EFFECT OF ETHANOLIC EXTRACT OF ALSTONIA BOONEI LEAVES ON SERUM ELECTROLYTE LEVELS IN WISTAR ALBINO RATS.

Afolabi B.A.1*, Obafemi T.O.2, Akinola T.T.1, Adeyemi A.S.1, Afolabi O.B.2

1Department of Biochemistry, Bowen University Iwo, Nigeria.
2Chemical Sciences Department, Afe Babalola University Ado-Ekiti, Nigeria.

*afolabiblessing@ymail.com

Abstract

This study evaluates the effect of methanol extract of Alstonia boonei leaves on levels of serum Mg2+, Ca2+, K+, Na+, HCO3-, urea and creatinine in wistar albino rats as a way of assessing the effect of the extract on kidney function. Twenty rats weighing 180-240 g were assigned to four groups (A, B, C and D) with five animals in each group. Group A served as the control while groups B, C and D served as the test groups and received 100 mg/kg, 200 mg/kg and 400 mg/kg of the extract respectively. All the animals were sacrificed after fourteen days of extract administration. Blood was collected by cardiac puncture for biochemical analysis of serum electrolytes. Serum chemistry showed that there was no significant difference (P<0.05) in the serum levels of bicarbonate, calcium, potassium and urea at all doses tested. Significantly raised sodium and decreased magnesium levels was observed in animals treated with 100 mg/kg, 200 mg/kg and 400 mg/kg of the extract respectively. All the animals were sacrificed after fourteen days of extract administration. Blood was collected by cardiac puncture for biochemical analysis of serum electrolytes. Serum chemistry showed that there was no significant difference (P<0.05) in the serum levels of bicarbonate, calcium, potassium and urea at all doses tested. Significantly raised sodium and decreased magnesium levels was observed in animals treated with 100 mg/kg, 200 mg/kg and 400 mg/kg of the extract respectively. However, the values were brought close to those of control at doses of 200 mg/kgbw and 400 mg/kgbw. The serum creatinine level was however significantly (P<0.05) higher than control at 400 mg/kgbw. From the result of this experiment, it is concluded that the administration of ethanolic extract of Alstonia boonei leaves does not alter the levels of serum electrolyte. However, care must be taken when exploring the medicinal value of the leaf because of the observed increased creatinine levels observed in the study.

Keywords – Biochemical, Electrolyte, Kidney, Serum, Alstonia boonei
Introduction
The use of plants as sources of remedies for the treatment of many diseases dates back to prehistory and people of all continents have this old tradition. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants [1]. In developing countries, notably West Africa, new drugs are not often affordable, thus, up to 80% of the population use medicinal plants as remedies [2,3]. Medicinal plants have contributed immensely to health care. This is due in part to the recognition of the value of traditional medical systems and the identification of medicinal plants from indigenous pharmacopoeias which have significant healing power [4]. The consumption of a variety of local herbs and vegetables by man is believed to contribute significantly to the improvement of human health in terms of prevention or cure of diseases because plants have long served as useful and rational sources of therapeutic agents [5].

The kidney is a chief regulator of all the body fluid and is primarily responsible for maintaining homeostasis or equilibrium of fluid and electrolytes of the body. Kidney’s main function include urine formation, regulation of acid-base balance, excretion of waste products of metabolism and toxic substances, protein conservation, secretory functions and recovery of useful metabolites which filters through them [6]. The ability of the kidney to regulate blood volume and chemical composition in accordance with the body’s changing needs requires great complexity of function. Homeostasis is maintained in large part by coordination of renal functions with those of the cardiovascular and pulmonary systems. The compromise of kidney functions may be the result of ischemia caused by reduced blood flow, it may also result from excessive use of certain drugs [7].

Alstonia boonei De Wild (Devil tree) Apocynaceae is a medicinal plant that is widely used in Africa for the treatment of various ailments. Numerous therapeutic properties have been attributed to A. boonei like antifungal, antibacterial, antiviral anti-thrombosis, anti-tumor, anti-inflammatory, analgesic, antioxidant and antipyretic activities [8,9]. A previous study detected the presence of secondary metabolites such as alkaloids, tannins, saponins, resins, flavonoids, steroids, glycosides and terpenoids in the pulverized dried leaves and the ethanol extract of A. boonei leaves [10]. However, the various species of Alstonia are highly rich in alkaloids, steroids and triterpenoids, and phenolic compounds which contribute to the toxicity of Alstonia boonei [8]. Moreover, the plant was found to contain poisonous alkaloids comprising ditamine, echitamine and echitamidine [11]. To the best of our knowledge the effect of ethanolic extract of Alstonia boonei leaves on kidney functions has not been evaluated, hence this study.

Materials and Methods
Plant material
Fresh mature leaves of Alstonia boonei were collected from a farmland in August in Iwo Osun State, Nigeria. The plant was authenticated by Mr Donatus in the Department of Botany, University of Ibadan, Nigeria. The leaf were picked, washed in distilled water and air-dried. The dried leaf was ground to powder using an electric grinder. The leaf powder (435 gram) was extracted in five liters of 95% ethanol by maceration for 72 hrs. The extract was filtered using Whatman No 1 filter paper and the filtrate was concentrated in a rotary evaporator, lyophilized and thereafter preserved for further use.

