LYCOPENE PROTECTS AGAINST DELTAMETHRIN-INDUCED OXIDATIVE RENAL DYSFUNCTION IN RATS

ABSTRACT:
Deltamethrin is α-cyano pyrethroid insecticide used extensively in pest control. Although initially thought to be least toxic, a number of recent reports showed its toxicity in mammalian and non-mammalian laboratory and wildlife animal species. Lycopene is the pigment that gives tomatoes their red color and is one of four main carotenoids normally found in human blood and tissue. The present study was carried out to investigate the effects of lycopene on deltamethrin-induced toxicity in the kidney of rats. In experimental rats, oral administration of deltamethrin (1.28 mg/kg/day) for 30 days significantly induced the renal damage which was evident from the increased levels of serum urea, uric acid and creatinine. A markedly increased levels of lipid peroxidation markers (thiobarbituric acid reactive substances and lipid hydroperoxides) and protein carbonyl contents with significant (p < 0.01) decrease in non-enzymatic antioxidants (total sulfhydryl groups, reduced glutathione, vitamin C and vitamin E) and enzymatic antioxidants (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) as well as elevation in the enzymatic activity of caspase-3 in the renal tissue were also observed in deltamethrin-treated rats. Co-administration of lycopene (1 mg/kg/day) along with deltamethrin resulted in a reversal of deltamethrin-induced biochemical changes in kidney accompanied by a significant decrease in lipid peroxidation and an increase in the level of renal antioxidant defense system. The histopathological studies in the kidney of rats also showed that lycopene administration markedly reduced the toxicity of deltamethrin and preserved the normal histological architecture of the renal tissue. The present study suggest that the nephroprotective potential of lycopene in deltamethrin toxicity might be due to its antioxidant properties which could be useful for achieving optimum effects in deltamethrin-induced renal damage.

KEY WORDS:
Deltamethrin, lycopene, kidney, oxidative stress

INTRODUCTION:
The use of synthetic pyrethroids as insecticidal and anti-parasitic formulations has markedly increased in the last two decades (Yousef et al., 2006). The main advantages of their use are their photostability, high efficacy at low concentrations, easy disintegration and low toxicity to birds and mammals (Maund et al., 1998). It constitute a unique group of insecticides having pyrethrum like structures with better performance characteristics and account for over 30% of insecticides used globally (Prasanthi et al., 2005). Based on the symptoms produced in animals, pyrethroids fall into two distinct classes: types I & II. While type I pyrethroids affect sodium channels in nerve membranes, producing repetitive neuronal discharge and prolonged negative after-potential, type II pyrethroids produce even longer delay in sodium channel inactivation leading to a persistent depolarization of the nerve membrane without repetitive discharge. They are more hydrophobic in nature (Michelangeli et al., 1990) and their target site is biological membrane. In addition, type II syndrome implicates the central nervous system, while type I involve the peripheral nerves (Lawrence and Casida, 1982).

Deltamethrin is a type II synthetic pyrethroid with strong insecticidal activity (Manna et al., 2004). Deltamethrin is an α-cyano pyrethroid with the chemical structure: (S-α-cyano-3-phenoxybenzyl-1R-cis-3,82,2-dibromovinyl)-2,2 dimethyl cyclo propane carboxylate (Csillik et al., 2000). Deltamethrin is globally used in crop protection and control of malaria and other vector borne diseases (Barlow et al., 2000). It has a potent insecticidal activity with an appreciable safety margin (Mestres and Mestres, 1992). However, a number of studies have demonstrated genotoxic, immunotoxic and tumorigenic effects of deltamethrin in mammalian and non-mammalian species (Husain et al., 1996; Shukla et al., 2001). Since intended use of deltamethrin involves spraying in the crop fields to control insect pests and impregnation of bednets to ward off the mosquitoes, concern has been expressed also about aquatic ecotoxicological implications of its use. Studies
describing the oxidative stress mechanisms in pyrethroid-induced toxicity are limited. Few reports have demonstrated the induction of oxidative stress by pyrethroids such as cypermethrin and fenvalerate (Giray et al., 2001; El-Demerdash et al., 2004; Prasanthi et al., 2005).

