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Evaluation of haematological and serum electrolyte changes in Wistar rats administered with ethanolic extract of whole fruit of *Lagenaria breviflora* Robert

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The effect of the ethanolic extract of the whole fruit of *Lagenaria breviflora* Robert was evaluated on haematological indices and serum electrolyte levels of Wistar rats. 25 adult rats were randomly but equally divided into 5 groups. The rats in group A (Control) were administered with 0.9% Physiological Saline, while rats in groups B to E were administered with the extract of 1000, 2000, 4000 and 8000 mg per Kg body weight respectively once daily for 14 days according to the acute and chronic toxicities studies conducted. The parameters evaluated were PCV, Hb concentration, MCV, MCH, MCHC values, RBC, WBC, neutrophils and lymphocytes counts. The serum electrolytes included Na\(^+\), Cl\(^-\), HCO\(_3\)\(^-\), K\(^+\), Ca\(^{2+}\) and HPO\(_4\)\(^2-\). The extract increased the mean PCV, RBC, WBC, Hb and MCV values of rats in the test groups, while MCH and MCHC decreased. The increase in the mean value of MCV, RBC and PCV coupled with decreased values of MCH and MCHC indicated increased production of reticulocytes (reticulocytosis), which suggest that the extract of the plant is capable of stimulating erythropoiesis. The mean lymphocyte values increased, while mean neutrophils values decreased for most of the test groups. There was evidence of electrolyte imbalance exhibited by significant (P < 0.05) reduction of HCO\(_3\)\(^-\) and Ca\(^{2+}\). This electrolytes imbalance was accompanied with significant (P < 0.05) elevation of BUN in the rats in the test groups, thus strongly incriminating renal injury as possible cause. It was concluded that prolonged administration of extract of *L. breviflora* elicit electrolyte imbalance and the extract of the plant is not haematoxic, rather, it stimulate erythropoiesis.

Key words: Haematology, serum electrolytes, extract, *Lagenaria breviflora*, Wistar rats.

INTRODUCTION

*Lagenaria breviflora* Robert is a medicinal plant of the family Cucurbitaceae (United States Department of Agriculture, 2001; Morimoto et al., 2005). It is used in West Africa for a wide range of gastrointestinal disorders and measles in man. It is also used with other medicinal plants as concoctions to aid parturition in humans (Sonaiya, 1999). Livestock farmers employ the fruit extract of the plant for the treatment of Newcastle disease and coccidiosis in animals (Sonaiya, 1999). Researchers have validated the use of the plant as an anti-implantation agent (Elujoba et al., 1985), abortificient (Elujoba and Hymete, 1986), miracicide and cercaricide (Ajayi et al., 2002), as well as an antibacterial agent with broad spectrum activity (Tomori et al., 2007). Phytochemical analysis of *L. breviflora* showed that it contains variety of chemical compounds ranging from saponins, phenolic acids (Elujoba et al., 1990) and cucurbitacins (Miro, 1995; Wakimoto, 2008). There are several types of cucurbitacins that have been discovered in plants of Cucurbitaceae family, with profound pharmacological actions. The primary cucurbitacins are types B and E and they have also been found outside the Cucurbitaceae family (Beutler et al., 2000; Momma et al., 2008). Currently there are many cucurbitacins and new ones are being discovered and many more are being synthesized from parent compounds (Chiu and Gao, 2003). Quite a number of cucurbitacins have been investigated for their cytotoxic (Seeram et al., 2007), anti-inflammatory (Escandell et al., 2007) hepato-protective and cardiovas-

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cicular effects (Miro, 1995).

Though there have been chemical characterization of different types of cucurbitacin from the natural flora aside their semi synthetic and synthetic analogues; clinical applications of these compounds have not been reported. In the mean time herbal medicine has becoming more popular as more people resort to use of medicinal plants in their crude preparations. Our focus in this study was to investigate the toxicological effects of L. breviflora using its ethanolic extract which is closest to the aqueous extract of the plant commonly administered in folklore medicine in West Africa.

