THE AMELIORATING EFFECTS OF GREEN TEA EXTRACT AGAINST CYROMAZINE AND CHLORPYRIFOS INDUCED LIVER TOXICITY IN MALE RATS

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Received: 1 October 2012, Revised and Accepted: 27 October 2012

INTRODUCTION

Pesticides are used daily and internationally on a massive scale. They have conferred immense benefits to mankind by improving health and nutrition. Pesticides fall into numerous chemical classes, which have widely differing biological activities and thus differing potential to produce adverse effects in living organisms, including humans 3. Chlordane (CYP), 4-Chlorophenylphenol (OP), utilized extensively in agriculture 2, 3. CPF is thought to be primarily metabolized in the liver by multiple, specific cytochrome P450 enzymes through several reaction pathways 4. CPF elicits a number of additional effects, including hepatic dysfunction, haematological and immunological abnormalities, embryotoxicity, genotoxicity and neurobehavioral changes 5-8.

Cyromazine (Cyr), N-cyclopropyl-1,3,5-triazine-2,4,6-triamine, is an insect growth regulator that acts by inhibiting the moulting processes. It is widely used as an agricultural insecticide and a feed additive to animal breeding against fly larvae from hatching in manure 10,11. The early investigation showed that Cyr is harmless to mammalian and poultry. It was proved to cause mammary tumors in the mouse 12. Besides the amount of parent drug excreted through feces, a portion of Cyr (2-14%) is metabolized to melamine in vivo 13. The main adverse effects shown in previous studies on animals were kidney related, including renal calculi, renal tubular necrosis, crystalluria, haematuria and renal dysfunction 5, 11. Also, Cyr toxicity included reduced body weight, high blood pressure, epithelial hyperplasia of the urinary bladder among others 5, 11, 13.

Pesticides are known to increase the production of reactive oxygen species (ROS), which in turn generate oxidative stress in different tissues 5, 14-16. Many studies have implicated oxidative damage as the central mechanism of toxicity 16-21. Oxidative damage primarily occurs through production of reactive oxygen species (ROS), including hydroxyl radicals and hydrogen peroxide that are generated during the reaction and react with biological molecules, eventually damaging membranes and other tissues 22. Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipids bilayers 22 and they may damage membranes by inducing lipid peroxidation (LPO) 17, 19.

In fact, herbal medicines derived from plant extracts are being increasingly utilized as adjunct treatment options for a wide variety of clinical disease. More attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities 24. The health benefits of green tea have been extensively studied in the past few decades. Nowadays, tea is considered as a source of dietary constituents endowed with biological and pharmacological activities with potential benefits to human health. The increasing interest in the health properties of tea extract and its main catechin polyphenols have led to a significant rise in scientific investigation for prevention and therapy in several diseases 25-27. Furthermore, many previous investigators reported that green tea extract (GT) displays antioxidants and free radicals scavenger properties 25, 26. Therefore the present study aimed to elucidate if Cyr has hepatotoxicity when administered orally to male rat separately or in combination with CPF as well as the hepatoprotective effect of GT against oxidative damage which may result from Cyr and/or CPF exposure using biochemical alterations and histopathological findings as criteria.

MATERIALS AND METHODS

Animals

Healthy male Wister rats weighing 120 ±5 g were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt, and maintained in clean plastic cages in the laboratory animal room (23 ± 2°C). On standard pellet diet, tap water ad libitum, and daily dark/light cycle (12/12 hrs.), the rats were acclimatized for 1 week 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 international guidelines for care and use of laboratory animals 27.

