Changes In Lipid And Haematological Profile Of Aqueous Ethanolic Extract Of Alstonia Boonei In Rats

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Abstract
This study investigated the effect of 50 and 200mg/kg extract of Alstonia boonei stem bark (ASBE) on serum lipids and blood parameters for 2 and 4 weeks in albino rats. The ASBE lowered serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) significantly (p<0.05) in animals orally administered with 50 and 200mg/kg of the extract for 2 weeks. The ratio of the atherogenic risk predator indices showed that 50 and 200mg/kg extract may possess antiatherogenic effect and therefore desirable. There was no significant difference in haematological parameters, except for WBC where the fall was significant (p < 0.05) at 200mg/kg by the 4th week of treatment. These results suggest possible hypolipidaemic effect of ASBE at short course of treatment, which also agrees with its local use in the management of hypertensive conditions.

INTRODUCTION
Alstonia boonei (Apocynaceae) is a deciduous tree found in the tropical and sub-tropical regions of the world. It is widely distributed in Africa especially south of the Sahara. Every component of the plant appears to possess one medicinal value or the other.

Phytochemical studies identified the principal constituents to be indole alkaloids from which echitamine, alstonine, alstonidine, amyrin, lupenol, porphyrine, triterpines and ursane have been isolated. Extracts of A. boonei are commonly employed in folk medicine to treat a wide range of disease conditions, especially in developing countries. In folk medicine practice, an infusion of the extract of the stem bark serves as anti-snake venom and as antidote to some arrow poisons. The extracts are also used locally as astringents and febrifuge for relapsing fever. Exudates of the leaf and latex are topically applied against inflammatory reactions, rheumatoid and muscular pains as well as raised blood pressure (Asuzu and Anyaga, 1991; Iwu, 1993).

Pharmacological screenings have been carried out on some constituents of the plant extract. The anti-inflammatory activity has been widely reported in different animal models. Ojewole, (1984) Raji et al. (2000), Salahedeen et al (2003) and Owoyele et. al., (2004) demonstrated the ability of the plant extract to inhibit carageanen-induced paw adema, cotton-pallet granuloma and other rheumatoid arthritis models. The extract of the stem bark is commonly used to treat malaria, and is listed in the African Pharmacopoeia as an antimalarial drug (Olajide et. al., 2002). It is usually referred to as “Australian Quinine” around the oceanics; further expressing its anti-malarial efficacy (lie-Fen et al, 2005). The extract of the stem bark have also been known to posses’ potent neuroleptic and anxiolytic properties in behavioral studies using mice (Elizabetsky & Costa-campos, 2006).

However, cardiovascular diseases present some of the main health problems across the globe today, the major ones being coronary heart diseases, stroke and hypertension (Alters & Shiff, 1997). Elevated plasma lipids are risk factors in cardiovascular problems. Hyperlipidaemia and other abnormal blood lipid profile are largely of genetic origin or due to unwholesome nutritional habits. Lipids and other substances accumulate on arterial wall, forming plaque, which occlude the vascular lumen and obstruct the blood flow to vital organs such as the heart, brain, liver, or kidney. Obstruction of blood supply to the heart, brain, liver or kidney cause coronary heart diseases, stroke or kidney failure, as the case may be.

The important lipids whose elevations are implicated in these disease conditions are cholesterol and triacylglycerols. Lipids are transported as lipid-protein complexes called lipoproteins, which are classified based on their density and charges. The high-density lipoprotein cholesterol (HDL-c) transports lipids out of blood cells to the liver, while the low
density lipoproteins cholesterol (LDL-c) mobilizes lipids against the cells and blood vessels. Triacylglycerols have been found to be elevated along with total cholesterol elevation. Therefore, elevated low-density cholesterol, triacylglycerols and total cholesterol with reduced HDL-c will enhance the development of atherosclerosis and related cerebrovascular disorders (Nwanjo, 2004). The clinical consequences of these disease conditions are serious; and meaningful research efforts to improve the knowledge and understanding of the pathogenesis is essential; in order to provide a more rational approach to their prophylaxis and treatment. (Kritchersky, 1970; kucera et al. 1972)

The blood is a vital fluid, which contains the Red Blood Cell (RBC), White blood cells (WBC) and platelets suspended in the serum in homeostatic concentrations. The circulatory blood volume makes up about 8% of the weight of an average man. The blood cells take up about 45% of the blood, while plasma constitutes about 55% (Guyton & Hall, 2000).

