THE ETHANOL LEAF EXTRACT OF ALSTONIA BOONEI (APOCYNACEAE) REDUCES HYPERGLYCEMIA IN ALLOXAN-INDUCED DIABETIC RATS


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ABSTRACT

Alstonia boonei (Apocynaceae) is a plant indigenous to Nigeria and locally used in the treatment of diabetes mellitus. The leaves of the plant was extracted using absolute ethanol and an experimental work was carried out to evaluate the effect of different doses (100, 200 and 400 mg/kg) of the extract, glibenclamide and distilled water on the blood glucose and lipid profile of both normal and alloxan-induced diabetic rats. Induction of experimental diabetes was done using alloxan (150 mg/kg) intraperitoneally. Oral dosing was done for a period of 14 days. Blood samples were obtained at the 2nd, 4th, 8th and 24th hour for blood glucose evaluation and on the 14th day for both blood glucose and for lipid profile analysis. The oral acute toxicity profile was determined and the extract’s phytochemical constituents were also investigated. The preliminary phytochemical screening revealed the presence of carbohydrate, cardiac glycoside, flavonoids, alkaloids, anthraquinone and saponin glycoside. The extract produced significant reduction (p<0.05) of the blood glucose level at all doses tested in the diabetic rats compared to the untreated diabetic rats. This effect was noted to be greatest at the 8th hour and was dose dependent. This effect also compares well with glibenclamide where significant (p<0.05) reduction in the blood glucose was also observed. The extract at all doses also significantly reduced (p<0.05) the LDL, while increasing the HDL (p<0.05) of the diabetic rats treated at all doses in comparison with the untreated diabetic rats. The oral acute toxicity screening revealed that the extract is safe at doses up to 4g/kg. From the results therefore, it was concluded that the ethanol leaf extract of Alstonia boonei reduced the alloxan-induced increase in blood glucose level and has a similar anti-diabetic effect as glibenclamide.

Keywords: Alloxan, Alstonia boonei, lipid profile, glibenclamide, hypoglycaemia, hyperglycaemia

INTRODUCTION

The use of herbs to treat diseases is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The WHO estimates that 80% of the populations of some Asian and African countries presently use herbal medicines for some aspect of primary healthcare. Studies in the United states and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicines have become more widely available. Herbal medicines are increasingly becoming the choice of most patients nowadays. The promise of preserving the natural qualities
that promote a healthier method of healing different ailments have contributed to the popularity of these medicines (WHO, 2010). Herbal medicines also play a significant role in the traditional management of different ailments, majorly as a result of their relative safety and low cost (Hui et al., 2009).

Alstonia is a genus of the family Apocynaceae to which many other medicinally important plants belong. Alstonia species are tropical plants growing in various parts of Africa and South Asia, the plant is named after professor Alston (1685–1760), at the Department of Botany in the University of Edinburgh. Alstonia boonei is commonly known as “ahun” in Yoruba, “ogbu-ora” in Igbo and “ukhu” in Urhobo (Majekodunmi et al., 2008). The plant parts are rich in various bioactive compounds such as echitamidine, Nα-formylechitamidine, boonein, loganin, lupeol, ursolic acid, and β-amyrin among which the alkaloids and triterpenoids from a major portion (John-Prosper et al., 2012). Studies on Alstonia boonei have focused on bioactivity of its chemical constituents, ethnobotany, pharmacology and taxonomy. It is used as therapeutics for dysentery, typhoid, gonorrhea and asthma and is also applied to ulcers, toothache, snakebites, rheumatic pain and sores and as a galactagogue. Therapeutically, the bark/leaves have been found to possess anti-rheumatic, anti-inflammatory, analgesic, anti-malaria, antipyretic, anti-diabetic (hypoglycemic), anti-helminthic, antimicrobial and antibiotic properties (John-Prosper et al., 2012).

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemic (high blood sugar) resulting from a reduction in the level of insulin produced by the pancreas. In some cases as is seen in type 2, there is resistance by cells to insulin’s effects (Shoback et al., 2003). It is a global health issue, and about 25 % of the world population is estimated to be affected by this disease (Maiti et al., 2004).