Chemicals

Chlordane (97%) and Cyromazine (99%) were obtained from TaGeuk Corp., South Korea. Pu-erh green tea of post-fermented tea produced in Yunnan province, China. The kits used for biochemical measurements of malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione

ABSTRACT

In the present study, the protective effect of an aqueous extract of green tea (GT) against hepatotoxicity and oxidative damage induced by cyromazine (Cyr), chlorpyrifos (CPF) and their combination in male rats was undertaken. Eight groups containing six rats each were selected. Group I served as control. Groups II, III and IV rats were given a single daily oral doses of Cyr (169.35 mg/kg, 1/20 LD50, in corn oil), CPF (6.75 mg/kg, 1/20 LD50, in corn oil) and their combination for 28 consecutive days, respectively. Group V permitted free access to solubilised GT (1.5%) as the sole drinking fluid. Groups VI, VII and VIII rats were given the same doses as groups II, III and IV and simultaneously permitted free access to solubilised GT as the sole drinking fluid. Insecticides administration to rats resulted in significant reduction in body weight and elevation in liver weight compared to control. Insecticides administration to rats resulted in significant elevation of serum transaminases (AST & ALT), alkaline phosphatase (ALP), total protein, lipid peroxidation (LPO) expressed as malondialdehyde (MDA), lactate dehydrogenase (LDH) and decrease of serum albumin (Alb). Furthermore, significant elevation of hepatic superoxide dismutase (SOD), catalase (CAT), reduction of hepatic lactate dehydrogenase (LDH), glutathione peroxidase (Gpx), depletion of hepatic glutathione reduced (GSH) and elevation of hepatic protein carbonyl (PC) content were noticed in insecticides-treated rats. Histopathological studies of liver revealed that supplementation of GT resulted in mild degeneration and necrosis of the hepatocytes. Furthermore, GT had normalized CAT, SOD, Gpx, ALT, AST, ALP, serum LDH, total proteins and PC content, whereas attenuated Alb, hepatic LDH, GSH and LOP. In conclusion, the use of green tea extract appeared to be beneficial to rats, to a great extent in attenuating and restoring the damage sustained by insecticide exposure.

Keywords: Biochemical, Cyromazine, Chlorpyrifos, Green tea, Lipid peroxidation, Liver, Oxidative stress, Histopathology.
peroxidase (GPx), transaminases (ALT & AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein and albumin were all purchased from Biodiagnostic Company, 29 Tahrir St, Dokki, Giza, Egypt. Protein carboxyl (PC) assay kit was purchased from Cayman Co. Cat. no. 10005020 (USA). All other chemicals were of reagent grades and were obtained from the local scientific distributors in Egypt.

Preparation of Green Tea Extract and Total Phenolic Content
Likewise, the crude aqueous extract of green tea was prepared according to Maity et al. and later adopted by El-Beshbishy by soaking 15 g of instant green tea leaves in 1 L of distilled water whose temperature did not exceed 90 °C, for 5 min to obtain soluble polyphenols dissolved in the aqueous extract. The solution was filtered to obtain the final 1.5% (w/v) green tea extract (1.2 g extract/L water). This solution was substituted in the place of water as the sole source of drinking fluid.

Total soluble phenolics in green tea extract (GT) were determined according to the method of Slinkard and Singleton with some modifications. Alkols [100 µl] of the extracts (2 mg/ml) were transferred into test tubes and combined with 100 µl of Folin-Ciocalteu reagent after 3 min, 100 µl of sodium carbonate solution (2% Na2CO3) was added and the volume was adjusted to a final volume of 2.5 mL. After 1 h of incubation in dark at room temperature, the absorbance was measured at 760 nm. The amount of total phenolic compounds was calculated as mg of gallic acid equivalents (mg Eq gallic acid) from the calibration curve of gallic acid standard solution (covering the concentration range between 6.25 µg/ml and 100 µg/ml). The data were presented as the average of five replicate analyses. The concentrations of phenolic compounds were calculated according to the following equation obtained from the standard gallic acid graph.

Absorbance (O.D.) = 0.046 gallic acid (µg) + 0.00342.

Experimental Design
Rats were randomly divided into 8 groups each containing 6 animals (6 rats/cage). The route of administration selected for the study was oral gavage for 28 consecutive days. Rats in Group I served as control and were given corn oil (0.5 ml/rat) and allowed distilate water ad libitum. Rats in Group II were given chlorpyrifos (CPF) in corn oil at a dose of 6.75 mg/kg b.wt. daily (1/20 LD50, Tomlin). Rats in Group III were given cyromazine (Cyr) in corn oil at a dose of 169.35 mg/kg b.wt. daily (1/20 LD50, Tomlin). Rats in Group IV were given a combination of CPF [6.75 mg/kg b.wt.] and Cyr [169.35 mg/kg b.wt.]. Rats in Group V were given aqueous green tea extract as the sole drinking fluid during the 28 days at a concentration of 1.5% (w/v) at a dose of 30 mg green tea extract/rat/day. Rats in Group VI, VII and VIII were given the same doses of pesticides as in groups I, II and III, respectively and simultaneously allowed to an aqueous green tea extract as the sole source of drinking fluid. During the experimental duration, body weights were recorded and the doses modulated according to weekly body weight gain.

