INTRODUCTION

Lead poisoning is one of the oldest and widely studied occupational and environmental hazards (Leonard and Harris, 2004). Lead because of its dense, ductile, malleable and corrosion resistant nature has found its pronounced use in human civilization (Florea and Busselberg, 2006). Lead is used extensively in building materials, pigments, to glaze ceramics, water pipes and glass, paints, dyes, artificial jewellery, cosmetics, protective coatings, acid storage batteries and also as gasoline additives. Exposure of humans to lead and its derivatives in day-to-day life is unavoidable because of its extensive use and application (Florea and Busselberg, 2006).

It has been reported that lead has deleterious effects in experimental animals including rat and human (Chia and Yap, 20004). Lead induces a broad range of physiological, biochemical, and behavioral dysfunctions in laboratory animals and humans (Flora et al. 2006), including central and peripheral nervous systems, haemopoietic system, cardiovascular system, kidneys, liver, and male, and female reproductive systems (Ronis and Bedger, 1998). It has been suggested that lead exerts its toxic effects through lead-induced oxidative stress and the pathogenesis of lead poisoning is due to oxidative stress induced disruption of the delicate pro-oxidant/antioxidant balance that exists within mammalian cells (Lima-Hermes and Bechara, 1991).

Acute lead toxicity is related to occupational exposure and is quite uncommon. Chronic toxicity on the other hand is much more common and occurs at blood lead levels of about 40–60 μg/dL. Lead toxicity can be much more severe if not treated in time and it is
characterized by persistent vomiting, encephalopathy, lethargy, delirium, convulsions and coma (Flora et al. 2006; Pearce, 2007).

Studies have shown that uptake of certain nutrients like mineral elements, flavonoids and vitamins can provide protection from the environmental lead as well as from the lead already present in the body (Hsu and Guo, 2002). These nutrients play a pivotal role in restoring the imbalanced pro-oxidant/antioxidant ratio that arises due to oxidative stress. Although the mechanism by which these nutrients restore the delicate pro-oxidant/antioxidant ratio is still unclear, significant data are available suggesting a protective role of nutrients against lead poisoning (Hsu and Guo, 2002).

Research findings have suggested that administration of various antioxidants can prevent or subdue various toxic effects of lead and generation of oxidative stress in particular by inactivating the generated reactive oxygen species (ROS) at molecular level, thereby terminating the radical chain reaction (chain breaking), chelating the lead ion and preventing further formation of ROS and by chelating lead and maintaining it in a redox state, which leads to its incompetency to reduce molecular oxygen (Garcia and Gonzalez, 2008). For example, vitamin B₆ (pyridoxine) and vitamin B₁ (thiamine) are reported to have essential characteristics that can cure the deleterious effects of lead toxicity. Pyridoxine is an important co-factor which participates in the metabolic trans-sulfuration pathway which is responsible for the synthesis of cysteine from dietary methionine. Vitamin B₁ acts also as an antioxidant by stimulating the production of reduced glutathione (GSH) and acts also as moderate chelator (Ahamed and Siddiqui, 2007). Also, flavonoid has been shown to cure or prevent oxidative stress by chelating redox active metal ions and also by terminating the free radical chain reaction (Terao, 2009; Rice-Evans, 2001).

Some spices have shown to have a curative and/or preventive action against toxic effect of lead. Such spices include ginger, garlic, curry leaf etc. Garlic can prevent oxidative stress by chelating lead ions and scavenging free radicals. (Senapati et al. 2001). The protective efficacy of aqueous garlic extract was studied against lead induced hepatic injury in rats. The results clearly indicated the ameliorative ability of garlic towards hepatic injury caused by lead due to generated oxidative stress (Kilikdar et al. 2011). Also, several studies reported the protective effects of ginger extracts against alcohol induced toxicity (Ali and Fahmy, 2009), bromobenzene induced hepatotoxicity (El-sharkey et al. 2009), fenitrothion or lead induced developmental toxicity (Farag et al. 2010), fungicide induced liver toxicity (Sakr, 2007), ethionine-induced toxicity (Habib et al. 2008), acetic acid-induced ulcerative colitis (El-abhar et al. 2008).

It was considered of interest to investigate whether the combination of calcium acting as an antagonist to lead and spices mixture acting as a cyto-protective agent was capable of ameliorating lead toxicity, when combined, or administered separately. The outcome of such investigation would be of significant relevance in view of the differences in their modes of action and could offer advantages over conventional therapies which have been associated with adverse effects.

**MATERIALS AND METHODS**

**Chemicals/Reagents**

Cadmium Chloride and calcium carbonate of analytical grades were purchased from Sigma-Aldrich Co. LLC, USA.

**Laboratory Animals**

Twenty five male Albino rats weighing 100-123.3 g were obtained from Department of Pharmacy University of Nigeria Nsukka, and kept in animal house of the Department of Biochemistry, Ebonyi State University Abakaliki, Nigeria. The animals were acclimatized for 7 days with free access to food and water. All the rats received human care in accordance with the National Institute of health guidelines for the care and use of laboratory animals (NRC, 1985).

