ABSTRACT

The aqueous extract of *Alstonia boonei* (*A. boonei*) has been shown to have antidiabetic activity using chemical drug. However there is no report on its effect on sucrose-induced insulin resistance and glucose tolerance in long term consumption. The current study investigates the effects of *A. boonei* aqueous extract on sucrose-induced glucose intolerance and insulin resistance in rats. Animals were fed with 20% sucrose solution and 5% sucrose in drinking water during ten weeks. Those with glucose tolerance abnormality were treated with the extract (100 or 200 mg/kg) for two more weeks after stopping or without stopping the sucrose. Animals were subjected to insulin sensitivity and glucose tolerance tests. Sera and homogenates of organs were used for biochemical analysis. Sucrose administration caused hyperglycemia (54.85%), glucose intolerance but failed to induce insulin resistance. Rats receiving sucrose for two more weeks showed hyperglycemia (71.39%), glucose intolerance and insulin resistance. When stopping sucrose, the extract significantly reduced blood glucose and improved sucrose tolerance. The extract significantly decreased blood glucose, prevented insulin tolerance and insulin resistance. Sucrose caused a significant increase in cholesterol, triglycerides, LDL-cholesterol, atherogenic index, ALT, AST, creatinine, MDA levels and a decrease of GSH concentration and SOD activity. The extract significantly improved the concentrations of all these parameters. The aqueous extract of *A. boonei* improves glucose tolerance and insulin sensitivity due to its hypoglycemic, hypolipidemic and antioxidant...
activities. It can be used to manage glucose intolerance and insulin resistance in diabetic patients.

**KEYWORDS:** Sucrose, glucose tolerance, insulin resistance, *Alstonia boonei*.

**INTRODUCTION**
Sucrose is a disaccharide made up of glucose and fructose. It is well known that, fructose consumption induced dislipidemia with an increase in visceral adiposity and a decrease in insulin sensitivity.\(^1\)\(^2\) Human studies demonstrate that fructose infusions can induce hepatic insulin resistance.\(^3\) In addition, in animal models, high consumption of fructose have been shown to induced, hyperglycaemia, hyperinsulinemia, hypertension, and insulin resistance.\(^1\) Hyperinsulinemia and Insulin resistance are common metabolic disorder that plays a critical role in the pathophysiology of diabetes. In 2013, the International Diabetes Federation (IDF) estimated that the prevalence of diabetes in the world population is 7.9% including glucose intolerant patients. The aim of diabetes management is to reduce hyperglycaemia by activating the endogenous insulin secretion (sulfonylureas and glitinides) or reinforcing its effects (biguanides and thiazolidinediones). Many people not adhere to conventional pharmacological treatments because of the high cost of treatment and a lack of health facilities.\(^4\) In addition, synthetic drugs (insulin, oral antidiabetic drugs) used to manage diabetes present several side effects including hypoglycaemia, Drug-resistance, weight gain.\(^5\) In recent decades attention has targeted the use of medicinal plants in the management of diabetes because of their fewer side effects. Thus there is an increase interest to search and demonstrate the effect of natural antidiabetic agents. *Alstonia boonei* is a plant belonging to the family Apocynaceae, and is commonly known as the “Ekuk” or “Emiens” (central Cameroon-region). This plant is used in traditional medicine for the treatment of several diseases including diabetes, malaria, jaundice and typhoid fever.\(^6\) Toxicological studies of the aqueous extract of *A. Boonei* barks revealed a toxicity at high doses.\(^7\) In addition the antihyperglycaemic effect of this plant extract using dexamethasone; a chemical drug induced-hyperglycaemic have been shown.\(^8\) However, chemical drug induced toxicity in some organs and variability of results on development of hyperglycaemia.\(^9\) To better understand the etiology of diabetes, several animal models have been developed, including the sucrose model.\(^10\) Hence, this study was carried out to evaluate the effect of the barks of *Alstonia boonei* on sucrose-induced hyperglycaemia and intolerance.
MATERIALS AND METHODS

Plant material and extraction
The fresh barks of *Alstonia boonei* (*A. boonei*) were harvested at Obeck (Centre Region of Cameroon) in November 2012 and authentication was done by comparing the sample No 1943 of the National Herbarium of Cameroon. The barks of *A. boonei* were dried in the shade at room temperature and were crushed into fine powder. The powder (1 kg) was soaked in 4 L of tap water for 24 hours according to the protocol of traditional healer. After filtration of the mixture, the filtrate collected was evaporated in an oven at 40°C. The yield of the extract was 4% (W/W).

