Evaluation of Ameliorative Effects of *Parinari Curatellifolia* on Wistar Rats Exposed to Lead

By

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**ABSTRACT**

This work was designed to determine the effect of methanolic leaf extract of *Parinari curatellifolia* on hematology and serum biochemistry of lead induced Wistar rats. *Parinari curatellifolia* leaf was shade dried for 2 weeks and pulverized into powder. 324 g powdered sample was extracted with the aid of methanolic solvent using soxhlet extraction. 48 healthy apparently 8 to 10-week old Wistar rats weighing between 109 and 210g were used in the study. They were randomly divided into six groups and control. The experiment was monitored for 28 days. Hematological and serum parameters were measured. Data was analyzed using one-way ANOVA at p<0.05 level of significance. There was no significant difference in PCV, Hb and neutrophil concentration. The highest and the lowest RBC (mg) was found in group IV (12.55) and II (6.95) respectively; group V (13.48) had the highest WBC (mg), while group III (5.20) had the least; and lymphocyte (mg) was also highest in group V (6.80), and lowest in group III (3.20). Serum biochemical parameters were significantly highest in group II and lowest in group IV. *Parinari curatellifolia* has a good ameliorative effect as it was observed in Wistar rats treated 14 days with lead solution and 14 days using plant extract.

**Key words**: Lead, *Parinari curatellifolia*, Wistar rats, Serum biochemistry, Hematology

**INTRODUCTION**

Lead (Pb) is one of heavy metal widely used in gasoline and lead-acid battery industries (1). Pollution as a result of lead may be released into air or waste mixtures into soil and waterways in the process of manufacturing, which were then up-taken through food, water and air (2). Lead poisoning usually results in brain and neurology defects, such as cognitive disorder, movement and coordination impairment, hearing and visual problems (3). Independent studies by other workers showed that Pb has effect on endocrine by disrupting chemical. Pb is not only associated with late-onset puberty (4, 5) but also acts on hypothalamic-pituitary-adrenal (HPA) axis, thus causing high stress-related cortisol levels (6, 7)

The last three decades were witness to several reports on the toxicity of heavy metals in human beings, due to the contamination in the fish and fishery organisms (8). In Nigeria reports indicate that lead contamination is raising serious medical concern to both humans and
animals. Zamfara lead poisoning is the worst and most recent heavy metals incidence in the Nigerian records that claimed the lives of over 500 children within seven months in 2010 (9). Studies by Dan-Azumi and Bichi (10) showed water samples from river Challawa situated along the industrial area of Kano state contain elevated levels of Pb, Cr, Cu, and Fe higher than the recommended WHO and FEPA guidelines.

The challenge is determining biomarkers that could aid early diagnosis as well as finding cheaper, readily available and effective alternative remedies to the conventional chelating therapy in lead poisoning. Parinari curatellifolia has already shown promise in mitigation of tissue damage in experimentally induced carbon tetrachloride toxicity (11), this compound has similar mechanism of toxicity as lead. In spite of its associated antioxidant properties, the use of Parinari curatellifolia to mitigate the toxicity associated with lead exposure is scanty. This study is therefore designed to determine the haematological and biochemical changes associated with lead toxicity in Wistar rats (Rattus norvegicus) and ameliorative effects, if any, of methanolic leaf extract of Parinari curatellifolia on the lead-induced changes.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Parinari curatellifolia leaf were collected from Karau karau along Birnin Gwari Local Government Area of Kaduna State and were authenticated by a taxonomist at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria; and assigned voucher number 903.

Processing of Plant Material

Fresh leaf of Parinari curatellifolia was shed dried under room temperature for two weeks and pulverized to powdered using mortar and pestle. 100g of Parinari curatellifolia leaf was extracted using soxhlet extractor with the aid of methanol as solvent. Plants extracts were preserved in a 500ml container in refrigerator at -4°C for further use.

Experimental Animal

A total of 48 apparently healthy 8 to 10-week old Wistar rats (Rattus norvegicus) weighing between 109 and 210 g were obtained from the Laboratory Animal Unit of National Institute for Trypanosomiasis Research (NITR), Angwan Rimi, Kaduna State. They were transported and kept in the Animal cages in the Postgraduate Laboratory in the Department of Biological Sciences, Ahmadu Bello University, Zaria, at a room temperature of 27°C and under normal photo period. The rats were allowed to acclimatize for fourteen days in the experimental animal house before the commencement of the experiment. All animals were handled in accordance with the standard guide for the care and use of laboratory animals (12).

