Assessment the Ameliorative Effect of Pomegranate and Rutin on Chlorpyrifos-ethyl-Induced Oxidative Stress in Rats

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Abstract: Oxidative stress is one of the possible mechanisms resulted from organophosphate toxicity. Therefore, the aim of this study is to evaluate the in vivo effects of chlorpyrifos-ethyl (CPF; 16.4 mg / kg / day body weight), on the serum and tissues antioxidant system of male Sprague Dawily rat and the efficacy of pomegranate peel extract (P; 500 mg/ kg/ day body weight) and rutin (R; 50 mg/ kg/ day body weight) as polyphenols to antagonize this response. The parameters were cholinesterase (ChE), acid phosphatase (ACP) and protein thiol (PrTh) in serum. Levels of malondialdehyde (MDA) as a marker of lipid peroxidation (LPO), reduced glutathione (GSH), glutathione-S-transferase (GST) and catalase (CAT) were estimated in liver, brain and kidney tissues. In addition, the activities of lysosomal enzymes (acid phosphatase, cathepsin D and RNase) in the liver were measured as early apoptosis marker. Administration of CPF orally by gavage for two weeks induced a significant increase in serum ACP activity, LPO levels and liver lysosomal enzyme s. Associated inhibitions in serum ChE activity and PrTh level were detected to CPF exposure. Also, results showed significant decreases in GSH content, GST and CAT activities in liver, brain and kidney. Supplementation with P or R to treated animals was significantly (P< 0.01) attenuated the toxicity and oxidative stress evoked by CPF. [Nature and Science. 2009;7(10):49-61]. (ISSN: 1545-0740).

Keywords: Oxidative stress, chlorpyrifos-ethyl, antioxidant system, pomegranate peel extract, rutin, acid phosphatase, cathepsin D, RNase

Introduction

Organophosphates (OPs) act mainly as acetylcholinesterase inhibitors (AChE) and can be an indicator of chronic toxicity of Ops (Tinoco and Halperine, 1998). Oxidative stress is one of the possible mechanisms that could be involved in the OPs toxicity. Oxidative stress is known to be a key factor in several diseases and was reported as a result of OPs exposure in human and experimental animals (Abou-Donia, 2003; Abdollahi et al., 2004; Milatovic et al., 2006; Dettbarn et al., 2006). Chlorpyrifos is a member of the most commonly used organophosphorus insecticide. As a result of widespread use, residues of chlorpyrifos have been detected in the air (Cattani et al., 2001) and in the crops (Sun et al., 2006; Atif Randhawa et al., 2007) which considered a risk for living organisms (Zhao et al., 2006). Chlorpyrifos is in particular chlorpyrifos-ethyl (CPF), resulted in deleterious effects including hepatotoxicity, genotoxicity, teratogenicity, immunotoxicity as well as neurochemical and neurobehavioural alterations (Thrasher et al., 1993; Bagchi et al., 1995; Song et al., 1998; Dam et al., 1999; Gomes et al., 1999; Hunter et al., 1999). Previous studies has been shown that there is a correlation between acetylcholine inhibition and lipid peroxidation level following subchronic exposure to OPs (Ranjar et al., 2002; Akhgari et al., 2003). Exposure to chlorpyrifos increased levels of lipid peroxides in the rat liver, kidney, brain, and erythrocytes (Bagchi et al., 1995; Gultekin et al., 2001; Verma and Srivastava, 2001; Oncu et al., 2002; Tuzmen et al., 2008) and altered antioxidant enzymes in rat blood, liver, and lung (Bebe and Panemangalore, 2003). Moreover, administration of CPF to pregnant rats induced oxidative stress and altered antioxidant system in liver, kidney, brain, and fetus (Zama et al., 2007).

Lysosomes are membrane bound structures that contain hydrolitic enzymes capable of degrading most of the cellular constituents. They are essential for controlled intracellular pathways such as autophagy, heterophagy and endocytosis. Apoptosis or programmed cell death which follows from moderate oxidative stress is preceded by partial lysosomal rupture and such lysosomal destabilization seems to be an initial event in apoptosis caused by a variety of other agents (Brunk et al., 2001). Exposure to organophosphorus insecticides has been shown to inhibit all cytoplasmic proteases and some of the lysosomal proteases in the liver tissue; the major site of insecticides metabolism (Mantle, 1997).

