations to malathion-induced rats tends to improve this injury and showed mild injury in liver,

DISCUSSION

In recent years there is wide spread concern over exposure to low levels of OPs in the diet over a long period of time. There are reports which suggest that OPs insecticides manifest their toxic effects by enhanced production of ROS which is a major cellular source of oxidative stress (Abdollahi et al., 2004; Kalender et al., 2010; Cemek et al., 2010). ROS can damage every major cellular component including membrane, lipids, carbohydrate and DNA (Altuntas and Delibas, 2002). The pathological consequence of such uncontrolled injury is widespread tissue damage (Banerjee et al., 2001; Kalender et al., 2010). The objective of this study was to evaluate the biochemical and pathological changes in hepatocytes of malathion induced rats. Also study the protective effect of vitamin E and/or selenium on liver injury induced by malathion .

Body weight and relative liver weight

In toxicological studies, organ and relative organ weights are important criteria for evaluation of toxicity (Crissman et al., 2004). In the present study, the body weight and relative liver weights of rats treated with malathion were significantly ($P<0.01$) lower than those of control group. The final body weights were decreased after malathion administration. This may be attributed to decrease food intake (anorexia or food avoidance) or poor food palatability due to treatment related toxicity. Furthermore, malathion may induce oxidative stress leading to generation of free radicals and alterations in antioxidant status or ROS which cause metabolic disorder and weight loss. For this reason, treatment with antioxidants and free radical scavengers (vitamin E and/or selenium) can decrease the oxidative stress and improve metabolic process of malathion treated rats, so improve rat food palatability, food intake and consequently their body weight and liver/body weight ratio. Also administration of vitamin E to Malathion induced rats significantly improve body weights than that of selenium which increase body weight marginally significant ($P<0.07$) compared to normal group.

Liver dysfunction

Liver play a control rule in the detoxification process and along with kidney faces the threat of maximum exposure to xenobiotics and their metabolic by-products. The susceptibility of liver tissues to this stress due to exposure to pesticides is a function of overall balance between the degree of oxidative stress and the antioxidant capacity (Khan et al., 2005). However, serum enzymes including ALP, ALT, AST, GGT and LDH are mainly used in the evaluation of hepatic damage. Results of the present study revealed that malathion treatment caused an increase in the activities of ALT, AST, GGT and LDH in serum of male rats. Whereas, there was a decrease in the activity of ALP enzyme compared to that in normal control. These results are confirmed with other studies (Khan et al., 2005; Ogutcu et al., 2008; Ncibi et al., 2008; Celik et al., 2009; Kalender et al., 2010) they mentioned that OP insecticides can elevate the enzymatic activity of ALT, AST and LDH.

Also, these results are consistent with the damage to the hepatic tissues in the malathion-treated rats seen by light microscopy. But, about ALP activity, there are

contradictory results. The previous authors found that OP increases the activity of this enzyme but in this study we found that malathion treatment decrease ALP activity. The decrease activity of ALP by malathion treatment may be attributed to food restriction or poor food palatability due to treatment related toxicity which loss of normally circulating intestinal fraction (Machlin, 1991). In this respect, the final body weight confirms this data, where malathion treatment decreased body weight and liver/body weight ratio. Awad et al. (1998) found that cell damage exhibited good correlation with the enzyme leakage. Also, the increase in serum LDH activity may be due to the hepatocellular necrosis leading to leakage of the enzyme into the blood stream (Wang and Zhai, 1988). It has been previously reported that during liver damage there was an observed decrease in anti oxidant defenses in the liver (Seven et al., 2004). The hepatic function test corroborated the histopathological lesions observed in the present study. These observations indicated marker changes in the overall histoarchitecture of liver in response to malathion, which could be due to its toxic effects primarily by the generation of ROS causing damage to the various membrane components of the cell.