Diabetes is characterized by some classical symptoms which include polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Current drugs used in the management of diabetes include insulin, oral hypoglycemic agents such as the sulphonylureas, anti-hyperglycaemic such as biguanides and thiazolinediones. These drugs are marred with adverse effects and are usually not curative. Hence the search for newer agents that are more tolerable with lesser adverse effects and better still, curative. From the foregoing, there has been research into herbal remedies, for the purpose of getting agents that can cure and that are better tolerated, as herbal products are well documented to have lesser adverse effects compared with orthodox drugs (Hui et al., 2009).

This research was thus drawn up to investigate and possibly provide a scientific basis for the use of Alstonia boonei on the basis of its ethno medicinal use in the management of diabetes. The leaves of the plant was extracted with ethanol and evaluated for anti-diabetic property on alloxan-induced diabetic rats. Ethanol was used as the extracting solvent based on how it is used locally by the traditional practitioners, who usually soak in alcohol for some days prior to oral intake. Its effect on the lipid profile of alloxan-induced diabetic rats was also evaluated, based on the influence of diabetes on the lipid profile and vice versa.

MATERIALS AND METHODS

Collection, Preparation and Extraction of Plant material

Mature leaves of Alstonia boonei were obtained from a secondary forest along Federal Government Girls College (FGGC) Road, Benin City, Edo State, Nigeria. Leaves were collected during the
harmattan period in January, 2013. Specimens of these leaves were authenticated by Mr Sunny Nweke, a herbarium curator and voucher specimens deposited in the herbarium at the Department of Pharmacognosy, University of Benin. Botanical authentication of the plant was further confirmed at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria where a voucher specimen (No FHI 109793) was deposited for future reference. Leaves of *Alstonia boonei* were air dried for a week and then pulverized into powder. A weight of 500g of the powdered samples was agitated in absolute ethanol for 72 hours, this was to simulate how the plant is locally used. Usually the crushed leaves are soaked in alcohol for a minimum of two days prior to use by the traditional practitioners. The macerated extract was filtered with a filter cloth and the filtrates concentrated in an oven at a low temperature (below 40°c). The percentage extract yield was calculated and gave 13.4% semisolid mass. The extract was reconstituted with 3% tween 80 prior to administration.

**Chemicals/Reagents**
All chemicals used in the study were of analytical grade. They include; Absolute ethanol (Sigma, UK), distilled water, alloxan (Qualikens laboratory reagent), glibenclamide (Hovid), tween 80.

**Analysis of Phytochemical constituents**
Qualitative chemical test were performed to assess the presence of various phytochemical constituents of the aqueous root extract of *Alstonia boonei* (Trease and Evans, 1989).

**Animals**
Adult albino rats and albino mice weighing 160-250g and 20-30g respectively of either sex were obtained from the animal house of the Department of Pharmacology, University of Benin, Nigeria. The animals were acclimatized for 14days under standard environmental conditions on a regular feed (standard growers mash, Ewu Feeds, Ewu, Edo State) and water ad libitum.

**Ethical approval**
Ethical approval for the study was obtained from the Ethical Committee on the Use of Animals for Experiments, Faculty of Pharmacy, University of Benin. The approval letter dated January, 2012. The animals were handled according to the standard protocols for the use of laboratory animals, National institute of health, USA: Public health service for human care and animal studies, 2012.

**Induction of experimental diabetes**
Experimental diabetes was induced by intraperitoneal injection of 150 mg/kg body weight of alloxan monohydrate in overnight-fasted animals after acclimatization (Katsumata et al., 1993). Experimental diabetes was confirmed three days later in the alloxan treated animals showing random blood glucose (RBG) level greater than or equal to 200mg/dl (11.1mmol/L). Experimental diabetes status determination was monitored via blood obtained from tail vein puncture. The determination of blood glucose level was done using the Accu-check active glucometer (Rheney and Kirk, 2000). The results were recorded in mg/dl and levels between 200 and 400 mg/dl were used for the study.

**Experimental protocol/design**
A total of thirty albino rats were divided into six groups of five rats each. The animals were grouped as follows: Group1 (reference/ normal control rats fed normal diet and administered distilled water, group 2: diabetic untreated rats administered distilled water (diabetic control), group 3: diabetic rats administered glibenclamide 5 mg/kg (Owolabi and Omogbai, 2013), groups 4, 5 and 6: diabetic rats administered 100, 200 and 400 mg/kg body weight of the ethanol leaf extract of *Alstonia boonei*, respectively. Doses were picked after an initial preliminary work. The different
The dose of the extract/distilled water was orally administered to the rats on a daily basis usually in the mornings after feeding for 14 days.

