www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Renal toxicity in the adult male mice exposed to methyle parathion and protective role of lycopene

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Abstract

Parathion is an insecticide has been demonstrated to be a highly toxic compound for animals and humans. It has been used widely in agriculture and domestic. Many cases of acute poisoning have been reported over the past few decades when exposed to parathion. The present work was designed to evaluate the protective role of lycopene against renal histopathological and biochemical changes due to exposed to insecticide parathion. The animals were divided into four groups with six in each: Group I: served as control animals received saline, Group II: receive lycopene (10 mg/kg b.w) orally. Group III: received once daily methyle parathion at a dose of 0.28 mg/kg b.w. (1/50 LD_{50} oral dose). Group IV: receive once daily methyle parathion at a dose of 0.28 mg/kg b.w. plus lycopene (10 mg/kg b.w.). Histological examinations revealed that parathion caused glomerular atrophy, dilated renal tubules, haemorrhage, oedema and necrosis. Immunohistochemical localization of Bax for apoptosis was performed. Methyle parathion treated animals showed positive reaction to Bax in glomerulii and renal tubules as compared with controls. Methyle parathion treated animals showed also and increased in lipid peroxidation and decreased antioxidant enzyme, glutathione. Coa-dmnistration with lycopene decrease pathological changes, apoptosis, lipid peroxidation and increase antioxidant enzyme.

Keywords: Methyle parathion; lycopene; kidney; histopatholgy; mice

To cite this article: Abdel-Rahman GH, AM Sliai and MS Al-Harbi, 2014. Renal toxicity in the adult male mice exposed to methyle parathion and protective role of lycopene. Res. Opin. Anim. Vet. Sci., 4(12): 701-706.

Introduction

Organophosphorus compounds (OP) are widely used in agriculture as insecticides and acaricides. Wide range application of OP pesticides can lead to penetration of these pesticides into hydrological systems or contamination of food crops. Excessive use of these compounds in agricultural has caused severe environmental pollution and health hazards (Konstantinou et al. 2006). Residual amounts of organophosphate (OP) pesticides have been detected in the soil, water, vegetables, grains and different foods products. Exposure to these pesticides can induce pathological changes in liver, kidney, heart and lung. Methyl parathion (C8H10NO5PS; O, O-dimethylO-4-nitrophe-nyl phosphorothioate) is a widely used organophosphate insecticide in agriculture (WHO, 1993). It is used in order to kill the insects in different products such as corn, apple, bean, rice, heat, peach, clover and sunflower (Ruckart et al., 2004; Uzunhisarciki, 2008). Due to it potentially causing damage to both human health and the environment, its use has been restricted in many countries. Methyl parathion is a highly toxic insecticide classified by the United States EPA as a class I toxicant (EPA, 1999). Consumption of vegetables-fruits and other foods including pesticide residuals having methyl parathion cause many acute and chronic poisoning. Its toxicity profile includes convulsions, dizziness, headaches, vomiting, cardiac arrest, and even death (WHO, 1993). Methyl parathion exposure has been linked to substantial adverse health effects on several organ systems, such as the reproductive system (Uzunhisarcikli et al., 2007), and the nervous system

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(Edwards and Tchounwou, 2005). The most prominent clinical effects of poisoning with methyl parathion result from the inhibition of acetylcholinesteras (Paudyal, 2008; Bharate et al., 2010).

Plant products are known to exert their protective effects by scavenging free radicals and modulating antioxidant defence system. A large number of natural products and dietary components have been evaluated as potential antioxidant agents. Lycopene which is found in tomato and watermelon is a natural pigment and the most prevalent carotenoid in the western diet (Mordente et al., 2011). Atessahin et al. (2006), Mure and Rossman (2001) stated that lycopene with strong antioxidant activity in cell protection against free radicals. Lycopene is one of the most effective antioxidants in the carotenoid family (Amarowicz, 2011; Yonar and Sakin, 2011; Yonar, 2012). Many studies has been demonstrated the anticancer activity of lycopene, such as prostate, stomach, breast and lung cancer (Mahmooduzzafar et al., 2007; Atessahin et al., 2006). It has been suggested that lycopene can prevent carcinogenesis by protecting vital biomolecules including DNA, proteins, enzymes and lipids (Scolastici et al., 2007). Lycopene was found to be protective against chemotherapeutic-induced renal damage in several studies (Wang et al., 2010; Dogukan et al., 2011). The aim of this study was to investigate the pathological and biochemical changes of the kidney after methyl parathion administration to male mice and protective effect of lycopene.

