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Cole (2000), Steddy et al. (2003), (Kelebeni, 1983), (Bane and Jake, 1992), (Chege, 1998; Cohen, 1987a,b; Tristan, 1993,1995), (Kumasi et al., 2001)

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

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Taurine alleviated biochemical alterations in male Wistar rats co-exposed to chlorpyrifos and lead

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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 (Yanet al., 2012). The main toxicity associated with exposure...
to OPs is acetylcholinesterase (AChE) inhibition at cholinergic synapses and the neuromuscular junctions (Oruc, 2010). CPF undergoes oxidative desulphuration to CPF oxon (CPF-O), the active metabolite which is more potent than CPF itself as an AChE inhibitor, and the nervous system is the main target of the acute toxic effects of CPO (Sultatos, 1994). In addition, CPF has been reported to evoke oxidative stress in biological systems (Elsharkawy et al., 2013).

Metals are naturally occurring elements with high atomic weights and they are found in the air, aquatic and terrestrial systems (Jones and Miller, 2008). They may concentrate through food webs, and species at the top of food chains can accumulate high levels of metals (Niecke et al., 1999). Lead (LA) is a naturally occurring systemic toxicant and heavy metal found in pipes, drains and soldering materials (Ansari et al., 2013). It is a widespread environmental toxic metal that poses serious threats to human health and it is mainly conveyed to humans through dietary and occupational sources (Chang et al., 2012; Flora et al., 2012). LA induces oxidative stress and disrupts the intracellular prooxidant/antioxidant balance through the generation of excess reactive oxygen species (ROS) in biological systems (Hsu and Guo, 2002). Consequently, LA has been implicated in oxidative damage to erythrocytes, the heart, liver, kidneys and brain (Ahamed et al., 2005).

It is noteworthy that OPs and heavy metal residues are common chemical food contaminants (Bhanti and Taneja, 2007; Sharma et al., 2008). Specifically, CPF and LA residues have been detected in leafy vegetables, fruits and some aromatic medicinal plants in several countries (Dogheim et al., 2004; Markovic et al., 2010; Harris et al., 2011). Although CPF and LA inhibit acetylcholinesterase (Richetti et al., 2011; Ambali and Aliyu, 2012) and delta-aminolevulinic acid dehydratase (ALAD) activities (Hernández et al., 2005; Rendón-Ramírez et al., 2007), oxidative stress has also been identified as a common molecular mechanism of toxicity of CPF and LA in biological systems (Khalaf et al., 2012; Lee et al., 2012). Oxidative stress occurs when a perturbation in the balance between oxidants and antioxidants culminates in damage to critical biomolecules such as DNA, lipids and proteins (Yonar and Sakin, 2011). It has been shown that antioxidants prevent or reduce the oxidation of other molecules by reactive oxygen species (ROS) in living organisms, scavenge free radicals and attenuate their deleterious effects (Koivula and Eeva, 2010; Ma et al., 2013).

Taurine (TA) is a sulphur containing β-amino acid and an antioxidant that is present in most animal tissues and it is essential for the normal functioning of different organs (Brosnan and Brosnan, 2006). The source of TA in the body is biosynthesis and dietary intake from meat and especially sea food (Ito et al., 2012). Moreover, TA has been shown to prevent toxin-mediated hepatic injuries by reducing oxidative stress, enhancing mitochondrial 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. 107-35-7; purity ≥ 99%) and lead (LA) acetate trihydrate (CAS No. 6800-56-4; purity ≥ 99.9%) 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 millilitres 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 Autoanalysers (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 Spender and Price (1977). Additionally, serum urea concentration was determined by using the diacetyl-monoximethiosemicarbazide procedure (Natelson 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 Schwartz 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 was calculated by using the absorbance coefficient of MDA-TBA complex 1.56 × 10^5/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 Specialities, 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 4.0 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 protein concentration of the CPF+LA group was significantly reduced (P < 0.01) relative to those of the DW and SO groups.
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>

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>

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.

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.
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 and SO 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 (Superoxide dismutase), CAT (Catalase) and GPx (Glutathione peroxidase).