Preparation of Solution Medium

Solution of lead acetate was prepared by dissolving 1g of lead acetate salt in 5 ml of deionised water, to give 200 mg/ml concentration; 1g of methanolic leaf extract of P. curatellifolia was also dissolved in 5mls of distilled water to produce 200 mg/ml concentration; while 1g of sodium EDTA was dissolved in 10mls of deionised water to give the 100mg/ml that was used for the study. The respective concentrations were administered to the experimental rats at 480
mg/kg body weight. Unused solutions were stored in the refrigerator at 4°C.

**Acute and Subacute Toxicity Study**

The median lethal dose (LD$_{50}$) for lead acetate and *P. curatellifolia* were carried out in rats in line with the method described by Lorke (13). Twelve rats were used in each trial to determine the acute toxicity of lead acetate and Methanolic leaf extract of *P. curatellifolia*.

In the initial phase, nine rats were divided at random into three groups with three rats in each group. Rats in groups I, II and III were given dose once 10, 100 and 1000 mg/kg, of the plant extract respectively, and monitored for 48–72 hours for toxicity signs and death. In the second phase, 3 rats were placed into 3 groups of 1 rat in each group. Rats in groups I, II and III were given by oral route 1600, 2900 and 5000 mg/kg, respectively, once. The rats were then observed for 48–72 hours for any sign of toxicity or mortality. This protocol was applicable for methanolic leaf extract of *P. curatellifolia*. A dose of 500 mg/kg for MLEPC (one tenth of the highest dose, 5000 mg/kg), was then selected for the study based on the absence of observable signs of toxicity and mortality at the highest administered dose. While a dose 480mg/kg was selected for Lead acetate to induce the toxicity.

**EXPERIMENTAL DESIGN**

Forty-eight (48) wistar rats were randomly divided into 6 groups of 8. The rats were maintained daily on pelleted grower’s marsh (Vital Feeds Ltd., Jos) and water provided *ad libitum*. Wistar rats were grouped into,

Group I: Rats served as negative control and received distilled water by oral gavage daily for 28 days.
Group II: Rats received lead acetate solution (480mg/kg) by oral gavage daily for 28 days.
Group III: Rats received lead acetate solution and 1/10$^{th}$ LD$_{50}$ of MLEPC concurrently by oral gavage.
Group IV: Rats in this group received lead acetate solution at a dose of 480mg/kg by oral gavage daily for the first 14 days and, subsequently, 1/20$^{th}$ LD$_{50}$ of MLEPC for next 14 days through the same route.
Group V: Rats in this group received 1/15$^{th}$ the LD$_{50}$ of MLEPC at a dose of 500mg/kg by oral gavage daily for 28 days.
Group VI: Rats in this group received lead acetate solution for 14 days and then 200mg/kg EDTA for the next 14 days.

**Haematological Screening**

Packed cell volume (PCV %), haemoglobin content (Hb g/dl), red blood cell (RBC), neutrophils and lymphocyte were investigated. Differential leukocyte (white blood cell, WBC) counts were determined on thin blood smears stained with Giemsa stain.

**Serum Biochemical analysis**

The serum samples were collected from the clotted blood that was allowed to clot at room temperature and then centrifuged at 3000 r/min for 15 minutes. The sera were carefully collected into appropriately labeled plastic tubes and then stored at -20°C until analysed. Serum samples were used to determine the concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea
nitrogen (BUN), creatinine, phosphate (PO$_4$), calcium (Ca), potassium (K), bicarbonate (HCO$_3$), sodium (Na) and chloride (Cl$^-$) using the automated Audiocomb analyser in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria.