Quantitative assay kits
Assay kits for creatinine, urea, bicarbonate and calcium were made by Randox, magnesium, sodium, and potassium were procured from Teco diagnostics. All other chemicals and reagents used were of analytical grade.

Laboratory animals
Twenty male wistar rats weighing 180-240 g were obtained from the Animal Care Facility, University of Ibadan, Nigeria. The Animal Ethics Committee of Department of Biochemistry, Bowen University Iwo, Nigeria approved all experimental protocols.

Experimental animals and procedure
The twenty male wistar rats were randomly grouped into four, comprising of five rats per group. The animals were housed in cages made of wooden frames and metal netting, acclimatized for 7 days in the animal house Biochemistry department, Bowen University Iwo, Nigeria and maintained under standard conditions. The rats were fed ad libitum with rat pellet and tap water with 12-hours light/dark cycle. The cages were cleaned every morning and disinfected at intervals of 3 days. The animal groups are as follows:
Group A: control, received distilled water
Group B: received 100 mg/kg body weight of ethanolic extract of Alstonia boonei leaves
Group C: received 200 mg/kg body weight of ethanolic extract of Alstonia boonei leaves
Group D: received 400 mg/kg body weight of ethanolic extract of Alstonia boonei leaves

Administration lasted for 14 days using metal cannula attached to a 2 ml syringe., after which the rats were fasted for 12 hours, body weights recorded and were humanely sacrificed by cervical dislocation. Blood was collected by cardiac puncture and placed in bottles with no anticoagulant.

Biochemical assays
Creatinine, urea, bicarbonate, calcium, magnesium, sodium, and potassium concentrations were estimated according to the manufacturer’s instruction on the assay kits.

Statistical analysis
The results were expressed as mean ± standard error of mean (SEM) and were subjected to one way analysis of variance (ANOVA), using statistical package for social sciences (SPSS-16) at 95% level of confidence. Values were considered statistically significant at (p<0.05) and denoted by different alphabets.

Results
Our results as presented in table 1 shows that there was no significant (P<0.05) difference in the bicarbonate, calcium, potassium and sodium levels between all the doses tested and the control. Likewise, there was no significant (P<0.05) difference between the magnesium level in all the doses tested. However the magnesium level in the control was significantly higher than the lowest dose (100 mg/kg).

Table 2 shows that there was no significant (P<0.05) difference between the serum urea levels of the control and all the doses tested. However, the creatinine level observed in the highest dose tested (400mg/kg) was significantly (P<0.05) higher than that of control while the levels observed in the control and the other doses are not significantly (P<0.05) different. These results showed that the ethanolic extract of Alstonia boonei is not toxic on the kidney to a reasonable extent at all the doses tested in the experimental animals as observed assays carried out.

Discussion
Sodium is the major extracellular electrolyte implicated in hypertension [12]. Extracellular Sodium electrolyte level is responsible for the extent to which vessel walls contract [13]. When the Sodium level is high, there is increased contraction of the blood vessels (especially in the kidney), and hence a greater force is required to pump blood, with a consequent hypertension [12]. Moreover, high levels of sodium in the blood causes the cells to be dehydrated leading to hypernatremia which can cause coma or death [14]. Increases or decreases in sodium concentration may contribute to fluctuations of blood pressure [15]. Different studies have demonstrated the ability of plant extracts to influence the blood concentrations of Na⁺. In our result presented in fig 1 there was no significant difference in serum Na⁺ levels after administration of the ethanolic extract of Alstonia boonei leaves for 14 days. Even though the Na⁺ level increased significantly (P<0.05) at a dose of 100 mg/kg the level was brought down to a value that is significantly (P<0.05) close to control at the highest dose of 400mg/kg. This is in line with the work of Odey et al. which reported on the anti-hypertensive property of Naucea latifolia and suggested that the extract causes sodium level to be maintained at a level in which no great force is required to pump the blood i.e not causing hypertension. This implies that the extract at a dose of 400 mg/kg might have antihypertensive effect [16].

Magnesium ion has numerous functions in the body. It serves as a cofactor for several enzymatic reactions. It is essential for the activity of hexokinase, creatine kinase, Na⁺/K+ ATPase, adenylate and guanylate cyclases. It also involved in cell adhesion, neurotransmitter release, and it also performs structural functions in proteins, nucleic acids and polyribosomes [17]. The most common test for the evaluation of magnesium levels and magnesium status in patients is serum magnesium concentration [18, 19], which is valuable in clinical medicine, especially for rapid assessment of acute changes in magnesium status [20]. Although serum magnesium concentration does not correlate with tissue pools, with the exception of interstitial fluid and bone [20, 21], our result however showed that ethanol extract of Alstonia boonei leaves does not have a significant effect on the magnesium levels of the test animals as compared with control. Although it was observed that the lowest dose of our extract (100 mg/kg) reduced the magnesium level significantly (P<0.05),
our extract increased the level to a value that is significantly (P<0.05) close to that of control at 400 mg/kg. This implies that all the biochemical reactions in which magnesium ion is involved as cited above would proceed unperturbed. Our result shows that there is no significant difference between the serum bicarbonate, calcium, and urea levels of the test groups when compared with the control group. The bicarbonate ion acts as a buffer to maintain the normal levels of acidity (pH) in blood and other fluids in the body. Bicarbonate levels are measured to monitor the acidity of the blood and body fluids. Our result shows that there is no significant (P<0.05) difference between the serum bicarbonate levels of the test groups when compared with the control group.