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).
superoxide dismutase (SOD) activity of the CPF+LA group compared to that of the DW group (Table 4). 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 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 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., Lichetershire, 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 myocardial 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 (Stojic 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 (Hazarakia 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 (Elsharkawy 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 glomerular 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 tubules, 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 chloramines 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


Moon SS, Lee YS, Kim JG, Lee IK (2013). Association of 119-


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


Full Length Research Paper

Undesirable effects of drinking water chlorination by-products

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The fundamental objective of water treatment is the protection of consumers from pathogenic microorganisms. Chlorination of drinking water is essential to prevent waterborne disease. However, chlorine reacts with organic matter present in surface waters to form various by-products suspected of being carcinogenic. In the last decade, several epidemiological studies have been conducted to determine the connection between exposure to these chlorination by-products and human health defects. The purpose of this paper is to evaluate the genotoxicity of drinking water of Annaba city. The study have been carried out in different points of water distribution and in the station of treatment, using two tests of determination of genotoxic risk by means of SOS chromotest (using the strain Escherichia coli PQ37). SOS chromotest showed genotoxic effect of the sample collected from the exit of treatment station.

Key words: Drinking water, genotoxicity, SOS chromotest, Escherichia coli PQ37.

INTRODUCTION

Water is the most essential element to life on earth that is why it is a subject of attentive surveillance to prevent waterborne disease. The objectives of the surveillance of the quality of water destined for consumption are numerous and vary depending on means and process possibilities (Bouziani, 2000). Disinfection of drinking water has been widely practiced in drinking water treatment. It is essential to protect the public health and ensure water quality from the water treatment plant outlet to the consumer’s tap (that is, during water distribution). Chlorine (Cl2) is used as the most common disinfectant due to its high efficiency to eliminate pathogens and protect human health against waterborne diseases (United States Environmental Protection Agency (US EPA), 2000; Liu et al., 2011). However, chlorine and other disinfectants react with natural organic matter and/or inorganic substances occurring in water to form various disinfection by-products such as trihalomethanes (THMs), haloacetic acids (HAAs) and other compounds (Legay et al., 2010). The presence of these compounds depends certainly on added quantities of chlorine but also on some organic matter and present organohalogenated by-products (AOX) in water, which can be of natural origin (humic and fulvic acids) or artificial one (residues of pesticides, phenols) (Zekkour and Beron, 2001; Potelon and Zysman, 1998).

The fundamental objective of the treatment of water is to protect the consumers of the pathogenic microorganisms and unpleasant or dangerous impurity for health (OMS, 1994; Jouany, 2000). Nevertheless, epidemiological studies suggested a possible link between the chlorination, chlorinated by products and increase risk of several types of cancer. Since the first research works about disinfection by-products (Rook, 1974; Bellar et al.,
1974), trihalomethanes accepted a lot of attention because chloroforms have been shown as carcinogenic, further to work on laboratory animals. The United States Environmental Protection Agency (US EPA) (2000) reported that these THMs are human carcinogens, of which CHCl₃, CHClBr and CHBr₂ are carcinogen class B2 (human carcinogen) and CHClBr₂ is carcinogen class C (probable human carcinogen). The cancer of the genital tract and the gastro-intestinal cancer would be more frequent after consumption of chlorinated water (Rook, 1974; Urien, 1986; Monod, 1989; Lafferriere et al., 1999; Zekkour and Beron, 2001).

The objective of this paper was to investigate the effects of seasonal variation on physicochemical and genotoxic parameters of water quality and on the formation and species distribution of organohalogenated by-products of the water plant of Annaba city.

MATERIALS AND METHODS

Treatment of water

The treatment plant treats surface water coming from the dam on the Bou-Namoussa raw and water of the Bouchet Bridge and Hnichet, and water of the saline. Treatments performed on surface water are: prechlorination, coagulation flocculation, decantation, filtration and sterilization.