After completion of treatment period, blood samples were withdrawn from the animals under light ether anesthesia by puncturing the retro-orbital venous plexus of the animals with a fine sterilized glass capillary tube. Blood samples were collected, subjected to serum separation and stored at -20 °C for biochemical analysis within one week.

Rats were then killed by decapitation. Livers were immediately isolated, cleaned and weighed for biochemical investigation. Small pieces of liver were cut and kept in 10% of formalin solution for histological studies. Other portions of liver washed with saline solution, weighed, cut in small parts, homogenized in 10% (w/v) ice-cold 100 mM phosphate buffer (pH 7.4) and used for the determination of GSH content and liver lactate dehydrogenase (LDH). Homogenates were centrifuged at 10,000 rpm at 4 °C for 15 min, then the supernatants were used for the measurements of antioxidant enzyme activities (CAT, SOD and GPx).

Biochemical Analysis
The sera obtained from different treatments were subjected to certain biochemical analyses by using Shimadzu UV-VIS Recording 2401 PC (Japan).

Liver function tests
Serum Transaminases (ALT & AST) activities were determined by a colorimetric method according to Reitman and Frankel. Serum alkaline phosphatase (ALP) activity was determined by enzymatic colorimetric method according to Young et al.

Oxidative stress parameter
Malondialdehyde (MDA) content as indicator of lipid peroxidation was determined in the serum, by a colorimetric method according to Satoh et al.

The reduced form of glutathione (GSH) was determined in the liver homogenate by colorimetric method according to Beutler et al.

Protein oxidation in the rat liver homogenate was measured as protein carbonyl content. The Cayman’s protein carbonyl (PC) assay kit utilizes the dinitrophenylhydrazine reaction to measure the PC content in homogenate according to Levine et al. The amount of protein-hydrazoone produced was quantified spectrophotometrically at 380 nm.

Liver antioxidant enzymes
Catalase (CAT) activity was measured according to the method described by Aebi by assaying the hydrolysis of H2O2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25 °C. The activity of CAT enzyme is expressed as nmol/mg protein.

Superoxide dismutase (SOD) activity was determined according to the method described by Marklund and Marklund by assaying the autoxidation and illumination of pyrogalol at 440 nm for 3 min. One unit of SOD activity was calculated as the amount of protein that caused 50% pyrogalol autoxidation inhibition. The SOD activity is expressed as U/mg protein.

Glutathione peroxidase (GPx) activity was measured using H2O2 as substrate according to the method described by Paglia and Valentine. The reaction was monitored indirectly as the oxidation rate of NADPH at 240 nm for 3 min. Enzyme activity was expressed as nmol/mg protein.

Total protein
The total protein concentrations were measured as described by Lowry et al. using bovine serum albumin as standard.

Indicators of liver cell necrosis
Liver and serum lactate dehydrogenase (LDH) activities and indicators of necrotic cell death was determined according to the method of Vassault et al.

Histopathological Examination
For light microscopic investigations, specimens from liver were fixed in 10% phosphate buffer formalin, dehydrated in alcohols and embedded in paraffin. Five micron tissue sections were stained with hematoxylin and eosin stain (H&E) for general histopathological examination. One slide was prepared for each rat. Scoring of histopathological changes was done as follows: (-) absent; (+) mild; (+++) moderate; (++++) severe, and (+++++) extremely severe (Bancroft et al. 1992).