**Preparation of Spices Mixture (SM)**

Ginger rhizomes, garlic bulbs and curry leaves were purchased from Abakpa market, Abakaliki, Ebonyi state. The ginger and garlic
were peeled and the three spices were washed with water. Exactly 200.0 g of each spice were pounded together and soaked overnight in distilled deionized water. It was then filtered and the filtrate was allowed to stand for 24 hours at room temperature (25°C) for sedimentation to occur. The supernatant was discarded and the sediment was dried using rotary evaporator.

**Preparation of Aqueous Extract of Spices Mixture and Chemicals**

Three hundred milligram (300 mg) of the extract was weighed in a weighing balance and dissolved in 100 ml of distilled water. This solution was used as stock. In the same manner, 50 mg of lead chloride (PbCl₂) was dissolved in 200 ml of distilled water and used as stock. Also 750 mg of calcium carbonate (CaCO₃) was dissolved in 100 ml of distilled water and used as stock. Appropriate concentrations of the extract, calcium carbonate were prepared from the stock.

**Experimental design**

The laboratory animals were grouped into five (I-V) with 5 rats in each group as follows:

- **Group I (Negative control):** The animals in this group were not administered any preparation.
- **Group II (positive control):** Animals were administered 25 mg/kg body weight of PbCl₂ only throughout the period of the experiment.
- **Group III:** Rats in this group were concurrently administered 750 mg/kg body weight of calcium carbonate and 25 mg/kg body weight of PbCl₂ for 6 weeks.
- **Group IV:** In this group, the animals were concurrently administered 300 mg/Kg body weight of extract of spice mixture (SM) and 25 mg/Kg body weight of PbCl₂ for 6 weeks.
- **Group V:** Rats in this group were concurrently administered 300 mg/Kg body weight of extract of SM, 25mg/Kg body weight of PbCl₂, and 750 mg/Kg body weight of CaCO₃, for 6 weeks.

**Sample collection**

After 6 weeks of administration, the animals were sacrificed under chloroform anaesthesia. Blood samples were collected into plain bottles for determination of renal function parameters. Blood samples were allowed to clot and retract at room temperature (25°C) and serum was isolated after centrifugation at 2000g for five minutes.

**Biochemical Analysis**

Renal function parameters (creatinine, urea and uric acid) were determined using commercial reagent kits from Randox Laboratories (UK). The analysis was done in strict compliance with the manufacturer's instruction.

**Determination of Creatinine**

Creatinine was determined using Jaffé-slot picrate method as described by Peake and Whiting (2006).

**Determination of Urea:**

Urea was determined by a method known as diacety monoxime method which was described by Dawson (1973).

**Determination of Uric acid:**

Uric acid is determined by the uricase method as described by Fossati et al. (1980).

**Determination of serum electrolytes**

The plasma/serum electrolytes were determined using Convergy® Ion Selective Electrodes Electrolyte Analyser (Convergent Technology, GmbH and CO, KG, Marburg, Germany).

**Data Analysis**

Data were analysed for mean and standard deviation. Parameters were compared among groups using one-way analysis of variance (one-way ANOVA) and values were considered statistically different at p-values less than 0.05.

**RESULT**

Figure 1 showed that all the animals gained weight throughout the experiment, but the least weight gain was observed in lead exposed animals treated with calcium alone, while the highest weight gain was observed in the lead exposed animals treated with spices mixture.
Table 1 shows the effect of calcium/spices mixture (SM) alone or in combination on the renal function parameters of lead exposed rats. Lead exposed rats were found to exhibit significantly (p<0.05) higher serum levels of urea, creatinine and uric acid in comparison to the non-exposed rats. Administrations of calcium carbonate (CaCO$_3$) or spices mixture (SM) or co-administration of both tended to restore the levels of the parameters to the values observed in the non-exposed rats but only the administration of SM was found to significantly restored urea and co-administration of CaCO$_3$ and SM to significantly restored serum uric acid. However, administration of both CaCO$_3$ and SM alone or co-administration of both had no significant effect at restoration of serum creatinine.

Table 1: Effect of calcium/spices mixture (SM) alone or in combination on the renal function parameters of lead exposed rats

<table>
<thead>
<tr>
<th>Parameters (mg/dl)</th>
<th>NC</th>
<th>PC</th>
<th>Pb+Ca</th>
<th>Pb+SM</th>
<th>Pb+Ca+SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>11.97±1.30a</td>
<td>22.60±1.61b</td>
<td>21.00±3.30b</td>
<td>14.90±1.79a</td>
<td>17.00±1.21b</td>
</tr>
<tr>
<td>Creatinine</td>
<td>5.80±0.70a</td>
<td>9.83±0.70b</td>
<td>10.70±0.47b</td>
<td>8.64±0.81b</td>
<td>9.83±0.95b</td>
</tr>
<tr>
<td>Uric acid</td>
<td>5.40±0.44a</td>
<td>10.77±0.69b</td>
<td>7.23±0.26b</td>
<td>8.60±0.31b</td>
<td>6.21±0.39a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of means. Values with different superscript are statistically significantly different (p<0.05).