Polyphenols are one of the most abundant groups in plant which have a high antioxidant activity. Phenolic compounds are a biologically active group of phytochemicals. They are
classified according to their chemical structure into flavonoids, phenolic acids, coumarins, and tannins (Tapiero et al., 2002; Mennen et al., 2005). Because of its potent antioxidant activity, pomegranate considers one of the commonly used natural antioxidants. Pomegranate fruit, juice, and peel extracts is a rich source of polyphenols and hence posses a potent antioxidant properties (Gil et al., 2000; Noda et al., 2003a; b). Murthy et al., 2002 added that methanolic extract of the peel has shown a higher antioxidant potential than that of seeds isolated antioxidants have been tested (Cedra et al., 2002). The effectiveness and safety of its antioxidant properties (Gil et al., 2000; Noda et al., 2002; Murthy et al., 2002; Singh et al., 2005). Because of its potent antioxidant activity, pomegranate considers one of the commonly used natural antioxidants. Pomegranate fruit, juice, and peel extracts is a rich source of polyphenols and hence posses a potent antioxidant potential than that of seeds and could prevent CCl4-induced hepatotoxicity. Recently, study by Elha lwagy et al., 2008 shown that supplementation with (60 mg/animal) green tea polyphenols, partially attenuate oxidative stress resulted from the toxic effect of fenitrothion insecticide, on the liver and kidney of rats. Also, tea polyphenols have been shown to protect against liver injury in animals intoxicated with chlorpyrifos insecticide (Khan and Kour 2007).

Flavonoids are a family of phenolic compounds (Harborne, 1986). It has become increasingly popular in terms of health protection because they possess a remarkable spectrum of biochemical and pharmacological activities. Flavonoids affect basic cell function such as growth, differentiation and apoptosis. Also, they were shows to be potent antioxidant because of their radical-scavenging activity; ability to complex heavy metal ion and to antagonize a broad spectrum of enzymes such as tyrosine protein kinase (Akiyama et al., 1987; Hollman et al., 1996; Knект et al., 2002; Mira et al., 2002). Rutin, a flavonoid, has shown pharmacological benefits including anti-tumor (Deschner et al., 1991), anti-inflammatory (Aleksandrov et al., 1986), anti-diarrhoeal (Di Carlo et al., 1993), anti-mutagenic (Bear and Teel, 2000), myocardial protecting (Pozin et al., 1996), immunomodulator (Chen et al., 2000) and hepatoprotective activities (Janbaz et al., 2002). Literature revealed that rutin increase of antioxidant capacity in the kidney of normal rats (Gao et al., 2002) as well as in a liposomal model (Nagasawa et al., 2003). Moreover, treatments of diabetic rats with rutin inhibit lipid peroxides while total protein and reduced glutathione were increased (Kamalkannan and Stanely, 2006). Based on our knowledge on free radicals and their involvement in several diseases (Hogg, 1998) and the dependence on traditional medicine to replace ineffective medications, in this context, more research should be conducted to investigate the effectiveness of natural antioxidants (Madhavi and Salumkhe, 1995). The aim of this study was to investigate the ameliorative effect of pomegranate peel extract and rutin on toxicity, oxidative stress and apoptosis induced by chlorpyrifos-ethyl intoxication in male rats.

**Material and methods**

**Plant Extraction**

Pomegranate fruit peel was purchased from local market, dried and powdered. Amount of 500 g of the powdered plant material was extracted three times with ethanol (80%). The extracts were filtered, concentrated and freeze dried. The residue yielded was stored at 4 ºC for further analysis.

**Chemicals**

Chlorpyrifos-ethyl (CPF) [O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridal) phosphorothioate] 40% emulsion concentrate (CariboTM, Egychem) was purchased from local market and reconstituted to 1% solution in distilled water. Rutin, reduced glutathione, 1-chloro-2, 4-dinitrobenzene (CDNB), bovine serum albumin and 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB), were purchased from Sigma (St Louis, MO, USA). All solvents used were HPLC grade (Merck, Darmstadt, Germany).

