Full Length Research Paper

Biochemical Taurine alleviated modification in male Wistar rats co-exposed to chlorpyrifos and lead

Nwokoye Okeke, Asomba Emeka and Chuks Johntel

Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, P.M.B 5025, Awka,

Accepted 30 August 2014

The aim of the study was to investigate the effects of taurine on biochemical parameters in male Wistar rats co-exposed to chlorpyrifos and lead. Fifty rats were divided into five groups of ten rats each. The distilled water (DW) group received distilled water and the soya oil (SO) group received soya oil (1 ml/kg). Other groups were treated sequentially with taurine (50 mg/kg), chlorpyrifos (4.25 mg/kg, 1/20th LD50) and lead (233.25 mg/kg, 1/20th LD50), and the last group received taurine (50 mg/kg), chlorpyrifos (4.25 mg/kg) and lead (233.25 mg/kg). The treatments were administered once daily by oral gavage. The rats were sacrificed and blood samples were collected after 16 weeks. The serum samples were analyzed for proteins, enzymes, urea and creatinine concentration. The hepatic and renal malondialdehyde concentration and activities of hepatic and renal antioxidant enzymes were evaluated. The results indicated that chronic co-administration of chlorpyrifos (CPF) and lead acetate (LA) induced biochemical alterations in the rats. It is proposed that taurine antioxidant (TA) decreased the alterations in the biochemical parameters partly through its antioxidant, hepatoprotective and nephroprotective properties. It is concluded that taurine is a useful prophylactic agent against biochemical toxicity in individuals that are constantly co-exposed to chlorpyrifos and lead in the environment.

Key words: Taurine, chlorpyrifos, lead, oxidative stress, biochemical effects.

INTRODUCTION

It has been shown that human beings and other living organisms in the environment are commonly subjected to mixtures of different environmental pollutants either concurrently, sequentially, or both (Lokke et al., 2013). Indeed, pesticides and heavy metals are regarded as the most pervasive environmental pollutants due to their widespread applications in all aspects of human endeavour (Ambali et al., 2011). Living organisms are exposed to pesticide residues in soil and water and this constitutes a risk to ecosystem health and integrity (Kulshrestha and Kumari, 2011). The intensive use of pesticides has resulted in serious environmental problems because they are either recalcitrant or biodegraded very slowly, and pesticide residues have been detected in human blood samples, livestock, drinking water and foods on a global scale (Fenske et al., 2002; Mehta et al., 2009). Organophosphate insecticides (OPs) are one of the most widely used classes of pesticides for both agricultural and landscape pest control (Wu et al., 2011). Chlorpyrifos O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothionate (CPF) is one of the effective OPs that is commonly used throughout the world for domestic and industrial applications (Mansour and Mossa, 2010; Lee et al., 2012). The routes of exposure to CPF are inhalation, ingestion of contaminated food and by dermal contact (Yan et al., 2012). The main toxicity associated with exposure

*Corresponding author. E-mail: Okere_e@yahoo.com
function and modulating cytoplasmic and mitochondrial calcium homeostasis (Asha and Devadasan, 2013). Besides, TA exerts nephroprotective effects, probably due to its antioxidant and membrane stabilization effects (Chesney et al., 2010; Han and Chesney, 2012).

In the present study, we hypothesized that chronic co-administration of CPF and LA in the male Wistar rats may elicit adverse effects on biochemical parameters including proteins, enzymes, urea and creatinine concentration, hepatic and renal malondialdehyde concentration, and activities of hepatic and renal antioxidant enzymes. Additionally, we surmised that TA may alleviate the adverse effects of chronic co-administration of CPF and LA on biochemical parameters in the rats based on its bioprotective effects on the liver and kidneys. The present study is important because metals and pesticides are priority chemical mixtures for risk assessment due to their multiple organ toxicity (Lokke et al., 2013), and there is a high probability of the co-exposure of humans and other living organisms in the ecosystem to CPF and LA due to their prevalence (Agency for Toxic Substances and Disease Registry (ATSDR), 2006).

The aim of the present study was to investigate the effects of TA on biochemical parameters in male Wistar rats co-exposed to CPF and LA.

MATERIALS AND METHODS

Chemicals

Chlorpyrifos [CPF, EXCEL TERMIKILL® 20% emulsifiable concentrate, Excel Crop Care Limited, Mumbai, India] was reconstituted in soya oil (Grand Cereals and Oil Mills Limited, Jos, Nigeria) to produce a 1% stock solution that was used for the study. Taurine (TA) (CAS No. 107-36-3; purity ≥ 99%) and lead (LA) acetate trihydrate (CAS No. 6080-56-4; purity ≥ 99.99%) were purchased from Sigma Aldrich®, Steinheim, Germany. TA was reconstituted as a 10% stock solution in distilled water and LA was dissolved in distilled water to obtain a 40% stock solution before daily administration.