Sampling and sampling frequency

Four samplings (one sampling by season) have been performed from 2003 to 2004. The directed months were: April, June, September and January. Sampling was accomplished to study the variation of physicochemical parameters of raw water that may influence water quality during seasons; and those which are in direct contact with the production of organohalogenated compounds, proven harmful to human health. The studied raw water parameters were: pH, temperature, turbidity, colour, alkalinity, hardness ISO 6059-1994 (F), organic matter ISO 8467:1993 (F), ammonium ISO 7150/1-1984 (F) and chlorine request ISO 7393-3:1990 (F).

The research of the complete organohalogenated products (AOX)

The AOX is a parameter used for regulatory purposes for water quality. It represents the totality of chlorides and organically linked bromides, adsorbed on active charcoal. Volatile halogenated compounds in suspension are also assayed. The proportion of AOX of raw and treated water in the different sampling points is determined according to international norm ISO 9562: 1989 (F), using the apparatus coulomat 702cl.

Determination of genotoxic activity (the SOS chromotest)

SOS chromotest is a quantitative procedure, based on the measurement of two enzymatic activities (β-galactosidase and alkaline phosphatase) in liquid medium. It uses the SOS repair system of Escherichia coli PQ37 strain, after 2 h of exposure. This assay is a simple, efficient and rapid test of genotoxicity that can be easily adapted to the study of environmental water samples. The genotoxic activity of the concentration C is expressed by the ratio

\[ I(C) = \frac{R(C)}{R_0} \]

where \( R_0 \) is the genotoxic activity measured in the absence of this compound. A compound is considered genotoxic if the induction factor is higher than 1.5 according to Olivier and Marzin (1987).

RESULTS AND DISCUSSION

For the system of water samples collected from the studied water plant, the effects of seasonal variation concentrations on water quality and on the formation and distribution of chlorinated by product have been investigated. Table 1 displays the results of the analysis of variance of two classification criteria (water and seasons). The results of the analysis of variance show the influence of seasons on the change of water quality. The content of organic matter, turbidity and the pH of drinking water vary from season to season.

The interpretation of these results points out that the seasonal variation significantly influences the temperature changing between hot season and cold season. This variation is highly significant and induces variation of turbidity that changes depending on the rate of pluviometry and the organic matter content of raw water. The change in the colour parameter which is in direct relation with the presence of humic and fulvic acids is also significant with seasons.

Turbidity, organic matter and colour introduce parameters linked to the presence of the potential forerunners of the chlorinated by-products and the temperature being key parameter in reaction chlorinates - organic forerunners. The same result was reported by Lafferriere et al. (1999). They demonstrated, by analyses of correlation, the effect of colour and temperature of raw water; as well as the effect of residual chlorine concentrations of treated water in the formation of trihalomethanes (a class of volatile chlorinated by-products).

The results reported in Figure 1 represent concentration of organohalogenated by-products (AOX) at different sampling sites. The analysis of bar charts representing the rates of organohalogenated by-products adsorbed on active charcoal apparently revealed that the autumn is the critical season of the apparition of organohalogenated by-products.
Table 1. ANOVA test of 2 controlled factors (values from P to IC = 95%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Season</th>
<th>Water</th>
<th>Interaction (water × season)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.836NS</td>
<td>0.000***</td>
<td>0.009**</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.000***</td>
<td>0.858NS</td>
<td>0.621NS</td>
</tr>
<tr>
<td>Turbidity</td>
<td>0.004**</td>
<td>0.001***</td>
<td>0.003**</td>
</tr>
<tr>
<td>Organic matter</td>
<td>0.728NS</td>
<td>0.001***</td>
<td>0.716NS</td>
</tr>
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<td>Colour</td>
<td>0.086NS</td>
<td>0.013*</td>
<td>0.104NS</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>0.431NS</td>
<td>0.579NS</td>
<td>0.524NS</td>
</tr>
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<td>Hardness</td>
<td>0.409NS</td>
<td>0.646NS</td>
<td>0.761NS</td>
</tr>
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<td>Conductivity</td>
<td>0.192NS</td>
<td>0.474NS</td>
<td>0.330NS</td>
</tr>
<tr>
<td>Ammonium</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>chlorine request</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

NS: no significant differences. - :The most part of data are less than the range (< 0.06), there is no statistical differences. / : insufficient data for statistical study.
P > 0.05, no statistical differences. P ≤ 0.05 *, significant differences. P ≤ 0.01**, significant increase. P ≤ 0.001***, very significant increase.