Statistical Analysis
The results were expressed as means ± S.E.M. All data were done with the Statistical Package for Social Sciences (SPSS 11.0 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 significance was set at p < 0.05.
RESULTS

Total Phenolic Content

In our study, the total polyphenolic content of GT in terms of gallic acid equivalents (GAEs) was 28.11 ± 0.18 µg GAEs/mg (un-tabulated data).

Clinical Signs

During the experiment, no death was observed in any of the experimental groups. Rats in the control group and in GT treated group did not show any sign of toxicity. However, CPF and Cyr+CPF treated rats showed varying degrees of clinical signs few minutes after dosing. The signs included huddling, mild tremor and diarrhea. The observed signs were related to the cholinergic crisis; a consistent sign in organophosphate poisoning. Except for the huddling, no other significant clinical manifestation was observed following GT supplementation.

Body and Organ Weights

At the end of the experimental course, there was no significant difference in body and relative liver weights between GT and untreated rats. However, a significant loss of weekly body weight gain accompanied by a significant increase in the relative liver weights were recorded in rats treated with CPF, Cyr and their mixture compared to the control. The administration of GT to pesticide treated groups has an ameliorated effect either in the loss of body weight or in the increase of relative liver weights (Table I).

Table 1. Effect of green tea consumption on body weight (g), liver weight (g), and relative liver weight (%) of rats treated with/cyromazine, chlorpyrifos and their binary mixture.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight</th>
<th>Absolute liver weight (g)</th>
<th>Relative liver weight (g/100g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (g)</td>
<td>Final (g)</td>
<td>% Change/week</td>
</tr>
<tr>
<td>Control</td>
<td>119.4±0.93</td>
<td>225.5±5.42</td>
<td>7.56±0.28ab</td>
</tr>
<tr>
<td>GT</td>
<td>122.1±1.16</td>
<td>221.9±4.19</td>
<td>7.85±0.16bc</td>
</tr>
<tr>
<td>Cyr</td>
<td>118.7±0.67</td>
<td>192.3±6.52</td>
<td>7.79±0.15ac</td>
</tr>
<tr>
<td>CPF</td>
<td>120.7±0.47</td>
<td>184.9±4.25</td>
<td>8.41±0.16ad</td>
</tr>
<tr>
<td>Cyr+CPF</td>
<td>119.6±0.93</td>
<td>174.5±7.50</td>
<td>8.27±0.30ad</td>
</tr>
<tr>
<td>Cyr+GT</td>
<td>120.8±0.55</td>
<td>202.9±2.55</td>
<td>7.65±0.50ab</td>
</tr>
<tr>
<td>CPF+GT</td>
<td>117.9±1.66</td>
<td>195.9±7.99</td>
<td>7.86±0.22ab</td>
</tr>
<tr>
<td>Cyr+CPF+GT</td>
<td>119.9±0.77</td>
<td>189.9±6.19</td>
<td>7.50±0.20bc</td>
</tr>
</tbody>
</table>

Each value is a mean of 6 rats ± S.E.M; a,b,c,d values are not sharing superscripts letters (a, b, c, d) differ significantly at p < 0.05; % of body weight change/week = [(final b.wt. – initial b.wt.)/ initial b.wt.]/no of weeks X 100; Cyr: Cyromazine; CPF: Chlorpyrifos; GT: Green tea.

Biochemical Measurements

Serum lipid peroxidation (LPO)

Administration of Cyr, CPF and Cyr+CPF led to a significant increment (p < 0.05) in lipid peroxidation as evidenced by the increase in serum MDA levels by 62.0%, 65.6%, and 85.8%, respectively as compared to the control group. However, co-administration of GT to treated rats reduced the augmentation in serum MDA levels to 13.6%, 34.3% and 42.7% for Cyr, CPF and Cyr+CPF-treated rats, respectively (Fig. 1A).

Liver glutathione (GSH)

Glutathione, in the reduced form (GSH), acts as one of the major detoxifiers in the body. A significant decrease of glutathione (GSH) level in liver was evident in Cyr, CPF and Cyr+CPF-treated groups by -32.7%, -39.6% and -48.8%, respectively when compared to control (Fig. 1B). However, co-administration of GT to treated rats ameliorated the decrease in liver GSH levels to -17.9%, -19.8% and -24.1% for Cyr, CPF and Cyr+CPF-treated rats, respectively (Fig. 1B). No significant changes were observed between control and GT-treated groups.