Induction of oxidative stress is one of the main mechanisms of many pesticides action (Abdollahi et al., 2004). The damage to membrane lipids, protein and DNA is the endpoint biomarker of oxidative stress-inducing effects of pesticides (Parvez and Raisuddin, 2005). Estimation of free radical generation and antioxidant defense has become an important aspect of investigation in mammals during the past few years. Many recent studies were carried out to evaluate the potential role of antioxidant, such as, Vitamin C, Vitamin E, isoflavones and folic acid (El-Demerdash et al., 2004; Yousef, 2006; Ogutcu et al., 2008) for the protection of cells against oxidative damage due to pesticides toxicity. Lycopene, an aliphatic hydrocarbon, is one of the 600 known naturally occurring carotenoids. It is the pigment that gives tomatoes their red color and is one of four main carotenoids normally found in human blood and tissue. There is about 5 mg of lycopene per 100 gram of ripe tomato fruit (Stahl and Sies, 1992). Although not considered an essential nutrient, research has shown that lycopene may have various benefits for human health. Recently, it was found that lycopene has attracted attention due to efficient antioxidant properties and free radical scavenging capacity (Velmurugan et al., 2004). It also known to decrease the risk of age-related chronic diseases, such as cancer (Rao et al., 2007) and cardiovascular disease (Sesso et al., 2004).

However, the mechanisms of deltamethrin-induced kidney injury and nephroprotective effects of lycopene are not yet completely understood. The main goal of this study was to investigate the possible nephroprotective mechanisms of lycopene against deltamethrin-induced oxidative damage in rat kidney and to explore the mechanism of its action.

MATERIAL AND METHODS:

Chemicals:
Technical grade deltamethrin (98.8% pure) was purchased from Mitchell Cotts Chemicals, West Uorkshyer, UK. The LD<sub>50</sub> of deltamethrin when given orally to rats was reported to be 128 mg/kg BW (Worthing, 1983). The tested dose of deltamethrin was 1.28 mg/kg BW (1/100 LD<sub>50</sub>). Lycopene was obtained from Sigma Chemical Co., St-Louis. Mo., USA. The dose 1mg/kg BW of lycopene was used. The doses of deltamethrin and lycopene used in this study were based on the efficacy of these drugs in rats (Kim, 1995; Yousef et al., 2006).

Animals and Administration Schedule of Deltamethrin and Lycopene:
Forty male Sprague–Dawely rats weighing 120–140 g were obtained from the animal house of Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Animals were housed ten per cage and kept on commercial standard pellet diet and tap water provided ad libitum. The rats were maintained under standard laboratory conditions (Temperature 24 ± 2ºC natural light-dark cycle). All animals received human care and this study complies with the instruction's guidelines.

After two weeks of acclimation, the groups were assigned at random to one of the following treatments: group 1 served as control were administrated vehicle (0.2 ml corn oil) orally, while groups 2–4 were orally treated with deltamethrin (1.28 mg/kg BW dissolved in corn oil), deltamethrin (1.28 mg/kg BW) plus lycopene (1mg/kg BW) and lycopene (1 mg/kg BW dissolved in saline solution), respectively. The animals of group 3 received lycopene orally shortly prior to the administration of deltamethrin. Rats were orally treated with repeated doses of deltamethrin and lycopene daily for 30 days.

After the experiment termination, rats were fasted overnight. Blood samples were taken by cardiac puncture into tubes without adding anticoagulant and were sacrificed by cervical decapitation under ether anesthesia. The serum obtained after centrifugation (1000 gm for 10 min at 4ºC) was used for further biochemical analysis.

The kidney was removed quickly, weighed and placed in ice-cold 0.9% NaCl solution, perfused with the physiological saline solution to remove blood cells, blotted on filter paper, then the removed kidney was minced and homogenised (10%, w/v) in appropriate buffer (pH 7.4) and centrifuged (3000 gm for 10 min). The resulting clear supernatant was used for various enzymatic and non-enzymatic biochemical assays. Ten rats from each group were sacrificed and used for analysing serum and tissue biochemical assays.