These results agree with those reported by Le et al. (1996). They found that contents of AOX in autumn, caused by the decomposition of organic matters notably after the fall of leaves, are 75 to 110% higher from those reported in spring.

**Standard SOS chromotest**

Results of the present test are cited in Figure 2. The analysis of the interpretative bar charts of the mailman of induction I (C) showed that the sample Exit of Treatment Station (ETS) have a genotoxic activity superior to the other two. This proves that chlorinated by-products present in ETS are more genotoxic than those present in water and last point of sampling. Therefore, chlorinated by-products changes in the course of distribution are depending on the added doses of chlorine, to the stocking and to seasonal variation; as it leads to changes in physicochemical characteristics of water (pH, temperature, colour, turbidity and organic matter), which is consistent with the results of this work and previous works elaborated across the world to identify the adverse effect, especially genotoxic issues induced by compounds produced from the disinfection of waters intended for human consumption (Urien, 1986; Lebel et al., 1995; Williams et al., 1995, 1998; Chen and Weisel, 1998).
Figure 2. Results of standard SOS chromotest.

**Conclusion**

The study of the genotoxic effects of drinking water, treated with chlorine, constitutes a new approach to assess the pollution of drinking water. The introduction of the genotoxic tests of water as a new quality criterion for drinking water, in addition to the already existent physicochemical and microbiological parameters, could be beneficial. Physicochemical identification, microbiological quantification and study of the genotoxicity of the potentially hazardous agents are important approaches to fix norms in order to minimize health risks.

In other words, the group efforts aiming to restrict the production of the genotoxic agents, notably by using less chlorine, must never put in danger the perfect disinfection of water intended for consumption.

**REFERENCES**

Bellard TA, Lichtenberg JJ, Kroner RC (1974). The occurrence of
Histopathological and hormonal disrupting effects of Escravos crude oil on the ovary of Chinchilla rabbits

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Crude oil is found in water and soil due to pollution resulting from oil exploration and exploitation. It is used as traditional medicine in some countries, especially among rural dwellers in the south-south and south-eastern Nigeria. The aim of this study was to investigate the effects of Escravos crude oil on serum cholesterol, estradiol and progesterone in the ovary of Chinchilla rabbits. A total of thirty female Chinchilla rabbits of age twelve to fourteen weeks and weighing 1.2 to 1.45 kg were used. Crude oil was orally given at the dose of 15, 20, 25 and 30 mg/kg body weight, corresponding to groups B, C, D and E, respectively for 28 days while group A was the Control. The results showed a significant increase in serum levels of estradiol, cholesterol and ovary weight (p < 0.05) while a significant decrease in serum level of progesterone (p < 0.05) was observed. The histological findings include: ovarian cysts, fibrosis, marked lymphocytic infiltrations and hydropic cells. Therefore, Escravos crude oil could be considered as a potential endocrine disruptor which can affect the tissue architecture and the endocrine functions of the ovary.

Key words: Chinchilla rabbits, Escravos crude oil, estradiol, fibrosis, hydropic cells, progesterone, ovarian cysts, total cholesterol.

INTRODUCTION

Oil spills are a common event in Nigeria and occurs due to a number of causes including: corrosion of pipelines and tankers (accounting for 50% of all spills), sabotage (28%) and oil production operations (21%), with 1% of the spills being accounted for by inadequate or non-functional production equipment. The largest contributor to the oil spill in total, besides corrosion of pipes and tanks, is the rupturing or leaking of production infrastructures that are described as “very old and lack regular inspection and maintenance” (Nwilo and Badejo, 2001). Between 9 and 13 million barrels have been spilled into the Niger delta since 1958 (Baird, 2010).