MATERIALS AND METHODS

Experimental animals

25 adult Wistar rats were used for the study. These were obtained from the Laboratory Animal Breeding Section of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, and housed at the Experimental Animal House of the same department. They were fed with pelletized rat ration (Guinea Feeds Nigeria Limited) and given water ad libitum. The rats were randomly but equally divided into 5 groups. The rats in Group A served as the control animals, while rats in groups B, C, D, and E were the test animals. The animals were stabilized for 4 weeks before commencement of the experiment. All the rats received humane care and the study protocols were in compliance with the University’s guidelines for the use of laboratory animals.

Extraction of the fruit

Fresh fruits of L. breviflora Robert were obtained from the Botanical Garden, University of Ibadan, Nigeria. The plant material was identified by qualified botanist from Department of Botany, University of Ibadan. 3.115 kg of fresh fruits were washed, cut into small pieces and weighed out in small portions. These were tied into cloth sieves and placed in plastic containers. Sufficient ethanol which covered each portion was poured into each container and this was left to extract. The ethanol was replaced with a fresh batch every 3 days. The extraction process was completed in a week. This was stored in plastic containers and refrigerated at 4°C. The extract obtained was clarified by filtration through celite on vacuum using a rotation evaporator at low temperatures. The remaining moisture was finally removed by placing small volumes in porcelain dishes in the oven set at low temperatures at 4°C. The extract came as semi-solid greenish brown paste. A total yield of 287g of dried extract was obtained (9.21% of the fresh fruit). A stock solution was afterward prepared by dissolving 100 g of the extract in 50 ml of distilled water.

Acute and chronic toxicity studies

25 adult rats divided into five groups (n = 5) were administered with ethanolic extract of L. breviflora for 16 days. Doses of extract given were 2,000, 4,000, 6,000, 8,000 and 10,000 mg/kg body weight according to their groupings. There was no mortality recorded in any group during the period and after.

Dosing of the experimental animals

The rats in group A (control) were administered with 0.9% physiological saline, while rats in the test groups B - E were administered with graded doses of the extract of 1000, 2000, 4000 and 8000 mg/ml of the extract per kilogramme body weight respectively in tandem with the toxicity studies conducted. They were dosed once daily for 14 days using oral canula.

Sample collection

The rats were anaesthetized using ether while blood samples were collected from each rat via the orbital sinus on day 14. About 3 ml of blood was collected into Lithium heparinized bottles for haematological analysis by Cole’s method (Cole, 1986). Also, 3 ml of blood was collected into another Lithium heparinized bottle for analysis of serum electrolyte levels. The rats were subsequently decapitated and organ samples of the kidney and liver were collected and preserved in 4% formalin for histopathology.

Statistical analysis

Student t-test was used to analyze the data (Steel and Torrie, 1996). The differences of the means were considered significant at p < 0.05.

RESULT

Haematology

Packed cell volume (PCV): There was an increase in the PCV of rats in all the treatment groups relative to that of the rats in the control group. The increase was significant (p < 0.05) for rats in groups C (41.20 ± 2.89%), D (45.20 ± 5.02%) and E (44.00 ± 2.92%) compared with those in the control group (38.00 ± 2.35%) (Table 1).

Red blood cell count (RBC): The mean total red blood cell count of rats in each of the test groups B (6.86 ± 1.08 X 10^{6} /µL), C (7.24 ± 0.54 X 10^{6} /µL), D (7.56 ± 0.93 X 10^{6} /µL) and E (7.52 ± 0.74 X 10^{6} /µL) were observed to have higher values compared with those in the control group (6.70 ± 0.39 X 10^{6} /µL), but these differences were not significant (p > 0.05) (Table 1).

Haemoglobin concentration (Hb): There was a non-significant (p > 0.05) increase in the mean Hb concentration recorded for rats in the test groups B (12.30 ± 1.33 g/dl), C (12.16 ± 0.64 g/dl), D (13.60 ± 1.27 g/dl), and E (13.50 ± 1.02 g/dl) relative to that observed in the control group (12.38 ± 0.61 g/dl) (Table 1).