Experimental animals
The experiments were performed on albinos male Wistar rats aged approximately two months and weighting between 130 to 140g. The animals were raised in the animal house of the Faculty of Science, University of Yaounde I. They were housed together (5 rats per cage), maintained at room temperature (22±2°C) with adequate ventilation and free access to tap water and food. These studies were conducted with the approval of the Cameroon National Ethical Committee (Ref n°.FW-IRB00001954).

Procedure
Impaired glucose tolerance was induced in normal rats by ingestion of sucrose (20% by gastric intubation and 5 % in drinking water) for 12 weeks. The baseline blood glucose of the fasting (12 hours) rats was determined and the rats were then divided into a normal control and a test control. The rats of the test group (40 rats) were given by gastric intubation a 20% sucrose solution (10mL/kg) and had free access to drinking water consists of a 5% sucrose solution. Normal control rats (5 rats) were given by gastric intubation distilled water and had for drinking, tap water without sucrose. At the end of this period, blood glucose levels were evaluated in different groups using a glucometer (Accuchek glucometer, Boehringer Mannheim, Germany). Animals with a fasting glucose between 110 and 126 mg/dL were submitted to glucose tolerance test. Rats presenting blood sugar between 140 and 200 mg/dL two hours after glucose ingestion were considered intolerant to glucose.

Animals with impaired oral glucose tolerance test were divided into 2 main groups each comprising 4 lots (5rats/lot) and treated as follow. The first group received different treatments after stopping sucrose intake thus, allowing to notice if the metabolic disorders persist once stopping sucrose administration. In the second group, animals received
simultaneously sucrose and different treatments to evaluate preventive effect of sucrose-induced metabolic disorders. In each main group, one lot received distilled water (10mL/kg) as negative control, another received metformin (200mg/kg) as positive control. Two lots received the extract at the doses of 100 and 200 mg/kg. One group beside these lots was a normal control group made up of five rats receiving distilled water (10mL/kg). Different substances were administered in a single daily dose for 14 days. All substances were dissolved in distilled water and administered by gastric intubation. After these 2 weeks of treatment, the blood glucose of animals of different groups was determined and the animals were subjected to glucose tolerance and insulin sensitivity tests.

**Insulin sensitivity test**
To evaluate insulin resistance in all animals, insulin tolerance test was performed.\(^{[13,14]}\) Briefly, after 12 hours of fasting, the glycaemia was evaluated (0h) and animals received 2 UI/kg of insulin, the blood was obtained from tail at 10, 20, 30 and 60 min after insulin injection, then, serum glucose was measured.

**Oral Glucose Tolerant Test**
Oral Glucose Test Tolerance was performed after 12 hours of fasting. The glycaemia was evaluated (0h) and animals received by oral route 5g/kg of glucose solution. The blood samples were collected from the tail vein at 30, 60, 120 and 180 min post-administration. Their glucose content were assessed using the glucose oxidase method (Accuchek glucometer, Boehringer Mannheim, Germany).\(^{[12,14]}\)

**Biochemical analysis**
At the end of experimental period, the animals were sacrificed by decapitation under ether anesthesia. The arterio-venous blood was collected into test tubes, allowed to stand for 30 minutes, and then centrifuged at 3000 rpm for 10 minutes. The collected serum was stored frozen at -20°C for subsequent assay of lipid parameters as total cholesterol, high density lipoprotein (HDL-cholesterol), low density lipoprotein (LDL-cholesterol) triglycerides, glucose, transaminases (ASAT and ALAT) and creatinin using commercial kits (Fortress Diagnostics).

The organs (heart, liver and kidney) were collected and weighed. Homogenate (20 %) of each organ was prepared using Tris-HCl buffer 50mM (pH 7.4). Mc Even solution was used for the heart homogenate. The mixture obtained was centrifuged at 3000 rpm for 20 min at 4°C.
The supernatant was collected and stored frozen at -20°C for determination of tissue markers of oxidative stress.

**Statistical analysis**

Results were expressed as mean ± standard error mean (SEM). Means were compared by the analysis of variance (ANOVA) followed by the Tukey's post test using Graph Pad Prism software version 5.03. p values < 0.05 were considered significant.

**RESULTS**

**Effects of aqueous extract of *Alstonia boonei* on blood glucose levels**

The administration of sucrose diet for 10 to 12 weeks induced a significant increase (p<0.001) in blood glucose levels compared to normal control rats (Figure 1). Figure 1A shows that blood sugar remained significantly high by 54.85 % (p<0.001) in rats after stopping sucrose diet compared to normal control. In rats treated with the extract, after stopping the sucrose diet, it was observed a significant decrease in blood glucose by 24.76 % and 14.08 % respectively at doses of 100 and 200 mg/kg compared to rats treated with distilled water. When sucrose ingestion is prolonged during two more weeks, blood glucose levels significantly increase by 71.39 % (Figure 1B). Concomitant administration of the plant extract with sucrose significantly prevented (p<0.01) the increase in blood glucose levels compared to negative control. The decrease in glucose was 22.88 % and 22.97 %, respectively at doses of 100 and 200 mg/kg. Metformin induced a significant decrease in blood glucose by 26.45% and 23.60% respectively without and with sucrose administration.