Experimental animals

Male Wistar rats weighing between 150 to 200 g were obtained from the animal house of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The rats were housed in steel cages at a temperature of 25°C in a 12 h light/dark cycle in the Toxicology Laboratory of the Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The animals were acclimatized for two weeks before the commencement of the study and they were maintained on standard rat chow and tap water ad libitum. The study was approved by Ahmadu Bello University Research Ethics Committee and it was conducted in accordance with the guidelines of the National Institute of Health Guide for Care and Use of Laboratory animals (Garber et al., 2011).

Experimental protocol

The Wistar rats were weighed and randomly allocated into five
groups, with 10 animals in each group. The rats in the DW group were administered with distilled water and those in the SO group received soya oil at 1 ml/kg. The TA group was treated with taurine (50 mg/kg) (Cetiner et al., 2005), while the CPF+LA group was sequentially administered with chlorpyrifos (CPF, 4.25 mg/kg, ~ 0.05% of LD₅₀) and then lead (LA, 233.25 mg/kg, ~ 0.05% of the LD₅₀). The median lethal dose (LD₅₀) of CPF is between 82 to 270 mg/kg according to some reports (Goel et al., 2007), and an LD₅₀ of 85 mg/kg was obtained for CPF in the present study according to the method of Lorke (1983). An LD₅₀ of 4665 mg/kg was applied for LA based on toxicity data obtained from the manufacturer (Sigma-Aldrich, 2012). The TA+CPF+LA group received TA, CPF and LA sequentially at the previously mentioned doses. The treatments were administered once daily by oral gavage for 16 weeks.

Evaluation of serum enzymes and proteins

The rats from each group were sacrificed after light ether anaesthesia by severance of the jugular veins at the end of the 16 weeks dosing period. Three millimeters of blood sample was collected from each rat into a centrifuge test tube that was devoid of anticoagulant. The blood samples were allowed to clot and were incubated at room temperature for 30 min. Subsequently, the blood samples were centrifuged at 1000 × g for 5 min to obtain a clear straw coloured serum that was used to evaluate biochemical parameters such as concentrations of total protein, albumin, urea and creatinine. Other biochemical parameters assayed in the serum Were the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH). The biochemical parameters were assayed with Bayer Express Plus Clinical Chemistry Autoanalyser (Bayer® Germany). Serum total protein concentration was estimated based on the Biuret method described by Henry et al. (1974), while serum albumin concentration was determined by the method of Spencer and Price (1977). Additionally, serum urea concentration was determined by using the diacetyl-monoximethiosemicarbazide procedure (Natlison et al., 1951), while serum creatinine concentration was measured using the method of Miller and Miller (1951). Serum globulin was obtained by the deduction of serum albumin concentration from total serum protein concentration, and the albumin/globulin ratio was also calculated. AST and ALT activities were evaluated as described by Schwarz et al. (1985) using the same auto-analyzer as stated. ALP concentration was estimated based on the enzymatic hydrolysis method described by King and Armstrong (1934), while GGT was evaluated based on the method described by Szasz et al. (1974). The concentration of LDH was assessed with the method of Zimmerman and Weinstein (1956).

Preparation of tissue homogenates

The liver and kidney samples were rinsed immediately with physiological saline, patted dry with filter paper and weighed following their excision from the rats. Portions of the tissues were mixed with phosphate buffered saline (PBS) pH 7.4 in a 1:10 (w/v) ratio and made into homogenates with pestles and mortars. The mixtures obtained were kept cold with ice baths and were centrifuged afterwards for the evaluation of the activities of hepatic and renal antioxidant enzymes and the concentration of hepatic and renal malondialdehyde.

The concentration of malondialdehyde (MDA) as an indication of lipid peroxidation was evaluated in the sera, liver and kidney samples with the method described by Draper and Hadley (1990). The principle of the method was based on the spectrophotometric measurement of the colour developed during the reaction of thiobarbituric acid (TBA) with MDA. The procedure was conducted for the sera, liver and kidneys of the rats as follows: 2.5 ml of 100 g/L trichloroacetic acid solution was added to 0.5 ml of the samples in centrifuge tubes that were placed in boiling water baths for 15 min. After cooling under tap water for 5 min, each mixture was centrifuged at 1000 × g for 10 min. Subsequently, 2 ml of each supernatant was added to 1 ml of 6.7 g/L TBA solution in test tubes placed in boiling water baths for 15 min. The solutions were cooled under tap water and the absorbance was measured with a UV spectrophotometer (T80° UV/VIS Spectrophotometer®). PG Instruments Ltd., Lichestershire, LE 175BE, United Kingdom) at 532 nm. The concentration of MDA in the samples were calculated by using the absorbance coefficient of MDA-TBA complex 1.56 × 10⁵/cm/M and expressed as μmol/L (in the serum) nmol/mg protein (in the liver and kidneys).