Biochemical Parameters:
Determination of Lipid Peroxidation and Protein Carbonyl Contents:
Lipid peroxidation in the renal tissue was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARSSs) and lipid hydroperoxides as described by Niehias and Samuelsson (1968) and Jiang et al. (1992), respectively. As a hallmark of protein oxidation, total protein carbonyl content was determined in the kidney by a spectrophotometric method described by Levine et al. (1990) and expressed as nmol/mg protein.
Measurement of Non-Enzymatic Antioxidants:

Reduced glutathione (GSH) was determined by the method of Moron et al. (1979) based on the reaction with Ellman’s reagent (19.8 mg dithionitrobenzoic acid in 100 ml of 0.1% sodium citrate). Total sulfhydryl (TSH) groups in the kidney homogenate were measured after the reaction with dithionitrobenzoic acid using the method of Ellman (1959). Vitamin C and vitamin E concentrations were measured by the methods of Omaye et al. (1979) and Desai (1984), respectively.

Assay of SOD activity:

Superoxide dismutase (SOD) kits were purchased from biodiagnostic commercial kits (Qu et al., 2009). SOD activity was expressed as units of SOD activity per milligram of protein.

Assay of CAT Activity:

Catalase (CAT) activity was assayed by the method of Aebi (1984). In brief, to a quartz cuvette, 0.85 ml of the phosphate buffer (50 mmol/l; pH 7.0) and 50 µl sample were added, and the reaction was started by addition of 0.3 ml of 30 mM hydrogen peroxide (H₂O₂). The decomposition of H₂O₂ was monitored spectrophotometrically at 240 nm at 25°C. CAT activity was calculated as nM H₂O₂ consumed/min/mg of tissue protein.

Assay of GPx Activity:

The glutathione peroxidases (GPx) activity assay was based on the method of Paglia and Valentine (1967). Tert-Butylhydroperoxide was used as substrate. The assay measures the enzymatic reduction of H₂O₂ by GPx through consumption of reduced glutathione (GSH) that is restored from oxidized glutathione GSSG in a coupled enzymatic reaction by GR. GR reduces GSSG to GSH using NADPH as a reducing agent. The decrease in absorbance at 340 nm due to NADPH consumption was measured in a quartz cuvette, 0.65 ml of the phosphate buffer (50 mmol/l; pH 7.0) and 50 µl sample were added, and the reaction was started by addition of 0.3 ml of 30 mM hydrogen peroxide (H₂O₂). The decomposition of H₂O₂ was monitored at 240 nm at 25°C. GPx activity was calculated as nM H₂O₂ consumed/min/mg of tissue protein.

Measurement of Serum Urea, Uric Acid and Creatinine Levels:

The levels of urea, uric acid and creatinine in serum were estimated spectrophotometrically using commercial diagnostic kits purchased from Stanbio laboratory Co. USA.

Caspase-3 Enzymatic Activity Assay:

The caspase-3 enzymatic activity in the nuclear fraction was carried out by caspase-3 colorimetric kit (Kuhad and Chopra, 2009). The analysis was done according to the manufacturer’s instructions. The results were expressed as ng/mg protein.

Histopathological Examinations:

The kidneys were excised after killed. The tissues were fixed in 10 % formalin, embedded in paraffin wax, sectioned at 5 mm and were stained with haematoxylin and eosin. Tubular injury was defined as tubular necrosis, tubular dilation, tubular degeneration and thickened basement membranes. Briefly, only cortical tubules were included in the following scoring system: 0, no tubular injury; 1, ≤ 10% of tubules injured; 2, 10–25% of tubules injured; 3, 26–50% of tubules injured; 4, 51–75% of tubules injured and 5, ≥75% of tubules injured (Liu et al., 2010)

Statistical Analysis:

All statistical analyses were performed using the SPSS software, version 11.5. A one-way analysis of variance (ANOVA; P < 0.01) was used to determine significant differences between groups and the individual comparisons were obtained by Turkey’s HSD post hoc test. Statistical significance was set at P ≤ 0.01.

