Impact of oxidative stress induced by chlorpyrifos in male rats: The ameliorating effect of rutin and vitamin E

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Abstract

The present study was designed to assess some toxicological effects in male rats following exposure to technical and formulated chlorpyrifos (CPF T. and CPF F.; at a dose of 10 mg a.i. kg⁻¹ b.wt.) and to evaluate the possible ameliorative effect of Rutin (RT, 50 mg/kg) or Vitamin E (Vit.E, 100 mg/kg) administration. Nine groups of rats were designated for: control; [Vit.E]; [RT]; [CPF.T]; [CPF.T.+Vit.E]; [CPF T.+RT]; [CPF F.]; [CPF F.+Vit.E] and [CPF.F+RT] treatments. Administration of CPF T. and CPF F. caused a significant increase in (LPO) level and decrease in the activities of antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), glutathione-s-transferase (GST), Glutathione peroxidase (GPx), and glutathione reductase (GR). Also, it caused a significant increase in serum aminotransferases, alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatinine and uric acid levels, whereas albumin, total protein and cholinesterase (ChE) were decreased. In contrast, the present results demonstrated that co-administration of Vit.E or Rutin minimized oxidative damage included by CPF as the levels of LPO, CAT, SOD, GST, GPx, GR, AST, ALT, ALP, LDH, creatinine, uric acid, ChE, albumin and total protein were maintained to levels not significantly different from that of the normal control. These results suggest the protective role of Vit.E and Rutin in alleviating the oxidative stress and the toxicity induced by CPF in male adult rats.

Keywords: Chlorpyrifos; Rutin; Vitamin E; Serum; Oxidative stress; Rats.

1. Introduction

Human are potentially exposed to pesticides either directly, as workers in green-houses and in agriculture, or indirectly, via food consumption. Organophosphate compounds (OPs) are occasionally used indiscriminately in large amounts causing environmental pollution [1]; [2]; [3]. Organophosphate insecticides induced toxic effects causing damage to various membranous components of the cell. Chlorpyrifos is an organophosphate insecticide (OPI) used to control household, public health and stored product insects. It is effective against mushroom flies, aphids, spider mites, caterpillars, thrips and white flies in greenhouse, outdoor fruit and vegetable crops [4]. The mechanism of acute CPF toxicity involves generation of reactive oxygen species (ROS) and alteration of antioxidant enzymes activity in the intoxicated rats. In fact, reactive oxygen species (ROS) are produced by univalent reduction of dioxygen to superoxide anion (O⁻₂), which in turn disproportionate to H₂O₂ and O₂ spontaneously or through a reaction catalyzed by superoxide dismutase (SOD). Endogenous H₂O₂ may be converted to H₂O either by catalase or glutatione peroxidase (GSH-Px). Otherwise, it may generate a highly reactive free hydroxyl radical (-OH) via a Fenton reaction, which is responsible for oxidative damage. GSH-Px converts H₂O₂ or other lipid peroxides to water or hydroxyl lipids, and during this process glutathione (GSH) is converted to oxidized glutathione [5]. Antioxidants are defense against free radical and oxidative attacks. They act as free radical scavengers and slow down not only radical oxidation but also the accompanying damaging effects in the body. Rutin (RT), a quercetin-3-rutinosidand sophorin or vitamin-P, is a well-known flavonoidal glycoside and set as an affective phenolic compound. RT has shown pharmacological benefits
including anti-tumor, anti-inflammatory and hepatic protective activities. It is an antioxidant, comprised of the flavonol quercetin and the disaccharide rutinose. RT has been demonstrated to scavenge superoxide radicals. Moreover, RT has inhibitory effects against membrane lipid peroxidation and generation of ROS [6].

Vitamin E (alpha-tocopherol) is a fat soluble vitamin which regulates different oxidation processes in the body as it acts as a powerful antioxidant. Previous studies revealed that dietary intake of vitamin E can normalize the damaging effect of oxidative stress induced by oxygen free radicals [7; 8]. A number of studies have concluded that Vit.E in combination with flavonoid synergistically inhibits oxidative damage both in vivo and in vitro [9]. Therefore the objective of this study was to investigate the protective potential effect of Vitamin E or Rutin on oxidative damage induced by chlorpyrifos in male rat.