Liver protein carbonyl (PC) content

The administration of GT to normal rats showed non-significant decrease in liver protein carbonyl content (PC). However, administration of Cyr, CPF and Cyr+CPF significantly increased the liver PC content by 71.4%, 65.7% and 95.7%, respectively as compared to the control group. Co-administration of GT to intoxicated rats attenuated the augmentation in liver PC content to 17.6%, 22.9% and 32.9% for Cyr, CPF and Cyr+CPF-treated rats, respectively (Fig. 1C).

Figures 1A, 1B, and 1C illustrate the effects of green tea consumption on body weight, liver weight, and relative liver weight of rats treated with cyromazine, chlorpyrifos, and their binary mixture. The data are expressed as mean ± S.E.M. of 6 rats; Columns are not sharing above letters (a, b, c) differ significantly at p < 0.05; Cyr: cyromazine; CPF: chlorpyrifos; GT: green tea extract; LPO: lipid peroxidation; GSH: glutathione; PC: protein carbonyl.

Figure 1: Effect of repetitive doses, for 28 consecutive days, of Cyr, CPF and their combination on serum LPO (Fig. 1A), liver glutathione (Fig. 1B) and liver protein carbonyl (Fig. 1C) levels of rat in the absence and presence of GT.
Enzymatic antioxidant status in liver

Results in Table 4 show the influence of Cyr, CPF and their combination on the activities of CAT, SOD and GPx. Subacute levels of the tested pesticides resulted in a state of liver injury and extensive oxidative damage in rats as manifested by the significant alteration in these enzymes. In fact, in treated rats, a significant increase was noted in the activities of CAT and SOD, whereas a significant decrease was observed in the activity of GPx. However, the co-administration of GT mitigated the significant alteration in the activities of SOD, GPx and CAT (Table 4).

Effects of treatments on biochemical parameters in serum and liver

The oral administration of GT to normal rats produced no changes in all the biochemical parameters tested compared to the normal control rats (Tables 2&3). Treatments with Cyr, CPF and Cyr+CPF induced a significant increase (p < 0.05) in the total protein levels by 13.6, 8.3 and 15.3%, respectively compared to the control. In contrast, exposure to Cyr, CPF and Cyr+CPF induced a significant decrease (p < 0.05) in albumin level by -28.4, -25.7 and -28.9%, respectively, compared to the control rats. However, co-administration of GT to the treated rats resulted in a partial recovery in the above mentioned parameters (Table 2). The estimated globulin concentrations in serum of treated rat as well as the A/G ratio were recorded in Table 2. The A/G ratio in control rats recorded 2.21. This ratio was declined in intoxicated rats by 0.72, 0.87 and 72 for Cyr, CPF and the mixture Cyr+CPF, respectively, however mitigation by 1.22, 1.32 and 1.41 was recorded following administration of GT (Table 2).

Data are expressed as mean ± S.E.M of 6 rats; Columns are not sharing above letters (a, b, c, d, e) differ significantly at p < 0.05; Cyr: Cyromazine; CPF: Chlorpyrifos; GT: Green tea.

Histopathological Examination

The representative pictures of histopathological examination in the liver tissue are shown in Figure 3 (A-F) and the semi-quantitative histological scoring of liver damage is presented in Table 4. Liver sections from the control group rats showed normal hepatic cytoarchitecture. They formed of hepatocytes radiating from central vein to the periphery of the lobules (Fig. 3A). Liver lobules of Cyr or Cyr+CPF treated rats showed degeneration and coagulative necrosis in hepatocytes. However, CPF-treated rat liver showing inflammatory cells infiltration in the portal area with diffuse Kupffer cells proliferation in between the hepatocytes (Fig. 3C). Inflammatory cells infiltration in the portal area was shown in CPF and Cyr+CPF treated rats (Fig. 3 D&E) and fatty change in hepatocytes was shown in Cyr+GT-treated rats (Fig. 3 F). Histopathological examination of liver of green tea treated rats hepatocytes were more or less similar to control group. However, the liver of the GT and other treated groups showed marked improvement in its histological structure in comparison to the treated groups (Cyr, CPF, Cyr+CPF) alone and represented by nil to