**Animal**

Sprague Dawily rats (200±30g) were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR), Egypt. The animals were kept under standard laboratory conditions of light/dark cycle (12h /12h) and temperature (25 ± 2°C). They were provided with a nutritionally adequate standard laboratory diet.

**Experimental Design**

Sixty rats were treated according to the standard procedures laid down by OECD guidelines 407 (1992) repeated dose 28 days oral toxicity study in rodents . They are randomly allocated to six groups of six rats each as follow:

Group 1 received distilled water by gavage orally and acts as control.

Group 2 received CPF (16.4 mg / kg / day bw) orally by gavage.

Group 3 received P (500 mg / kg / day bw) orally by gavage.

Group 4 received P (500 mg / kg / day bw) and CPF (16.4 mg / Kg / day bw) orally by gavage.

Group 5 received R (50 mg / kg / day bw) orally by gavage.

Group 6 received R (50 mg / kg / day bw) and CPF (16.4 mg / Kg / day bw) orally by gavage.

**Sampling**
The animals were sacrificed by cervical decapitation after one and two weeks of exposure. Blood was collected and the separated serum was used for the estimation of ChE, ACP activities and PrTh content. Liver, brain and kidney tissues were removed quickly, washed in cold isotonic saline and homogenized in 50 mM phosphate buffer (pH 7) using an electronic homogenizer to prepare 10% W/V homogenate. The homogenate was centrifuged at 3000 rpm for 10 min at 4°C by cooling centrifuge (Sigma 3K 30) to separate the nuclear debris. The supernatant were used for biochemical analysis.

Isolation of liver lysosomal fraction

A portion of liver homogenate was centrifuged at 4°C and 1000 xg for 10 min. The supernatant was centrifuged at 16,000 xg for 20 min to obtain lysosomal fraction. The residual pellets were then resuspended in phosphate buffer, pH 7.4 (Galvin Jr. et al., 1980).

Biochemical Analysis

The procedure used for the determination of cholinesterase activity in serum is a modification of Ellman et al. (1961) method as described by Gorun et al., 1978. Enzymatic activity of serum acid phosphatase was determined according to Moss (1984) using readymade kits by QCA, Spain. While serum protein-SH was measured by spectrophotometric method using 5’-5’-dithio-bis-2-nitrobenzoic acid according to Motchink et al., 1994. Lipid peroxidation (LPO) was measured by estimation of thiobarbituric acid reactive substances (TBRAS) by the method of Ohkawa et al., 1979. Reduced glutathione (GSH) and glutathione -S-transferase (GST) contents in tissues were measured by the methods of Ellman, 1959; Habig et al. 1974 respectively. Catalase (CAT) activity was estimated by the method of Takahara et al., 1960. Protein was assayed by the method of Lowry et al (1951) using bovine serum albumin as standard. The activities of lysosomal enzymes (acid phosphatase, cathepsin D and RNase) were measured according to the method of Barrett and Heath (1977); Gianetto and De Duve (1955).

Statistical Analysis

Data were expressed as mean ± standard error (SE). Differences in experimental groups were determined by one-way analysis of variance (ANOVA) followed by Dunnett’s t test. $P<0.01$ was considered to be statistically significant.

Results

Serum parameters

The present results revealed that chlorpyrifos ethyl-treated rats showed a significant ($P<0.01$) increase in the activity of acid phosphatase (ACP) when compared with the normal rats. On the other hand, associated inhibition in ChE activity and Prth level were detected to CPF exposure. Supplementation with pomegranate peel extract (P) significantly countered this effect; while supplementation with rutin (R) significantly ($P<0.01$) restored ACP activity near the control values (Table 1).