2. Material and methods

2.1. Chemicals and reagents

Chlorpyrifos Technical (purity >99.9%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Chlorpyrifos Formulated (Trade name: Pestban® 48 % EC) was purchased from Agrochemical, Alwatneia Company, Alex., Egypt. Rutin were procured in their highest grades available from Sigma-Aldrich (St. Louis, MO, USA). Vitamin E manufactured by PHARCO pharmaceutical company, obtained from local pharmacy. Kits of SOD, CAT, GST, GSH, GR and GPx were obtained from Bio-diagnostic, Dokki, Giza, Egypt. All chemicals were of the highest quality grade. All other reagents used were of analytical reagent grade and obtained from the local scientific distributors in Egypt.

2.2. Animals

Albino male rats (weighing 150–170g) were obtained from the animal house of the Faculty of Medicine, Alexandria University, Egypt. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health. Animals were caged in groups of eight, given food and water ad-libitum. The animal room was maintained at (23 ± 2 °C) and 40–60% relative humidity with 12-h light–dark cycles, the light cycle coinciding with the day light hours. Rats were acclimatized for 2 weeks prior to the start of experiments. The experimental work on rats was performed with the approval of the Animal Care & Experimental Committee, National Research Centre, Cairo, Egypt, and according to the guidance for care and use of laboratory animals [10].

2.3. Experimental groups

Animals were segregated into 9 groups of rats; each group comprised 8 animals. Chlorpyrifos technical or formulated was administered at a dose 10 mg kg⁻¹ b.wt. [11]. Rutin was administered at a dose 50 mg kg⁻¹ b.wt. [12], and Vitamin E was administered at a dose 100 mg kg⁻¹ b.wt. Animals were treated with the tested compounds by oral gavages day by day for 30 days (the time of the experiment). The following outlines the 9 experimental groups.

Group 1: Rats in this group served as control and received corn oil, 0.5 ml /rat, (control group); Groups 2; 3; 4; 5; 6; 7; 8 and 9 rats were orally administered: [Vit. E, 100 mg kg⁻¹ b.wt.]; [RT, 50 mg kg⁻¹ b.wt.]; [CPF T, 10 mg kg⁻¹ b.wt.]; [CPF E, 10 mg + Vit. E, 100 mg kg⁻¹ b.wt.]; [CPF T, 10 mg + RT, 50 mg kg⁻¹ b.wt.]; [CPF F, 10 mg kg⁻¹ b.wt.]; and [CPF T, 10 mg + RT, 50 mg kg⁻¹ b.wt.], respectively. Dosages of each administered compound were freshly prepared and adjusted weekly for body weight changes and given at approximately the same time each morning.

2.4. Blood collection

The animals were starved overnight for 12h before blood was collected. Rats were anaesthetized with light ether and venous blood samples were collected by direct heart puncture into sterilized vials and allowed to stand for 30 min at room temperature to clot before being centrifuged at 3000 × g for 15 min. Serum was obtained by centrifugation and stored at −60°C. Serum samples were a liquored in Eppendorf tubes to use each one for one time.

2.5. Biochemical analysis

Thiobarbituric acid-reactive substances (TBARS): Lipid peroxidation levels were measured by the thiobarbituric acid (TBA) reaction using the method of [13]. This method was used to measure spectrophotometrically the color produced by the reaction of TBA with malondialdehyde (MDA). 1.5 ml of 0.8% thiobarbituric acid was added to 1 ml of serum sample. Then 0.4 ml of 8.1% sodium dodecyl sulfate and 1.5 ml of acetic acid were added. The mixture was finally made up to 5 ml with distilled water and placed in a hot water bath at 95 °C for 1 h. After cooling, 1.0 ml of distilled water and
5 ml of the mixture of n-butanol and pyridine (15:1, v/v) were added. The mixture was vortexed and after centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer (upper layer) was measured by the spectrophotometer at 532 nm against blank using distilled water. Thiobarbituric acid when allowed to react with MDA aerobically formed a colored complex [MDA - (TBA) 2 complex] which was measured by the spectrophotometer.

2.5.1. Reduced glutathione (GSH) activity was determined by the method of Ellman [14]. One ml of supernatant was taken and 0.5 ml of Ellman’s reagent (0.0198% DTNB in 1% sodium citrate) and 3 ml of phosphate buffer (pH 8.0) was added. The color developed was read at 412 nm.