Assessment of activities of antioxidant enzymes in the liver and kidneys

Superoxide dismutase (SOD) activity was assessed with the NWLSS™ SOD activity assay kit and the principle of the method was based on autoxidation of haematoxylin (Martin et al., 1987). Catalase (CAT) activity was analyzed with the NWLSS™ CAT activity assay kit and the method used was based on the consumption of H₂O₂ substrate as described by Beers and Sizer (1952). The activity of glutathione peroxidase (GPx) was evaluated with the NWLSS™ GPx activity assay kit and the procedure was based on the oxidation of reduced GSH to form oxidized GSH (Paglia and Valentine, 1967). The assays were conducted according to the manufacturer's (Northwest Life Science Specialties, LLC, Vancouver, WA 98662) instructions.

Statistical analysis

Data were presented as mean ± standard error of the mean (SEM). The biochemical parameters were analyzed with one-way analysis of variance followed by Tukey’s multiple comparison post-hoc test. Statistical analysis was conducted with GRAPHPAD PRISM VERSION 4.00 FOR WINDOWS (Graphpad Software, San Diego, California, USA). Values of P < 0.05 were considered significant. The differences in the mean values of the data obtained between each group were expressed in percentages where necessary.

RESULTS

Effects of treatments on serum proteins

Taurine increased total protein concentration

The total protein concentration of the TA+CPF+LA group was significantly increased (P < 0.05) compared to that of the CPF+LA group (Table 1). Additionally, the total pro-tein concentration of the CPF+LA group was significantly reduced (P < 0.01) relative to those of the DW and SO groups.
respectively (Table 1). The total protein concentration of the TA group was increased by 7% compared to that of the CPF+LA group.

**Taurine improved albumin concentration**

There was a significant improvement ($P < 0.05$) in the serum albumin concentration of the TA group compared to that of the CPF+LA group (Table 1). Although not significant, the serum albumin concentration of the TA+CPF+LA group increased by 19% compared to that of the CPF+LA group. There was an increase ($P < 0.05$) in the serum albumin concentration of the DW and SO groups relative to that of the CPF+LA group.

**Co-exposure of rats to chlorpyrifos and lead decreased serum globulin concentration**

There was no significant change in the serum globulin concentration in between the groups (Table 1). However, increases were recorded in the serum globulin concentration of the TA+CPF+LA (17%), TA (18%), DW (19%) and SO (9%) groups relative to that of the CPF+LA group.

**Taurine increased albumin/globulin ratio**

A significant increase was recorded in the albumin/globulin ratio of the TA ($P < 0.01$) group compared to that of the CPF+LA group (Table 1). The albumin/globulin ratio of the SO group was also higher ($P < 0.01$) compared to that of the CPF+LA group. There were increases in the albumin/globulin ratios of the TA+CPF+LA (20%) and DW (30%) groups compared to that of the CPF+LA group.

### Effects of treatments on serum enzymes

#### Chlorpyrifos and lead co-treatment in rats elevated aspartate aminotransferase concentration

There was no significant difference in the aspartate aminotransferase (AST) activity in between the groups (Table 2). The highest AST activity was observed in the CPF+LA group while the lowest was recorded in the TA group. The AST activity was relatively higher in the CPF+LA group compared to those in the TA+CPF+LA (16%), TA (11%), DW (10%) and SO (7%) groups.

#### Co-treatment of rats with chlorpyrifos and lead increased alanine aminotransferase activity

There was a significant increase in the alanine aminotransferase (ALT) activity of the CPF+LA group compared to those of the DW ($P < 0.05$) and SO ($P < 0.01$) groups (Table 2). The ALT activity of the CPF+LA group was relatively higher compared to those of the TA+CPF+LA (17%) and TA (16%) groups.

### Table 1. Effects of the treatments on serum protein concentration.

<table>
<thead>
<tr>
<th>Concentration (g/dl)</th>
<th>DW</th>
<th>SO</th>
<th>TA</th>
<th>CPF+LA</th>
<th>TA+CPF+LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>67.6±2.2</td>
<td>68.4±1.6</td>
<td>63.2±2.3</td>
<td>59±1.2</td>
<td>66.2±1.1</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>37.4±2</td>
<td>40.6±1.8</td>
<td>37.8±1.7</td>
<td>29.4±1.6**</td>
<td>36.2±1.4</td>
</tr>
<tr>
<td>Serum globulin</td>
<td>30.2±1.5</td>
<td>27.8±1.2</td>
<td>30±1</td>
<td>25.4±1.2</td>
<td>29.6±1</td>
</tr>
<tr>
<td>Albumin/globulin ratio</td>
<td>1.3±0.1</td>
<td>1.5±0.1</td>
<td>1.6±0.1</td>
<td>1±0.1#</td>
<td>1.2±0.1</td>
</tr>
</tbody>
</table>

DW (Distilled water), SO (Soya oil), TA (Taurine), CPF+LA (Chlorpyrifos+Lead), TA+CPF+LA (Taurine +Chlorpyrifos + Lead). $^*P < 0.01$ versus DW group, $^*P < 0.01$ versus SO group, $^*P < 0.01$ versus TA group, $^*P < 0.05$ versus TA+CPF+LA group $P < 0.05$ versus DW, SO and TA groups.