Materials and Methods

Animals and experimental design

Sexually mature male albino mice weighing 35 ± 5 g were used. The animals were housed in cages, fed a standard laboratory diet and water *ad libitum*. The animals were exposed to a 12 h light/dark cycle at a room temperature and left to acclimatize for one week before the experiment. Mice were divided into four groups, six for each.

Group I (control group): received corn oil at a dose of 1 ml/kg (b.w.) per day was given orally via gavage once a day.

Group II (lycopene group): received orally lycopene (10 mg/kg b.w) suspended in corn oil.

Group III (Methyl parathion group): received oral doses of methyl parathion (0.28 mg/kgbw (1/50 LD50 oral dose; Gains, 1960) in corn oil via gavage.

Group IV (Methyl parathion and lycopene group): received oral doses of methyl parathion (at a dose of 0.28 mg/kg b.w plus lycopene (10 mg/kg b.w).

Histopathological examination

At the end of the experiment, animals were killed by cervical dislocation. Kidneys from animals were carefully separated and cut into small pieces. A part of tissue immediately fixed in 10% neutral buffered formalin for histopathological and immunohistochemical studies. The samples were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections (5 μm thickness) were cut and stained with hematoxylin and eosin.

Immunohistochemistry

Immunolocalization for Bax was performed using the avidin-biotin complex method. Slides were deparaffinized and blocked for endogenous peroxidase with hydrogen peroxide in methanol for 20 mm, antigen retrieval for 15 mm. The slides were allowed to cool. The monoclonal antibody was applied overnight followed by the biotinylated secondary antibody and the ABC complex. Diaminiobenzidine (DAB) was applied for 20 min at room temperature as chromogen. Slides were counterstained with hematoxylin, dehydrated, and covered by coverslip. In negative control slides, the same system was applied with replacement of the monoclonal antibody by diluted normal bovine serum. Bax immunostaining was performed using polyclonal rabbit-anti-human at a dilution of 1:50.

Oxidative stress and antioxidant enzyme assays
Lipid peroxidation

Malondialdehyde (MDA) levels have been widely used as a measure of lipid peroxidation. MDA were determined using by a method of (Ohkawa et al., 1979). Results were expressed as nmol/g tissue.

Reduced glutathione (GSH)

Kidney homogenate, reduced glutathione (GSH) was determined spectrophotmetrically according to the methods of Ellman (1959) using Ellman'sreagent [5, 50-dithio-bs- (2 nitrobenzoic acid)]. The results were expressed as µmol/g tissue.

Statistical analysis

The results are expressed as mean ± SE. The results were statistically analyzed using an ANOVA test among different experimental groups (P<0.05).

Table 1: Effect of Lycopene on methyle parathion (MP) induced changes in the Lipid peroxidation (LPO) (nmole/g tissue) and reduced glutathione (GSH) of male mice. Values represents as mean ± SE.

	Control	Lycopene	MP	MP + Lycopene
(LPO) (nmol/g tissue)	22.36 ± 2.7	18.75 ± 1.5	27.32 ± 2.3*	23.12 ± 2.03
(GSH) (umol/g tissue)	4.35 ± 0.98	4.19 ± 0.87	2.92 ± 0.62*	3.77 ± 0.45

* P<0.05 compared with the control group; P<0.05 compared with the methyle parathion group.

Results

Histological changes in the kidney

The kidney in the control and lycopene animals showed normal histological structuren glomeruli, proximal and distal tubules (Figs. 1A & B). After 4 weeks of methyl parathion exposure (Fig. 1C), most of glomeruli showed dilatation of Bowman's space with glomerular atrophy. Some of the renal tubules were degenerated and others were dilated. Also, heamorhage, edema, necrosis were observed in kidney tissues. After 4 weeks of lycopene plus methyl parathion-treatments to mouse, there are improvements in both glomerular and renal tubules pathological changes (Fig. 1D).

Immunohistochemical observation

In control and lycopene treated animals, kidney sections showed negative immune reaction to Bax (Figs. 2A&B). Methyle parathion treated animals (Fig. 2C) showed more positive reaction to Bax in glomerulii and renal tubules as compared with controls. Methyle parathion plus lycopene treated animals showed less reaction to Bax (Fig. 2D).

Oxidative stress and antioxidant enzymes

Lipid peroxidation (LPO)

The data presented in Table1 shows significant changes in the concentrations of LPO during the treatment of mice with Methyle parathion and Lycopene alone or in combination. The results showed that LPO concentration significantly increased in kidneys of mice treated with Methyle parathion ($P<0.05$) in comparison to controls. Treatment with Lycopene significantly lowered LPO concentration ($P<0.05$).