2.5.2. Superoxide dismutase activity (SOD; EC 1.15.1.1) was determined spectrophotometrically according to the method of [15]. The assay procedure involves the inhibition of epinephrine auto-oxidation in an alkaline medium to adrenochrome, which is markedly inhibited by the presence of SOD. Samples were added to reaction mixture made in sodium carbonate buffer, pH 10.2, and the absorbance change was recorded at 480 nm.

2.5.3. Catalase (CAT; EC 1.11.1.6) activity was determined according to the method of [16]. The method is based on the decomposition of H2O2 by catalase. The reaction mixture was composed of 50 mM phosphate buffer of pH 7.0; 110 mM H2O2 and erythrocyte lysate. The reduction rate of H2O2 was followed at 240 nm for 30 s.

2.5.4. Glutathione peroxidase (GPx; EC 1.11.1.7). Peroxidase determination was done according to the method of [17]. The assay is an indirect measure of the activity of c-GPx. Oxidized glutathione (GSSG), produced upon reduction of organic peroxide by c-GPx, and is recycled to its reduced state by the enzyme glutathione reductase (GR). The oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm (A340) providing a spectrophotometric means for monitoring GPx enzyme activity.

2.5.5. Glutathione S-transferase (GST; EC 2.5.1.18) activity was measured spectrophotometrically by the method of [18]. Using S-2, 4-dinitrophenyl glutathione (CDNB) as a substrate. The principle of the method is based on measurement of the conjugation of S-2, 4-dinitrophenyl glutathione (CDNB) with reduced glutathione. The formation of adduct of CDNB, S-2, 4-dinitrophenyl glutathione was monitored by measuring the net increase in absorbance at 340 nm against the blank.

2.5.6. Glutathione reductase (GR; EC 1.6.4.2) activity was estimated by measuring the change in absorbance at 340 nm due to NADH utilization and GR activity was expressed as n moles NADPH oxidized/min/ mg proteins using an extinction coefficient of 6.22 mM-1 cm-1 by the method of [19].

2.5.7. Acetylcholinesterase (AChE; EC 3.1.1.7) activity was determined spectrophotometrically, at 405 nm and 25 C, using acetylthiocholine iodide as a substrate according to the method of [20]. The method is based on reaction of thiocholine with 5, 59-dithiobis-2-nitrobenzoic acid (DTNB) to yield yellow 5-thio-2-nitrobenzoate anion which could be measured. AChE activity was expressed in l mol/ml.

2.5.8. Alkaline phosphatase (ALP; EC 3.1.3.1) activity was determined according to the method of [21].

2.5.9. Aspartate aminotransferase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) activities were determined by colorimetric method of [22].

2.5.10. Lactate dehydrogenase (LDH; EC 1.1.1.27) was determined by the method of [23]. Total Protein content was determined using the method of [24]. Folin and Ciocalteus phenol reagent was used to develop the blue color that was measured spectrophotometrically at 750 nm. Bovine serum albumin was used as a standard. Uric acid determination was carried out according to the method of [25].

2.5.11. Creatinine was determined according to the method of [26].

2.6. Statistical Analysis

The results were expressed as means ± S.E.M. All data were done with the Statistical Package for Social Sciences (SPSS 12.0 for windows). The results were analyzed using one way analysis of variance (ANOVA) followed by Duncan’s test for comparison between different treatment groups. Statistical analysis was significance at p ≤ 0.05.
3. Results

3.1. Effects on the activities of AST, ALT, ALP and LDH in male rats

There was no significant changes in the activity of serum biomarkers ALT, AST, ALP and LDH of Rutin - or Vit E - treated rats compared to control, while chlorpyrifos , CPF T. ; CPF F., treated rats showed significant increase (P ≤ 0.05) in these biomarkers , indicating the occurrence of hepatic injury. Co- administration of Vit.E or RT significantly (P ≤ 0.05) decreases the levels of these biomarkers in [CPF T. + Vit.E] ; [CPF T. + RT]; [CPF F. + Vit.E]; [CPF F.+ Rutin] treated rats when compared with CPF treated rats. Thus, the activity of such antioxidants, Vit E or RT to decrease the hepatotoxicity signs resulted from chlorpyrifos was significantly observed (Table 1).