### Table 2. Effects of the treatments on the activities of serum enzymes.

<table>
<thead>
<tr>
<th>Enzyme (IU/L)</th>
<th>DW</th>
<th>SO</th>
<th>TA</th>
<th>CPF+LA</th>
<th>TA+CPF+LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>22±1.4</td>
<td>22.8±2.3</td>
<td>21.8±2.5</td>
<td>24.4±0.7</td>
<td>20.5±1.6</td>
</tr>
<tr>
<td>ALT</td>
<td>28±2.1</td>
<td>26±1.4</td>
<td>31.6±2.8</td>
<td>37.4±1.3**</td>
<td>31±1.8</td>
</tr>
<tr>
<td>ALP</td>
<td>58±3.3</td>
<td>56.6±1.6</td>
<td>51.6±3.9</td>
<td>71.6±3.4**</td>
<td>64±3.2</td>
</tr>
<tr>
<td>GGT</td>
<td>33.6±2.1</td>
<td>33.8±1.2</td>
<td>41.8±3</td>
<td>50.4±2.6**</td>
<td>44.2±2.9</td>
</tr>
<tr>
<td>LDH</td>
<td>170.4±11</td>
<td>184±25.8</td>
<td>209.6±16</td>
<td>245.2±7.6**</td>
<td>223.2±6.7</td>
</tr>
</tbody>
</table>
Table 3. Effects of the treatments on the activities of hepatic antioxidant enzymes.

<table>
<thead>
<tr>
<th>Enzymes (IU/L)</th>
<th>DW</th>
<th>SO</th>
<th>TA</th>
<th>CPF+LA</th>
<th>TA+CPF+LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver SOD</td>
<td>2.1±0.1</td>
<td>2.2±0.1</td>
<td>2.5±0.1</td>
<td>2±0.1*</td>
<td>2.2±0.1*</td>
</tr>
<tr>
<td>Liver CAT</td>
<td>54.2±1.4</td>
<td>57.8±1.5</td>
<td>52.8±1.7</td>
<td>50.6±1.4#</td>
<td>55±1.5</td>
</tr>
<tr>
<td>Liver GPx</td>
<td>32.2±2.8</td>
<td>32±2.6</td>
<td>34.2±2.5</td>
<td>30.4±2.4</td>
<td>31.8±2.5</td>
</tr>
</tbody>
</table>

DW (Distilled water), SO (Soya oil), TA (Taurine), CPF+LA (Chlorpyrifos + Lead), TA+CPF+LA (Taurine + Chlorpyrifos + Lead).

*P < 0.01 versus TA group, #P < 0.05 versus SO group. SOD (Superoxide dismutase), CAT (Catalase) and GPx (Glutathione peroxidase).

**Taurine decreased alkaline phosphatase activity**

There was a marked decrease (P < 0.001) in the alkaline phosphatase (ALP) activity of the TA group compared to that of the CPF+LA group (Table 2). Additionally, the ALP activity of the TA+CPF+LA group was decreased by 11% compared to that of the CPF+LA group. The co-exposure of the rats to CPF and LA also caused a significant increase (P < 0.05) in the ALP activity compared to those of the DW and SO groups.

**Taurine reduced gamma glutamyl transferase activity**

The gamma glutamyl transferase (GGT) activity of the TA group was significantly reduced (P < 0.05) compared to that of the CPF+LA group (Table 2). In addition, a reduction of 12% was recorded in the GGT activity of the TA+CPF+LA group compared to that of the CPF+LA group. There was a significant increase (P < 0.001) in the GGT activity of the CPF+LA group compared to those of the DW (5%) and SO (9%) groups.

**Chronic co-administration of chlorpyrifos and lead in rats increased lactate dehydrogenase activity**

The lactate dehydrogenase (LDH) activity of the CPF+LA group was increased compared to those of the DW (P < 0.01) and SO (P < 0.05) groups (Table 2). There were reductions in the LDH activity of the TA+CPF+LA (9%) and TA (15%) groups compared to that of the CPF+LA group.

**Chlorpyrifos and lead co-administration increased serum urea concentration**

There was a significant increase (P < 0.05) in the serum urea concentration of the CPF+LA group compared to that of the DW group (Figure 1). The serum urea concentration of the CPF+LA group was also relatively higher compared to those of the TA+CPF+LA (8%), TA (13%) and SO (20%) groups.

**Chronic co-exposure of rats to chlorpyrifos and lead increased serum creatinine concentration**

There was no significant difference in the serum creatinine concentration in between the groups (Figure 1).

The serum creatinine concentration of the CPF+LA group was higher compared to those of the TA+CPF+LA (6%), TA (9%), DW (15%) and SO (13%) groups.