**Determination of blood glucose level**
The blood glucose level of the alloxan-induced diabetic rats was tested using a glucometer by cutting the tip of the tails to obtain blood from the tail vein. Blood glucose determination was done at 0, 2nd, 4th, 8th and 24 hour intervals. The 14th day which was the day of sacrifice was the last day for blood glucose determination. The results obtained were recorded as mg/dl (Rheney and Kirk 2000).

**Collection of blood samples**
On the day of sacrifice, food and water were withdrawn 6 hours prior to the time of sacrifice. This was done to minimize the glycogen stored in the body. The animals were sacrificed under chloroform anaesthesia, blood samples were collected using 2ml and 5ml syringes from the abdominal aorta and left ventricle of the heart. The blood samples were introduced into lithium heparinized bottles.

**Determination of lipid profile**
Total plasma cholesterol (TC), high density lipoproteins (HDL), and triglycerides (TG) were estimated in all groups of animals by collecting blood via terminal bleeding, 24 hours after drug or normal saline administration. Blood samples (100, 10 and 200 μl for total cholesterol, triglycerides and HDL respectively were introduced into lithium heparin tubes. The samples were then centrifuged at 5,000 g for 15 minutes to obtain the plasma.

**Total cholesterol**
Total cholesterol in plasma was determined by enzymatic method, using wet reagent diagnostic kits, which is a modification of the method of Abell et al., 1952. The reagent (1000 μl) was pipetted into 3 different test tubes A, B and C. Distilled water, a standard solution (standard) and plasma (sample) of 100 μl each were pipetted into same test tubes A, B and C respectively. After mixing, the test tubes were left to stand for 10 minutes at room temperature to allow for colour change, after which the absorbance of the blank, standard and samples were read at 500 nm using a colorimeter. This was repeated for all the plasma samples. Concentration of cholesterol in sample was then calculated and expressed in mg/dl.

**Triglycerides**
Total triglyceride in plasma was determined by the enzymatic method, using wet reagent diagnostic kits, a modification of the method of Jacobs and Vandemark, 1960. The reagent (1000 μl) was pipetted into 3 different test tubes A, B and C Distilled water, a standard solution (standard) and plasma (sample) of 10 μl each were pipette into same test tubes A, B and C respectively. After mixing, the test tubes were left to stand for 10 minutes at room temperature to allow for colour change. The absorbance of the blank, standard and sample were read 500 nm using a colorimeter. This was repeated for all the plasma samples. Concentration of the triglyceride in sample was then calculated and expressed in mg/dl.

**High density lipoprotein**
Accurate measurement was done by the enzymatic method, using wet reagent diagnostic kits. A precipitating reagent was used. This was pre-diluted in the ratio 4 to 1 with distilled water. This reagent composition is phosphotungstic acid: (0.55mmol/l) and magnesium chloride: (25 mmol/l) (Frieldwald et al., 1972). The reagent (500 μl) was pipetted into 2 different test tubes A and B. A standard solution (standard) and plasma (sample) of 200 μl each were pipetted into same test tubes A and B respectively.
After mixing, the test tubes were left to stand for 10 min at room temperature to allow for colour change, after which the absorbance of the standard and sample were read at 500 nm using a colorimeter. This was repeated for all the plasma samples. Concentration of HDL in sample was then calculated and expressed in mg/dl.

**Low density lipoprotein**

This was obtained from the values of total cholesterol, HDL and triglycerides for each of the sample using the formula below (Frieldwald et al., 1972) In mg/dl:

\[
LDL = \text{Total cholesterol} - \text{Triglycerides} - \frac{5}{\text{HDL}}
\]

**Acute toxicity study**

The acute toxicity of the plant extract was conducted using a modified method of Lorke, 1983. Mice were divided into 4 groups of 4 mice each. The four groups were each administered 1000, 2000, 4000 mg/kg and 2ml/kg of distilled water which served as control respectively. All administration was done via the oral route using an orogastric syringe. General symptoms of toxicity and mortality were observed for 24 h and thereafter for 14 days.