Our results are confirmed by other studies conducted on OP insecticides (Wang and Zhai, 1988; Mansour and Mossa, 2005; Mansour et al., 2008). Interestingly, our results indicated that co-administration of selenium or vitamin E or combination of vitamin E and selenium to malathion intoxicated male rats reverted most of these altered biochemical parameters levels to be within normal limits and improved liver dysfunction. A partial amelioration of this damage by a combination of vitamin E and selenium or vitamin E alone or selenium alone would be attributed to antioxygenic role of selenium as a component of glutathione peroxidase (GPx) and vitamin E as a free radical scavenger and an effective inhibitor of autocatalytic process of lipid peroxidation (Sodhi et al., 2008). Ozden et al. (2009) reported that vitamin E is the most important lipophilic antioxidant and exists mainly in the cellular membranes, thus helping to maintain membrane stability. Kaneko (1989) mentioned that tissues of animals deficient in selenium and vitamin E have a greater tendency to peroxidize both *in vivo* and *in vitro* than those of animals adequate in these nutrients.

Histopathological studies

In the present study, malathion caused histopathological alterations in hepatic tissue. Free radicals produced from LPO in the liver, were probably responsible for sever tissue damage, leading to necrosis of hepatocytes, marked damage of the liver tissues in the form of dilated veins, hemorrhagic spots and some degenerative signs of the hepatocytes. These changes are entirely consistent with the changes in various biochemical parameters that were also observed. In the same respect, Gokcimen et al. (2007) mentioned that, such liver damage may arise from the toxic effect of malathion, which disturbs the detoxification mechanisms of the liver. In addition, it is possible that malathion, like several other insecticides, adversely affects the cytochrome P450 system or the mitochondrial membrane transport system of hepatocytes. These results are coincide with El-Halwgy et al. (2008) who found that administration of fenitrothion OP to the rats showed pathological signs in liver tissues varying from dilated veins, hemorrhagic spots and distorted nuclear membrane.

In this study all of biochemical and histological changes that were induced by malathion exposure were at least partially normalized when vitamin E and selenium were given together with malathion. Moreover, our light microscopic analysis revealed that the vitamin treated malathion-exposed animals did not exhibit the hepatic calcification, vacuolar degeneration and necrosis seen in the liver of the malathion treated group. Thus, vitamin E could ameliorate the liver damage induced by malathion exposure. Also, Kim and Mahan (2003) have reported a clear cut relation between reduced GPx levels, which related to selenium reduction, and development of liver necrosis. In the same aspect, Sulak et al. (2005) reported that a toxic dose of methidathion OP in rats stimulates peroxidation and dietary supplementation of vitamin E and C alleviate both peroxidation and toxic effects. These observations are in accordance with Bottje et al. (1995) who reported the protective effect of vitamin E in ameliorating oxidative stress in liver and lung of broilers. Giray (2001) observed ameliorating effect of vitamin E in quenching free radicals in liver induced by cypermethrin OP by attenuating processes leading lipid peroxidation and improve antioxidant capacity.

Conclusions

Administration of malathion in rats induce body weight and relative liver weight losses, increase the activities of ALT, AST, GGT and LDH while decrease ALP activity also caused histopathological alteration in hepatic tissues which appear in the form of necrosis and damage of liver, dilated veins, hemorrhagic spots and some degenerative signs. Co-administration of vitamin E, or selenium or combination of vitamin E and selenium to malathion induced rats improve body weights, arrange the enzyme activities near to the control and ameliorate the histopathological alteration. This may be due to the anti-oxygenic role of selenium or free radical scavenger of vitamin E. Thus it may be suggested that vitamin E and selenium, the potent 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 vitamin E and/or selenium is evident in present study.

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REFERENCES

Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezale A (2004). Pesticides and oxidative stress: a review. Medical Science Monitor. 10: RA141- RA147.

Altuntas I, Delibas N (2002). Effects of organophosphate imedicide fenthion on lipid peroxidation and antioxidant enzymes in rat erythrocytes: the role of vitamins E and C. Biomed. Res., 13: 43-47.

Awad ME, Abdel-Rahman MS, Hassan SA (1998). Acrylamide toxicity in isolated rat hepatocytes. Toxicology. 12: 699-704.

Banerjee BD, Seth V, Ahmed RS (2001). Pesticide induced oxidative stress-perspectives and trends. Rev. Environ. Health, 16: 1-40.

Bottje W, Enkvetchakul B, Moore R (1995). Effect of α-tocopherols on antioxidants, lipid peroxidation and the incidence of pulmonary hypertension syndrome (Ascites) in broilers. Poult. Sci., 74: 1356-1369.

Bustos-Obregon E, Gonzales-Hormazabal P (2003). Effect of a single dose of malathion on spermatogenesis in mice. Asian J. Androl., 5: 105-107.