Within some Nigerian rural population, crude oil is orally ingested for medicinal purposes. It is claimed to be an antidote to poisoning and a cure for various gastrointestinal disturbances (Eyong et al., 2004). According to Dede et al. (2002), cases of misuse of this substance by individuals have been reported, as it is known to be used liberally by some of the indigenes who believe that it can repel witches when applied topically or given orally to afflicted individuals, while other countries such as Kenya, Tanzania, Zimbabwe, Ghana and Tunisia depend on crude oil for unorthodox treatment of ailments such as stomach ache, diarrhoea, respiratory distress.
and convulsion. The impact of crude oil spillage and discharge on the ecosystem as a result of oil exploration activities is an obvious problem of environmental concern (Otitoju and Onwurah, 2007; Ovuru et al., 2004). The Shell Petroleum Development Company (SPDC) since 1989 recorded an average of 221 spills per year in its operational area, involving 7,350 barrels annually (SPDC Nigeria Brief, May 1995:3). From 1976 to 1996, a total of 4,647 oil spill incidences spilling approximately 2,369,470 barrels of oil into the environment of which 1,820,410.5 (77%) were not recovered. Most of these oil spill incidences in the Niger Delta occur on land, swamp and the offshore environment (Nwilo and Badejo, 2005a, 2004; Twumasi and Merem, 2006).

Effects of petroleum hydrocarbons on reproductive and development processes can include interference of hydrocarbon derivatives with hormone synthesis (Truscott et al., 1983) and an increase in incidence of developmental abnormalities (Hawkes and Stehr, 1982). Polycyclic aromatic hydrocarbons (PAHs) found in Nigerian crude oil(s) such as the Benin light crude oil act as estrogenic endocrine disruptors (EED) and can cause disruption on both the reproductive tract and endocrine organs (Zhang and Qiao, 2004). PAHs, the major constituents of crude oil, have also been described as endocrine disruptors in fish, especially as modulators of steroidogenesis (Evanson and Van der kraak, 2001). Benzone arene oxide produces destructive and mutagenic effects on various organ systems of test group animals and is implicated in the etiology of cancer (Nwankwoala and Zuwortin, 2002).

Commonly reported effects of acute exposure to crude oil through inhalation or ingestion include: difficulty in breathing, headaches, nausea, confusion and other central nervous system effects (Akpofture et al., 2000). Chronic exposure of animals to crude oil produces signs and symptoms of toxicity involving the central nervous system (Onuoha and Nwadukwe, 1990), the reproductive system (Nte et al., 1997) as well as genotoxic (Kalf et al., 1987; John et al., 1996). Aslani et al. (2000) reported bloody stools, coughing, constipation, infertility and sudden death in female goats exposed to West Texas intermediate crude oil, while studies conducted by Igwebuike et al. (2007) revealed that exposure of male rats to Nigerian Qua-ibo crude oil, resulted in reduced packed cell volume, increased total leukocytes count and reduced cauda epididymal sperm reserves.

Cholesterol is a lipid synthesized by virtually all cells, especially the liver. It functions as structural component of membranes, precursors of bile salts, steroid hormones and vitamin D (Morrissey, 2006). Estradiol is a steroid hormone; it stimulates an increase in the size of the fallopian tubes, uterus, vagina and ovaries. Its deficiency is characterized by underdevelopment of the female reproductive organs. Progesterone is a steroid hormone. It reduces the frequency and intensity of uterine contractions and thus helps in preventing the expulsion of the implanted ovum. Its deficiency may cause expulsion of implanted ovum. Defective synthesis of the steroid hormones produced by the adrenal cortex and the gonads can have profound effects on reproduction, human development and homeostasis. The aim of this study was to investigate the effect of Escravos crude oil on serum cholesterol, estradiol, progesterone concentrations and the weight and architecture of the ovary.

MATERIALS AND METHODS

Test sample

The Escravos blend crude oil (with reference number 863) used in this study was provided by Warri Refining and Petrochemical Company Effurun, Delta State. The crude oil was exposed to sunlight in shallow pans (25 cm × 25 cm × 5 cm) for 24 h at the site of the project to allow the extremely light and volatile fractions to evaporate, leaving behind the stable components. This product simulates the naturally occurring condition following spillage (Neff et al., 2000).