The Blood is important for pulmonary and tissue respiration, as a medium of endocrine and neurohumoral transmissions, biotransformation and metabolic excretion (Adebayo et al., 2005), nutritional and immunological processes, as well as homeostatic responses (Oze, 1992). The laboratory determination of blood products and parameters for the purpose of disease diagnosis is highly accurate, sensitive and reliable; and remained the bed-rock of ethical and rational research, disease diagnosis; prevention and treatment (Murrey et al., 2000, Okonkwo et al., 2004).

Despite the several studies on the different pharmacological activities of Alstonia boonei, not much has been done on its lipid and haematological profile; notwithstanding its wide spread use in folk medicine. This study is aimed at investigating the validity or otherwise of the use of the plant extract in atherosclerotic conditions in folk medicine and the likely effect of the extract on haematological indices.

**MATERIALS AND METHODS**

**PLANT MATERIALS**

The stem bark of Alstonia Boonei was collected from the growing tree in the forest of Ukhu, Ekpoma, Edo State, Nigeria, in October 2005. It was identified by Dr. S. C Okeke, (Plant Taxonomist), Department of Crop Science and Biotechnology, Imo state University, Owerri. The voucher specimen is deposited in the departmental herbarium.

**PREPARATION OF PLANT EXTRACT**

The shaded stem bark was cut into pieces and dried in an oven (Grant instruments, Cambridge, England) at 50 °C to constant weight (2.60kg approx). It was ground using a Thomas Contact Mill (Pye Unicam, Cambridge, England). 500g of the powdered stem bark were soaked in 7.75 L of 70% ethanol (BDH) for 24 hours in a soxhlet extractor. The resulting aqueous ethanolic extract was concentrated using a rotatory evaporator (Laborato 400, China). A 16.0g residue was obtained and stored in a refrigerator (4 °C). Appropriate measures and dilutions of the residue were made with normal saline (using Metler analytical weighing balance, 0.DPI-200g) to obtain the required doses.

**ACUTE TOXICITY TEST**

The acute toxicity of the extract was done using 30 albino rats divided into 5 groups of 6 rat each, with each group receiving a dose of the extract intraperitonally (i.p.) as described by Miller and Tainter (1994). The number of death in each group within 24 hours was recorded. The lethal dose-50 (LD50) was estimated from the graph of percentage mortality (converted to probit) against log-dose of the extract; probit 5 being 50%.

**ANIMALS**

Thirty albino rats (200-350g) of both sexes were obtained from the Animal House of the College of Medicine and Health Sciences, Imo state University, Owerri. The animals were haboured in stainless steel cages under standard laboratory condition of 12 hours light /dark cycle. They had access to feed (Top Fed, Sapele) and water ad libitum.

**EXPERIMENTAL DESIGN**

The thirty rats were randomly assigned to 6 experiment groups of 5 rats each. In addition to feed and water;

Group 1 - Received Normal Saline (0.5ml/kg), and Served as control for the 2 Weeks treatment. Group 2 - Received 50mg/kg extract for two weeks Group 3 - Received 200mg/kg extract for two weeks Group 4 - Received Normal saline (0.5ml/kg), and served as control for the 4 weeks treatment groups. Group 5 - Received 50mg/kg extract for four weeks Group 6 - Received 200mg/kg extract for four weeks.

The extracts were also given by oral tubation.

**SAMPLE COLLECTION**

By the end of each experimental period, the rats were
Changes In Lipid And Haematological Profile Of Aqueous Ethanolic Extract Of Alstonia Boonei In Rats

reweighed, starved for 24 hours and sacrificed under chloroform anaesthesia. 5ml of blood was collected from each animal by cardiac puncture using sterile needle and syringe. Part of the blood sample was put into test tubes and allowed to clot for 30 minutes before centrifuging at 800g (Wisperfuge, 1384, Samson, Holland) for 5 minutes. The supernatant was used for the lipid analysis. The remaining blood sample was put in an EDTA bottles for haematological determinations.

ANALYTICAL PROCEDURE

The serum total and HDL-cholesterol were estimated by the method of Lopez –Vitrella et al (1995). Serum triglycerides was estimated by the method of Mendez et al. (1975). LDL-cholesterol values were calculated by modification of Friedewald Formular (Friedewald, et. al.,1972).

The haemoglobin (Hb) level was measured by the cyanomethaemoglobin method. The Red Blood Cell (RBC) and Reticulocyte counts were determined by visual method (Baker and Siverton, 2000). Packed cell volume (PCV) was measured using microhaematocrit method and total White Blood Cell (WBC) count was estimated by visual method (Cheesbrough 2000). The RBC indices were calculated from the RBC count, Hb level and PCV estimations (Baker and Siverton 2000, Cheesbrough, 2000).