Mean corpuscular volume (MCV): There was a slight increase observed in the mean MCV values of rats in groups D (58.40 ± 3.21 fl) and E (58.80 ± 2.77 fl) compared with those rats in the control group. (56.80 ± 1.10 fl), but these increases were non-significant (p > 0.05). The mean values of rats in groups B (56.00 ± 2.12 fl) and C (56.00 ± 0.14 fl) were non-significantly (P > 0.05) lower than the mean MCV values of rats in the control group (Table 1).

Mean corpuscular haemoglobin (MCH): Rats in each of the test groups were observed to have decrease mean
MCH values compared with the rats in the control group. Significant (p < 0.05) decreases were observed for rats in groups D (18.20 ± 0.84 g) and E (18.20 ± 0.84 g) compared with the rats in the control group (18.40 ± 0.55 g). The difference of the means between the rats of control group and group B (18.00 ± 1.22 g) or C (18.00 ± 0.71 g) is non-significant (p < 0.05). Rats in group B were also observed to have significantly (p < 0.05) lower mean MCH values (18.00 ± 1.22 g) compared with rats in groups B (32.20 ± 0.84 g/dl), C (32.20 ± 0.84 g/dl), D (32.20 ± 1.20 g/dl) or E (30.80 ± 0.45 g/dl) relative to the mean MCHC values of rats for each of the test groups (MCHC): (Table 1).

### Mean corpuscular haemoglobin concentration (MCHC):
There was a non-significant (p > 0.05) decrease in the mean MCHC values of rats for each of the test groups B (32.20 ± 0.84 g/dl), C (32.20 ± 0.84 g/dl), D (32.20 ± 1.20 g/dl) or E (30.80 ± 0.45 g/dl) relative to the value observed in rats in the control group (32.60 ± 0.55 g/dl) while the difference of means was significant (P < 0.05) for group E (Table 1).

### White blood cell count (WBC):
The mean WBC values increased non-significantly (p > 0.05) in the test rats in groups B (11.76 ± 3.29 x 10^3/µL), C (14.34 ± 6.92 x 10^3/µL), D (12.16 ± 4.86 x 10^3/µL) and E (14.64 ± 4.69 x 10^3/µL) compared with the value obtained for the rats in the control group (9.34 ± 2.32 x 10^3/µL) (Table 1).

### Lymphocyte
The mean lymphocyte value of rats in the control group (71.80 ± 5.97%) was lower (P > 0.05) than the values obtained for rats in groups B (74.00 ± 8.00%), C (75.40 ± 2.97%) or D (75.80 ± 4.55%) but slightly higher than 70.00 ± 10.26% recorded for group E (Table 1).

### Neutrophils
The mean neutrophils values of rats in the control group (25.40 ± 4.10%) was higher (P > 0.05) than the value obtained for rats in group B (21.60 ± 8.65%), C (22.00 ± 4.00 %) or D (23.40 ± 4.16 %) but lower (p > 0.05) than 29.60 ± 10.16 % recorded for rats in group E (Table 1).

### Serum electrolyte

#### Sodium ion (Na⁺) levels:
The mean serum Na⁺ level in the control rats (88.6 ± 1.72 mg/dl) was higher than those of the rats in groups B (86.6 ± 3.71 mg/dl), C (85.2 ± 2.40 mg/dl) or E (87.0 ± 2.85 mg/dl) but lower than that of group D (89.2 ± 1.43 mg/dl). However, the difference of the means was non-significant (p > 0.05) between all the means (Table 2).

#### Chloride ion (Cl⁻) levels:
The mean serum Cl⁻ level in the control rats (103.6 ± 1.46 mg/dl) was higher than that of rats in groups B (97.00 ± 1.18 mg/dl), C (92.80 ± 2.91 mg/dl), D (97.60 ± 1.08 mg/dl) or E (96.4 ± 3.80 mg/dl) but lower than that of group D (97.6 ± 1.08 mg/dl) (Table 2).