LPO level

Exposures to CPF (16.4 mg/kg body weight) up to two weeks elicited a significant increase in lipid peroxidation of liver, brain and kidney of rat as measured by the estimation of thiobarbituric acid reactive substances (TBRAS). After one week, the increase in LPO levels ranged from 256.3%, 167.6% and 219.6%, in the liver, brain and kidney respectively. While after two weeks, the levels estimated as 391.9%, 317.1% and 252.1%, in the same tested tissues. Co-administration of pomegranate or rutin significantly ($P<0.01$) modulated this increase. However, treatment with P and R showed a noticeably decrease in LPO levels comparing to the CPF -treated group. Thus this study shows that both of the P and R protected cells from oxidative damage by decreasing LPO levels elevated as a result of CPF treatment as showed in Figures 1, 2 and Table 2.
**Table (1):** Effect of pomegranate peel extract or rutin on serum activities of some enzyme as well as protein-thiol level induced by chlorpyrifos ethyl intoxication.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Time</th>
<th>ChE (µmol SH/hr/ml)</th>
<th>ACP (U/L)</th>
<th>Prth (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>W1</td>
<td>247.02±15.12</td>
<td>41.05±2.07</td>
<td>926.47±12.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>308.76±17.34</td>
<td>39.98±1.41</td>
<td>955.59±40.58</td>
</tr>
<tr>
<td></td>
<td>CPF</td>
<td>W1</td>
<td>174.94±7.45</td>
<td>50.70±1.12</td>
<td>494.41±35.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>191.52±7.27</td>
<td>49.27±1.72</td>
<td>535.00±17.92</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>W1</td>
<td>212.34±10.16b</td>
<td>37.43±1.58</td>
<td>1044.62±78.53b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>256.68±14.14</td>
<td>40.32±1.51b</td>
<td>1025.59±55.6b</td>
</tr>
<tr>
<td></td>
<td>P+CPF</td>
<td>W1</td>
<td>176.82±15.58</td>
<td>34.60±1.79</td>
<td>836.47±67.00b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>250.2±19.51b</td>
<td>36.54±2.13b</td>
<td>836.76±66.99b</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>W1</td>
<td>225.96±11.11b</td>
<td>37.41±2.24b</td>
<td>836.18±46.88b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>253.44±10.38b</td>
<td>37.94±2.24b</td>
<td>836.76±66.99b</td>
</tr>
</tbody>
</table>

**Table (2):** Effect of pomegranate peel extract or rutin on some oxidative stress parameters induced by chlorpyrifos ethyl intoxication in kidney.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Time</th>
<th>LPO (nmol/ mg protein)</th>
<th>GSH (nmol/ mg protein)</th>
<th>CAT (nmol/min/mg protein)</th>
<th>GST (nmol/min/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>W1</td>
<td>1.40±0.09</td>
<td>4.73±.015</td>
<td>172.10±1.92</td>
<td>199.80±3.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>1.20±0.10</td>
<td>4.86±0.16</td>
<td>175.20±2.60</td>
<td>200.30±2.01</td>
</tr>
<tr>
<td></td>
<td>CPF</td>
<td>W1</td>
<td>5.10±0.12a</td>
<td>2.40±0.06a</td>
<td>109.70±3.61a</td>
<td>115.00±3.62a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>6.10±0.23a</td>
<td>4.12±0.21b</td>
<td>98.20±2.21a</td>
<td>97.60±4.83a</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>W1</td>
<td>0.96±0.05b</td>
<td>5.00±0.07b</td>
<td>169.80±2.70b</td>
<td>156.90±5.13b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>0.90±0.06b</td>
<td>1.96±0.10a</td>
<td>172.30±4.91b</td>
<td>176.20±5.98 b</td>
</tr>
<tr>
<td></td>
<td>P+CPF</td>
<td>W1</td>
<td>2.70±0.13b</td>
<td>3.94±0.08b</td>
<td>138.10±1.34b</td>
<td>197.40±4.65b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>2.50±0.13b</td>
<td>5.28±0.11b</td>
<td>145.20±2.61b</td>
<td>201.10±3.12b</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>W1</td>
<td>0.94±0.09b</td>
<td>5.24±0.12b</td>
<td>170.50±1.87b</td>
<td>190.80±5.66 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>0.80±0.06b</td>
<td>5.44±0.15b</td>
<td>175.30±5.30b</td>
<td>200.80±4.93b</td>
</tr>
<tr>
<td></td>
<td>R+ CPF</td>
<td>W1</td>
<td>2.10±0.09b</td>
<td>3.62±0.17b</td>
<td>129.70±2.75b</td>
<td>172.40±1.84b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>1.90±0.11b</td>
<td>3.92±0.15b</td>
<td>132.40±4.27b</td>
<td>184.10±3.24b</td>
</tr>
</tbody>
</table>