![Figure 1](image-url)

**Figure 1:** Effects of the aqueous extract of *Alstonia boonei* on sucrose-induced glycaemia.

Each bar represents the mean ± SEM, n = 5, **p<0.01: significant difference compared to group SU+DW, +++p <0.001: significant difference compared to group DW, #p <0.05, ###p
<0.001: as compared to the normal control. A: animals received sucrose during 10 weeks then plant extract for 2 weeks without sucrose. B: animals received sucrose during 10 weeks with concomitant administration of sucrose and plant extract for 2 more weeks. NC=normal control; SU=Sucrose; groups SU+DW, SU+MET 200, SU+E100, SU+E200= Sucrose for 12 weeks with concomitant treatments during the last 2 weeks.

Effects of aqueous extract of Alstonia boonei on glucose tolerance and insulin resistance

Figure 2 shows the change in blood glucose after an oral glucose overload (5g/kg). The administration of sucrose during 10 and 12 weeks induced glucose intolerance. In rats that received sucrose for 10 weeks and distilled water for 2 more weeks (Fig. 2A), serum glucose levels was significantly higher than normal control by 28.23 % and 22.75 % respectively after 120 and 180 minutes. In rats treated with the plant extract for 2 weeks after stopping the sucrose diet, it was observed compared to the negative control, a significant anti-hyperglycaemic activity of the plant extract at 120 and 180 minutes respectively at the doses of 100 mg/kg and 200 mg/kg. Twelve weeks after sucrose ingestion, there was a significant increase (P<0.001) in blood glucose by 23.21 % after the 60th minute (Fig. 2B). This increase in blood glucose levels remained significantly higher (P<0.001) throughout the experimental period compared to normal control. Simultaneous administration of Alstonia boonei plant extract and sucrose during 2 weeks at the doses of 100 mg/kg and 200 mg/kg produced at 120 min a significant inhibition of hyperglycaemia respectively by 26.05 % and 38.39%. At 180 min, the reduction of blood glucose was 21.22 % and 29.04 % at the respective doses of 100 and 200 mg/kg compared to control rats. Administration of insulin to animals which received sucrose during ten weeks induced a progressive but not a significant decrease in blood glucose compared to normal control (Fig. 2C). In rats treated with the plant extract at the doses of 100 and 200 mg/kg, insulin did not induce a significant change in the blood glucose levels. In animals treated with sucrose for 12 weeks and submitted to insulin sensitivity test, it was observed a progressive decrease in blood sugar that nevertheless remained significantly (P<0.001) higher after 20 minutes (80.00 %), 30 minutes (141.54 %) and at 60 minutes (104.08 %) as shown in Figure 2D. In comparison with negative control (SU+DW), animals which concomitantly received the extract with sucrose for 2 weeks, presented a significant decrease in blood glucose. This reduction was 33.99 % and 33.79 % at 20 minutes, 46.4% and 47.4% at 30 minutes, and 38.00% and 32.75% at 60 minutes for the respective doses of 100 and 200 mg/kg. Metformin (200 mg/kg) also induced in rats treated for 12 weeks with sucrose a significant decrease in blood glucose levels compared to the negative control.
Figure 2: Effects of the aqueous extract of *Alstonia boonei* on sucrose-induced glucose intolerance and insulin insensibility.

Each point represents the mean ± SEM, n = 5, +++p <0.001: significant difference compared to group DW, ###p <0.01: as compared to the normal control. ***p<0.001: significant difference compared to group SU+DW, A: animals received sucrose during 10 weeks then plant extract for 2 weeks without sucrose. B: animals received sucrose during 10 weeks with concomitant administration of sucrose and plant extract for 2 more weeks. SU = Sucrose; NC=normal control; groups DW, MET, E100, E200= Sucrose for 10 weeks and treatments during the last 2 weeks. SU+DW, SU+MET 200, SU+E100, SU+E200= Sucrose for 12 weeks and concomitant treatments with plant extract or metformin during the last 2 weeks.