Celik I, Yilmaz Z, Turkoglu V (2009). Hematotoxic and hepatotoxic effects of dichlorvos at sublethal dosages in rats. Environ. Toxicol., 24: 128-132.

Cemek M, Büyükben A, Büyükokuroğlu ME, Aymelek F, Tür L (2010).Protective roles of vitamin E (tocopherol), Selenium and vitamin E plus Selenium in organophosphate toxicity in vivo: A comparative study, Pesticide Biochemistry and Physiology. Pesticide Biochem. Phys., 96: 113-118.

Crissman JW, Goodman DG, Hildebrandt PK, Maronpot RR, Prater DA, RileyJH, Seaman WJ, Thake DC (2004). Best practice guideline: toxicologic histopathology. Toxicol. Pathol., 32: 126-131.

Douglas R, John J, Hyman B, Richard P, Paula B, Virginia H, Leonard R, Robert J (1979). Serum Alkaline Phosphatase Determination Value in the Staging of Advanced Breast Cancer. J. Am. Med. Ass., 242: 1147-1149.

El-Halwagy M, Darwish N, Zaher E (2008). Prophylactic effect of green tea polyphenols against liver and kidney injury induced by fenitrothion insecticide. Pesticide Biochem. Phys., 91: 81-89.

Fortunato JJ, Feier G, Vital AM, Petronilho FC, Dal-Pizzol F, Quevedo J (2006). Malathion-induced oxidative stress in rat brain regions. Neurochem. Res., 31: 671-678.

Garcia-Repetto R, Martinez D, Repetto M (1995). Malathion and dichlorvos toxicokinetics after the oral administration of malathion and trichlorfon. Vet. Human Toxicol., 37a: 306-309.

Giray B (2001). Cypermethrin induced oxidative stress in rat brain and liver is prevented by vitamin E or allopurinol, Toxicology Letters, 118: 139-146.

Gokcimen A, Gulle K, Demirin H, Bayram D, Kocak A, Altuntas I (2007). Effects of diazinon at different doses on rat liver and pancreas tissues. Pesticide Biochem. Phys., 87: 103-108.

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.

Jalali N, Pajoumand A, Abdollahi M, Shadnia S (2003). Epidemiological survey of poisoning mortality in Tehran during 1997-1998. Toxicol. Lett., 1: 309.

Jalali N, Pajoumand A, Abdollahi M, Shadnia S, Pakravan N (2003). Pesticides poisoning: one-year report of Loghman-Hakim Hospital Poison Center. Progress Med. Res., 1(52): 1-9.

Jingtao W, Guiwen Y, Wujun W, Fumiao Z, Jinduo Y, Liguo A (2011). Cholesterol metabolism regulation and antioxidant effect of platycodin D on hyperlipidemic emulsion-reduced rats. Afr. J. Pharm. Pharmacol., 5(22): 2444-2453.

Kalender S, Uzun FG, Durak D, Demir F, Kalender Y (2010). Malathion-induced hepatotoxicty in rats: the effects of vitamins C and E. Food Chem. Toxicol., 48: 633-638.

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. Toxicol., 202: 227-235.

Kaneko JJ (1989). Clinical Biochemistry of Domestic Animals. (4th ed.). San Diego: Academic Press. p. 24.

Kerem M, Bedirli N, Gurbuz N, Ekincl O, Bedirli A, Akkaya T, Sakrak O, Pasaoglu H (2007). Effects of acute fenthion toxicity on liver and kidney function and histology in rats, Turk. J. Med. Sci., 37: 281-288.

Khan SM, Sobti RC, Kataria L (2005). Pesticide-induced alteration in mice hepatooxidative status and protective effects of black tea extract. Clinica Chimica Acta. 358: 131-138.

Kim XY, Mahan DC (2003). Biological aspects of selenium in farm animals Asian Australian. J. Anim. Sci., 16: 433-444.

Machlin JJ (1991). Vitamin E Handbook of Vitamins. New York: McGraw Hill.

Mansour SA, Heikal TM, Mossa AH, Refa A (2008). Toxic effects of five insecticides and their mixture on male albino rats. J. Egyptian Soc. Toxicol., 39: 85-94.

Mansour SA, Mossa AH (2005). Comparative effects of some insecticides as technical and formulated on male albino rats. J. Egyptian Soc. Toxicol., 32: 41-54.

