Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin–induced diabetic Wistar rats

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**Abstract**

**Objective:** To investigate the antidiabetic properties of aqueous extract of stem bark of *Afzelia africana* (*A. africana*) and its beneficial effect on haematological parameters in streptozotocin induced diabetic rats.

**Methods:** A total of 30 rats including 24 diabetic and 6 normal rats were used for this study. Diabetes was induced in male Wistar rats by intraperitoneal injection of streptozotocin. After being confirmed diabetic, animals were orally treated with distilled water or extracts at 100 or 200 mg/kg body weight daily for 10 days. The haematological parameters including red blood and white blood cells and their functional indices were evaluated in diabetic treated groups compared with the controls.

**Results:** The extract significantly reduced the blood glucose levels while the best result was obtained at 200 mg/kg body weight. The feed and water intake in diabetic rats were significantly reduced while weight loss was minimized at both dosages. Similarly, the levels of red blood, white blood cells and their functional indices were significantly improved after extract administration at both doses.

**Conclusions:** It can be concluded that the aqueous extract of bark of *A. africana* possesses antihyperglycemic properties. In addition, the extract can prevent various complications of diabetes and improve some haematological parameters. Further experimental investigation is needed to exploit its relevant therapeutic effect to substantiate its ethnomedicinal usage.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder of carbohydrates, proteins and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance. It is characterized with chronic high blood glucose that could lead to morbidity and mortality[1]. The number of people suffering from diabetes worldwide is increasing at an alarming rate. It is predicated that about 366 million people are likely to be diabetic by the year 2030[2]. This is because that none of the antidiabetic drugs could give a long term glycaemic control without causing any adverse side effects[3]. Meanwhile, medicinal plants that are effective in controlling plasma glucose level with minimal side effects are commonly used in under developed countries as alternative therapy. In Africa, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus. Unfortunately, only a few of such medicinal plants have been scientifically validated[4].

One of the plants commonly used in Africa traditional medicine for the management of diabetes mellitus is *Afzelia africana* (*A. africana*) Smith[5]. This plant is generally known as mahogany, widely distributed in Africa and Asia, where it is used as food as well as folklore remedies[6]. Previous studies have reported the anti-inflammatory and analgesic bioactivities of this plant[7]. Trypanocidal activities of leaves and stem bark extract of the plant has been reported against *Trypanosoma brucei*[8]. The mixture of the root powder with millet beer was used traditionally for the treatment of hernia among some tribes in Cote d’Ivoire[9]. Recently, phytochemical and antimicrobial properties of the crude extract of stem bark of *A. africana* were reported by Akinpelu *et al*[10].
However, there is no data on scientific literature to justify its folkloric usage. Therefore, this study is to investigate the antidiabetic and beneficial effect of aqueous extract of stem bark of *A. Africana* on haematological parameters in diabetic rats induced with streptozotocin.

### 2. Materials and methods

#### 2.1. Plant materials

Fresh stem bark of *A. africana* was collected in Abeokuta, Nigeria in April 2008. The plant was identified and authenticated by Dr. Illoh HC of the Department of Botany, Obafemi Awolowo University, Ile Ife, Nigeria. The voucher sample was prepared and deposited in the herbarium of the Botany Department.

#### 2.2. Preparation of the extract

The bark material of *A. africana* was air-dried to constant weight in the laboratory, powdered and stored in an air-tight container for further use. The dried material was then pulverized using an electric blender (Waring Products Division, Torrington, USA). About 40 g of the powdered plant material was extracted in 1 L of cold sterile distilled water maintained on a mechanical shaker (Stuart Scientific Orbital Shaker, UK) for 48 h. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was quickly frozen at -40 °C and dried for 48 h using a freeze dryer (Savant Refrigerated vapor Trap, RV T41404, USA) to give a yield of 6.4 g of dry extract with brownish colour. The resulting extract was reconstituted with cold distilled water to give desired doses of 100 and 200 mg/kg body weight.

#### 2.3. Animals

Male Wistar rats weighing between 150 and 180 g were obtained from the animal house of the Agricultural and Rural Development Research Institute, University of Fort Hare. They were kept in well ventilated house conditions [temperature (28±1) °C; photoperiod: 12 h light and 12 h dark cycle; humidity: 45%–50%]. The animals were allowed free access to food and water for 10 days. The experiment was approved by the Animal Ethics Committee of the University of Fort Hare.

#### 2.4. Induction of diabetes in the rats

Diabetes was induced in overnight fasted male Wistar rats by a single intraperitoneal injection (i.p.) of freshly prepared solution of streptozotocin (50 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5). The animals were confirmed diabetic by the elevated plasma glucose levels after 72 h of injection. The rats with stable glycosuria and hyperglycemia (blood glucose >8.1 mmol/L) were used for the experiment.