**Effects of aqueous extract of *Alstonia boonei* on lipid profile**

Total cholesterol, triglycerides, LDL-cholesterol and atherogenic index remained significantly higher (p<0.001) respectively by 51.55 %, 45.11 %, 86.79 % and 58.18 % in rats treated for 10 weeks with sucrose compared with normal control rats (Table 1). However, the administration of sucrose did not significantly affect HDL-cholesterol compared to normal control rats. When administered the plant extract during the last 2 weeks, it was observed at the dose of 100 mg/kg a significant decrease of total cholesterol (23.41 %), triglycerides (57.10 %), LDL-cholesterol (27.90 %) and atherogenic index (55.60 %). Where as a significant increase in HDL-cholesterol by 78.33% was observed as compared to the negative control. The plant extract at the dose of 200 mg/kg also induced a significant
reduction of triglycerides, LDL-cholesterol and the atherogenic index respectively by 41.44 %, 34.25 % and 63.08 %, and significantly increase (p<0.001) in HDL-cholesterol by 136.16 %. Metformin administered in the same conditions as the extract improved these different parameters except HDL-cholesterol levels which did not vary significantly.

On other hand, when rats received sucrose during twelves weeks, the levels of total cholesterol, triglycerides, LDL-cholesterol and atherogenic index significantly rises respectively by 56.73%, 47.55%, 100.85%, 75.65% as compared to normal control. Simultaneous administration of sucrose and plant extract after 2 weeks provoked a significant decrease of these parameters at all doses. The plant extract at the dose of 100 mg/kg induced a decrease of 15.05% in total cholesterol, 32.00 % in triglycerides levels, 30.88 % of LDL-cholesterol, 57.04 % of atherogenic index. At the dose of 200 mg/kg the decrease of total cholesterol, triglyceride, LDL-cholesterol and the atherogenic index were 21.81 %, 42.41 %, 32.91 % and 55.57 % respectively as compared with the control. The concentration of HDL cholesterol did not vary significantly in rats treated with sucrose during twelve weeks. However, rats treated simultaneous with sucrose and extract showed a significant increase (p<0.01) of HDL-cholesterol 98.55% and by 81.34% at the respective doses of 100 and 200 mg/kg compared to the negative control. Metformin administered under the same conditions as the extract induced a significant increase (p<0.05) of HDL-cholesterol by 73.55 %.

Table 1: Effects of the aqueous extract of *Alstonia boonei* on lipid profile in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>LDL-Cholesterol (mg/dL)</th>
<th>HDL-Cholesterol (mg/dL)</th>
<th>Atherogenic Index</th>
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<tbody>
<tr>
<td>NC</td>
<td>23.82 ± 1.66</td>
<td>42.52±1.82</td>
<td>10.09±0.35</td>
<td>5.22±0.35</td>
<td>4.71±0.59</td>
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<tr>
<td>DW</td>
<td>36.11±0.41</td>
<td>61.71±3.21</td>
<td>18.85±0.30</td>
<td>4.91±0.30</td>
<td>7.45±0.63</td>
</tr>
<tr>
<td>MET 200</td>
<td>27.46±0.54</td>
<td>41.63±4.02</td>
<td>13.01±0.73</td>
<td>6.13±0.25</td>
<td>4.51±0.51</td>
</tr>
<tr>
<td>E100</td>
<td>27.65±0.12</td>
<td>26.46±1.16</td>
<td>13.59±0.80</td>
<td>8.77±0.94</td>
<td>3.30±0.44</td>
</tr>
<tr>
<td>E200</td>
<td>31.23±0.12</td>
<td>36.13±1.04</td>
<td>12.39±0.75</td>
<td>11.61±0.85</td>
<td>2.75±0.27</td>
</tr>
<tr>
<td>SU+ED</td>
<td>37.34±1.17</td>
<td>62.75±3.49</td>
<td>20.27±0.11</td>
<td>4.52±0.15</td>
<td>8.27±0.30</td>
</tr>
<tr>
<td>SU+MET</td>
<td>29.01±1.04</td>
<td>36.72±1.65</td>
<td>13.81±0.79</td>
<td>7.85±0.75</td>
<td>3.80±0.31</td>
</tr>
<tr>
<td>SU+E100</td>
<td>31.66±2.63</td>
<td>42.67±2.68</td>
<td>14.14±0.35</td>
<td>8.98±0.90</td>
<td>3.55±0.12</td>
</tr>
<tr>
<td>SU+E200</td>
<td>29.50±0.79</td>
<td>36.13±3.14</td>
<td>13.76±1.26</td>
<td>8.20±0.74</td>
<td>3.67±0.35</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n = 5, *p <0.05, **p<0.01, ***p <0.001: significant difference compared to group SU+DW, +p <0.05, ++p<0.01, +++p <0.001: significant difference compared to group DW, #p <0.05, ##p <0.01, ###p <0.001: as compared to the normal control. NC=normal control; SU=Sucrose; groups SU+DW, SU+MET, SU+E100, SU+E200= Sucrose for 12 weeks with concomitant treatments during the last 2 weeks.
Effects of aqueous extract of *Alstonia boonei* on liver and kidney functions