**DATA ANALYSIS**

**RESULTS**

Table I: Haematological characteristics of *Rattus norvegicus* administered different treatments of methanolic leaf extract of *Parinari curatellifolia* at day 28

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PCV</th>
<th>Hb</th>
<th>RBC</th>
<th>WBC</th>
<th>NEUT</th>
<th>LYMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grp I</td>
<td>43.50±2.47</td>
<td>15.30±0.59</td>
<td>7.18±0.42</td>
<td>6.15±0.90</td>
<td>1.43±0.33</td>
<td>4.43±0.69</td>
</tr>
<tr>
<td>Grp II</td>
<td>41.50±0.65</td>
<td>12.38±2.66</td>
<td>6.95±0.19</td>
<td>7.18±0.83</td>
<td>1.28±0.17</td>
<td>5.54±1.03</td>
</tr>
<tr>
<td>Grp III</td>
<td>43.00±2.74</td>
<td>13.80±0.22</td>
<td>9.85±2.94</td>
<td>5.20±0.59</td>
<td>1.69±0.23</td>
<td>3.29±0.56</td>
</tr>
<tr>
<td>Grp IV</td>
<td>42.50±1.32</td>
<td>14.13±0.44</td>
<td>12.55±3.17</td>
<td>5.73±1.01</td>
<td>1.57±0.19</td>
<td>3.69±0.83</td>
</tr>
<tr>
<td>Grp V</td>
<td>44.75±1.80</td>
<td>14.27±0.66</td>
<td>10.60±2.45</td>
<td>13.48±4.47</td>
<td>2.80±0.86</td>
<td>6.80±0.87</td>
</tr>
<tr>
<td>Grp VI</td>
<td>43.25±1.60</td>
<td>14.40±0.54</td>
<td>7.15±2.29</td>
<td>8.95±1.90</td>
<td>2.12±0.58</td>
<td>6.77±1.20</td>
</tr>
</tbody>
</table>

Means± SE with different super script along the column were significantly different (p<0.05)

Key:
I, II, III, IV, V and VI represent the experimental groups as follows; GRP I Control group, GRP II (administered Pb acetate), GP III (co-administered Pb acetate and MLEPC), GP IV (administered Pb acetate for 14 days and MLEPC for the subsequent 14 days), GPV (administered MLEPC), Group VI (administered Pb acetate for 14 days and diNa EDTA for the subsequent 14 days) at 480mg/kg orally

Table II: Serum biochemical characteristics of *Rattus norvegicus* administered different treatments of methanolic leaf extract of *Parinari curatellifolia* at day 28

<table>
<thead>
<tr>
<th>Treatments</th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
<th>UREA</th>
<th>CREAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grp I</td>
<td>87.00±6.18</td>
<td>132.00±35.5</td>
<td>360.25±28.7</td>
<td>5.45±0.67</td>
<td>24.50±4.52</td>
</tr>
<tr>
<td>Grp II</td>
<td>130.00±9.93</td>
<td>170.50±11.5</td>
<td>691.00±139.14</td>
<td>8.52±1.09</td>
<td>46.25±13.63</td>
</tr>
<tr>
<td>Grp III</td>
<td>101.25±9.64</td>
<td>154.25±12.8</td>
<td>531.25±36.66</td>
<td>7.78±0.36</td>
<td>30.75±4.13</td>
</tr>
<tr>
<td>Grp IV</td>
<td>102.50±8.45</td>
<td>139.25±20.8</td>
<td>512.50±38.08</td>
<td>7.65±0.63</td>
<td>30.25±2.32</td>
</tr>
<tr>
<td>Grp V</td>
<td>92.00±13.33</td>
<td>129.00±21.8</td>
<td>472.25±45.08</td>
<td>7.23±0.75</td>
<td>26.50±4.66</td>
</tr>
<tr>
<td>Grp VI</td>
<td>112.50±15.03</td>
<td>140.50±28.8</td>
<td>562.25±44.68</td>
<td>7.35±0.69</td>
<td>30.25±2.72</td>
</tr>
</tbody>
</table>

Means± SE with different super script along the column were significantly different (p<0.05)

**Key:** I, II, III, IV, V and VI represent the experimental groups as follows; GRP I Control group, GRP II (administered Pb acetate), GP III (co-administered Pb acetate and MLEPC), GP IV (administered Pb acetate for 14 days and MLEPC for the subsequent 14 days), GPV (administered
MLEPC), Group VI (administered Pb acetate for 14 days and diNa EDTA for the subsequent 14 days) at 480mg/kg orally

**Table III:** Serum biochemical ions of *Rattus norvegicus* administered different treatments of methanolic leaf extract of *Parinari curatellifolia* at day 28