RESULTS:

Effects of Lycopene on Levels of Lipid Peroxidation Products in Deltamethrin-Treated Rat Kidney:

The levels of renal TBARS, lipid hydroperoxides and protein carbonyl contents were significantly (p < 0.01) increased in deltamethrin-treated rats by 76.42%, 57.83%, and 149%, respectively as compared with vehicle controls (Table 1). Interestingly, lycopene decreased the levels of these parameters in the kidney of lycopene + deltamethrin rats by 38.48%, 19.84%, and 45.39%, respectively as compared to the deltamethrin-treated rats. There was no significant difference in level of these parameters among the control group, the lycopene group and the lycopene + deltamethrin group.

Table 1. Effect of lycopene on deltamethrin-induced alterations in the levels of lipid peroxidation, lipid hydroperoxides and protein carbonyl content in kidney of control and experimental rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TBARS (mg/g tissue)</th>
<th>Hydroperoxides (mmol/g tissue)</th>
<th>Protein carbonyls (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>± 0.25</td>
<td>± 0.06</td>
<td>± 0.25</td>
</tr>
<tr>
<td>Deltamethrin (1.28 mg/kg)</td>
<td>± 0.45**</td>
<td>± 0.08</td>
<td>± 0.41**</td>
</tr>
<tr>
<td>Deltamethrin + Lycopene (1 mg/kg)</td>
<td>± 0.18**</td>
<td>± 0.06**</td>
<td>± 0.24**</td>
</tr>
<tr>
<td>Lycopene (1 mg/kg)</td>
<td>± 0.22**</td>
<td>± 0.05**</td>
<td>± 0.17**</td>
</tr>
</tbody>
</table>

Values are mean ± SE for ten rats in each group. *p<0.01, compared with the control group; **p<0.01, compared with the deltamethrin-treated group.
Effects of Lycopene on Levels of Non-Enzymatic Antioxidants in Deltamethrin-Treated Rat Kidney:

As shown in table 2, levels of reduced glutathione, total sulfhydryl groups, and vitamin C were markedly decreased (p < 0.01) in rats treated with deltamethrin by 43.83%, 34.22%, and 42.06%, respectively as compared with that in the vehicle controls. However, the level of vitamin E showed insignificant decrease by 9.45% in deltamethrin-treated group. Administration of lycopene along with deltamethrin significantly (p < 0.01) increased the levels of non-enzymatic antioxidants in kidney. In addition, there was no significant difference in the levels of these non-enzymatic antioxidants among the control group, the lycopene group and the lycopene + deltamethrin group.

Table 2. Effect of lycopene on deltamethrin-induced alterations in the levels of non-enzymatic antioxidants status in kidney of control and experimental rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH (µg/mg protein)</th>
<th>TSH (µg/mg protein)</th>
<th>Vitamin C (µmol/mg tissue)</th>
<th>Vitamin E (µmol/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.81 ± 0.37</td>
<td>14.17 ± 1.12</td>
<td>1.26 ± 0.07</td>
<td>0.74 ± 0.05</td>
</tr>
<tr>
<td>Deltamethrin (1.28 mg/kg)</td>
<td>2.14 ± 0.21**</td>
<td>9.32 ± 0.76**</td>
<td>0.73 ± 0.05**</td>
<td>0.67 ± 0.02**</td>
</tr>
<tr>
<td>Deltamethrin + Lycopene (1 mg/kg)</td>
<td>3.11 ± 0.28##</td>
<td>11.12 ± 0.83##</td>
<td>0.93 ± 0.06##</td>
<td>0.45 ± 0.02##</td>
</tr>
<tr>
<td>Lycopene (1 mg/kg)</td>
<td>4.25 ± 0.31##</td>
<td>15.23 ± 1.38##</td>
<td>1.40 ± 0.07##</td>
<td>0.81 ± 0.05##</td>
</tr>
</tbody>
</table>

Values are mean ± SE for ten rats in each group. **p<0.01, compared with the control group; ##p<0.01, vs. deltamethrin-treated group.