#### Bicarbonate ion (HCO₃⁻) levels:
The mean serum bicarbonate ion level was significantly (p < 0.05) lower in rats in the test groups B (17.4 ± 0.245 mg/dl), C (15.6 ± 0.60 mg/dl), D (16.2 ± 0.58 mg/dl) or E (17.2 ± 0.37 mg/dl) compared with the rats in the control group (20.2 ± 1.02 mg/dl) (Table 2).

#### Potassium ion (K⁺) levels:
The mean serum potassium level of the control group (3.10 ± 0.23 mg/dl) was higher (p > 0.05) than that of rats in groups B (3.02 ± 0.40 mg/dl), D (2.86 ± 0.21 mg/dl) and E (2.78 ± 0.12 mg/dl) but lower (p > 0.05) than that of group C (4.48 ± 1.54 mg/dl) (Table 2).

#### Calcium ion (Ca²⁺) levels:
The mean serum calcium level of the control group (8.12 ± 0.22 mg/dl) was non-significantly higher (p > 0.05) than that of rats in groups B (7.66 ± 0.11 mg/dl), C (6.92 ± 0.50 mg/dl), D (7.84 ± 0.00 mg/dl) and significantly (p < 0.05) higher than that of group E (7.44 ± 0.13 mg/dl) (Table 2).

#### Phosphate ion (HPO₄²⁻) levels:
The mean serum hydrogen phosphate ion level of the rats in control group (4.02 mg/dl) was significantly (p < 0.05) higher than that of rats in groups B (3.92 ± 0.60 mg/dl), C (3.52 ± 1.08 mg/dl) or E (3.52 ± 0.90 mg/dl) but lower (p > 0.05) than that of group C (4.48 ± 1.54 mg/dl) (Table 2).

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**Table 1.** Mean (± standard error of mean) levels of haematological parameters of control and test Wistar rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>1000 mg/kg</th>
<th>2000 mg/kg</th>
<th>4000 mg/kg</th>
<th>8000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>38.00 ± 2.35</td>
<td>38.20 ± 4.92</td>
<td>41.20 ± 2.39</td>
<td>45.20 ± 5.02</td>
<td>44.00 ± 2.92</td>
</tr>
<tr>
<td>RBC (X10^6/µL)</td>
<td>6.70 ± 0.39</td>
<td>6.86 ± 1.08</td>
<td>7.24 ± 0.54</td>
<td>7.56 ± 0.93</td>
<td>7.52 ± 0.74</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.38 ± 0.61</td>
<td>12.30 ± 1.33</td>
<td>12.16 ± 0.64</td>
<td>13.60 ± 1.27</td>
<td>13.50 ± 1.02</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>56.80 ± 1.10</td>
<td>56.00 ± 2.12</td>
<td>56.00 ± 1.14</td>
<td>58.40 ± 3.21</td>
<td>58.80 ± 2.77</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>18.40 ± 0.55</td>
<td>18.00 ± 1.22</td>
<td>18.00 ± 0.71</td>
<td>18.20 ± 0.84</td>
<td>18.20 ± 0.84</td>
</tr>
<tr>
<td>MCHC(g/dl)</td>
<td>32.60 ± 0.55</td>
<td>32.20 ± 0.84</td>
<td>32.20 ± 0.84</td>
<td>32.20 ± 1.20</td>
<td>32.20 ± 1.20</td>
</tr>
<tr>
<td>WBC (X10^3/µL)</td>
<td>9.34 ± 2.32</td>
<td>11.76 ± 3.29</td>
<td>14.34 ± 6.92</td>
<td>12.16 ± 4.86</td>
<td>14.64 ± 4.69</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>71.80 ± 5.97</td>
<td>74.00 ± 8.00</td>
<td>75.40 ± 2.97</td>
<td>75.80 ± 4.55</td>
<td>70.00 ± 10.22</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>25.40 ± 4.10</td>
<td>21.60 ± 8.65</td>
<td>22.00 ± 4.00</td>
<td>23.40 ± 4.16</td>
<td>29.6 ± 10.16</td>
</tr>
</tbody>
</table>
Blood urea nitrogen (BUN): The mean BUN levels in the test rats in groups B (120.75 ± 7.08), C (141.00 ± 8.31), D (132.75 ± 2.78) and E (120.00 ± 10.25) were significantly higher than the rats in the control groups (Table 2).