**Statistical analysis**

All data were expressed as mean ± SEM. Where applicable, the data were analyzed statistically by Student’s t-test using Graph pad instat version 2.05a. P values less than 0.05 were considered as significant.

**RESULTS**

**Phytochemical analysis**

The results of the phytochemical screening are as shown in Table 1. It revealed that the following secondary metabolites were present in the leaves of *Alstonia boonei*: simple sugars, reducing sugars, cardiac glycosides, flavonoids, alkaloids, anthraquinone and saponins.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple Sugars</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>+</td>
</tr>
<tr>
<td>Cyanogenetic glycoside</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>+</td>
</tr>
<tr>
<td>Saponin glycoside</td>
<td>+</td>
</tr>
</tbody>
</table>

**The effect of the ethanol leaf extract of *Alstonia boonei* on alloxan-induced diabetic rats.**

The results are presented in Table 2. In diabetic rats treated with 100, 200 and 400 mg/kg of ethanol leaf extract of *Alstonia boonei*, there was a significant reduction in the blood glucose concentration (p<0.05) compared to the untreated diabetic rats. This reduction increased with increasing dose and as time proceeded. This reduction was most significant at the 8th hour.

Also for the diabetic rats treated with glibenclamide, there was a significant reduction in the blood glucose level (p<0.05) compared to the untreated diabetic rats between the second and the eight hour.
The negative control groups treated with distilled water had normal blood glucose levels. The levels were significantly (p<0.05) lower than the untreated diabetic group. The essence of this negative control group was to serve as a basis for the establishment of diabetes in the other groups based on comparisons of the blood sugar level at the 0 time before extract administration. The effect of all the doses of the extract compares well with glibenclamide, the standard. The effect seems to be similar with that on blood glucose level of diabetic rats treated with glibenclamide. At the dose of 200 and 400 mg/kg of the extract, significant (p<0.05) reduction in the blood glucose level was observed at the second and fourth hour respectively.

Table 2: The blood glucose level of diabetic rats administered different doses of ethanol leaf extract of *Alstonia boonii* and glibenclamide

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>0hr</th>
<th>2hrs</th>
<th>4hrs</th>
<th>8hrs</th>
<th>24hrs</th>
<th>14th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>106±16.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.8±8.7</td>
<td>93±6.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.4±6.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>109.6±3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.2±9.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Untreated</td>
<td>390.8±59.6</td>
<td>417.7±46.5</td>
<td>373±66.6</td>
<td>323.7±49.2</td>
<td>381.0±87.0</td>
<td>181.3±37.2</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>294.6±78.6</td>
<td>263.8±91.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>207.2±62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180.6±51.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>302±89.3</td>
<td>176.8±63.4</td>
</tr>
<tr>
<td>200mg/kg</td>
<td>254.8±56.4</td>
<td>199.4±60.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>251.6±65.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>195.6±60.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>237.8±51.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124.8±56.8</td>
</tr>
<tr>
<td>400mg/kg</td>
<td>298.8±93.6</td>
<td>250.2±80.6</td>
<td>178.7±47.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>157.8±61.7</td>
<td>191.8±77.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>138.5±47.1</td>
</tr>
<tr>
<td>Gli (5mg/kg)</td>
<td>270.8±10.9</td>
<td>172.3±88.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>159.3±78.0</td>
<td>113.3±48.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>305.5±70.3</td>
<td>112±15.0</td>
</tr>
</tbody>
</table>

Values are mean blood glucose levels ± SEM (n = 5, per group). <sup>a</sup>p<0.05 significantly different from the untreated diabetic rats. The data were analyzed statistically by Student's t-test using Graph pad instat version 2.05a. Gli---Glibenclamide

Effect of the ethanol leaf extract of *Alstonia boonii* on the lipid profile of alloxan induced diabetic rats.

Table 3 shows the results of the lipid profile. The low density lipoprotein cholesterol was significantly reduced (p<0.05) when 200mg/kg dose of extract was administered to the diabetic rats.

There was no significant difference in the triglyceride level of the diabetic rats treated with the different doses of *Alstonia boonii* when compared with that of the untreated rats but there was a significant (p<0.05) increase in the high density lipoprotein of the diabetic rats treated with 100mg/kg of the extract when compared with all other doses of the extract and untreated diabetic rats (p<0.05).