3.2. Effects on Albumin, Total protein, Cholinesterase, Creatinine and Uric Acid in male rat’s serum

Results of control and Vit. E or RT treatments were of no significant differences in albumin, total protein, ChE, creatinine and uric acid, while a significant increase in uric acid and creatinine with a significant (P ≤ 0.05) decrease in the total protein, albumin and ChE levels in the serum CPF-treated rats. Co- administration of Vit.E or RT with chlorpyrifos (CPF T. and CPF F.) were able to preserve ChE activity and albumin, total protein, creatinine, uric acid levels near to the normal state for each. Thus co-administration of Vit E or RT to chlorpyrifos treated rats resulted in a partial recovery in the above mentioned parameters (Table 2).

3.3. Effects on SOD, CAT, GPx, GR, GST activities and LPO level

Five antioxidant enzymes and lipid peroxidation were determined in the serum of CPF T. - and CPF F. - treated rats either with or without Vit.E or RT administration. Results in Tables 3 and 4 revealed that there was no significant differences between control and Vit.E or RT treatments. However, serum SOD , CAT, GPx, GR, GST activities displayed a significant decrease in CPF-treated rats, while serum LPO level was significantly (P ≤ 0.05) increase in insecticide-treated rats as compared with control group . Co-administration of Vit E or RT with both technical and formulated chlorpyrifos caused significant (P ≤ 0.05) increase in the activities of SOD, CAT, GPx, GR, GST and decrease in the level of LPO nearby the values in the control group (Tables 3 and 4). In all of the previous results the formulated chlorpyrifos (CPF.F) had slightly more toxic than the technical pesticide (CPF T.)

4. Discussion

Organophosphorus insecticides have been demonstrated to cause oxidative damage in several studies conducted in various animal species [27; 28]. The increase in lipid peroxidase (LPO) level and the impairment of antioxidant enzyme activities (SOD, CAT, GPx, GR, GST) clearly indicated that chlorpyrifos had the potency to cause oxidative damage. Also, an observable reduction in GSH levels in serum is an early consequence of oxidative stress as expressed by increasing LPO. In recent decades, there are several studies that have shown the neuroprotective effects of RT [29; 30]. They indicated that RT has neuro protective effect in brain ischemia and its administration attenuates ischemic neural apoptosis by inhibition of neurological deficit, lipid peroxidation and increase in endogenous antioxidant defense enzymes [31]. Rutin has beneficial effects on hypoxic, glutamate and oxidative stress on retinal ganglion cell. It appears that the sugar side chain of flavonoids may be important for neuroprotective activities [32]. The neuroprotective effect of vitamin E is related to its antioxidant activity [33]. It seems that pretreatment with vitamin E in the presence of chlorpyrifos after oral administration with doses of tested pesticide have protective and therapeutic effects. It is possible that RT by inhibiting neurological deficit could improve behavioral index of animals. Moreover, it was shown that RT might be effective in treatment of tardive dyskinesia, an extra pyramidal movement disorder, through communicating the imbalance of dopaminergic transmission [34]. Pretreatment of RT for 30 days caused behavioral and neurochemical changes in male rats. It is possible that RT may exert the toxicity by affecting brain dopaminergic and adrenergic systems [35]. Thus, there is another possibility that RT could improve behavioral index of animals via modulating dopaminergic and adrenergic transmissions. The beneficial effect of Vit.E is mostly due to its antioxidant properties. Vit.E as a lipid soluble antioxidant plays a major protective role against oxidative stress and prevents the production of lipid peroxides by scavenging free radicals which are toxic byproducts of many metabolic processes in biological membranes. Moreover, Vit.E is essential in maintaining the physiological integrity of testis, epididymis and accessory glands [36]. After pre-administration of Vit.E and RT, serum testosterone level significantly increased parallel to the reduction in LPO concentration [37]. OPs insecticides are related to accumulation of acetylcholine within the cholinergic synapses resulting inhibition of acetyl cholinesterase by active Oxon metabolites [38; 39; 40]. Present study confirms the pervious findings, Also, CPF treatments reduced ChE activity as a biomarker of OP neurotoxicity. Co- treatment with Vit.E and RT restored ChE activity near to control level indicating their ameliorating effect [41; 42; 43].
Table 1 Effects on some biomarkers (AST, ALT, ALP, and LDH) related to hepatotoxicity induced by chlorpyrifos (CPF T.; CPF F.) in the serum of male rats and the ameliorative effect of Vit (E) and RT supplementation.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>LDH (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>% control</td>
<td>Mean ± SE</td>
<td>% control</td>
</tr>
<tr>
<td>Control</td>
<td>36.7±0.305</td>
<td>100</td>
<td>31.39±0.255</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin (E)</td>
<td>36.59±0.413</td>
<td>99.67</td>
<td>30.96±0.306</td>
<td>98.63</td>
</tr>
<tr>
<td>Rutin</td>
<td>37.15±0.054</td>
<td>101.19</td>
<td>32.38±0.231</td>
<td>103.15</td>
</tr>
<tr>
<td>(CPF T.)</td>
<td>62.56±0.589</td>
<td>170.41</td>
<td>57.13±0.332</td>
<td>182.01</td>
</tr>
<tr>
<td>(CPF T.) + Vit (E)</td>
<td>42.12±0.261</td>
<td>114.73</td>
<td>41.49±0.272</td>
<td>132.17</td>
</tr>
<tr>
<td>(CPF T.) + Rutin</td>
<td>38.57±0.160</td>
<td>105.06</td>
<td>43.18±0.033</td>
<td>137.55</td>
</tr>
<tr>
<td>(CPF F.)</td>
<td>65.28±0.200</td>
<td>177.82</td>
<td>58.28±0.420</td>
<td>185.66</td>
</tr>
<tr>
<td>(CPF F.) + Vit (E)</td>
<td>43.28±0.124</td>
<td>117.89</td>
<td>41.95±0.426</td>
<td>133.64</td>
</tr>
<tr>
<td>(CPF F.) + Rutin</td>
<td>40.91±0.521</td>
<td>111.44</td>
<td>42.67±0.248</td>
<td>135.93</td>
</tr>
</tbody>
</table>