STATISTICS

The results were analyzed using Duncan multiple range test. All data were expressed as mean ± SD. Differences between groups were considered at 95% confidence limit and probability level of 0.05. Probability < 0.05 was taken as significant.

RESULTS

Table 1 shows the result of the mean values of body weight changes in the treatment and control groups. The extract had no effect on the weight of the animals. In tables 2 both doses of the extract produced significant reduction (p<0.05) in serum total cholesterol and LDL-C concentrations after two weeks. But the reduction after the second week was not significant (Table 3). There was a transient but progressive increase in HDL-C levels throughout the duration of the experiment. (Tables 2 and 3).

The serum triglycerides level was significantly reduced at 50mg/kg and 200mg/kg after the second week. The changes after the second week were marginal decreases (Table 3). The ratio of the atherogenic risk predictor indices showed that the extract may posses antiatherogenic effect and therefore desirable. The haematological parameters were not affected by the plant extract, except for WBC where the fall was significant (p < 0.05) at 200mg/kg by the 4th week treatment.

Figure 1

Table 1: Mean values of body weights of the different groups of rats (kg).

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial weight</th>
<th>Final weight</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Control)</td>
<td>0.31±0.05</td>
<td>0.34±0.04</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.32±0.05</td>
<td>0.34±0.05</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.32±0.04</td>
<td>0.33±0.04</td>
<td>0.01±0.01</td>
</tr>
</tbody>
</table>

Table 2: Mean values of serum lipids and arthrogenic risk predictor indices of different groups after treatment for two weeks (n=5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3 (n=5)</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mmol/L)</td>
<td>1.39±0.48</td>
<td>0.75±0.18</td>
<td>0.93±0.27</td>
<td>1.45±0.43</td>
<td>1.39±0.28</td>
<td>1.44±0.35</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>6.6±0.26</td>
<td>7.2±0.20</td>
<td>9.2±0.19</td>
<td>8.9±0.23</td>
<td>8.9±0.25</td>
<td></td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>1.2±0.22</td>
<td>0.91±0.14</td>
<td>0.94±0.35</td>
<td>1.18±0.96</td>
<td>1.08±0.39</td>
<td>1.18±0.30</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.3±0.19</td>
<td>0.72±0.19</td>
<td>0.51±0.19</td>
<td>0.90±0.11</td>
<td>0.42±0.12</td>
<td>0.59±0.12</td>
</tr>
<tr>
<td>LDL to HDL ratio</td>
<td>2.3±0.21</td>
<td>4.0±0.34</td>
<td>0.90±0.59</td>
<td>2.81±1.51</td>
<td>1.49±0.60</td>
<td>1.21±0.50</td>
</tr>
</tbody>
</table>

Figure 2

Table 3: Mean values of haematological parameters of the different groups after treatment for four weeks. (Mean ± SD) (n = 5).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (Control)</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4 (Control)</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10^6/μL)</td>
<td>4.9±0.42</td>
<td>1.7±0.80</td>
<td>1.0±0.46</td>
<td>4.2±0.42</td>
<td>1.8±0.34</td>
<td>2.7±0.30</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.4±1.46</td>
<td>17.1±1.17</td>
<td>11.1±1.03</td>
<td>12.3±1.13</td>
<td>9.5±0.10</td>
<td>7.7±0.07</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>0.7±0.03</td>
<td>0.7±0.03</td>
<td>0.7±0.03</td>
<td>0.7±0.03</td>
<td>0.7±0.03</td>
<td>0.7±0.03</td>
</tr>
<tr>
<td>WBC (10^6/μL)</td>
<td>9.4±5.35</td>
<td>10.1±7.07</td>
<td>8.1±5.25</td>
<td>9.0±5.11</td>
<td>10.7±3.32</td>
<td>12.8±6.72</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>7.1±3.13</td>
<td>7.1±3.13</td>
<td>8.0±3.07</td>
<td>8.0±3.07</td>
<td>7.1±3.13</td>
<td>8.0±3.07</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>32±6.6</td>
<td>32±6.6</td>
<td>32±6.6</td>
<td>32±6.6</td>
<td>32±6.6</td>
<td>32±6.6</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>1.7±1.5</td>
<td>2.4±6.5</td>
<td>1.2±1.5</td>
<td>1.7±0.6</td>
<td>1.5±0.5</td>
<td>1.2±0.6</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>15.6±1.7</td>
<td>13.9±1.8</td>
<td>16.0±2.1</td>
<td>15.0±2.1</td>
<td>17.3±1.3</td>
<td>18.0±0.9</td>
</tr>
</tbody>
</table>

Figure 3

Table 3: Mean values of haematological parameters of the different groups after treatment for four weeks. (Mean ± SD) (n = 5).