Table 2 shows the effects of aqueous extract of *A. boonei* on some hepatic and renal parameters in rats fed with a supplement in sucrose. Ingestion of sucrose during 10 weeks provoked a significant increase in creatinine levels and ALAT activity respectively by 42.26 % and 26.79 % compared to the normal control. AST activity did not vary in these animals. The extract administered at the dose of 100 mg/kg after stopping sucrose diet, caused a significant decrease (p<0.01) of creatinine (34.40 %), and the activity of ASAT (45.83 %). At the dose of 200 mg/kg, there was a significant decrease of creatinine levels, activity of ASAT and ALAT respectively by 30.73 %, 37.50 % and 57.69% as compared to the control. Metformin administered in the same conditions as the extract induced a significant reduction in ALAT activity of 46.15% compared to the negative control. When sucrose administration is prolonged for 12 weeks, a significant increase was observed in serum creatinine levels and ALAT activity by 112.41% and 78.57% respectively in comparison with normal control. However, there was no significant modification in ASAT activity in these animals. Concomitant administration of the plant extract at the doses of 100 and 200 mg/kg caused a significant inhibition in the increase of serum creatinine by 42.46 % and 46 %, while the ALAT activity was inhibited by 28.00 % and 44.00 % and ASAT activity by 53.57 % and 53.00 % respectively when compared with SU+ED group.

Table 2: Effects of aqueous extract of *A. boonei* on liver and kidney functions

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Creatinin (mg/mL)</th>
<th>ASAT (U/L)</th>
<th>ALAT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td></td>
<td>0.97 ± 0.05</td>
<td>17.76 ± 0.60</td>
<td>14.52 ± 0.13</td>
</tr>
<tr>
<td>DW</td>
<td></td>
<td>1.38 ± 0.13</td>
<td>17.60 ± 0.60</td>
<td>18.41 ± 0.79</td>
</tr>
<tr>
<td>MET 200</td>
<td></td>
<td>1.12 ± 0.03</td>
<td>15.49 ± 0.39</td>
<td>14.52 ± 0.27</td>
</tr>
<tr>
<td>E100</td>
<td></td>
<td>0.90 ± 0.08</td>
<td>14.20 ± 0.64</td>
<td>16.47 ± 0.51</td>
</tr>
<tr>
<td>E200</td>
<td></td>
<td>0.95 ± 0.06</td>
<td>14.80 ± 0.51</td>
<td>13.55 ± 0.32</td>
</tr>
<tr>
<td>SU+DW</td>
<td></td>
<td>2.06 ± 0.20</td>
<td>19.05 ± 0.39</td>
<td>18.08 ± 0.27</td>
</tr>
<tr>
<td>SU+MET 200</td>
<td></td>
<td>0.93 ± 0.06</td>
<td>14.52 ± 0.79</td>
<td>17.11 ± 0.09</td>
</tr>
<tr>
<td>SU+E100</td>
<td></td>
<td>1.18 ± 0.07</td>
<td>14.20 ± 0.64</td>
<td>15.82 ± 0.21</td>
</tr>
<tr>
<td>SU+E200</td>
<td></td>
<td>1.10 ± 0.24</td>
<td>14.21 ± 0.39</td>
<td>14.52 ± 0.32</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n = 5, *p <0.05, **p<0.01, ***p <0.001: significant difference compared to group DW, *p <0.05, **p <0.01: as compared to the normal control. *p<0.05, **p<0.01, ***p<0.001: significant difference compared to group SU+DW, *p<0.05, **SU = Sucrose; NC=normal control; groups DW, MET, E100, E200= Sucrose for 10 weeks and treatments during the last 2 weeks. SU+DW, SU+MET 200, SU+E100, SU+E200=
Sucrose for 12 weeks and concomitant treatments with plant extract or metformin during the last 2 weeks.