#### 2.5. Experimental design

Thirty male rats were randomized into five groups consisting of six animals in each group. Group I: normal control rats administered with drinking water daily for 10 days; Group II: diabetic animals received 0.5 mL of distilled water; Group III and IV: diabetic rats, treated daily with 0.5 mL of 100 and 200 mg/kg body weight of *A. africana* extract, respectively; Group V: diabetic animals received 0.5 mL of glibenclamide only. All animals from each group were sacrificed by halothane 24 h after their respective daily dosages of the extract and distilled water.

#### 2.6. Preparation of serum

The method of Yakubu et al.[11] was adopted for the preparation of serum. Briefly, under halothane anaesthesia, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to prevent blood contamination by interstitial fluid) were sharply cut with sterile scalpel blade and an aliquot (2 mL) of the blood was collected into EDTA sample bottles (BD Diagnostics, preanalytical systems, Midrand, USA) for the haematological analysis. Another 5 mL of the blood was allowed to clot for 10 min at room temperature and then centrifuged at 1 282 g × 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 h of preparation for the assay.

#### 2.7. Determination of haematological parameters

The Horiba ABX 80 Diagnostics (ABX pentra Montpellier, France) was used for the determination of hematological parameters including red blood cells (RBC) and its related indices following manufacturer’s instruction. These include hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RCDW), White blood cell (WBC), neutrophils, monocytes, lymphocytes, eosinophils, basophils and platelet were also analyzed.

#### 2.8. Effect of extract on the weight, feed and water intake of the rats

Feed and water intakes were measured everyday at the same hour during the experimental periods while the body weight of the animals were measured at zero day and every fifth day for the period of 10 days.

#### 2.9. Statistical analysis
Data were expressed as (mean ± SD) of six replicates and were subjected to one way analysis of variance (ANOVA). Means were separated by the Duncan multiple test using SAS. Values were considered statistically significant at $P <0.05$.

3. Results

The blood sugar levels of both normal and experimental rats before and after 10 days of treatment are shown in Table 1. Intraperitoneal administration of streptozotocin into the rats causes significant diabetogenic response in Wistar rats with significant increase in the levels of blood sugar as compared with normal rats. The blood glucose level was increased from 5.60 mmol/L to 28.30 mmol/L (Table 1). Following oral administration of extract at the dose of 200 mg/kg the blood glucose level was significantly reduced ($P<0.05$). Meanwhile, the dose at 100 mg/kg also had significant effect on the blood glucose level as compared with diabetic untreated rats. The data obtained at 200 mg/kg compared favourably well with that of glibenclamide treated group (10.43 ± 3.30 mmol/L).

The significant decrease in the levels of RBC, Hb, PCV, MCH, MCV, RCDW and MCHC observed in the diabetic animals was drastically increased to near normal level as well as glibenclamide treated group after administration of extract especially at the dose of 200 mg/kg body weight (Table 2).

Table 1 shows the levels of serum WBC, basophils, neutrophils, eosinophils, lymphocyte and monocytes. The level of WBC was slightly increased after oral administration of the extract at 100 mg/kg while the dose of 200 mg/kg did not have any effect as compared with the diabetic groups. The plant extract significantly increased the level of lymphocyte, eosinophils, monocytes and platelet at both dosages while the best result was observed at the lower dose

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma glucose level (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Normal control</td>
<td>5.60 ± 0.40</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>18.20 ± 0.30</td>
</tr>
<tr>
<td>Diabetic + A. africana (100 mg/kg)</td>
<td>23.00 ± 1.14*</td>
</tr>
<tr>
<td>Diabetic + A. africana (200 mg/kg)</td>
<td>29.90 ± 0.09*</td>
</tr>
<tr>
<td>Diabetic + glibenclamide (0.6 mg/kg)</td>
<td>19.30 ± 3.20</td>
</tr>
</tbody>
</table>

*: $P < 0.05$ as compared with diabetic control group.

Table 2

The effect of aqueous extract of A. africana bark on red blood cells and the differentials in STZ induced diabetic rats ($n = 6$, mean ± SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>A. africana extract at 100 mg/kg bw group</th>
<th>A. africana extract at 200 mg/kg bw group</th>
<th>Glibenclamide group</th>
<th>Diabetes group</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (×10^12/L)</td>
<td>8.94 ± 0.04</td>
<td>8.13 ± 0.35*</td>
<td>8.82 ± 1.23*</td>
<td>8.15 ± 0.40*</td>
<td>7.50 ± 0.60*</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>15.03 ± 0.06</td>
<td>15.10 ± 0.84*</td>
<td>16.20 ± 1.97*</td>
<td>15.33 ± 1.03*</td>
<td>13.43 ± 1.02*</td>
</tr>
<tr>
<td>PCV (L/L)</td>
<td>0.50 ± 0.02</td>
<td>0.56 ± 0.02*</td>
<td>0.60 ± 0.07*</td>
<td>0.48 ± 0.02*</td>
<td>0.36 ± 0.03*</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>55.83 ± 1.11</td>
<td>68.30 ± 2.52*</td>
<td>68.50 ± 0.14*</td>
<td>62.65 ± 1.02*</td>
<td>52.13 ± 0.38*</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>16.93 ± 0.35</td>
<td>18.60 ± 0.69*</td>
<td>18.30 ± 0.21*</td>
<td>17.80 ± 0.46*</td>
<td>15.20 ± 0.40*</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>30.27 ± 0.82</td>
<td>27.30 ± 0.66*</td>
<td>26.80 ± 0.35*</td>
<td>28.50 ± 1.25*</td>
<td>18.23 ± 0.83*</td>
</tr>
<tr>
<td>RCDW (%)</td>
<td>13.63 ± 0.90</td>
<td>14.50 ± 3.13*</td>
<td>13.50 ± 2.47*</td>
<td>15.13 ± 1.20*</td>
<td>12.43 ± 0.55*</td>
</tr>
</tbody>
</table>