Effects of Lycopene on Activities of the Antioxidant Enzyme in Deltamethrin-Treated Rat Kidney:

In order to determine whether lycopene can attenuate the oxidative damage in the kidney of deltamethrin-treated rats, the activities of major antioxidant enzymes, including SOD, CAT and GPx in rat kidney were measured. The results showed that lycopene renewed the activities of these antioxidant enzymes in the kidney of deltamethrin-treated rats (Fig. 1). In deltamethrin-treated rat, renal SOD activity was significantly (P<0.01) decreased by 42.57% as compared with vehicle controls. However, the decreased activity of GPx in deltamethrin-treated rats was significantly (P<0.01) elevated (77.41%) by lycopene (Fig. 1C). Interestingly, there was no significant difference in renal activities of SOD, CAT and GPx among the control group, the lycopene group and the lycopene + deltamethrin group.

Effects of Lycopene on Serum Urea, Uric Acid, and Creatinine Levels in Deltamethrin-Treated Rats:

The levels of serum urea, uric acid and creatinine were considered to serum biochemical markers of renal damage. In order to determine whether lycopene can
attenuate the kidney damage in the deltamethrin-treated rat, the levels of serum urea, uric acid and creatinine were measured (Fig. 2). In deltamethrin-treated rats, the levels of serum urea, uric acid and creatinine significantly \((P < 0.01)\) increased by 50%, 109%, and 83.87% as compared with vehicle controls, respectively. However, the levels of serum urea, uric acid and creatinine in the rats cotreated with deltamethrin and lycopene decreased by 17.47%, 45.65%, and 36.84% as compared with the deltamethrin-treated rats, respectively. However, there was no significant difference in levels of serum urea, uric acid and creatinine among the control group, the lycopene group and the lycopene + deltamethrin group (Fig. 2).

**Effect of Lycopene on the Level of Caspase-3 in the Kidney of Deltamethrin-Treated Rats:**

As shown in figure 3, caspase-3 activity was significantly elevated in the deltamethrin-treated rat kidney. In the present study, it was found that lycopene markedly decreased the activity of caspase-3 in deltamethrin-treated rat kidney. In addition, there was no significant difference in renal activity of caspase-3 among the control group, the lycopene group and the lycopene + deltamethrin group.

**Effects of Lycopene on Histology of the Kidney of Deltamethrin-Treated Rats:**

The normal kidney possess a number of nephrons, the nephrons is made up of renal tubules and renal corpuscles (Fig. 4A). The renal corpuscles consist of a cluster of capillaries (glomerulus) surrounded by Bowman’s capsule. The microscopic picture of the glomerulus showed capillary space covered by endothelial cells on the inner side, which in their lumen contained blood cells. The space between capillaries is filled with mesangial cells. On the inner side of Bowman’s capsule, epithelial cells are found. The convoluted renal tubules are lined by tall columnar cells with a weak eosinophilic cytoplasm. Examination of kidney of animals treated with deltamethrin revealed many histological changes. Table 3 shows the percentage of tubular changes in different experimental groups. The glomeruli of deltamethrin-treated animals showed mesangial matrix expansion (Fig. 4B) and compressed capillaries. Furthermore, degeneration, necrosis \((p<0.05)\) of renal tubules and thickened basement membrane \((p<0.05)\) are evident. Some tubules appears empty, other show separation of the epithelial cells from its membrane causing a wide space in between the renal tubules. In another sections, the glomerulus shows frequent shrinkage (Fig. 4C) and destruction increase of mesangial tissue and widening of Bowman’s space. In addition, severe damage of the renal tubules displaying cytoplasmic vacuolation of their lining epithelia. However treatment with
Lycopene ameliorated the tubular necrosis, tubular degeneration and thickening of basement membrane. However it remained unaffected on tubular dilation (Fig. 4D). There were no histopathological alteration observed in kidney of rats treated with lycopene alone (Fig. 4E) when compared to control.