**Table (3):** Effect of pomegranate peel extract or rutin on some lysosomal enzymes induced by chlorpyrifos ethyl intoxication in liver.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Time</th>
<th>ACP (nmol/min/ mg protein)</th>
<th>Cathepsin D (U/ mg protein)</th>
<th>RNase II (U/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>W1</td>
<td>0.48±0.01</td>
<td>35.6±1.14</td>
<td>0.36±0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>0.51±0.02</td>
<td>36.9±0.89</td>
<td>0.38±0.01</td>
</tr>
<tr>
<td></td>
<td>CPF</td>
<td>W1</td>
<td>0.98±0.04a</td>
<td>74.0±1.75a</td>
<td>1.07±0.04a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>1.34±014a</td>
<td>82.1±2.22a</td>
<td>1.42±0.08a</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>W1</td>
<td>0.45±0.02b</td>
<td>35.4±1.86b</td>
<td>0.38±0.02b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>0.53±0.01b</td>
<td>36.1±1.49b</td>
<td>0.39±0.01b</td>
</tr>
<tr>
<td></td>
<td>P+CPF</td>
<td>W1</td>
<td>0.68±0.02b</td>
<td>52.1±1.33b</td>
<td>0.72±0.01b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>0.63±0.02b</td>
<td>49.7±0.93b</td>
<td>0.61±0.01b</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>W1</td>
<td>0.45±0.02b</td>
<td>33.8±1.47b</td>
<td>0.38±0.01b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>0.50±0.01b</td>
<td>35.5±1.38b</td>
<td>0.37±0.02b</td>
</tr>
<tr>
<td></td>
<td>R+ CPF</td>
<td>W1</td>
<td>0.64±0.02b</td>
<td>53.1±0.83b</td>
<td>0.71±0.01b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W2</td>
<td>0.53±0.03b</td>
<td>48.4±2.3b</td>
<td>0.61±0.02b</td>
</tr>
</tbody>
</table>

n=6; values are expressed as mean
a indicates significant difference against control group at P <0.01
b indicates significant difference against chlorpyrifos-ethyl treated group (CPF) at P <0.01.

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Figure 1: Effect of pomegranate peel extract (P) or rutin (R) on LPO content in liver of rats-treated with chlorpyrifos-ethyl (CPF).

Figure 2: Effect of pomegranate peel extract (P) or rutin (R) on LPO content in brain of rats-treated with chlorpyrifos-ethyl (CPF).

* Significant difference is indicated by \(^* P < 0.01\) when compared with control group (C). \(^b P < 0.01\) indicates significant difference when compared with chlorpyrifos-methyl-treated group (CPF).

**GSH Level**

In order to evaluate endogenous antioxidant system, glutathione levels were examined. Figures 3, 4 and Table 2 showed the decrease in GSH levels in the same previous tissues after treatment with CPF recording its maximum value at the second week. While administration of P extract or R along with CPF significantly increase GSH levels \((P<0.01)\) compared to CPF-group.

Figure 3: Effect of pomegranate peel extract (P) or rutin (R) on GSH level in liver of rats-treated with chlorpyrifos-ethyl (CPF)
**CAT activity**

Highlights the activity of the scavenger enzyme (CAT) in the same tissues, it was clearly demonstrated that tissues CAT inhibited significantly (P<0.01) as a result of CPF treatment when compared to the controls. Administration of P or R (500 mg/ kg , 50 mg/ kg respectively) along with CPF caused a significant increase (P<0.01) in CAT activity as compared to CPF-treated rats and restored its activity near that of the control (Figures 5, 6 and Table 2).

![Brain GSH](image1)

**Figure 4:** Effect of pomegranate peel extract (P) or rutin (R) on GSH level in brain of rats-treated with chlorpyrifos-ethyl (CPF).

* Significant difference is indicated by $^a P<0.01$ when compared with control group (C). $^b P<0.01$ indicates significant difference when compared with chlorpyrifos-methyl-treated group (CPF).

![Liver CAT](image2)

**Figure 5:** Effect of pomegranate peel extract (P) or rutin (R) on CAT activity in liver of rats-treated with chlorpyrifos-ethyl (CPF).