**Effects of treatments on antioxidant enzyme activity**

**Taurine augmented hepatic superoxide dismutase activity**

The hepatic superoxide dismutase (SOD) activity was augmented in the TA group (P < 0.01) compared to that of the CPF+LA group (Table 3). There was a 9% increase in the hepatic SOD activity in the TA+CPF+LA group compared to that of the CPF+LA group. Additionally, the rats co-exposed to CPF and LA showed decreased hepatic SOD activity compared to those of the DW (5%) and SO (9%) groups.

**Chronic co-exposure of rats to chlorpyrifos and lead reduced hepatic catalase activity**

There was a significant reduction (P < 0.05) in the hepatic catalase (CAT) activity of the CPF+LA group compared to that of the SO group (Table 3). The hepatic CAT activity of the TA+CPF+LA and TA groups were higher than that in the CPF+LA group by 9 and 4%, respectively. A decrease of 7% was recorded in the hepatic CAT activity in the CPF+LA group compared to that in the DW group.

**Taurine ameliorated hepatic glutathione peroxidase activity**

There was no significant change in the hepatic glutathione peroxidase (GPx) activity in between the groups (Table 3). However, the highest hepatic GPx activity was recorded in the TA group, while the lowest hepatic GPx activity was observed in the CPF+LA group. There were increases in the hepatic GPx activity of the DW (6%), SO (5%), TA (13%) and TA+CPF+LA (5%) groups compared to that of the CPF+LA group.

**Chronic chlorpyrifos and lead co-treatment in rats reduced renal superoxide dismutase activity**

There was a significant reduction (P < 0.05) in the renal
Table 4. Effects of the treatments on the activities of renal antioxidant enzymes.

<table>
<thead>
<tr>
<th>Enzyme (IU/L)</th>
<th>DW</th>
<th>SO</th>
<th>TA</th>
<th>CPF+LA</th>
<th>TA+CPF+LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal SOD</td>
<td>2.1±0.2</td>
<td>1.9±0.1</td>
<td>1.8±0.1</td>
<td>1.5±0.2*</td>
<td>1.6±0.1*</td>
</tr>
<tr>
<td>Renal CAT</td>
<td>46±2.5</td>
<td>47.8±2.6</td>
<td>50±3.3</td>
<td>42.8±3.6</td>
<td>48.6±1.3</td>
</tr>
<tr>
<td>Renal GPx</td>
<td>29.4±1.5</td>
<td>28.5±1.3</td>
<td>31.2±1</td>
<td>27.5±1</td>
<td>28.1±1.1</td>
</tr>
</tbody>
</table>

DW (Distilled water), SO (Soya oil), TA (Taurine), CPF+LA (Chlorpyrifos+Lead), TA+CPF+LA (Taurine + Chlorpyrifos + Lead). *P < 0.05 versus DW group SOD.

Figure 1. Effects of the treatments on serum urea concentration. DW (Distilled water), SO (Soya oil), TA (Taurine), CPF+LA (Chlorpyrifos+Lead), TA+CPF+LA (Taurine + Chlorpyrifos + Lead). *P < 0.05 compared to the DW group.

Figure 2. Effects of the treatments on serum creatinine concentration. DW (Distilled water), SO (Soya oil), TA (Taurine), CPF+LA (Chlorpyrifos+Lead), TA+CPF+LA (Taurine + Chlorpyrifos + Lead).
The liver samples were rinsed immediately with physiological saline following excision from the bodies of the rats, patted dry with filter paper and then weighed. Portions of the liver samples were mixed with phosphate buffered saline (PBS) pH 7.4 in a 1:10 (w/v) ratio and made into fine homogenates with pestles and mortars. The mixtures were kept cold with ice baths and were centrifuged afterwards. The concentration of malondialdehyde was evaluated in the liver by using the method of Draper and Hadley (1990). The absorbance was measured with a UV Spectrophotometer (T80+ UV/VIS SPECTROMETER, PG Instruments Ltd, Leicestershire, LE7 3BE, United Kingdom) at 532 nm. The concentration of MDA in the liver tissues was calculated by using the absorbance coefficient of malondialdehyde-thiobarbituric acid complex \(1.56 \times 10^5\)/cm/M and expressed as nmol/mg protein. The protein contents of the supernatant were evaluated using the method of Dacie and Lewis (1991).

**Figure 3.** Effects of the treatments on hepatic malondialdehyde concentration.

- DW (Distilled water), SO (Soya oil), TA (Taurine), CPF+LA (Chlorpyrifos + Lead), TA+CPF+LA (Taurine + Chlorpyrifos + Lead). *P < 0.01 compared to the DW and TA groups.

**Superoxide dismutase (SOD) activity**

There was no significant difference in the renal SOD activity in between the groups (Table 4). The highest SOD activity was observed in the TA group, while the lowest SOD activity was recorded in the CPF+LA group. The renal SOD activity of the CPF+LA group was relatively lower than those of the TA+CPF+LA (7%), TA (20%) and SO (27%) groups.