**Effects of aqueous extract of A. boonei on some tissue parameters of oxidative stress**

**Effects on the concentration of reduced glutathione**

Sucrose administration in rats during ten weeks induced a significant decrease in reduced glutathione (GSH) of the liver by 24.90 % (p<0.01), but in the kidney and the heart the concentration in reduced glutathione did not change significantly (Fig. 3A). Daily administration of the plant extract for 14 days induced a significant increase in reduced glutathione concentration of 127.86 % and 107.42 % in the liver, and 44.52 % (p <0.001) and 50.31 % (p<0.01) in the heart, respectively, at doses of 100 and 200 mg/kg. Metformin induced a significant increase (p<0.001) in the concentration of reduced glutathione in the liver of 30.85 % and in the heart of 29.85 % compared to rats receiving distilled water after stopping sucrose diet. GSH concentration did not change significantly in the organs of rats treated with sucrose for 12 weeks as compared to normal control (Fig. 3B). In comparison with control group, concomitant administration of the extract with sucrose during the last two weeks produced, a significant increase in glutathione concentration by 50.10 % and 43.30 % in the liver, by 20.57 % and 17.24% in the kidney, and by 34.80% and 26.91 % in the heart at the respective doses of 100 and 200 mg/kg. Metformin administered in the same conditions caused a significant increase (p <0.05) in the concentration of glutathione in the heart by 72.31 % compared to animal control.

![Figure 3: Effects of the aqueous extract of Alstonia boonei on reduced glutathione concentration.](image)

A: animals received sucrose during 10 weeks then plant extract for 2 weeks without sucrose.  
B: animals received sucrose during 10 weeks with concomitant administration of sucrose and
plant extract for 2 more weeks. Each bar represents the mean ± SEM, n = 5, +p <0.05, **p<0.01, +++p <0.001: significant difference compared to group DW, #p <0.05, ###p <0.01: as compared to the normal control. *p<0.05, ***p<0.001: significant difference compared to group SU+DW, SU= Sucrose; NC=normal control; groups DW, MET, E100, E200= Sucrose for 10 weeks and treatments during the last 2 weeks. SU+DW, SU+MET 200, SU+E100, SU+E200= Sucrose for 12 weeks and concomitant treatments with plant extract or metformin during the last 2 weeks.

**Effects on the activity of superoxide dismutase**

In rats treated with distilled water after stopping sucrose diet, it was observed a significant decrease in superoxide dismutase (SOD) activity in the kidney (11.31%, p<0.05), the heart (84.86%, p<0.001) and a non significant decrease in the liver compared to the normal control group (Fig. 4A). The extract at the dose of 100 mg/kg, significantly increased the activity of SOD in the liver (44.53%, p<0.001) and in the heart (280.28 % p<0.01) compared to the negative control. At the dose of 200 mg/kg, SOD activity was significantly increased in the kidney (34.88%, p<0.001) and in the heart (236.18 %, p<0.05). Metformin at the dose of 200 mg/kg used in the same conditions as the extract induced a significant decrease in SOD activity in the liver (22.66 %, p<0.05) and in the heart (308.75 %, p<0.01) compared to negative control rats. When sucrose was administered to normal rats for 12 weeks there was a significant decrease in SOD activity by 47.75 % in the liver, 53.16 % in the kidney and 72.81 % in the heart compared to the control normal (Fig. 4B). The extract administered at the dose of 100 mg/kg concurrently with sucrose during the last 2 weeks, led to a significant increase (p<0.01) of SOD activity in the liver (100.05 %), kidney (67.58 %) and in the heart (111.80 %) compared to rats receiving sucrose and distilled water. At the dose of 200 mg/kg, the extract induced a significant increase in the activity of SOD in the kidney (61.83 %, p<0.01) and in the heart (71.81 %, p<0.05). As compared to the control, metformin administered with sucrose during the last 2 weeks also caused a significant increase in SOD activity in the liver (136.66, p<0.01), the kidney (157.51, p<0.001) and in the heart (159.59 %, p<0.001).
Figure 4: Effects of the aqueous extract of Alstonia boonei on superoxide dismutase activity.

A: animals received sucrose during 10 weeks then plant extract for 2 weeks without sucrose.
B: animals received sucrose during 10 weeks with concomitant administration of sucrose and plant extract for 2 more weeks. Each bar represents the mean ± SEM, n = 5, +p <0.05, ++p<0.01, +++p <0.001: significant difference compared to group DW, # p <0.05, ## p <0.01: as compared to the normal control. *p<0.05, **p<0.01, ***p<0.001: significant difference compared to group SU+DW, #p<0.05, ## SU = Sucrose; NC=normal control; groups DW, MET, E100, E200= Sucrose for 10 weeks and treatments during the last 2 weeks. SU+DW, SU+MET 200, SU+E100, SU+E200= Sucrose for 12 weeks and concomitant treatments with plant extract or metformin during the last 2 weeks.