Moghadamnia AA, Abdollahi M (2002). An epidemiological study of poisoning in Northern Islamic Republic of Iran. Eastern Mediterranean Health J., 8: 1-6.

Ncibi S, Othman MB, Akacha A, Krifi MN, Zourgi L (2008). Opuntia ficus indica extract protects against chlorpyrifos-induced damage on mice liver. Food Chem. Toxicol., 46: 797-802.

Ogutcu A, Suludere Z, Kalender Y (2008). Dichlorvos-induced hepatotoxicity in rats and the protective effects of vitamins C and E. Environ. Toxicol. Pharm., 26: 355-361.

Olorunnisola OS, Bradley G, Afolayan AJ (2011). Antioxidant properties and cytotoxicity evaluation of methanolic extract of dried and fresh rhizomes of Tulbaghia violacea. Afr. J. Pharm. Pharmacol., 5(22): 2490-2497.

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.

Possamai FP, Fortunato JJ, Feier G, Agostinho FR, Quevedo J, Wilhelm Filho D, Dal-Pizzol F (2006). Oxidative stress after acute and sub-chronic malathion intoxication in Wistar rats. Environ. Toxicol. Pharm., 23: 198-204.

Pournourmohammadi S, Ostad SN, Azizi E, Hossein Ghahremani M, Farzami B, Minaie B, Larijani B, Abdollahi M (2007). Induction of insulin resistance by malathion; evidence for disturbed islets cells metabolism and mitochondrial dysfunction. Pesticide Biochem. Phys., 88: 346-352.

Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.

Rezg R, Mornagui B, El-Fazaa S, Gharbi N (2008a). Biochemical evaluation of hepatic damage in subchronic exposure to malathion in rats: effect on superoxide dismutase and catalase activities using native PAGE. Comptes Rendus Biologies, 331: 655-662.

Rezg R, Mornagui B, El-Fazaa S, Gharbi N (2008b). Caffeic acid attenuates malathion induced metabolic disruption in rat liver, involvement of acetylcholinesterase activity. Toxicol., 250: 27-31.

Seven A, Güzel S, Seymen O, Civelek S, Bolayirh M, Uncu M, Burak G (2004). Effects of vitamin E supplementation on oxidative stress in streptozotocin induced diabetic rats: investigation of liver and plasma. Yonsei Med. J., 45: 703-710.

Sodhi S, Sharma A, Brar APS, Brar RS (2008). Effect of α tocoferol and selenium on antioxidant status, lipid peroxidation and hepatopathy induced by malathion in chicks. Pesticide Biochem. Phys., 90(2): 82-86.

Sulak O, Altuntas I, Karahan N, Yildirim B, Akturk O, Yilmaz HR, Delibas N (2005). Nehrotoxicify in rats induced by organophosphate insecticide methidathion and ameliorating effects of vitamin E and C. Pesticide Biochem. Phys., 83: 21-28.

Szasz G (1969). Kinetic photometric method for serum gammaglutamyl transpeptidase. Clin. Chem., 15: 124-136.
Tietz NM (1995). Clinical Guide to Laboratory Tests. (3rd ed.). Philadelphia: WB Saunders Company. p. 384.
Traber MG, Atkinson J (2007). Vitamin E, antioxidant and nothing more. Free Radic. Biol. Med., 43: 4-15.
Verma RS, Srivastava N (2001). Chlorpyrifos induced alterations in levels of thiobarbituric acid reactive substances and glutathione in rat brain. Indian J. Exp. Bio., 39: 174-177.
Wang X, Zhai W (1988). Cellular and biochemical in bronchoalvelar lavage fluids of rats exposed to fenvalerate. Zhongguo Yaolixue YuDulixue Zoghi, 2: 271-276.
WHO (1997). Guidelines for Poison Control. WHO in collaboration with UNEP and ILO, Geneva. pp. 3-10.
Yan-Bin W, Li-Jun Z, Jun Y, Jian-Guo W, Chun-Jiang T, Ti-Qiang C, Jin-Zhong W, Ka-Hing W (2011). A comparative study on antioxidant activity of ten different parts of Nelumbo nucifera Gaertn. Afr. J. Pharm. Pharmacol., 5(22): 2454-2461.
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.