Table 3. Score for tubular injury in kidney of control and experimental rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tubular necrosis</th>
<th>Tubular degeneration</th>
<th>Tubular dilation</th>
<th>Thickened basement membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Deltamethrin (1.28 mg/kg)</td>
<td>3.56 ± 0.24**</td>
<td>4.21 ± 0.20**</td>
<td>2.74 ± 0.32**</td>
<td>2.23 ± 0.32**</td>
</tr>
<tr>
<td>Deltamethrin + Lycopene (1 mg/kg)</td>
<td>2.04 ± 0.32##</td>
<td>2.23 ± 0.42##</td>
<td>2.31 ± 0.22</td>
<td>0.91 ± 0.27##</td>
</tr>
<tr>
<td>Lycopene (1 mg/kg)</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

Values are mean ± SE for ten rats in each group. **p<0.01, compared with the control group; ##p<0.01, vs. deltamethrin-treated group.

DISCUSSION:

The kidney is a highly specialized organ that maintains the internal environment of the body by selectively excreting or retaining various substances according to specific body needs (Liu et al., 2010). Fundamentally, the main mechanism of the toxic effects of pesticides involves the formation of a high level of free radicals. Deltamethrin is metabolised in the liver through hydrolytic ester cleavage by cytochrome P450’s and the oxidative route (Eraslan et al., 2007). The induction in the levels of thiobarbituric acid-reactive substances (the marker of extent of...
lipid peroxidation), lipid hydroperoxides and protein carbonyl content observed in the present study after orally administered deltamethrin exposure suggesting the increased renal oxidative stress, which has been previously reported by Sayeed et al. (2003) and El-Demerdash et al. (2004) in deltamethrin-intoxicated rats. In most of the in vivo toxicity studies in mammals oral route of administration of deltamethrin has been employed, as gastrointestinal tract is the main site of its absorption. Oxidative damage has been recognized as one of the primary causes of subcellular toxicity of pesticides (Banerjee et al., 2001). Studies on pyrethroid insecticides have also suggested a putative role for free radicals in LPO and other oxidative stress-mediated injuries (Sayeed et al., 2003). LPO is caused by the action of reactive oxygen species. ROS also cause damage to DNA and proteins resulting in various harmful consequences (Abdollahi et al., 2004). El-Gohary et al. (1999) reported deltamethrin-induced LPO and nitric oxide (NO) production in plasma of rats and its role in testicular apoptosis. Moreover, Li et al. (2005) advocated a role for oxidative stress in deltamethrin-induced neurotoxicity. Manna et al. (2004) showed that deltamethrin exposure (15 mg/kg for 30 days) led to 13-fold increase in LPO in rat liver. ROS have been implicated in the pathogenesis of renal injury by direct cellular toxicity, partly through liberation of vasoconstrictor-bioactive lipids and inactivation of nitric oxide (Banerjee et al., 2001). In addition, oxidized LDL is injurious to renal tubular epithelial cells and may contribute to tubulo-interstitial disease (Agarwal et al., 1996). Also, Abdollahi et al. (2004) suggested that increased level of protein carbonylation in deltamethrin toxicity cause oxidative modification of many enzymes.

The current data show a marked influence of lycopene on renal lipid peroxidation and protein carbonyl content as evidenced by decrease TBARS and lipid hydroperoxide. The inhibition of LPO by lycopene is mainly attributed to the ability of scavenger free radicals (Velmurugan et al., 2004). Carotenoids are well known as highly efficient scavengers of singlet-oxygen ($O_2^*$) and other excited species. During $O_2^*$ quenching, energy is transferred from $O_2^*$ to the lycopene molecule, converting it to the energy-rich triplet state. Trapping of other ROS, like OH$^-$, NO$_2^-$ or peroxynitrite, in contrast, leads to oxidative breakdown of the lycopene molecule. Thus, lycopene may protect in vivo against oxidation of lipids, proteins and DNA (Stahl and Sies, 2003; Wertz et al., 2004). Lycopene has been shown to have the highest antioxidant activity among the carotenoids in cell protection against hydrogen peroxide and nitrogen dioxide radicals. In this line, Hosomi et al. (2007) reported that HgCl$_2$-induced increase of lipid peroxidation in kidney was prevented by lycopene. In addition, lycopene has been reported to attenuate oxidative stress and exert anticancer effects both in vitro and in vivo (Jonker et al., 2003; Wertz et al., 2004).