DISCUSSION

Atherogenicity with subsequent cardiovascular manifestations is one of the major causes of death and morbidity in the world (Raju and Binda, 2005). Various studies indicate that high serum cholesterol levels are strongly related to coronary atherosclerosis and increased risk of cardiovascular diseases. Clinical studies have also shown that lowering levels of serum cholesterol using diet or drugs decreases the incidence of coronary heart disease (Steiner & Li, 2000, Treasure, et al., 1995).

Increased LDL cholesterol with decreased HDL cholesterol usually increases the serum total cholesterol. This is because the plasma clearance of cholesterol is often impaired in the presence of low HDL –C. Triacylglycerols levels have also been found to increase with increase in plasma cholesterol. Atherogeneity therefore develops when LDL cholesterol, triacylglycerols and total cholesterol are elevated relative to plasma HDL-C. Elevated HDL-cholesterol improves the transportation of cholesterol from the plasma to the liver for biotransformation and excretion, thereby preventing atheroma formation and blood vessel occlusion (Ojiako and Nwanjo, 2005).

The administration of 50 and 200mg/kg Alstonia boonei aqueous extract in rats did not produce any significant weight changes in the animals. The extract however produced hypolipidaemic and antiatherogenic effect after two weeks of treatment with 50mg/kg, which significantly (p < 0.05) reduced total cholesterol and LDL-C ratio of atherogenic risk predictor indices to desirable levels, and also reduce the LDL-c/HDL-C and HDL-C/T-C ratios in the rats (Table 2).

Echitamine, the major constituent of the plant extract has been reported to be responsible for most of the pharmacological activities observed in the plant extract. Echitamine is an alkaloid with antioxidative and free radical scavenging properties (Elisabetsky & Costa-campos, 2006). Antioxidants prevent the oxidative modification of lipoproteins before their incorporation into the fatty streaks of the arterial wall. Studies have shown that oxidation of lipids increases their deposition on arterial walls, hence atherosclerosis and atherogenicity. However, the exact mechanism by which antioxidants lower blood cholesterol is not properly established. Galton & Krone (1991) suggested that it could be by promoting the stimulation of cholesterol excretion in the faeces via its biotransformation to bile acids.

However, Baliga et. al. (2004) in a study on Alstonia scholaris (which also contains echitamine) reported that the alkaloid lowered plasma cholesterol. The study also showed that the alkaloid exhibited a dose and time dependent cholesterol antiperoxidative effect. This supports the current findings that the extract appeared to lose its hypolipidaemic activity at higher doses and prolonged administration. The aqueous ethanolic extract of Alstonia boonei stem bark produced hypcholesterolaemic and antiatherogenic effects after two weeks of treatment with 50mg/kg, which was not observed at 200mg/kg. This may imply that low doses of the plant extract could be used for short periods to manage hyperlipidaemic and atherogenic conditions. These findings agree with the current use of the plant extract by folk medicine practitioners as antihypertensive agent.

The blood is the vital fluid that transports gases and nutrients to the tissues of the body. The biochemistry of the blood is directly linked to the functional capacity of the blood. The functional capacity of the blood is associated with the status of the blood components. And the health of the individual is affected by different disease conditions such as anemia, foreign bodies, including drugs and drug products, which find their way into the blood and other tissues.

The aqueous ethanolic extracts of Alstonia boonei are widely employed in traditional medicine practice where they are claimed to alleviate a wide range of ailments. Irrespective of their target organs, the extracts are conveyed to their sites of action via the blood stream. This makes it imperative to study the influence of this extract on blood parameters.

There were no significant changes (p>0.05) in the heamatological parameters except for WBC where the fall was significant (p<0.05) at 200mg/kg by the 4 th week of treatment. Costa-campos, et.al, (1999) and Baliga (2004) reported that echitamine alkaloid from A. boonei reduced the total WBC count in a similar pattern as in this study. The reduction of WBC count may lead to a compromise of systemic immunity and predisposition to opportunistic diseases and infections.

It is concluded that A. boonei stem extract caused hypolipidaemic effect at the tested doses. Further studies are suggested to confirm the validity or otherwise of these finding.

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