### Table 3: Effect of green tea consumption on serum levels of protein, albumin and globulin of male rats treated with/without cyromazine, chlorpyrifos and their binary mixture

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50.0</td>
<td>20.0</td>
<td>30.0</td>
<td>0.40</td>
</tr>
<tr>
<td>GT</td>
<td>51.0</td>
<td>20.5</td>
<td>30.5</td>
<td>0.41</td>
</tr>
<tr>
<td>Cyr</td>
<td>52.0</td>
<td>21.0</td>
<td>31.0</td>
<td>0.43</td>
</tr>
<tr>
<td>CPF</td>
<td>53.0</td>
<td>21.5</td>
<td>31.5</td>
<td>0.45</td>
</tr>
<tr>
<td>Cyr+CPF</td>
<td>54.0</td>
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<td>32.0</td>
<td>0.45</td>
</tr>
<tr>
<td>Cyr+GT</td>
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<td>22.5</td>
<td>32.5</td>
<td>0.48</td>
</tr>
<tr>
<td>CPF+GT</td>
<td>56.0</td>
<td>23.0</td>
<td>33.0</td>
<td>0.48</td>
</tr>
<tr>
<td>Cyr+CPF+GT</td>
<td>57.0</td>
<td>23.5</td>
<td>33.5</td>
<td>0.49</td>
</tr>
</tbody>
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Each value is a mean of 6 rats ± S.E.M; a, b, c, d, e values are not sharing superscripts letters (a, b, c, d, e) differ significantly at p < 0.05; Cyr: Cyromazine; CPF: Chlorpyrifos; GT: Green tea.

### Figure 2: Effect of repetitive doses, for 28 consecutive days, of Cyr, CPF and their combination on serum LDH (Fig. 2A) and liver LDH (Fig. 2B) of rat in the absence and presence of GT.

### Table 2. Effect of green tea consumption on serum levels of protein, albumin and globulin of male rats treated with/without cyromazine, chlorpyrifos and their binary mixture.

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<td>0.49</td>
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moderate degree in degeneration of hepatocytes, fatty change in hepatocytes, coagulative necrosis in hepatocytes, diffuse kupffer cells proliferation in portal area, diffuse kupffer cells proliferation in between hepatocytes, and dilatation in central vein (Table 5).

Table 4: Effect of green tea consumption on liver enzymatic antioxidant parameters of male rats treated with/without cyromazine, chlorpyrifos and their binary mixture.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Catalase (CAT) (nmole/min/mg protein)</th>
<th>Superoxide dismutase (SOD) (U/mg protein)</th>
<th>Glutathione peroxidase (GPx) (nmoles/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.169±0.007a</td>
<td>11.93±0.59a</td>
<td>4.80±0.216a</td>
</tr>
<tr>
<td>GT</td>
<td>0.173±0.006a</td>
<td>12.10±0.54a</td>
<td>4.86±0.185a</td>
</tr>
<tr>
<td>Cyr</td>
<td>0.258±0.006b</td>
<td>18.16±0.86b</td>
<td>3.30±0.128b</td>
</tr>
<tr>
<td>CPF</td>
<td>0.286±0.006a</td>
<td>19.92±0.82b</td>
<td>2.95±0.102a</td>
</tr>
<tr>
<td>Cyr+CPF</td>
<td>0.302±0.009d</td>
<td>21.35±1.15a</td>
<td>2.77±0.114d</td>
</tr>
<tr>
<td>Cyr+GT</td>
<td>0.183±0.005a</td>
<td>12.49±0.73a</td>
<td>4.52±0.173ab</td>
</tr>
<tr>
<td>CPF+GT</td>
<td>0.189±0.007ab</td>
<td>11.87±0.65a</td>
<td>4.37±0.234ab</td>
</tr>
<tr>
<td>Cyr+CPF+GT</td>
<td>0.204±0.005b</td>
<td>13.16±0.61a</td>
<td>4.19±0.192b</td>
</tr>
</tbody>
</table>

Each value is a mean of 6 rats ± S.E.M; a,b,c,d values are not sharing superscripts letters (a, b, c, d) differ significantly at p < 0.05; Cyr: Cyromazine; CPF: Chlorpyrifos; GT: Green tea.