Glutathione is a crucial component of the antioxidant defense mechanism and it functions as a direct reactive free-radical scavenger (Romão et al., 2006). The decreased level of renal GSH in deltamethrin-treated rats of the present study may be due to enhanced utilization during detoxification of deltamethrin. GSH and other thiol-containing proteins play a very crucial key role in cellular defense against pesticidal toxicity. It is well established that the non-enzymatic antioxidants such as vitamin C and vitamin E concomitantly decreased along with GSH in pyrethroid toxicity (Prasanthi et al., 2005). It has been suggested that the depletion of intracellular sulphhydryl group (TSH) by deltamethrin is the prerequisite for ROS generation as well as disruption of intracellular organelles (Valko et al., 2005). In consistence with this in the present study a significant decrease in the level of non-enzymatic antioxidants in deltamethrin toxicity could lead to increased susceptibility of the renal tissue to free-radical damage. The antioxidant ability of lycopene to inhibit radical generation could further reduce the oxidative stress elicited by deltamethrin, which could prevent the further consumption of endogenous non-enzymatic antioxidants and increase their levels in the renal tissue. In this line, Bhuveneswari et al. (2001) suggest that lycopene-induced increase in the levels of GSH in the buccal pouch mucosa treated with DMBA.

Antioxidant enzymes can also protect cellular compounds against damage induced by free radicals. SOD, CAT and GPx are important antioxidant enzymes (Boots et al., 2008). The SOD decomposes superoxide radicals ($O_2^{**-}$) and produce $H_2O_2$. $H_2O_2$ is subsequently removed to water by CAT in the peroxisomes, or by GPx oxidizing GSH in the cytosol (Dröge, 2002; Lee and Choi, 2003). So the activities of these enzymes have been used to assess oxidative stress in cells (Fan et al., 2009). Many studies have shown that the excessive ROS induced by pyrethroid would break the balance between ROS production and antioxidant defenses. The antioxidant enzymes may be exhausted and its concentration depletions (Eraslan et al., 2007). In the present study, the activities of antioxidant enzymes in rat kidney, including SOD, CAT and GPx, were dramatically decreased by the treatment of deltamethrin. It indicated that deltamethrin induced the oxidative stress by the inhibiting the activities of antioxidant enzymes. The decrease in the activities of these antioxidant enzymes is in agreement with the results of Kale et al.
(1999) who showed oxidative stress and alteration in antioxidant enzymes in erythrocytes and kidney of pyrethroid intoxicated rats. As reported in literature, this may be the result of $O_2^-$ production or a direct action of pesticides on the synthesis of the enzyme (Oruc and Uner, 2000). Yu and Nguyen (1996) reported that alkyl or aryl halogen groups contained in the molecules of pyrethroid insecticides can be attacked by peroxidases (GPxs). Kostaropoulos et al. (2001) found that the molecule of GPx binds pyrethroid insecticides in a sequestering mechanism adding to the defense of the organism either as a passive way of detoxification or as a facilitating one. Therefore, it is reasonable to suggest that the GPx functions as a binding protein contributing to the action of other enzymes involved in degradation of pyrethroids. The health benefits of lycopene including the antioxidative effects are still under evaluation. Interestingly, the present study revealed that lycopene could markedly increase the activities of those antioxidant enzymes in the kidney of deltamethrin-treated rats. It suggested that lycopene could at least partly attenuate oxidative stress by renewing the activities of those antioxidant enzymes in deltamethrin-treated rat kidney. Lycopene is one of the most potent singlet oxygen quenchers, which suggests that it may have comparatively stronger antioxidant properties than other major plasma carotenoids (Di Mascio et al., 1989). According, Hosomi et al. (2007) reported that glutathione peroxidase and catalase activities were enhanced, while superoxide dismutase activity was depressed in HgCl$^2$-treated rats when compared to control and these effects were prevented by lycopene. Moreover, Atessahin et al. (2005) reported that lycopene administration produced amelioration in biochemical indices of nephrotoxicity in both plasma and kidney tissues as MDA, GSH, GPx and CAT activities when compared to cisplatin, which induced nephrotoxicity and oxidative stress in rat.