Animals/experimental design

A total of 30 female Chinchilla rabbits aged 12 to 14 weeks and weighing 1.2 to 1.45 kg was obtained from the Faculty of Agriculture, Ebonyi State University Abakaliki (ESBU). The animals were examined, treated for ectoparasites using Lymectin (Hebei New Century Pharmaceutical Co. Ltd) by a veterinarian and allowed to acclimatize for two weeks at the Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. The rabbits were randomly divided into five groups containing 6 female rabbits each. The research plan consisted of four groups designated Group A (control), B, C, D and E. Group B to E were orally given a sub-lethal dose of 15, 20, 25 and 30 mg/kg body of the Escravos crude oil, respectively with due consideration of their body weight (those with greater body weight have their dose divided into two; one in the morning one at night). The different doses of the liquid Escravos crude oil were measured in weight on an electronic weighing balance and given orally (oral gavage) for 28 days.

Animal treatment

Overnight, prior to exposure, the animals (rabbits) were starved of solid food and their body weights were taken weekly and for the duration of the study to check for weight loss or gain which is associated with toxicity. The rabbits were fed vital grower pallets and water ad libitum for 28 days.

Sample collection, organ harvest and histology

On the 29th day morning, animals were anaesthetized using cotton wool damped in chloroform with due consideration of their body weights. The blood samples, obtained by marginal ear vein puncture, were drawn into tubes using 22 gauge sterile needles. For biochemical analyses, blood samples collected into plain test
Figure 1. Group A (control): Ovarian section with a section of a developed (arrow at the top) and developing follicles (arrow head). Stained by H&E Technique. ×200

Figure 2. Group B (15 mg/kg): Ovarian section with interlacing bundles of closely packed fibrocystic cell type with elongated spindle-shaped basophilic nuclei (arrows). The follicles are enlarged and cystic (arrow head). Stained by H&E Technique. ×200

tubes were centrifuged (Rotofix 32®-Hettich) at 3000 g for 10 min; the serum was collected and kept at -20°C until analysis. Animals were sacrificed and both ovaries were excised, trimmed of all fat, blotted dry to remove traces of blood and weighed using an electronic weighing balance (using 210/0.1 mg digital balance ESJ-210-4). The excised ovaries were fixed in 10% formal saline processed through paraffin wax. Ovary slices of 3 µm thickness were stained using Haematoxylin and Eosin (H&E) staining technique (Awvioro, 2002) and photomicrograph of the stained tissue sections were taken for documentation. The processing of the ovary was made at Histopathology Unit in the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

Biochemical analysis

Serum concentrations of estradiol (E2) and progesterone were estimated using the Microplate enzyme immunoassay as described by Radwanska (1976) using a kit from Monobind Inc., USA, and total cholesterol was estimated using the enzymatic end point kit from Randox Laboratories, United Kingdom. These biochemical analyses were done using enzyme linked immunosorbent assay (ELISA) machine, MR 96 USA, and spectrophotometer. The biochemical analyses were carried out using the facilities of Reene Laboratories Onitsha and Immaculate Hospital Nkpor, Anambra State.

Statistical analyses

Mean values ± standard deviation (SD) of the sex hormones, cholesterol, body and ovary weights were taken for analysis. The data was tested for homogeneity of variance and significantly different results were established by one-way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS) software application (version 16). The multiple comparisons were made using the Post hoc test. The accepted level of significance was set at p < 0.05. The Pearson’s correlation was made to compare the blood levels of sex hormones and the accepted level of significance set at 0.01.

RESULTS

Behavioural effect

After two days of the crude oil administration, the animals in the treated groups D and E became restless. The latter was followed by loss of appetite and decreased locomotion. Soon after the tenth day, they regained their appetite.

Biochemical findings

The mean ± SD change in body weight per week, ovary weights, and concentrations of serum cholesterol, estradiol and progesterone in the control group were 0.12 ± 0.004, 0.027 ± 0.003 kg, 1.54 ± 0.15 mmol/L, 37.38 ± 13.29 pg/ml and 23.88 ± 6.88ng/l, respectively as shown in Tables 1 and 2. The values of the parameters were significantly affected across the treated groups in a dose dependent manner (p < 0.05), especially group C, D and E.