Values are means ± SE; n=8; Statistical difference from the control: * indicates significant at P ≤ 0.05.

Table 2 Effects on some biomarkers (albumin, total protein, ChE, creatinine, uric acid) induced by chlorpyrifos (CPF T.; CPF F.) in the serum of male rats and the ameliorative effect of Vit. (E) and RT supplementation.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Albumin (g/dl)</th>
<th>Total protein (g/dl)</th>
<th>Cholinesterase (U/L)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric Acid(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>% control</td>
<td>Mean ± SE</td>
<td>% control</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Control</td>
<td>4.73±0.075</td>
<td>100</td>
<td>8.54±0.133</td>
<td>100</td>
<td>2517.73±26.3</td>
</tr>
<tr>
<td>Vitamin (E)</td>
<td>4.71±0.088</td>
<td>99.57</td>
<td>8.61±0.190</td>
<td>100.81</td>
<td>2542.34±18.9</td>
</tr>
<tr>
<td>Rutin</td>
<td>4.75±0.068</td>
<td>100.42</td>
<td>8.51±0.248</td>
<td>99.64</td>
<td>2499.55±14.5</td>
</tr>
<tr>
<td>(CPF T.)</td>
<td>2.83±0.011</td>
<td>59.83</td>
<td>5.12±0.066</td>
<td>59.95</td>
<td>1528.80±12.5</td>
</tr>
<tr>
<td>(CPF T.) + Vit (E)</td>
<td>4.25±0.023</td>
<td>89.85</td>
<td>7.51±0.152</td>
<td>87.93</td>
<td>2290.87±23.0</td>
</tr>
<tr>
<td>(CPF T.) + Rutin</td>
<td>3.96±0.015</td>
<td>83.72</td>
<td>7.12±0.080</td>
<td>83.37</td>
<td>2095.56±11.4</td>
</tr>
<tr>
<td>(CPF F.)</td>
<td>2.81±0.005</td>
<td>59.41</td>
<td>5.09±0.105</td>
<td>59.60</td>
<td>1556.69±12.5</td>
</tr>
<tr>
<td>(CPF F.) + Vit (E)</td>
<td>4.22±0.030</td>
<td>89.21</td>
<td>7.49±0.211</td>
<td>87.70</td>
<td>2191.82±32.9</td>
</tr>
<tr>
<td>(CPF F.) + Rutin</td>
<td>3.89±0.017</td>
<td>82.24</td>
<td>7.15±0.066</td>
<td>83.72</td>
<td>2265.62±18.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n=8; Statistical difference from the control: * indicates significant at P ≤ 0.05.
In line with the previous studies we found decrease in protein thiol due to CPF administration, which may be due to increased degradation of protein or increased consumption of this antioxidant in stress environment which confirm the role of OPs in disruption of body total antioxidant capacity [44; 45; 40]. Our results indicated that supplementation with RT or vitamin E can reduce both the toxicity and oxidative stress of CPF treatment. Recent literature pointed to the role of OPs in interference with metabolism of carbohydrate, biosynthesis of protein as well as respiration of mitochondria. Increased serum ALT activity was also shown to CPF treatment indicated damage to any or all of the organs producing this enzyme, while its activity was significantly lower in the groups treated with RT or vitamin E indicating their protective effects on the damage induced by CPF. This finding was in agreement with those of [27; 40; 46] using phenolic antioxidants against organophosphate toxicity. In addition, previous studies have been shown a correlation between