ORAL ACUTE TOXICITY STUDY

Twenty four hours following extract administration, signs of restlessness, decreased feed intake, urination, defecation, slowed movement were observed with the lower dose and death occurred in the group administered 4000mg/kg of the plant extract (table 4).
Table 3: The effects of *Alstonia boonei* and Glibenclamide on the lipid profile of alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TC</th>
<th>HDL</th>
<th>TG</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.4±4.4</td>
<td>77.8±17.2</td>
<td>40.0±8.1</td>
<td>4.8±4.1</td>
</tr>
<tr>
<td>Untreated</td>
<td>51.3±6.5</td>
<td>40.8±7.4</td>
<td>30.1±9.6</td>
<td>13.0±4.5</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>54.4±5.6</td>
<td>68.6±3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.1±5.9</td>
<td>8.7±3.4</td>
</tr>
<tr>
<td>200mg/kg</td>
<td>62.6±15.1</td>
<td>49.2±8.2</td>
<td>29.7±5.4</td>
<td>3.36±10.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>400mg/kg</td>
<td>61.2±7.3</td>
<td>42.4±11</td>
<td>42.7±12.5</td>
<td>19.6±5.6</td>
</tr>
<tr>
<td>Gli (5mg/kg)</td>
<td>68.8±3.7</td>
<td>47.1±2.8</td>
<td>15.3±4.7</td>
<td>11.0±5.7</td>
</tr>
</tbody>
</table>

Values are mean blood glucose levels ± SEM (n = 5, per group). <sup>a</sup>p<0.05 significantly different from the untreated diabetic rats. The data were analyzed statistically by Student’s t-test using Graph pad instat version 2.05a. Gli----- Glibenclamide

Table 4: Oral acute toxicity study of the ethanol Extract of *Alstonia boonei*

<table>
<thead>
<tr>
<th>Treatment (g/kg)</th>
<th>Log-dose</th>
<th>Percentage mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AB (1)</td>
<td>3.000</td>
<td>0</td>
</tr>
<tr>
<td>AB(2)</td>
<td>3.301</td>
<td>0</td>
</tr>
<tr>
<td>AB(4)</td>
<td>3.602</td>
<td>20</td>
</tr>
</tbody>
</table>

Control animals received distilled water (2ml/kg).

LD<sub>50</sub> was indeterminable, as the highest dose produced 20 % death.

AB is the ethanol extract of *Alstonia boonei*

**DISCUSSION**

The ethanol extract of *Alstonia boonei* lowered the blood glucose level effectively as glibenclamide. The extract may be acting via several mechanisms commensurate with the different chemical groups in the extract. *Alstonia boonei* may have produced hypoglycemic action by being insulinomimetic. This is the mode of action of some anti-hyperglycemic agents such as metformin(a biguanide),which produce a reduction of blood glucose by decreasing hepatic gluconeogenesis, increasing skeletal muscle glucose uptake, and reducing plasma triacylglycerol (Scott, 2004).

The extract may also have lowered the blood glucose levels in rats by stimulating
glucose catabolizing enzymes and inhibiting gluconeogenic enzymes as was seen by Singh et al., 2001, who observed the same mode of action by *C. roseus* in streptozotocin-induced diabetic rats. Alloxan and the product of its reaction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration thereby causing a massive destruction of the beta cells resulting in reduced synthesis and release of insulin (Colcha, 1983). The untreated diabetic rats had a significantly higher (p< 0.05) fasting blood glucose level than normal rats. This confirms induction of diabetes by alloxan. Sulphonylureas reduce blood glucose by increasing insulin secretion from pancreatic beta cells in patients with residual beta cell function (Scott, 2004). This means that they are active in mild alloxan-induced diabetes and are inactive in intense alloxan-induced diabetes.

In this study, some alloxan-induced diabetic rats that received the extract showed rapid normalization of blood glucose levels compared to untreated. This could be due to the possibility that some beta cells were still intact and were therefore stimulated to synthesize and release insulin by the extract. In addition, the possibility that the extract showed hypoglycemic action by enhancing tissue glucose utilization cannot be ruled out in this study.