Histological findings

This can be seen in Figures 1 to 5 and Tables 1 to 3.
DISCUSSION

The results of this study highlight the potential susceptibility of Chinchilla rabbits to reproductive endocrine disruption following exposure to Escravos crude oil, in relation to the measurements of ovary weight, serum concentrations of cholesterol, estradiol and progesterone, and histology of the ovary.

The significant increase in the weight of the ovary observed in this study could be linked to the enlarged follicles (Figure 2) and the hydropic cells (Figures 3 to 5) seen in the ovary, while the decreased change in body weight (Table 1) in the treated groups could be due to the loss of appetite at the beginning of the study.

Cholesterol is an unsaturated steroid alcohol. The major function of cholesterol is as a metabolic precursor for the biosynthesis of bile acids, and steroid hormones which include male and female sex steroids (androgens and oestrogens) and adrenal steroids (aldosterone and corticosterone) and liver, ovaries, testes and adrenal glands are the main producers of these hormones using cholesterol as the main precursor (Morrissey, 2006). The significant increase in serum cholesterol concentration observed in the treated test group (Table 2) is in concordance with the report of Otitoju et al. (2011) who administered Bonny light crude oil (BLCO) to female albino Wistar rats. This increase in cholesterol may be an indication of renal retention disease resulting in diminished removal of lipoprotein from the plasma, thus causing the concentration of cholesterol to increase markedly.

Vertebrates synthesize steroids via a pathway that involves the sequential degradation of cholesterol to progestin, then androgens (example testosterone) by the enzyme 17β hydroxysteroid dehydrogenase and finally oestrogens (example, 17-oestradiol) by the enzyme
Aromatase (Guillet et al., 1994). Progesterone is produced in the ovaries (by the corpus luteum), the adrenal glands and during pregnancy (by the placenta). It plays a vital role in the maintenance of pregnancy in part because it suppresses uterine contractility and most importantly because it contributes to a state of “transplantation immunity” and prevents immunological rejection of the fetus (Siiiteri and Macdonald, 1973). The reports by Alvarez et al. (2000) stated that crude oil acts as an anti-androgenic compound, and thereby inducing spontaneous abortion, stillbirths and reproductive malfunction, following the ingestion of hexachlorobenzene (a component of crude) by female rats.

These reproductive malformation and developmental disorders are the results of adverse effects on endocrine system (Indarto and Izawa, 2001). Previous study has shown that ingestion of crude oil contaminated water and feed by rats produced endometrial changes; thick endometrial epithelium and cornification as well as keratinized papilloma resulted in foetal abortion (Oveh and Nwankwoala, 2009). In this study, the significant decrease in the level of progesterone (Table 2) observed in the female test groups is in concordance with the findings of Georgewill and Nwankwoala (2006) who worked on BLCO. The decrease in progesterone may be due to formation of cystic spaces in the ovary; a pathologic replacement of functional hormone producing cells and the formation of interlacing bundles of closely packed fibrocystic type cells as shown in the photomicrographs (Figures 2 to 4).

Animals orally given the Nigerian Qua-iboe Brent crude oil exhibited irregular oestrous cycle when assessed via vaginal cytology, with subsequent significant effects on the conception rates (decrease), gestation length (increase) and litter size (decrease) in the test group when compared with the control group (Nwaigwe et al., 2012).

In this study, the test animals had significantly high concentration of estradiol (Table 2). This is not in accordance with the results of Georgewill and Nwankwoala (2006), who reported a decrease in the level of oestrogen in female guinea pigs which were given 3 kg feed/500 ml Nigerian Agip oil. The increase in estradiol is related to the increased serum cholesterol level, as there is a strong correlation between both variables. It could be adduced that the cholesterol glut made the production of more estradiol feasible. The concentration of progesterone was found to be inversely proportional to the concentration of cholesterol, a negative correlation (Table 3).

This could mean that the concentration of the Escravos crude oil causes a dose dependent inhibition of the enzyme responsible for the production of progesterone. According to the findings of Wagner et al. (1994), alopecic ferrets had high serum estradiol concentration. Some of the animals in the treated groups D and E showed signs of alopecia (hair loss) which could be associated with the high serum estradiol concentration observed.