Effects on malondialdehyde

Figure 5 shows the effects of the extract of A. boonei on malondialdehyde (MDA) levels in rats receiving sucrose. MDA concentration were significantly higher by 21.80 % (p<0.001) in the liver of rats treated for 10 consecutive weeks with sucrose as compared to the normal control (Fig. 5A). The extract administered for 2 weeks after stopping the sucrose diet induced a significant decrease (p<0.001) in the concentration of MDA by 24.57 % and 24.21 % in the liver, 52.35 % and 57.88 % in the kidney at the respective doses of 100 and 200
mg/kg, compared to negative control rats. Metformin used in the same conditions as the extract induced a significant decrease (p<0.001) in the concentration of MDA in the liver of 51.45 % and in the kidney of 50.48 % compared to the group treated with sucrose for 10 weeks and with distilled water during the last 2 weeks. Chronic administration of sucrose during 12 weeks caused a significant increase in the concentration of MDA in the liver and kidney by 27.81 % and 24.61 % respectively compared to the normal control (Fig. 5B). In comparison with the control treated with sucrose for 12 weeks, concomitant administration of the extract with sucrose during the last 2 weeks induced, a significant decrease in the concentration of MDA in the liver by 75.95 % and in the kidney by 13.84 % at the dose of 100 mg/kg, by 59.30 % in the liver, and 13.72 % in the kidney at the dose of 200 mg/kg. Metformin caused a significant decrease in the MDA levels of the liver and kidney respectively by 37.05 % and by 34.03 % compared to control treated sucrose for 12 weeks.

Figure 5: Effects of the aqueous extract of *Alstonia boonei* on MDA levels.

A: animals received sucrose during 10 weeks then plant extract for 2 weeks without sucrose. B: animals received sucrose during 10 weeks with concomitant administration of sucrose and plant extract for 2 more weeks. Each bar represents the mean ± SEM, n = 5, +++p <0.001: significant difference compared to group DW, ###p <0.01, ###p <0.001: as compared to the normal control. **p<0.01, ***p<0.001: significant difference compared to group SU+DW.
SU = Sucrose; NC = normal control; groups DW, MET200, E100, E200 = Sucrose for 10 weeks and treatments during the last 2 weeks. SU+DW, SU+MET 200, SU+E100, SU+E200 = Sucrose for 12 weeks and concomitant treatments with plant extract or metformin during the last 2 weeks.

3. DISCUSSION
The effects of barks aqueous extract of *Alstonia boonei* on glucose intolerance and insulin resistance induced by sucrose in Wistar rats were evaluated in this study. Our results showed that the ingestion of sucrose during 12 weeks resulted in a significant increase in blood glucose levels compared to normal control. The elevation of this parameter in rats receiving sucrose is in accordance with the work of Wilson and Islam (2012).\(^{[15]}\) Sucrose-induced hyperglycemia could be explained by the fact that once in the gut, sucrose is degraded into fructose and glucose. These compounds when arrived into the bloodstream induced hyperglycaemia which is responsible for dyslipidemia and hyperinsulinemia resulting to insulin resistance and the depletion of insulin secretion which will contribute to the installation of glucose intolerance and can lead to type II diabetes in long term.\(^{[16,17]}\) In this study it was observed intolerance to glucose in both type of treatment however, it is important to mention that insulin resistance is absent when stopped sucrose administration. These results attest that glucose intolerance and insulin resistance cohabit when sucrose consumption is maintained. The administration of the aqueous extract of *Alstonia boonei* for 14 days resulted in a significant decrease in blood sugar and consequently glucose tolerance and the improvement of insulin sensitivity. These results could be explained by the presence in the extract of compounds possessing hypoglycemic properties such as tannins and flavonoids.\(^{[18]}\) It is known that these compounds act by reducing the activity of liver enzymes such as glucose -6-phosphatase and fructose -1,6- bisphosphatase, causes a reduction of the synthesis of glucose by the liver and therefore reduced the blood glucose concentration.\(^{[19]}\)

In this study sucrose induced a significant increase in some markers of cardiovascular complications as total cholesterol, triglycerides, LDL-cholesterol and atherogenic index. These results are similar to those obtained by Njamen et al.\(^{[10]}\), Wilson & Islam\(^{[15]}\), Kamgang et al.\(^{[17]}\) In chronic hyperglycaemia, insulin deficiency or insulin resistance are implicated in dyslipidemia\(^{[20]}\) by promoting lipolysis which causes an increase in total cholesterol, triglycerides and LDL-cholesterol.\(^{[21]}\) Sucrose ingested in this study consists mainly of glucose and fructose. The absorbed fructose will cause stimulation of hepatic lipogenesis,
resulting in dyslipidemia characterized by hypertriglyceridaemia.\textsuperscript{[10]} It is known that the fructose from the breakdown of sucrose is metabolized in the liver to glycerol-3-phosphate and acetyl-CoA. These two intermediate metabolites are used as substrates for the synthesis of triglycerides, and contribute to the production of VLDL in liver. Consumption of the extract for 2 weeks concurrently with sucrose resulted in a significant decrease in total cholesterol, triglycerides, LDL-cholesterol, atherogenic index and an elevation of HDL-cholesterol. These results, similar to those obtained by\textsuperscript{[22]} suggest that \textit{Alstonia boonei} have a curative effect against certain risk factors for cardiovascular disease resulting from excessive consumption of carbohydrates. In addition, the aqueous extract of \textit{A. boonei} contains substances that can reduce serum total cholesterol by stimulating hepatic catabolism of LDL-cholesterol. These results are consistent with the work of Baliga et al.\textsuperscript{[23]} who have shown that alkaloids found in the aqueous extract of \textit{Alstonia scholaris}, have the ability to reduce the concentration of serum cholesterol.