**Taurine increased renal catalase activity**

There was no significant difference in the renal CAT activity in between the groups (Table 4). The highest renal CAT activity was observed in the TA group, while the lowest renal CAT activity was recorded in the CPF+LA group. The renal CAT activity of the CPF+LA group was lower compared to those of the TA+CPF+LA (12%), TA (17%), DW (8%) and SO (12%) groups.

**Taurine augmented renal glutathione peroxidase activity**

The effect of treatments on renal GPx activity is shown in Table 4. There was no significant difference in the renal GPx activity in between the groups. The highest renal GPx activity was noted in the TA group, while the lowest renal GPx activity was recorded in the CPF+LA group. However, there was a reduction in the renal GPx activity of the CPF+LA group relative to those of the TA+CPF+LA (2%), TA (12%), DW (7%) and SO (4%) groups.

**Effects of treatments on malondialdehyde concentration**

**Taurine reduced hepatic malondialdehyde concentration**

The hepatic malondialdehyde (MDA) concentration of the TA group was significantly reduced (P < 0.01) compared to that of the CPF+LA group (Figure 3). Additionally, a significant reduction (P < 0.01) was recorded in the hepatic MDA concentration of the DW group compared to that of the CPF+LA group. There were also reductions in the hepatic MDA concentration of the TA+CPF+LA (27%)
Figure 4. Effects of the treatments on renal malondialdehyde concentration. DW (Distilled water), SO (Soya oil), TA (Taurine), CPF+LA (Chlorpyrifos+Lead), TA+CPF+LA (Taurine + Chlorpyrifos + Lead). *P < 0.01 compared to the TA group. The kidney samples were rinsed immediately with physiological saline following excision from the bodies of the rats, patted dry with filter paper and then weighed. Portions of the kidney samples were mixed with phosphate buffered saline (PBS) pH 7.4 at a 1:10 (w/v) ratio and made into fine homogenates with pestles and mortars. The mixtures were kept cold with ice baths and were centrifuged afterwards. The renal malondialdehyde concentration was evaluated by using the method of Draper and Hadley (1990). The absorbance was measured with a UV Spectrophotometer (T80® UV/VIS SPECTROMETER, PG Instruments Ltd., Lichestershire, LE 175BE, United Kingdom) at 532 nm. The renal malondialdehyde concentration was calculated by using the absorbance coefficient of malondialdehyde-thiobarbituric acid complex 1.56 × 10^5/cm/M and expressed as nmol/mg protein. The protein contents of the supernatant were evaluated using the method of Lowry (1951).

and SO (36%) groups compared to that of the CPF+LA group.

**Taurine decreased renal malondialdehyde concentration**

There was a significant decrease (P < 0.01) in the renal MDA concentration of the TA group compared to that of the CPF+LA group (Figure 4). In addition, there were reductions in the renal MDA concentration of the TA+CPF+LA (40%), DW (40%) and SO (30%) groups compared to that of the CPF+LA group.

**DISCUSSION**

The present study indicated that chronic co-exposure of the rats to CPF and LA elicited a decrease in total protein concentration due to hypoalbuminaemia and hypoglobulinaemia. Hypoalbuminaemia may be attributed to a reduction in serum albumin synthesis as a result of hepatic impairment and/or increased urinary excretion caused by impaired renal function (Ambali et al., 2011). In addition, CPF and LA have been shown to evoke hepatic and renal damage (Ibrahim et al., 2012; Ma et al., 2013) partially through the induction of oxidative stress in rodents. It is noteworthy that albumin (an antioxidant) is usually depleted during oxidative stress and its cysteine-34 residue participates directly in the scavenging of free radicals in biological systems (Atmaca, 2004; Roche et al., 2005; El-Neekety et al., 2009).

The results revealed a reduction in globulin concentration in the CPF+LA group. It has been demonstrated that hypoglobulinaemia may ensue in biological systems following the induction of apoptotic damage to immune cells by pesticides and heavy metals (Rabideau, 2001; Simsek et al., 2009). Although apoptosis was not evaluated in the present study, it is possible that the hypoglobulinaemia recorded in the CPF+LA group was due to the induction of apoptotic damage to the immune cells by the chronic co-treatment of the rats with CPF and
LA. In contrast, TA increased total protein, albumin, globulin and albumin/globulin concentration in the current study. It is known that TA protects the immune system from oxidative stress by preventing DNA damage and apoptosis in lymphocytes (Schuller-Levis and Park, 2004; Sokol et al., 2009), and this may have contributed to its ability to normalize the total protein, albumin, globulin and albumin/globulin concentration in the TA+CPF+LA group. In addition, TA exhibits hepatoprotective (El-Sayed et al., 2011) and nephroprotective (Das and Sil, 2012) effects, and this may have contributed to the normalization of the serum protein parameters in the TA+CPF+LA group.