Table 5: Semiquantitative scoring of architectural damage on histopathological examination of the rat livers in the different treatment groups.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>GT</th>
<th>Cyr</th>
<th>CPF</th>
<th>Cyr+CPF</th>
<th>Cyr+GT</th>
<th>CPF+GT</th>
<th>Cyr+CPF+GT</th>
</tr>
</thead>
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<td>Degeneration of hepatocytes</td>
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<td>Fatty change in hepatocytes</td>
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<td>Coagulative necrosis in hepatocytes</td>
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<td>Diffuse kupffer cells proliferation in portal area</td>
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<td>Diffuse kupffer cells proliferation in between hepatocytes</td>
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<td>Dilated central vein</td>
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Cyr: Cyromazine; CPF: Chlorpyrifos; GT: Green tea. (–) indicates normal, (+) indicates mild, (++) indicates moderate, (+++) indicates severe, and (++++) indicates extremely severe.

Figure 3: Histopathological examination of rat liver. (Fig. 2A) (80×) showing normal histological structure of the central vein (CV) and surrounding hepatocytes, Cyr-treated animal liver showing degeneration (d) and coagulative necrosis (c) in the hepatocytes (Fig. 2B-80×), CPF-treated rat liver showing inflammatory cells infiltration in the portal area (m) with diffuse Kupffer cells proliferation in between the hepatocytes (Fig. 2C-80×), Cyr+CPF-treated rat liver (Fig. 2D-F) showing inflammatory cells infiltration surround the bile duct (m) with diffuse Kupffer cells proliferation in between the degenerated hepatocytes (d) (Fig. 2D-80×), Cyr+GT- treated rat liver showing alveolisation in hepatocytes indicated accumulation of lipids (arrows) (Fig. 2E-80×), showing dilatation in the central vein (cv) with diffuse Kupffer cells proliferation (arrow) in between degenerated hepatocytes (d) (Fig. 2F-80×). Specimens stained with hematoxylin and eosin.
DISCUSSION

The liver is the largest organ in the vertebrate body, and is the major site of xenobiotic metabolism and excretion. Hepatic injury is a common pathological feature which exists in many liver diseases. Since liver fibrosis, cirrhosis and even liver cancer could result from the long existence of hepatic injury. Therefore, prevention and treatment of hepatic injury is a key to treat liver diseases clinically. 

In toxicological studies, body, organ and relative organ weights are important criteria for evaluation of organ toxicity. In the present study, oral administration of CPF and/or Cyr resulted in a significant reduction and elevation in body and liver weights of rats, respectively. The reduction in body weight may be due to the combined action of cholenergic and oxidative stress, and/or due to the overall increased degradation of lipids and proteins as a result of the direct effects of CPF as an organophosphate compound. Moreover, the increase in liver weight could be attributed to the relationship between liver weight increase and various toxicological effects or to the reduction in body weight gain of experimental animals. Results are consistent with many previous investigators. However, co-administration of GT attenuated body and liver weights of intoxicated rats.

The activities of ALT, AST and ALP enzymes are the most sensitive biomarkers directly implicated in the extent of hepatic damage and toxicity. In our findings, we demonstrated that CPF and/or Cyr administration to rats provoked a marked elevation in serum AST, ALT and ALP activities which indicating hepatocellular damage as previously reported by El-Demerdash and Kalender et al. This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation, indicating a necrosis and inflammatory reactions. The increase of total protein in treated groups may be due to (a) due to high plasma globulin levels reflecting high protein in treated rats. In fact, elevation in LDH activity indicates cell lysis and death as well as the switching over of anaerobic glycolysis to aerobic respiration. In the present study, LDH activity was increased in the serum of treated rats, while was decreased in the liver tissue. The change in LDH activity resulted from overproduction of superoxide anions and hydroxyl radicals, which cause oxidative damage to the cell membrane and increase in membrane permeability.