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PO₄</th>
<th>Ca</th>
<th>K</th>
<th>HCO₃⁻</th>
<th>Na</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grp I</td>
<td>2.16±0.18ᵃ</td>
<td>2.40±0.09ᶜ</td>
<td>6.73±0.32ᵇ</td>
<td>24.75±1.60ᵇ</td>
<td>140.25±1.44ᵇ</td>
<td>102.75±1.25ᵇ</td>
</tr>
<tr>
<td>Grp II</td>
<td>2.40±0.12ᵃ</td>
<td>.76±0.45ᶜ</td>
<td>8.20±0.39ᵇ</td>
<td>26.00±0.41ᵃ</td>
<td>144.50±1.44ᶜ</td>
<td>107.00±1.08ᵃ</td>
</tr>
<tr>
<td>Grp III</td>
<td>2.18±0.05ᵃ</td>
<td>2.68±0.07ᵇ</td>
<td>7.70±0.35ᵃᵇ</td>
<td>25.25±0.63ᵃᵇ</td>
<td>144.00±1.35ᵃᵇ</td>
<td>103.25±1.65ᵇ</td>
</tr>
<tr>
<td>Grp IV</td>
<td>2.38±0.15ᵃ</td>
<td>2.62±0.08ᵃᵇ</td>
<td>7.25±0.37ᵃᵇ</td>
<td>24.25±0.85ᵃᵇ</td>
<td>142.50±0.87ᵃᵇ</td>
<td>102.25±0.75ᵇ</td>
</tr>
<tr>
<td>Grp V</td>
<td>2.17±0.21ᵃ</td>
<td>.36±0.11ᶜ</td>
<td>6.88±0.19ᶜ</td>
<td>22.25±1.38ᶜ</td>
<td>141.00±0.82ᵃᵇ</td>
<td>103.25±0.63ᵇ</td>
</tr>
<tr>
<td>Grp VI</td>
<td>2.35±0.11ᵃ</td>
<td>2.60±0.10ᵃᵇ</td>
<td>7.15±0.23ᵃᵇ</td>
<td>23.75±1.32ᵃᵇ</td>
<td>142.50±0.29ᵃᵇ</td>
<td>97.00±0.41ᵇ</td>
</tr>
</tbody>
</table>

Means± SE with different super script along the column were significantly different (p<0.05)

**Key:**

I, II, III, IV, V and VI represent the experimental groups as follows; Grp I Control group, Grp II (administered Pb acetate), Grp III (co-administered Pb acetate and MLEPC), Grp IV (administered Pb acetate for 14 days and MLEPC for the subsequent 14 days), Grp V (administered MLEPC), Grp VI (administered Pb acetate for 14 days and diNa EDTA for the subsequent 14 days) at 480mg/kg orally.

**DISCUSSION**

The result of the haematological investigation showed a non-significant decrease in the mean values of PCV, Hb, and RBC in all the test groups except groups IV and V that were administered methanolic leaf extract of *Parinari curatellifolia* only and that treated with DiNaEDTA after two weeks of lead acetate administration. An increase in packed cell volume (PCV), a slight decrease in haemoglobin concentration (Hb) and a relatively unchanged red blood cell count (RBC) was observed in group IV. The rats in group V had relatively unchanged red blood cell parameters. It therefore shows that methanolic leaf extract of *Parinari curatellifolia* possess the potential to improve red blood cell parameters. This is so because of the increase in the red blood cell parameters observed in the group administered only methanolic leaf extract of *Parinari curatellifolia* and the slight decrease observed in the groups co-administered lead and MLEPC and that treated with MLEPC two weeks post exposure to lead acetate. This is in consonance with the work of Ogbonnia *et al.* (14) where oral administration of *Parinari curatellifolia* increased the WBC, RBC and haemoglobin concentration of the animals. Although, there were no statistically significant (P > 0.05) changes in these haematological parameters, lead has been reported to cause a decrease in these parameters (15). The decrease was attributed to the morphologic, mechanic and osmotic fragility of erythrocytes as well as inhibition of the enzymes which are central to heme biosynthesis (16, 17, 18).

A slight leukocytosis due to lymphocytosis was observed in the group administered lead acetate daily for 28 days.
Similarly, there was marked decrease in leukocyte count in the rats administered methanolic leaf extract of *Parinari curatelifolia*, and that treated with DiNaEDTA two weeks post exposure to lead acetate. Leukocytosis could be physiologic or pathologic. Physiologic leukocytosis occurs as a result of fear, excitement or stress while pathologic leukocytosis is observed in inflammatory conditions, steroid administration etc. Therefore, the non-significant increases in the neutrophils and lymphocytes in the group administered only methanolic leaf extract of *Parinari curatelifolia* and that treated with DiNaEDTA could suggest an inflammatory response to the test agent.