![Brain CAT](image3)

**Figure 6:** Effect of pomegranate peel extract (P) or rutin (R) on CAT activity in brain of rats-treated with chlorpyrifos-ethyl (CPF).

* Significant difference is indicated by $^a P<0.01$ when compared with control group (C). $^b P<0.01$ indicates significant difference when compared with chlorpyrifos-methyl-treated group (CPF).
**GST activity**

A consistent and significant decrease was observed in GST activity (GSH dependent antioxidant enzyme) in the tissues of rats treated with CPF. Supplementation with P or R significantly countered this decrease which restored GST values close to the control level as shown in Figures 7, 8 and Table 2.

![Liver GST](image)

**Figure 7:** Effect of pomegranate peel extract (P) or rutin (R) on GST activity in liver of rats-treated with chlorpyrifos-ethyl (CPF).

![Brain GST](image)

**Figure 8:** Effect of pomegranate peel extract (P) or rutin (R) on GST activity in brain of rats-treated with chlorpyrifos-ethyl (CPF).

* Significant difference is indicated by $^a P<0.01$ when compared with control group (C). $^b P<0.01$ indicates significant difference when compared with chlorpyrifos-methyl-treated group (CPF).

**Lysosomal Enzymes activities**

Effect of CPF treatment on liver lysosomal enzymes (acid phosphatase, cathepsin D, and RNase II) was presented in Table (3). It concluded that CPF treatment increases the activities of the three enzymes significantly ($P<0.01$) compared to control group. Administration of P or R along with CPF significantly ($P<0.01$) ameliorated the effect of CPF when compared to pesticide treated group.
Discussion

Accumulated data from in vitro and in vivo studies shown that the primary mechanism of action and most acutely life threaten effect of OPs insecticides are related to accumulation of acetylcholine within the cholinergic synapses resulting inhibition of acetyl cholinesterase by active oxon metabolites (Milesson et al., 1998; Karanth et al., 2006; Gokcimen et al., 2007; Elhalwagy and Zaki, 2009). The present study conform the pervious finding, CPF treatment reduced ChE activity as a marker of OPs toxicity. Pomegranate peel extract and rutin combined treatments restored ChE activity near to control. The increase of serum lipid peroxidation and defense mechanisms in rats. The obtained results show that CPF may have properties to induce oxidative stress indicated by enhancement of MDA production, decrease in GSH content, GST and CAT activities in rat tissues. The increase of free radicals and lipid peroxidation may result from the inhibition of GSH levels induced by CPF toxicity. The present findings are in agreement with other investigations indicating that accumulation of lipid peroxides has been resulted after exposure to acute dose of chlorpyrifos in rat liver (Bagshi et al., 1995), kidney (Oncu et al., 2002), brain (Gultekin et al., 2001), and erythrocytes (Verma and Srivastava, 2001). However, repeated doses increased LPO levels as well as antioxidant enzymes in blood, liver, and lung of rat (Ahmed et al., 2000; Bebe and Panemangalore, 2003; Akhgari et al., 2003). In vitro studies have been reported that accumulation of lipid peroxidation in human erythrocytes (Gultekin et al., 2000) and PC12 cells (Qiao et al., 2005). Supplementation with antioxidants effectively suppressed the oxidative damage induced by OPs (Ahmed et al., 2000; Gupta, 2001; Gultekin et al., 2001; Karaöz et al., 2002; Oncu et al., 2002; Camkayali et al., 2005; Elhalwagy et al., 2008). Antioxidant activity of pomegranate is referred to its polyphenolic capacity such as ellagic acid and ellagittannins (Seeram et al., 2005), which may suggest its role as an electron donor in scavenging free radicals (Kaur et al., 2006). Previous investigation revealed the ability of pomegranate fruit extract (Sudheesh and Vijayalakshmi, 2005; Noda et al., 2002) and peel extract (Singh et al., 2002) to suppress lipid peroxidation. Pretreatment with pomegranate flower for a period of one week significantly protected against oxidative damage and hepatic injury induced by Ferric nitrolotriaacetate (Fe-NTA) by modulation of LPO levels and GSH content as well as antioxidant enzymes CAT and GST (Kaur et al., 2006). The same result has been obtained using the peel extract in mice treated with CCl4 (Murthy et al., 2002). Ingestion of pomegranate juice for 4 weeks reduced hepatic oxidative stress (Faria et al., 2007) and improved the antioxidant enzyme activity in elderly subjects (Guo et al., 2008). Oral administration of pomegranate fruit extract inhibit LPO and increase GSH content as well as CAT activity in liver, kidney, and heart (Sudheesh and Vijayalakshmi, 2005).