A drop in progesterone level could be one step that facilitates the onset of labor (Luoma et al., 2012; Herson et al., 2009). Asara et al. (2013) reported that the loss of pregnancy following ingestion of BLCO contaminated feed might be explained by the crude oil induced destruction of the lining of epithelial from the fallopian tubes and on the ovarian stroma that was seen in the ovaries. Progesterone modulates the activity of CatSper (cation channels of sperm) voltage-gated Ca²⁺ channels. Since eggs release progesterone, sperm may use progesterone as a homing signal to swim toward eggs (chemotaxis). Hence substances that block the progesterone binding site on CatSper channels or decrease its concentration in the blood could potentially cause infertility in female (Strünker et al., 2011).

The histological assessment of the sectioned ovaries in this study shows multiple cysts (immature follicles) of various sizes and features of necrosis (ballooning degeneration/hydropic cells) and fibrosis (Figure 5).
Table 2. Mean ± SD serum and pairwise comparisons of the concentrations of sex hormones and cholesterol.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (control)</th>
<th>Group B (15 mg/kg)</th>
<th>Group C (20 mg/kg)</th>
<th>Group D (25 mg/kg)</th>
<th>Group E (30 mg/kg)</th>
<th>f-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>1.54±0.15</td>
<td>2.18±0.47 (0.007)</td>
<td>2.31±0.27 (0.002)</td>
<td>2.33±0.28 (0.001)</td>
<td>2.96±0.17 (0.000)</td>
<td>12.166</td>
<td>0.000</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>37.38±13.29</td>
<td>38.68±6.45 (0.875)</td>
<td>41.42±15.05 (0.626)</td>
<td>45.86±6.44 (0.312)</td>
<td>68.86±11.96 (0.001)</td>
<td>5.089</td>
<td>0.009</td>
</tr>
<tr>
<td>Progesterone (ng/l)</td>
<td>23.88±6.88</td>
<td>21.57±3.41 (0.454)</td>
<td>15.45±0.17 (0.000)</td>
<td>9.80±3.29 (0.000)</td>
<td>10.45±4.50 (0.000)</td>
<td>20.293</td>
<td>0.000</td>
</tr>
</tbody>
</table>

P-value is significant at (p < 0.05). The pairwise comparison was made between the control group and the treated groups. The post hoc test (LSD) showed significant decrease in serum progesterone concentration with significant increase in serum cholesterol and estradiol concentrations (p<0.05), in a dose dependent manner across the treated group.

Table 3. Correlation between cholesterol, estradiol and progesterone.

<table>
<thead>
<tr>
<th>Variable</th>
<th>r-value</th>
<th>p-value</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol correlated with Estradiol in females</td>
<td>0.539*</td>
<td>0.014</td>
<td>Positive correlation</td>
</tr>
<tr>
<td>Cholesterol correlated with Progesterone in females</td>
<td>-0.381</td>
<td>0.098</td>
<td>Negative correlation</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.01 level (2-tailed). Pearson’s correlation. The correlation between the concentrations of sex hormones and cholesterol showed that the increase in cholesterol concentration led to a proportional increase in the concentration of estradiol but led to an inverse decrease in the serum progesterone concentration.

Conclusion

Hormones act at extremely low levels (part per trillion), therefore exposure to low levels of hormonally active agents as found in the crude oil may be of major health concern, particularly during sensitive periods of development and reproduction. The elevated cholesterol, estradiol levels and ovary cysts found in this study are suggestive of polycystic ovarian syndrome. The increased weight of the ovary might be related to the increased number of follicles found in the ovaries. This study suggests that Escravos crude oil, a variant of the Nigerian crude oil, might possess estrogenic property and may be a potential toxic substance to the female reproductive organs.

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REFERENCES


Nwilo CP, Badejo TO (2004). Management of Oil Dispersal along the Nigerian Coastal Areas. Department of Survey GeoInformatics, University of Lagos, Lagos, Nigeria.

Nwilo CP, Badejo TO (2005a). Impacts and Management of Oil Spill Pollution along the Nigerian Coastal Areas. Department of Survey & GeoInformatics, University of Lagos, Lagos, Nigeria.


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