Glutathione (GSH)

The data presented in Table 1 shows significant changes in the concentrations of GSH during the treatment of mice with Methyle parathion and Lycopene alone or in combination ($P<0.05$). The GSH content decreased in the Methyle parathion treated group. However, the GSH content was significantly increased in mouse treated with Methyle parathion along with Lycopene as compared to the Methyle parathion group ($P<0.05$).

Discussion

Organophosphate insecticides are some of the most useful classes of insecticides in use for almost five decades in agriculture. It has been reported that OP compounds induce oxidative or toxic stress in animals and humans, both in acute and chronic poisonings (Ghafour et al., 2007). Pesticides cause various histopathological and cytopathological changes in the male rat kidney (Solecki, et al., 1996; Sulak et al., 2005). In the present study, methyl parathion administration to mice resulted in oxidative stress by significant decrease in the mean values of glutathione and significant increase in MDA levels when compared with control. However, pre-treatment with lycopene supplementation resulted in modulation of these measured parameters (but still not as controls. These results are in agreement with Suke et al. (2008) who stated that when rats exposed to methyl parathion, leads to increase MDA and decrease GSH levels. The present results also are in accordance with many authors who reported that methyl parathion decreased the levels of glutathione and increase MDA levels (Monteiro et al., 2006; Guney et al., 2007; Kalender et al., 2007). Increase of lipid peroxidation and free radicals seem to be the main causes of kidney damage induced by methyl parathion. Methyl parathion has toxic effects on the mammals it also has toxic effects on fishes, birds and invertebrates (Solecki et al., 1996; Fanta et al., 2003). In the present study, methyl parathion caused increase of MDA level. The increase of MDA level in this study is an indicator of free radical formation caused by methyl parathion in kidney tissue of mice.

The results of the present study demonstrated that mice treated for 4 weeks with methyl parathion showed many histopathological changes such as the glomerular atrophy and dilated renal tubules. These findings are in accordance with Kalender et al. (2007) who stated that methyl parathion in the rate of 1/50 LD50 caused pathological alterations (necrosis, infiltration and glomerular atrophy in kidney tissues after 4 and 7 weeks administration. The present results are also in agreement with Dikshith et al. (1991) who found that the toxicity of repeated dermal applications of MP to rats induced degenerative changes in the liver and kidney. It was found that MP, as a result a single-exposure, was the most hazardous tested organophosphate showing definite pathology in the livers of treated rats.

Apoptosis is a common feature of renal toxicity induced by chemicals or drugs. The Bax gene was the first identified pro-apoptotic member of the Bcl-2 protein family. In this study, parathion treatment produced high Bax protein expression. This is in agreement with Garcia et al. (2003) in which methyl parathion through oral, dermal and inhalation exposure pathways. Inspection of the data obtained from the present work regarding the histopathology and biochemistry displayed the occurrence of good improvement by lycopene against methyl parathion-induced kidney damage.

In summary mice exposed to methyl parathion showed marked oxidative stress as well as kidney injuries at the end of the 4th week. Treatment with