Previous studies revealed the effect of rutin on the activity on gene expression of...
antioxidant enzymes in different experimental models both in vivo and in vitro (Lores-Arnaiz et al., 1995). In this context, treatment with rutin increased the activity of enzymatic antioxidants and also levels on non-enzymatic antioxidants in liver, kidney and brain of CPF intoxicated rats. Inhibition of MDA levels in the group treated with both of CPF and rutin was referred to the ability of rutin to transfer electrons and free radicals (Ferrali et al., 1997) in addition to activation of antioxidants enzymes (Elliott et al., 1992). However administration of rutin alone did not show any significant effect. The same finding has been obtained after pretreatment with rutin to isoproterenol-treated rats for 42 days (Karthick and Stanely Mainzen, 2006). Also, it has been reported that rutin has effectively reversed the biochemical, behavioral, and neurochemical changes in rat treated with haloperidol (Bishnoi et al., 2007) and improved the antioxidants enzymes system in human hepatoma cell line (Hep G2) by inhibition MDA levels and increasing CAT activity and therefore preventing or delay oxidative damage and its adverse effects (Alia et al., 2006). Moreover the antioxidant enzyme status was increased after rutin feeding in normal liver and in diabetic liver and kidney (Kamalakkannan et al., 2006).

In the present study CPF intoxicated rats showed significant increase in the liver lysosomal enzymes activity (acid phosphatase, cathepsin D and RNase II). We suggested that reactive oxygen species (ROS) generated from CPF pesticide may be responsible for the release of lysosomal enzymes, as a result decrease in membrane integrity and the leakage of enzymes from the enclosed sacs. These lead to intracellular dysfunction, disruption of potential substrates and organelles such as mitochondria, sarcosome etc. (Kennett and Wegglicki, 1978 and Mayanskaya et al., 2000). Also, the present study showed that induction of lysosomal enzymes was associated with a decrease in serum protein thiol level. Early study by Teimouri et al., 2006 confirms our finding and concludes that ROS can induce oxidation of critical sulfhydryl (SH) groups in protein and DNA, which will alter cellular integrity and function. The phospholipids-rich lysosomal membrane is a potential site of free radical attack subsequently causing loss of membrane stability. However, Brunk et al., 2001 added that apoptosis or programmed cell death is preceded by partial lysosomal rupture and such lysosomal destabilization seems to be an initial event in apoptosis. Treatment with pomegranate peel extract or rutin were able to decrease the release of lysosomal enzymes which could be due to the membrane stabilizing affect of P and R on the lysosomal membrane. The antioxidant properties of P and R scavenge the oxygen free radicals and preservation of cellular viability serving secondarily to preserve lysosomes, thereby retaining near normal functioning of the lysosomes. Indeed, cathepsin D is lysosomal proteases possibly involved in autophagic of discrete areas of cytoplasm, myofibrillary and mitochondria proteins (Zak et al., 1976). Cell death causing by oxidants may be initiated by lysosomal rupture and that this latter event may involve intralysosomal iron which catalyze Fenton-type chemistry and resultant peroxidative damage to lysosomal membrane. The antioxidant activity of rutin and pomegranate peel extract stopped this reaction by iron chelating activity. According to our results, pomegranate peel extract and rutin have shown abilities to preserve the activity of antioxidant enzymes and lysosomal membrane which may be referred to its role in modulating the levels of H$_2$O$_2$ and O$_2$. Furthermore, the contents of the extract have been suggested to induce the de novo synthesis of antioxidant enzymes by acting as several loci in the metabolic pathway.

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**Conclusion**
The present study highlights the protective role of pomegranate and rutin against OPs pesticides but the mechanism involved is still unclear. Further work is required to clarify how the plants extract works to enhance the antioxidant enzymes or their gene expression.

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