Furthermore, this extract may have achieved hypoglycemic activity by decreased rate of carbohydrate absorption into the portal hepatic circulation, increased glucose transport and uptake, increased glycogen storage, and modulation of insulin secretion. Therefore from the results obtained, the ethanol leaf extract of *Alstonia boonei* reduced the blood glucose level of diabetic rats and has a similar antidiabetic effect as glibenclamide. Lipids are group of naturally occurring molecules of fats, waxes, sterols, fat soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids and others. They play many important roles in the body but can also lead to cardiovascular disease when their concentration is abnormal in an organism (Ugwu et al., 2013) Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose levels and increased cholesterol concentration (Murugan et al., 2009). Hyperlipidemia is another recognized complication of diabetes mellitus characterized by elevated levels of cholesterol, triacylglycerol and changes in lipoprotein composition. In severe diabetic conditions, the kidneys lose their ability to remove wastes products, such as creatinine and urea, from the blood.

Results of the lipid profile show that the extract had neither significant hypotriglyceridemia nor hypocholesteric activity on the normal and diabetic rats. The HDL of the diabetic rats were increased at all doses but was only significant at the 100mg/kg dose, while the LDL of same rats were significantly decreased at the 200 mg/kg dose of *Alstonia boonei*. This is an added advantage for the diabetic patient (Owolabi and Omogbai, 2013). Previous studies carried out by Ginsberg, 1991 have shown that patients with type 2 diabetes have less HDL cholesterol than non-diabetics. In this research, the HDL-cholesterol concentration in the diabetic rats treated with all doses of the extract was increased compared to the untreated
diabetic rats; although significance was only observed at 100 mg/kg dose. According to American Heart Association in 2012, total cholesterol is made up of HDL-cholesterol, LDL-cholesterol and triglyceride. Elevated levels of LDL-cholesterol circulate in the blood, and can slowly build up in the inner walls of the arteries that feed the heart and brain. Together with other substances, this can form plaque that narrows the arteries and makes them less flexible, a condition known as arteriosclerosis, hence the need for a reduction in the concentration of triglyceride, LDL-cholesterol and total cholesterol in the blood. Hence a decrease in the LDL-cholesterol is an advantage. In addition HDL-cholesterol helps to scavenge for LDL-cholesterol and transports it to the liver, where it can be processed, hence the need for an increase in the concentration of HDL-cholesterol (Owolabi and Omogbai, 2013).

From the study carried out, the extract caused a decrease in the concentration of LDL-cholesterol at the 200mg/kg dose.

On the phytochemical screening, the hypoglycemic action of the extract tested in this study could be attributed to the presence of flavonoids, cardiac glycosides and bound anthraquinones, which have been shown to be hypoglycemic. A flavonoid rich fraction isolated from guava (Psidium guajava) leaves given at a daily dose of 7.2-14.4g lowered blood glucose in humans (Kimura et al., 1985). The presence of saponins in this extract could also be responsible for the hypoglycemic activity. For instance, ginseng and its saponins have been shown to lower blood glucose in alloxan- treated, genetically diabetic, and normal mice (Kimura et al., 1985). In elderly patients with hyperglycemia, saponins also reduced serum glucose (Chen et al., 1987). Anthraquinones in root bark extracts of Salvia miltiorrhiza have been shown to prolong the hypoglycemic effect in diabetic mice (Dong et al., 1990). In addition, the presence of alkaloids in the ethanol leaf extract of Alstonia boonei could also be responsible for the hypoglycemic activity. From the result of the toxicity study, the plant can be said to be safe as death was seen only at the highest dose, while lower doses produced neither signs of toxicity nor death. The LD50 was indeterminable, as the highest dose produced 20% death.

CONCLUSION

In conclusion, Alstonia boonei used in herbal medicine practice showed significant antidiabetic activity. The plant extract lowered the blood sugar levels in the alloxan-induced diabetic rats at the different dose levels used. The plant extract was as effective as glibenclamide. The extract also reduced low density cholesterol and increased high density lipoprotein. Also, it is rich in phytochemicals and this could be the reason why Alstonia boonei leaves have been used in numerous ethno-medicinal treatments.

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COMPETING OF INTEREST

The authors declare no conflict of interest.

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immunoglobulin A by lung cells of mice that were immunized intragastrically with inactivated influenza virus vaccine. Journal of Virology 61:2150-2154.