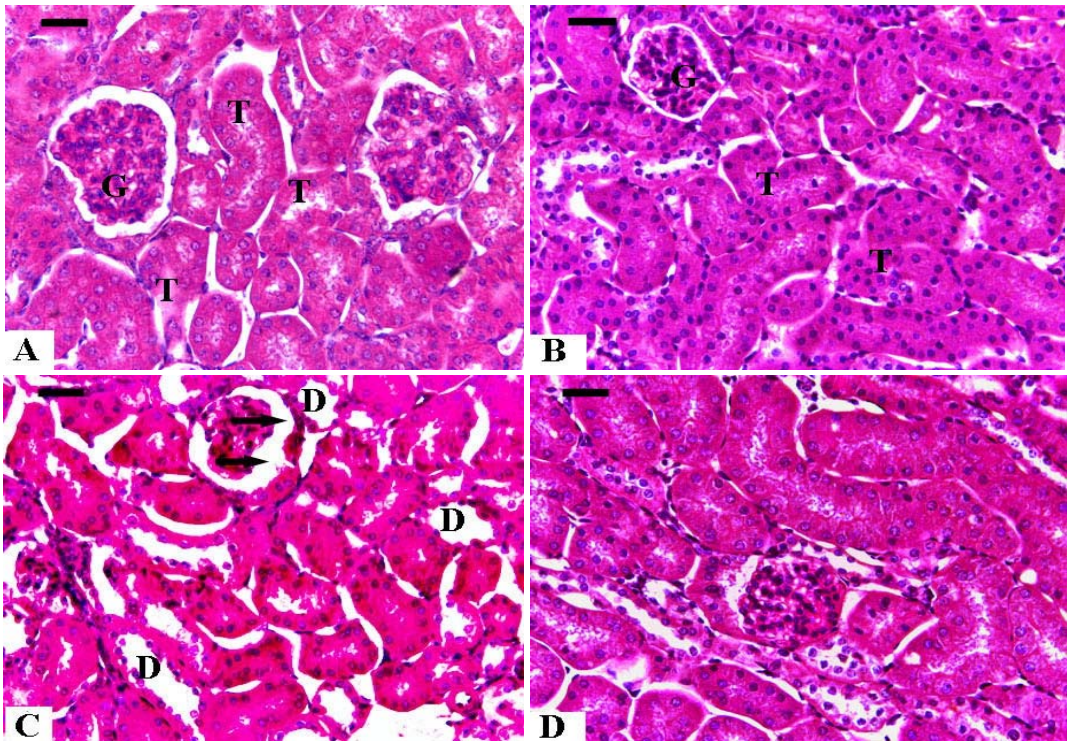

Fig. 1: (A&B) Kidney section of control and lycopene treated mice showing normal glomeruli (G) and tubules (T). (C) 4 weeks after methyl parathion treatment to mice illustrating glomerular atrophy (arrows) and tubules dilatation. (D) 4 weeks after methyl parathion plus lycopene treatment. Scale Bar = 25 um.

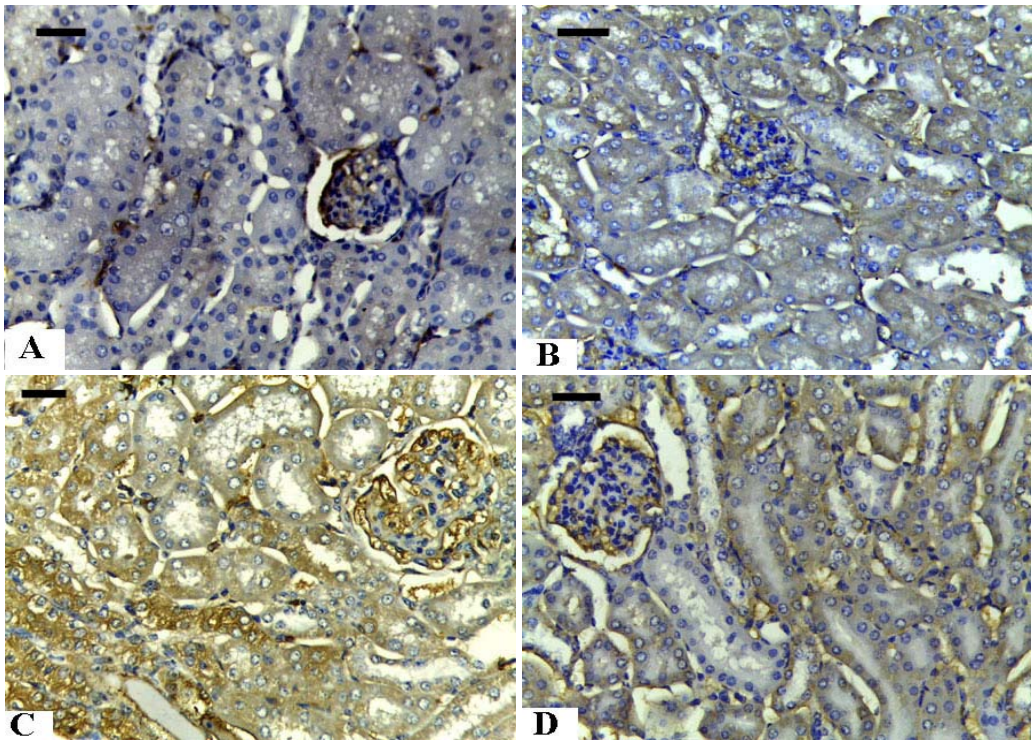

Fig. 2: Immunohistochemical localization of Bax in the renal tissue of mice. (A&B) Control and lycopene treated animals kidney sections. (C) mice treated with methyl parathion. Sections appeared with staining of Bax. (D) mice treated with methyl parathion plus Lycopene. Scale Bar = 25 um.

lycopene provided protective effects against this toxicity. Immunohistochemical study using Bax antibody showed that lycopene can decrease apoptosis caused by methyl parathion.

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