Total protein and A/G ratio were done as a routine test to evaluate the toxicological nature of various chemicals. Increases of total protein and decrease of A/G ratio were observed in the present study following Cyr, CPF and CPF+Cyr treatments. In relation to decrease in the A/G ratio, the albumin level was also decreased, suggesting high plasma globulin levels reflecting high protein in treated rats. The increase of total protein in treated groups may be due to (a) due to production of enzymes lost as a result of tissue necrosis (b) to meet increased demand detoxifying the pesticide might necessitate enhanced synthesis of enzyme proteins.

Oxidative damage primarily occurs through production of reactive oxygen species, including hydroxyl radicals and hydrogen peroxide that are generated during the reaction and react with biological molecules, eventually damaging membranes and other tissues. Lipid peroxidation and the oxidative protein damages, provoked by free radicals' attack on biological structures, have been demonstrated to play a significant role in several pathological events. Indeed, measurement of protein carbonyl content (PC) and MDA have been used as a sensitive assay for oxidative damages of proteins and lipids, respectively. From the other side, GSH is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with chemicals. Also, it is central to the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes including GPx and GST. The results of the present study indicated that exacerbation of oxidative injury in liver of CPF and/or Cyr treated rats was more than control group as evidenced via increased serum MDA level and liver PC and via decreased liver GSH content. Our results are consistent with many previous studies following OPIs exposure. The significant increase in lipid peroxidation and PC of Cyr, CPF, Cyr+CPF-treated rats, explains observed leakage of cellular ALT, AST and ALP to circulation due to liver injury.

The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress. Hepatotoxicity could also be explained by the impaired antioxidant enzyme activities in the liver of the rats. Indeed, the antioxidant enzymes SOD, GPx and CAT limit the effects of oxidant molecules in tissues and act in the defense against oxidative cell injury by means of their being free radical scavengers. These enzymes work together to eliminate active oxygen species. In this respect, SOD accelerates the dismutation of H$_2$O$_2$, also termed as a primary defense, as it prevents further generation of free radicals whereas, CAT helps in the removal of H$_2$O$_2$ formed during the reaction catalyzed by SOD. Our results indicated that CPF and/or Cyr exposure altered SOD, CAT and GPx activities in liver of rat. These results were in line with previous studies which have shown that exposure to CPF generates lipid peroxidation and alters the antioxidant status of several tissues in rats. This alteration may be due to the decreased synthesis of enzymes or oxidative inactivation of enzyme protein.

The histopathological alterations in the present study could be summarized as follows: degeneration and coagulative necrosis in the hepatocytes, inflammatory cells infiltration, and Kupffer cells proliferation were observed in CPF, Cyr-, and CPF+Cyr-treated groups which sustained the leakage of liver enzymes.

Hepatoprotective potentials of medicinal plants against pesticides induced hepatotoxicity remain an area that needs extensive scientific research. Results of the current study revealed that GT extract reversed the elevation of lipid peroxidation. Hence, it is possible that the mechanism of hepatoprotection of GT extract may be attributed to polyphenolic compounds (e.g. epicatechins) that scavenge a wide range of free radicals including the most active hydroxyl radical, which initiate lipid peroxidation. Therefore, it may decrease the concentration of lipid free radicals. It was reported previously that phenolic compounds chelate metal ions, especially iron and copper, which, in turn inhibit generation of hydroxyl radicals and degradation of lipid hydroperoxides.

The biochemical alterations accompanied by histopathological changes resulted from CPF and/or Cyr exposures were alleviated following GT administration. This could be attributed to the antioxidant capacity of GT that attenuates the lipid peroxidation and liver antioxidant enzymes capacity which in turn restore the integrity of the cell membrane and improve the disturbance in permeability.

In conclusion, the results of the present study showed that Cyr and CPF induced oxidative damage and hepatotoxicity in rats. The ultimately effects was observed in their histopathological sections.

ACKNOWLEDGEMENT

The authors are grateful of Prof. Dr. Adel Mohamed Beker Kholoussy, Professor of Pathology, Faculty of Veterinary Medicine, Cairo University for interpreting the histopathological sections.

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