Furthermore, there were elevations in the activities of the enzymes (AST, ALT, ALP, GGT and LDH) in the rats co-treated with CPF and LA. Ambali et al. (2011) reported that subchronic co-administration of CPF and LA elicited a significant increase in the activity of AST (an enzyme found in the liver, skeletal muscle and myocardiad cells). The enhanced AST activity in the CPF+LA group may be a result of the increased release of the enzyme into the peripheral circulation following hepatic or muscular damage caused by both toxicants. Moreover, the significant increase in ALT activity in the CPF+LA group indicated hepatic damage because the enzyme is regarded as a sensitive and specific index of hepatocellular injury (Moon et al., 2013). It has been shown that CPF (Uzun and Kalender, 2013) and LA (Dewanjee et al., 2013) generate ROS, evoke lipid peroxidation and cell membrane damage, and cause the leakage of enzymes into the blood.

Accordingly, these adverse effects may have resulted in the elevation of the activities of the enzymes in the CPF+LA group. Additionally, ALP activity was increased in the CPF+LA group in the present study. It is known that ALP activity increases in hepatic cell damage and bile duct obstruction (Kalender et al., 2005) and the activity of the enzyme also increases in CPF and LA intoxication, respectively (Lukaszewicz-Hussain, 2013; Thenmozhi et al., 2013). In the current research, there was also an increase in the activity of GGT in the CPF+LA group. GGT activity is an excellent indicator of hepatobiliary disease and it is mainly used to confirm if increased ALP activity is of hepatic aetiology (Stojacic et al., 2008). Therefore, the increased GGT activity recorded in the CPF+LA group implied that the increased ALP activity resulted mainly from lesions in the liver and bile duct. The liver is an important site of multiple oxidative reactions and maximum free radical generation (Hazari et al., 2003), and it is plausible that the induction of oxidative stress by the co-administration of CPF and LA to the rats enhanced oxidative damage in the liver.

CPF has been shown to adversely affect the cytochrome P450 system or the mitochondrial membrane transport of hepatocytes (Elshehrawy et al., 2013), and this may have contributed to the increased ALP activity in the CPF+LA group. Besides, the LDH activity was increased in the CPF+LA group in the present investigation. CPF has been reported to increase LDH activity in male rats (Uzun and Kalender, 2013), while LA has also been shown to increase LDH activity in male rats according to Ibrahim et al. (2012), as demonstrated in the current study. Increased serum LDH activity in experimental animals is associated with hepatocellular necrosis that may result in the leakage of the enzyme into the blood stream (Mansour and Mossa, 2010).

In contrast, TA alleviated the alterations in the activities of the enzymes (AST, ALT, ALP, GGT and LDH) in the TA+CPF+LA group. Although the activities of the enzymes were insignificantly reduced in the TA+CPF+LA group, it was apparent that chronic supplementation with TA attenuated the increase in the activities of the enzymes. It is notable that the hepatoprotective property of TA is due to its ability to decrease oxidative stress, enhance mitochondrial function and amend cytoplasmic and mitochondrial Ca\(^{2+}\) homeostasis in biological systems (Asha and Devadasan, 2013).

In the current study, the serum urea concentration was increased in the CPF+LA group and this suggested that the chronic co-administration of both toxicants to the rats evoked renal impairment since the kidney primarily eliminates urea in the urine. Increased urea concentration has been reported following CPF intoxication in rodents (Ambali et al., 2007) and it was associated with glo-merular and renal tubular degeneration, partially evoked by oxidative stress. Similarly, some researchers (El-Neekety et al., 2009; Abdel-Moneim et al., 2011) reported increased urea concentration in rats exposed to subacute LA intoxication. It is postulated that the increased urea concentration recorded in the CPF+LA group may be attributed to the induction of renal damage by the chronic co-treatment of the rats with both toxicants. On the contrary, TA pretreatment evoked decreased urea concentration in the TA+CPF+LA group and this may be a demonstration of its nephroprotective role (Roy et al., 2009; Das et al., 2010).

Moreover, there was an elevation in the serum creatinine concentration in the CPF+LA group, and this may be an indication of renal damage. Goel et al. (2005) reported an increase in creatinine concentration as well as glomerular and renal tubular degenerative changes following CPF exposure in rats. It has been shown that CPF elicits renal damage by enhancing the ROS level in kidney tissues and this culminates in ROS accumulation, oxidative stress and renal tissue damage (Ma et al., 2013). Similarly, LA has been reported to increase creatinine concentration in rats and it was associated with renal parenchymal damage and impaired glomerular infiltration (Krishna and Ramachandran, 2009; Abdel-Moneim et al., 2011; Karamala et al., 2011). LA evokes
renal damage by inducing oxidative stress and altering the expression of apoptosis related proteins (such as Bax) in rat kidneys (Abdel-Moneim et al., 2011). Bax is a pro-apoptotic member of the Bcl-2 family that is essential for the regulation of intrinsic apoptotic signalling through oxidative injury to the mitochondria (Wei et al., 2001).