From Table 2, significantly (p<0.05) lower plasma levels of Na$^+$ and HCO$_3^-$ were observed in lead exposed rats in comparison to the non-exposed rats. Co-administration of both calcium carbonate and spices mixture (SM) restored the values to the levels observed in non-exposed rats. However, while there was significant (p<0.05) elevation in Cl$^-$ in lead exposed rats, which was restored to the value in non-exposed rats by co-administration of CaCO$_3$ and SM, K$^+$ was neither affected by lead exposure nor treatment with CaCO$_3$, or SM or co-administration of both as comparable levels were found in all the groups (Table 2).
Table 2: Effect of calcium/spices mixture (SM) alone or in combination on the plasma electrolytes of lead exposed rats

<table>
<thead>
<tr>
<th>Parameters (mmol/l)</th>
<th>NC</th>
<th>PC</th>
<th>Pb+Ca</th>
<th>Pb+SM</th>
<th>Pb+Ca+SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium ion K⁺</td>
<td>2.60±0.25</td>
<td>2.30±0.15</td>
<td>2.80±0.29</td>
<td>2.69±0.30</td>
<td>2.40±0.06</td>
</tr>
<tr>
<td>Sodium ion (Na⁺)</td>
<td>128.7±0.9a</td>
<td>117.3±4.2b</td>
<td>113.3±3.9b</td>
<td>127.7±2.7a</td>
<td>124.0±3.6a</td>
</tr>
<tr>
<td>Chloride ion (Cl⁻)</td>
<td>95.7±3.5a</td>
<td>100.5±0.9b</td>
<td>103.3±3.9b</td>
<td>92.3±2.6a</td>
<td>90.7±1.5b</td>
</tr>
<tr>
<td>Bicarbonate ion (HCO₃⁻)</td>
<td>28.0±1.5a</td>
<td>20.3±1.2b</td>
<td>20.3±2.0b</td>
<td>19.3±0.9b</td>
<td>28.7±0.9a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of means. Values with different superscript are statistically significantly different (p<0.05).

**DISCUSSION**

In the present study, exposure of rats to lead chloride has significant deleterious effects on body weight, renal function parameters and serum electrolytes, which were ameliorated with the administration of either calcium carbonate or spices mixture alone or when both are co-administered. The significantly (p<0.05) lower body weights gain in lead exposed rats might be due to the interruption in absorption and metabolism of nutrients essential for health (Rastogi et al. 2010). On the contrary, the comparable body weight gain in rats administered either spices mixture (SM) or calcium carbonate (CaCO₃) and SM after lead exposure with those of the non-exposed rats, suggests that both SM and CaCO₃ tended to reverse the growth depressive effect of lead in rats. This might be as a result of the protective and therapeutic role of both calcium and SM against lead toxicity (Sharma et al. 2010). This finding is similar to that of Youdim et al. (2002) who reported that spice mixture can reverse growth depression of lead exposed rat. Chia and Yap (2004) had also reported that spice mixture can reverse growth depression caused by lead. The significantly (p<0.05) higher serum levels of urea, creatinine and uric acid observed in lead exposed rats in comparison to their non-exposed counterparts in the present study is in corroborations with the findings of Mohammed et al. (2007) and Chia and Yap (2004) where it is reported that lead has deleterious effects in the kidney of experimental animals including rat and human. Similarly, Ashour et al. (2007) have also reported raised level of serum creatinine in lead acetate treated rats.

The restoration of altered renal function parameters observed in lead exposed rats by administrations of calcium carbonate (CaCO₃) or spices mixture (SM) or co-administration of both to values in non-exposed rats is similar to the findings of Kilikdar et al. (2011); Ali et al. (2008) and Tachibana et al. (2001) where garlic, ginger and curry were found to possess the ability to reduce toxic effect of lead in the kidney by preventing oxidative stress through chelation of lead ions and scavenging free radicals. The present findings are also similar to the findings of Djukic-Cosic (2007) who reported that calcium reduces the effect of lead in lead exposed rat by competing with lead for intestinal absorption and prevent heavy metal induced tissue damage by competitive binding to active sites of the enzymes. Similarly, Peter et al. (1991) reported that doubling of dietary calcium with Ca₃(PO₄)₂ significantly decreased lead absorption in the kidney.

Significantly (p<0.05) lower plasma levels of Na⁺ and HCO₃⁻ observed in lead exposed rats in comparison to the non-exposed rats observed in the present study is similar to the report in ATSDR (1999) that lead lowers the plasma level of serum electrolytes, although the mechanism is not known. However, the altered electrolytes were restored to the levels observed in non-exposed rats by co-administration of both calcium and spices mixture (SM). Although no similar study was encountered, the present findings, may be attributed to the protective effect of both calcium and spice mixture.

**CONCLUSION**

From the present study, it may therefore be concluded that administration of spices mixture or calcium alone or SM (spice mixture) plus calcium has ameliorative effect on kidney...
dysfunctions induced by lead toxicity.

REFERENCES