The effects of aqueous extract of \textit{A. boonei} were evaluated on the liver and kidney function. Our results showed that sucrose increased significantly the transaminases (ASAT and ALAT) levels. These results corroborate those of Wilson & Islam\textsuperscript{[15]}, which showed that 10 \% fructose, altered liver function. Transaminases are enzymes with significant metabolic activity in liver cells and they give information about membranes damage such as cytolysis and cellular necrosis\textsuperscript{[24]} Administration of fructose may cause oxidative stress inducing membrane lipid peroxidation and thus hepatocytes damage\textsuperscript{[25]} which thus reflect hepatic injury.\textsuperscript{[24]} \textit{Alstonia boonei} aqueous extract administered for 14 days in rats treated with sucrose resulted in a significant decrease in serum transaminase activities (ALAT and ASAT) due to the presence of compounds such as flavonoids in the extract \textsuperscript{[26]} with benefic properties on prevention of cell damage. Sucrose also induced a significant increase in creatinin levels compared to normal rats, indicating impaired renal function. Creatinin is produced from creatin, a molecule essential for energy production in muscles. Normally, creatin is transported by the blood and eliminated by the kidneys in the urine. It increase in the blood is associated with the reduction of renal function.\textsuperscript{[27]} The administration of the aqueous extract of \textit{Alstonia boonei} significantly decreased creatinin levels compared to control treated with sucrose. These results suggest that \textit{Alstonia boonei} may reduce renal failure in rats intolerant to glucose by improving glomerular filtration.
In this study, it was observed a decrease in SOD activity, an increase in the concentration of MDA in the investigated organs; and a decrease in the concentration of GSH in the liver of animals receiving distilled water after stopping the sucrose diet compared to normal control. These results are similar to those obtained by Ngo-lemba et al.\textsuperscript{[28]} who observed a decrease in the GSH concentration, the activity of SOD and catalase due to hyperglycaemia induced by glucose 10\%. SOD and catalase are important antioxidant enzymes SOD catalyzes the dismutation of the superoxide radical to form hydrogen peroxide, which will be converted to water by catalase; and GSH reduces the hydrogen peroxide and/or organic peroxides through the reaction catalyzed by glutathione peroxidase (GPx). It is recognized that high concentrations of glucose in extra and intracellular environments induce oxidative stress.\textsuperscript{[29]} In this study, sucrose induced oxidative stress involved a decrease in SOD activity and GSH levels, and an increase in MDA levels. It has been shown that in diabetes or even in glucose intolerance status, the beta cells are depleted in SOD, catalase, glutathione peroxidase and reduced glutathione.\textsuperscript{[30]} This depletion can be explained by their destruction thus decreased their activity in case of persistent oxidative stress.\textsuperscript{[28]} Furthermore, malondialdehyde is used as an index of lipid peroxidation resulting from the reaction of active oxygen species with the lipid membrane.\textsuperscript{[31]} Lipid peroxidation induced a change in the fluidity, and the permeability of excitable membranes.\textsuperscript{[32]} The increase in MDA levels is a sign of cell membrane damage. The aqueous extract of \textit{Alstonia boonei} significantly increased the concentration of GSH and SOD activity in the investigated organs (liver, kidney and heart) and significantly decreased tissue malondialdehyde. The improvement of these parameters could result to hypoglycaemic effect of the plant extract and to its ability to eliminate sucrose-induced reactive oxygen species.

CONCLUSION

At the end of this work, it appears that the administration of sucrose to normoglycemic rats induced glucose intolerance and insulin resistance which is associated to dyslipidemia, impaired hepatic and renal functions, and installation of oxidative stress. \textit{Alstonia boonei} barks aqueous extract administered to rats after induction of glucose intolerance caused a significant decrease in glucose levels, improved glucose tolerance, insulin sensitivity and the lipid profile. Our results suggest that this plant would possess hypoglycaemic properties and antioxidant activity that protect the liver, kidney and heart against deleterious effects caused by long period consumption of sucrose.
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