In the present study, the creatinine concentration was normalized in the TA+CPF+LA group, and TA may have alleviated the renal damage induced by chronic co-exposure of the rats to CPF and LA through its free radical scavenging and nephroprotective properties (Manna et al., 2009; Das et al., 2010). The protective property of TA may also reside in its ability to become chlorinated in the presence of hypochlorous acid, thereby preventing the direct attack of this oxidant on cell membranes of organs, including the kidney (Roy et al., 2009). It is known that TA also exhibits nephroprotection by regulating blood flow in the renal vasculature and Na⁺ transport in the proximal tubes, maintaining osmoregulation and scavenging ROS in the glomerulus (Chesney et al., 2010; Karbalay-Doust et al., 2012).

Furthermore, there was increased hepatic and renal MDA concentration in the CPF+LA group in the current study. The individual administration of CPF and LA has been shown to elicit lipid peroxidation in the liver and kidneys of rodents and it was manifested as increased MDA concentration (Dewanjee et al., 2013; Ma et al., 2013). It may be deduced that the chronic co-administration of CPF and LA to the Wistar rats elicited the elevation in MDA concentration in the liver and kidneys based on their separate hepatotoxic and nephrotoxic effects. MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids and increased MDA content is a crucial indicator of oxidative stress and lipid peroxidation (Demir et al., 2011). Lipid peroxidation entails oxidative degradation of poly-unsaturated fatty acids and its occurrence in biological membranes engenders impairment of membrane fluidity and inactivation of several membrane-bound enzymes that are crucial for numerous biological processes (Goel et al., 2005). Conversely, there was augmentation of the activities of the hepatic and renal antioxidant enzymes and attenuation of lipid peroxidation in the liver and kidneys of the rats in the TA+CPF+LA group. It is known that TA offsets lipid peroxidation either by scavenging ROS directly or by binding to ferrous ion or copper ion through its sulphonic acid group (Franconi et al., 2004; Hagar, 2004). These mechanisms of action of TA may have contributed to the mitigation of lipid peroxidation in the liver and kidneys of the rats in the TA+CPF+LA group.

The results of the current study also indicated reductions in the activities of the hepatic and renal antioxidant enzymes (SOD, CAT and GPx) in the CPF+LA group. Antioxidant enzymes comprise the antioxidant defence system of the body against oxidative stress evoked by various xenobiotics (Ojha et al., 2011). SOD catalyzes the conversion of superoxide radicals to H₂O₂, while CAT converts H₂O₂ into H₂O (El-Demerdash, 2011). Besides, GPx converts H₂O₂ into H₂O in the presence of oxidized GSH (Kanbur et al., 2009). It has been observed that the exposure of rats to CPF elicits oxidative stress in the liver and kidneys through the modification of endogenous antioxidant enzymes including SOD, GPx and GSH (Ma et al., 2013; Uzun and Kalender, 2013). Likewise, it has been reported that LA increased ROS generation, enhanced lipid peroxidation and reduced the activities of SOD, CAT and GPx in the liver and kidneys of rats (Dewanjee et al., 2013; Wang et al., 2013). Conceivably, the depletion of the activities of the hepatic and renal antioxidant enzymes in the CPF+LA group may be attributed to chronic CPF and LA-induced oxidative stress in the rats. However, there was improvement in the activities of the hepatic and renal antioxidant enzymes in the TA and TA+CPF+LA groups in the present study. It has been shown that TA exhibits its antioxidant capacity by enhancing the antioxidant system, forming chlora-mines with hypochlorous acid and replacing glutathione (GSH) in biological systems during oxidative stress (Eppler and Dawson, 2002; Devi and Anuradha, 2010).

Conclusion

The results of the present study indicated that chronic co-administration of CPF and LA induced biochemical alterations in the male Wistar rats. It is proposed that TA attenuated the alterations in the biochemical parameters partly by alleviating oxidative stress, augmenting the activities of the antioxidant enzymes and exhibiting protective effects on the liver and kidneys in the TA and TA+CPF+LA groups. It is concluded that TA may be a useful prophylactic agent against biochemical toxicity in individuals that are constantly co-exposed to CPF and LA in the environment.

ABBREVIATIONS

ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BAX, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; CAS, Chemical Abstract Service; CAT, Catalase; Cl⁻, Chloride; CPF, Chlorpyrifos; DW, Distilled water; GGT, Gamma-glutamyl transferase; GPx, Glutathione peroxidase; GSH, Glutathione; H₂O₂, Hydrogen peroxide; HCO₃⁻, Bicarbonate; K⁺, Potassium; LA, Lead acetate; LDH, Lactate dehydrogenase; MDA, Malondialdehyde; Na⁺, Sodium; NADPH, Nicotinamide adenine dinucleotide phosphate hydrogen; Ops, Organophosphate pesticides; SO, Soya oil; SOD, Superoxide dismutase; TA, Taurine antioxidant.
REFERENCES


Sigma-Aldrich Corporation (2012). Lead acetate trihydrate ACS reagent. 215902.