Table 3 Effects on some biomarkers (SOD, CAT, GPx) related to oxidative stress induced by chlorpyrifos (CPF T.; CPF F.) in the serum of male rats and the ameliorative effect of Vit (E) and RT supplementation.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Superoxide dismutase (SOD) (µmol/min/ml)</th>
<th>Catalase(CAT) (µmol/min/ml)</th>
<th>Glutathione peroxidase (GPx)(µmol/min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>% control</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Control</td>
<td>104.33±0.881b</td>
<td>100</td>
<td>0.53±0.012ab</td>
</tr>
<tr>
<td>Vitamin (E)</td>
<td>109.61±0.743a</td>
<td>105.06</td>
<td>0.54±0.005a</td>
</tr>
<tr>
<td>Rutin</td>
<td>105.18±1.742b</td>
<td>100.81</td>
<td>0.53±0.026ab</td>
</tr>
<tr>
<td>(CPF T.) + Vit E</td>
<td>62.73±0.371e</td>
<td>60.12</td>
<td>0.34±0.005d</td>
</tr>
<tr>
<td>(CPF T.) + Vit E</td>
<td>96.50±0.866d</td>
<td>92.49</td>
<td>0.48±0.004abc</td>
</tr>
<tr>
<td>(CPF T.) + Rutin</td>
<td>94.36±0.683dabc</td>
<td>90.44</td>
<td>0.47±0.005bcd</td>
</tr>
<tr>
<td>(CPF F.) + Rutin</td>
<td>60.73±0.933fabc</td>
<td>58.20</td>
<td>0.33±0.003d</td>
</tr>
<tr>
<td>(CPF F.) + Vit E</td>
<td>96.13±1.162dabc</td>
<td>92.14</td>
<td>0.48±0.014abc</td>
</tr>
<tr>
<td>(CPF F.) + Rutin</td>
<td>92.83±0.166eabc</td>
<td>88.97</td>
<td>0.46±0.005cabc</td>
</tr>
</tbody>
</table>

Values are means ± SE; n=8; Statistical difference from the control: * significant at P ≤ 0.05.

Table 4 Effects on some biomarkers (GR, GST, and LPO) related to oxidative stress induced by chlorpyrifos (CPF T.; CPF F.) in the serum of male rats and the ameliorative effect of Vit (E) and RT supplementation.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Glutathione reductase (nmol/min/ml)(GR)</th>
<th>Glutathione-S-transferase (µmol/min/ml)(GST)</th>
<th>lipid peroxidation (LPO) (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>% control</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Control</td>
<td>83.86±0.953a</td>
<td>100</td>
<td>1.63±0.028a</td>
</tr>
<tr>
<td>Vitamin (E)</td>
<td>81.87±1.850a</td>
<td>97.62</td>
<td>1.62±0.015a</td>
</tr>
<tr>
<td>Rutin</td>
<td>84.20±2.795a</td>
<td>100.40</td>
<td>1.64±0.023a</td>
</tr>
<tr>
<td>(CPF T.) + Vit E</td>
<td>49.95±1.523b</td>
<td>59.56</td>
<td>1.12±0.011d</td>
</tr>
<tr>
<td>(CPF T.) + Vit E</td>
<td>80.87±1.430a</td>
<td>96.43</td>
<td>1.50±0.020cd</td>
</tr>
<tr>
<td>(CPF T.) + Rutin</td>
<td>85.13±2.209a</td>
<td>101.51</td>
<td>1.40±0.023e</td>
</tr>
<tr>
<td>(CPF F.) + Vit E</td>
<td>51.05±1.608b</td>
<td>60.87</td>
<td>1.23±0.005f</td>
</tr>
<tr>
<td>(CPF F.) + Rutin</td>
<td>81.24±1.712a</td>
<td>96.87</td>
<td>1.49±0.011cd</td>
</tr>
<tr>
<td>(CPF F.) + Rutin</td>
<td>84.75±1.413a</td>
<td>101.06</td>
<td>1.42±0.015c</td>
</tr>
</tbody>
</table>