Potassium ion is a major cation of the intracellular fluid and only about 10% of the total body potassium is found extracellular. Fluctuations in serum potassium level are known to have serious health implications. Hypokalemia can lead to muscular weakness, hypotonia and cardiac arrhythmias [22] while hyperkalemia predisposes to cardiac arrest [15]. Our result in fig 1 shows that the extract at all the concentrations did not alter the serum potassium levels when compares with the control group.

However, a significantly higher levels of creatinine was observed in the study. The presence of increasing creatinine concentration in the blood is used in the evaluation of the effects of chemicals on the kidney and it also serves as an indicator of the glomerular filtration rate of the kidney [23]. Rise in creatinine levels is majorly observed if there is marked damage to functional nephrons [24]. This is in consonance with the study of Avila et al that reported the cytotoxic effect of aloe which suggests that aloe promotes nephrotoxicity, thus causing impaired renal function evident by an increase in serum creatinine concentration. However, serum creatinine levels are also known to increase when angiotensin converting enzyme inhibitors (ACE inhibitors) or angiotensin receptor blocker (ARB) are taken. The increased serum creatinine levels observed in our study may therefore be because the ethanol extract of Alstonia boonei leaves mimics either ACE inhibitors or ARB [25]. Moreover Singh and Singh reported that various species of Alstonia are highly rich in alkaloids, steroids and triterpenoids, and phenolic compounds which contribute to the toxicity of Alstonia boonei [8]. This fact might also be responsible for the increase in creatinine level observed in the animals after administration of ethanolic extract of Alstonia boonei leaves for 14 days.

Conclusion
These results suggest that the extract does not have any adverse effect on the functions of the kidney. Although the lowest dose of the extract caused significant perturbations in few of the serum electrolytes assayed for, the levels of the electrolytes were brought to values that are significantly close to those observed in the control group. This implies that the extract might have some toxicity effects at a low dose which were reversed with higher doses of the extract. Moreover, the increase in serum levels of creatinine observed in the study might be a consequence of the presence of some compounds which were reported to confer toxicity on the extract. Therefore, great caution should be exercised while utilizing Alstonia boonei leaves for its numerous health benefits.

References
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15. Enemor VH, Okaka AN. Sub-acute effects of ethanol extract of Sarccephalus latifolius root on some physiologically important electrolytes in serum of normal wistar albino rats. Pakistan Journal of Biological Sciences 2013;10;3923
Table 1: Effect of ethanol extract of *Alstonia boonei* leaves on serum of levels magnesium, bicarbonate, calcium, potassium and sodium.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Magnesium</th>
<th>Bicarbonate</th>
<th>Calcium</th>
<th>Potassium</th>
<th>Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>.22 ± 0.22b</td>
<td>36.52 ± 4.17a</td>
<td>16.44 ± 1.08a</td>
<td>4.86 ± 0.60a</td>
<td>189.81 ±13.07a</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>2.00 ± 0.43a</td>
<td>44.64 ± 5.10a</td>
<td>22.01 ± 3.62a</td>
<td>5.82 ± 0.37a</td>
<td>227.86 ± 8.00b</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>2.38 ± 0.29a,b</td>
<td>28.69 ± 9.13a</td>
<td>18.14 ± 1.52a</td>
<td>5.50 ± 0.51a</td>
<td>207.81 ±12.42a,b</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>2.61 ± 0.25a,b</td>
<td>32.61 ± 6.37a</td>
<td>15.49 ± 1.44a</td>
<td>4.85 ± 0.70a</td>
<td>213.41 ± 3.59a,b</td>
</tr>
</tbody>
</table>

Values are means± SD of three replicate determinations. Means on the same column not followed by same superscript differ significantly (P<0.05).

Table 2: Effect of ethanol extract of *Alstonia boonei* leaves on levels of some serum urea and creatinine

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.73 ± 2.73a</td>
<td>117.81 ± 11.21a</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>23.13 ± 3.50a</td>
<td>70.35 ± 4.94a</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>28.67 ± 3.76a</td>
<td>266.47 ± 6.23a,b</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>19.23 ± 2.23a</td>
<td>418.06 ± 22.57b</td>
</tr>
</tbody>
</table>

Values are means ± SD of three replicate determinations. Means on the same column not followed by same superscript differ significantly (P<0.05).