Elevated blood urea is correlated with an increased protein catabolism in mammalian body or from more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production (Rodwell, 1979). Also, the high levels of blood urea results from either increased breakdown of tissue or dietary or impaired excretion (Bush, 1991). In consistent with these explanations, the present results showed that total protein concentrations were decreased in kidney of animals treated with deltamethrin, as urea is the end product of protein catabolism (Rodwell, 1979). The presented study indicated that treatment with deltamethrin caused significant increase in serum creatinine and urea. This indicates diminished ability of the kidneys to filter these waste products from the blood and excrete them in the urine. An abnormally elevated blood creatinine is a specific and sensitive indicator of impaired kidney function (Cameron, 1996). These changes in urea and creatinine levels may indicate a reduction in the glomerular filtration rate (GFR) as a result of deltamethrin intoxication, since the serum concentration of these two parameters depends largely on the glomerular filtration (Gavin, 1995). On the other hand, lycopene administration for 30 days caused improvement in kidney parameters. The efficiency of lycopene treatment to improve the serum concentrations of urea, uric acid and creatinine was provoked. It has been well established that lycopene attenuates the nephrotoxicity induced by several toxicants such as CCl$^4$ and lead (Moawad, 2007). Also, Kim (1995) found that carotenoids (alpha-carotene, beta–carotene, lycopene and lutein) have protective effects on oxidant-induced injury of kidney in rats and suppression of serum uric acid. Supporting this explanation, Karahan et al. (2005) found that lycopene as a novel natural antioxidant might have protective effects against gentamicin-induced nephrotoxicity and oxidative stress in rats.

Cell apoptosis or necrosis can inevitably affect glomerular filtration rate and endothelial function, resulting in renal failure (Bonegio and Lieberthal, 2002; de Vries et al., 2003). Several reports suggest that DNA becomes an easy target for both apoptosis and necrosis, and the stability of the cellular genomic apparatus is constantly challenged by a wide-spectrum of environmental toxicants which generate DNA lesions (Hickey et al., 2001). Moreover, high ROS concentrations contribute to the apoptotic cell death whenever they are generated in the context of the apoptotic process (Xu et al., 2008; Franco et al., 2009; Liu et al., 2010). Caspase-3 is one of the key executioners of apoptosis, capable of cleaving or degrading many key proteins such as nuclear lamins, fodrin, and the nuclear enzyme poly(ADPribose) polymerase (PARP) (Lawen, 2003; Kaul et al., 2003). In order to determine whether lycopene can attenuate the oxidative DNA damage and apoptosis in the kidney of deltamethrin-treated rats, the activity of caspase-3 was also examined. Based on determination of the activity of caspase-3 in the present study, caspase-3 activity was significantly elevated in the deltamethrin-treated rat kidney, suggesting that the caspase-3 activity was involved in the apoptosis pathway induced by deltamethrin (Hossain et al., 2005). Whereas, the activity of caspase-3 in the kidney of deltamethrin + lycopene treated rats was markedly decreased as compared with deltamethrin-treated rats. Thus, it suggests that lycopene has a potential nephroprotective effect against deltamethrin-induced DNA damage and apoptosis by its antioxidant ability.
The histopathological observation in deltamethrin-treated rats showed tubular necrosis, tubular degeneration, swelling of tubules and vacuolization in kidney of treated animals. This could be due to the accumulation of free radicals as the consequence of increased lipid peroxidation by deltamethrin in the renal tissues. Previous reports have suggested that lycopene reduced the histological alterations caused by cisplatin-induced nephrotoxicity (Atessahin et al., 2005). The present study also reported that lycopene significantly reduces the histological changes induced by deltamethrin.

In conclusion, this study demonstrates that lycopene has the protective effect against deltamethrin-induced oxidative damage in the kidney of rats. The mechanisms contributing to its effectiveness involves the quenching of free radicals and antioxidant ability of lycopene. Recently much attention has been focused on the protective biochemical functions of naturally occurring antioxidants in biological systems against environmental toxicants. The present study therefore provides biological evidence supporting the usefulness of lycopene against deltamethrin-induced toxic oxidative stress on the kidney tissue of rat.

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