DISCUSSION

Relative to the rats in the control group, the mean PCV values of rats in all the treatment groups were observed to have increased dose-dependently. The observed increase in the mean PCV can be attributed to increases in both red and white cell indices.

The mean RBC and Hb concentration of rats were increased, especially for rats administered the higher doses. Mean MCV values were also increased, while MCH and MCHC values on the other hand decreased. Increase in circulating RBC occurs in response to stimulation of the erythropoietic system. Reticulocytosis occur as the initial response to stimulation of the erythropoietic system (Adamson and Longo, 2001) and is often characterized by macrocytic and hypochromic red blood cells with increased MCV and decreased MCH and MCHC values (Adamson and Longo, 2001), which compares with the findings in the test rats in this study. Plants in the Cucurbitaceae family, especially Telfaria occidentalis have also been reported to have haematinic effect and have proven to be of therapeutic value in conditions of anaemia (Alada, 2000; Dina et al., 2000). The efficacy of the extract of fruit of L. breviflora on treatment of anaemia can thus be investigated.

The differential cell analysis showed that there was an increase in the mean lymphocyte values. Increase in circulating lymphocyte values is associated with enhanced immunological status of the body (Guyton and Hall, 2006a) especially the cell-mediated immune response (Lowenthal et al., 1994). CuE, a primary cucurbitacin was reported to induce and maintain high proliferation rates of lymphocytes (Attard et al., 2004) but when co-cultured with cancer cells, an interesting lymphocyte-mediated cytotoxicity was observed (Attard et al., 2005). These corroborate the immunomodulatory action of ethanolic extract of L. breviflora observed in this study.

The value of circulating neutrophils was reduced in the rats administered with the extract in this study. Increased circulating neutrophils serve as an index of bacterial infection in the body (Guyton and Hall, 2006b). It is believed that the reduction of mean value of circulating neutrophils in this study is indirect, probably due to the antibacterial action of L. breviflora by Tomori et al. (2007).

Serum electrolyte levels

Serum sodium ion level was higher in the test rats studied. Kang et al. (2002) reported that hypernatremia is rare but does occur when there is loss of body fluids containing less sodium than plasma along with water intake restriction or if there is excessive sodium intake with limited liquid intake. Nduka (1999a) concluded that hypernatremia almost always indicates water depletion. Water was not however restricted in this study thus, this increase is suspected to be due to the inability of the kidneys to excrete adequate sodium from the tubular fluid or the fruit extract may contain some sodium based compounds. These may have led to the excess sodium ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats. Potassium and chloride ion levels in the test rats.

Losses of bicarbonate ($HCO_3^-$) values were significant in this study. Bicarbonate buffer system is the most important amongst blood buffers when the pH of the blood is considered (Fisher, 1969). Reduction in serum bicarbonates implies that the pH of the blood was lowered. This reduction can be due to two mechanisms:
excessive respiratory excretion via hyperventilation, or increased renal excretion of bicarbonates (Shirley et al., 2003). There was no hyperventilation observed in the experimental rats, the renal route of excretion appears as the factor for the probable loss of bicarbonates.

Calcium level was reduced, with a dose-dependent pattern observed. Osborne et al. (1972) reported that hypocalcaemia is most commonly found in terminal stages of chronic generalized renal failure when values of 5 - 8 mg/dl of serum calcium may occur in man. L. breviflora - induced electrolyte imbalance has not featured in the literature; we therefore associated it with derangement of renal function resulting from interference with ions transport across the renal tubules (Shirley and Unwin, 2005). Though the serum creatinine level was increased non-significantly, the BUN was significantly (p < 0.05) increased, as a strong indication of renal impairment (Nduka, 1999b).

Conclusion

Prolonged administration of extract of L. breviflora elicited electrolyte imbalance but it is not haematoxic, rather, it stimulate erythropoiesis and enhance immunity especially the cell-mediated immunity.

REFERENCES