Values are means ± SE; n=8; Statistical difference from the control: * significant at P ≤ 0.05.
inhibition of ChE and lipid peroxidation following sub chronic and chronic exposure to OP [41]. Lipid peroxidation has
considered one of the molecular pathways involved in the toxicity of OPs [47]. Organophosphates have been suggested
to induce lipid peroxidation in vivo by enhancement of MDA production [48; 49; 50; 51]. The present study shows that
CPF may have properties to induce oxidative stress indicated by enhancement of MDA production, decrease in GST and
CAT activities in rat serum. The increase of free radicals and lipid peroxidation may result from the inhibition of GSH
levels induced by CPF toxicity. This present findings are in agreement with other investigations indicating that
accumulation of lipid peroxides has been resulted after exposure of rats to acute dose of chlopyrifos in their liver [52],
kidney [50], brain [48], and erythrocytes [53]. However, repeated doses increased LPO levels as well as antioxidant
enzymes in blood, of rat [54]. Supplementation with antioxidants effectively suppressed the oxidative damage induced
by OPs [54; 55; 48; 50; 40]. Antioxidant activity of RT is referred to its polyphenolic capacity such as ellagic acid and
ellagitanis [56], which may suggest its role as an electron donor in scavenging free radicals [57].

Many studies revealed the effect of RT on the activity of antioxidant enzymes in different experimental models both in
vivo, in vitro. In this context, treatment with RT increased the activity of enzymatic antioxidants and also levels on non-
enzymatic antioxidants in serum of CPF intoxicated rats this finding was in agreement with [58]. Inhibition of MDA
levels in the group treated with both of CPF and RT was referred to the ability of RT to transfer electrons and free
radicals [59] in addition to activation of antioxidants enzymes [60]. However, administration of RT alone show
significant effect. The same finding has been obtained after pretreatment with RT to isoproterenol-treated rats for 42
days [61]. Also, it has been reported that RT has effectively reversed the biochemical, behavioral, and neurochemical
changes in rat treated with haloperidol [34] 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 [62]. In the present study CPF intoxicated rats showed significant increase in AST, ALT, ALP.
Results suggested that reactive oxygen species (ROS) generated from CPF pesticide may be responsible for the release
of such enzymes, as a result decrease in membrane integrity and the leakage of enzymes from the enclosed sacs. Also,
the present study showed that induction of lysosomal enzymes was associated with a decrease in serum protein thiol
level. Early study [63] confirms our finding and concludes that ROS can induce oxidation of critical sulphhydryl (SH)
groups in protein, which will alter cellular integrity and function. The antioxidant properties of RT and vitamin E
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 autophagy of discrete areas of cytoplasm, and mitochondria proteins [64]. The antioxidant activity of RT
and vitamin E stopped this reaction by iron chelating activity. According to our results, pretreatment with Vitamin E or
RT 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 H2O2 and O2. Furthermore, the contents of such substances have been
suggested to induce the de novo synthesis of antioxidant enzymes by acting as several loci in the metabolic pathway.

5. Conclusion

In conclusion, the present study has shown that the oxidative stress plays an essential role in chlopyrifos mediated
injury and the co-administration of Rutin or Vit.E ameliorated the injury through its free radical scavenging effect.
Therefore, the administration of Rutin or Vit.E may be of value to farmers and other workers who are frequently exposed
to the organophosphorus insecticides such as chlopyrifos, CPF, in reducing injury mediated by these compounds.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The experimental work on rats was performed with the approval of the Animal Care and Experimental Committee,
Faculty of Agriculture, Damanhur University, Egypt, and according to the Guide for Care and Use of Laboratory
Animals,(NRC, Acad. Press; Washington, DC, USA,1996.

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