The significant increase in serum ALT concentration in all the test groups might be indicative of hepatocellular necrosis. Similarly, increase in serum AST concentration in the group administered lead acetate only and that co-administered lead acetate and MLEPC are suggestive of liver and muscular damage. However, the decrease in serum levels of AST in groups II, III and IV shows that MLEPC and DiNaEDTA possibly possess the potential to prevent muscular damage induced by lead acetate. This is in agreement with the work of Yakubu et al., (11) were administration of *P. curatelifolia* methanolic extract, disrupted hepatic and serum ROS metabolism associated with hepatic injury progression in rats intoxicated with CCl4. The serum concentrations of ALP were significantly high in all the test groups. This finding may indicate hepatic bile duct obstruction, hepatocellular necrosis or damage to the bones.

Increase in serum BUN concentration was observed in all the test groups. This increase may suggest increase in protein catabolism or decreased glomerular filtration rate. Urea has been reported to be a byproduct of protein catabolism (19). Mansouri et al. (20) reported a significant increase in uric acid level as a result of lead exposure in rats. Even the group supplied with dandelion showed a significant increase despite the diuretic effect of this herb. Uric acid is a substance which results from the degradation of nucleic acids. It has been confirmed that lead reduced the urinary excretion of uric acid and there was a positive correlation between blood lead levels and uric acid of their study.

Conversely, the serum creatinine concentration was increased in all the test groups compared to control group. Increases in serum creatinine concentration are usually observed in muscle mass, severe liver disease or decreased creatinine production. This study is in agreement with Sujatha et al. (21) who reported a significant increase in serum creatinine values they observed throughout the experimental period in lead treated rats of GP II (5.39mg/dl) and GP III (5.08mg/dl) as dose dependent manner when compared to control (2.67mg/dl).

The serum electrolyte concentration of PO$_4^{2-}$ and K$^+$ increased in groups II, IV, and VI but not statistically significant (P > 0.05). There was decrease in the serum electrolyte concentration of calcium in all the treatment groups as compared to the control and this decrease was statistically significant (P > 0.05). The results of acid and alkaline phosphate showed that activities of both enzymes in intoxicated rats with lead were stimulated relative to non-toxicated
control group. There was no significant change in bicarbonate level of the treated group and the control except wistar rats in group V where marked reduction was observed. Sodium concentration was significantly higher in group II and III compared to control. Also, wistar rats in group II had significantly higher Cl\(^-\). The groups treated with lead acetate solution and combination of lead acetate and MLEPC respectively had the ability to react to the intoxication. The toxic nature of the lead and MLEPC could have necessitated the production of PO\(_4^{2-}\), Ca\(^{2+}\), K\(^+\), HCO\(_3^-\), Na\(^+\) and Cl\(^-\) in higher amount by the system of the wistar rats, since ions play role in transmitting the information in nervous system. The ameliorative effect of the plant extracts against biochemical ions and chemical substances of animal origin have been reported to confer protection. There are reports from some quarters on the protective effect of plant extracts against lead induced toxicities which can be attested to by the work of Halawa et al. (22). He and colleagues showed that administration of 1.5 ml/kg of plant extract orally for 4 weeks improved liver function and architecture in lead treated rats. Elmenofi (23) reported similar observation when he administered 200mg/kg of lead to animals and observed impairment in biomarkers of renal function and renal architectural damage. Administration of honey significantly ameliorated these lead induced changes.

CONCLUSION

Toxicities due to lead exposure have been attributed to the ability of lead to induce oxidative stress through the generation of reactive oxygen species (ROS). The ability of lead to induce reactive oxygen species could be supported by the fact that lead induced toxicities were found to be mitigated by chemical agents like vitamin C, vitamin E, N-acetyl cysteine dimercaptosuccinic acid, calcium disodium ethyldiaminetetraacetic acid, melatonin and selenium which have antioxidant properties. Parinari curatellifolia extracts has demonstrated its ability to protect against lead induced toxicity in the experimental animals. The abilities of these extracts to ameliorate these toxicities were attributed to the antioxidant properties of principles contained in these extracts. Some of these ameliorative agents may need more evaluation if they could be of clinical application.

REFERENCES