a: $P < 0.05$ vs control group; b: $P < 0.05$ vs diabetes group.

Table 3

The effect of aqueous extract of A. africana on white blood cells and its differentials in STZ induced diabetic rats ($n = 6$, mean ± SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>A. africana extract at 100 mg/kg bw group</th>
<th>A. africana extract at 200 mg/kg bw group</th>
<th>Gliben group</th>
<th>Diabetes group</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10^3/L)</td>
<td>17.00 ± 3.20</td>
<td>6.96 ± 2.03*</td>
<td>3.16 ± 3.72*</td>
<td>6.00 ± 5.60*</td>
<td>2.53 ± 0.93*</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>25.37 ± 1.17</td>
<td>19.80 ± 0.01*</td>
<td>40.60 ± 0.30*</td>
<td>23.30 ± 0.16*</td>
<td>2.59 ± 0.51*</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>17.46 ± 6.11</td>
<td>14.70 ± 0.41*</td>
<td>9.60 ± 0.01*</td>
<td>19.00 ± 0.64*</td>
<td>4.69 ± 1.00*</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>65.40 ± 6.86</td>
<td>60.90 ± 2.20*</td>
<td>47.30 ± 3.10*</td>
<td>61.40 ± 5.10*</td>
<td>5.36 ± 0.33*</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>5.70 ± 1.18</td>
<td>4.50 ± 0.22*</td>
<td>2.50 ± 0.60*</td>
<td>1.20 ± 0.32*</td>
<td>1.03 ± 0.78*</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.53 ± 0.21</td>
<td>0.10 ± 0.02*</td>
<td>0.10 ± 0.03*</td>
<td>0.25 ± 0.15*</td>
<td>0.03 ± 0.03*</td>
</tr>
<tr>
<td>Platelets (×10^9)</td>
<td>851.00±78.58</td>
<td>201.00±68.00*</td>
<td>90.00±20.00*</td>
<td>176.00±55.20*</td>
<td>55.00±31.11*</td>
</tr>
</tbody>
</table>

a: $P < 0.05$ vs control group; b: $P < 0.05$ vs diabetes group.
The significant effect depicted at 100 mg/kg compared favourably well whereas that of 200 mg/kg significantly boosted the level of neutrophils (Table 3). The extracts at both dosages did not have any beneficial effect on the level of basophils.

The feed and water intake of the diabetic rats were increased throughout the study period as compared with the normal control group. However, after plant extract administration the feed and water intake was markedly reduced as compared with the diabetic untreated rats (Figure 1 and 2). The effect of extracts on both feed and water intake was not dose related.

A significant decrease in the body weights (28–33 g) of diabetic animals was observed 10 days after induction of streptozotocin into the animals. The oral administration of plant extract markedly increased the body weight of the animals but the effect was not dose related (Figure 3). The percentage increase in the body weight at 200 mg/kg was 8.82% while that of 100 mg/kg did not show any significant difference as compared with the initial body weight.

4. Discussion

Streptozotocin is used as an agent to induce diabetes mellitus by selective cytotoxicity effect on pancreatic beta cells. Thus it affects endogenous insulin release and as a result increases blood glucose level[12]. The continuous administration of aqueous extract of *A. africana* at 200 mg/kg or glibenclamide for 10 days significantly reduced the blood glucose concentration in STZ induced diabetic rats. The plant extract (200 mg/kg) showed a comparable activity with the glibenclamide treated groups. Glibenclamide is a standard antidiabetic drug that stimulates insulin secretion from beta cells of islets of Langerhans. The probable mechanisms of action of the plant extract at higher dose could be linked to potentiation of insulin from beta cells or by increasing peripheral glucose uptake[13].

The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds including plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology, normal functioning and histomorphology of the organs[14].

The occurrence of anaemia in diabetes mellitus has been reported due to the increased non–enzymatic glycosylation of RBC membrane proteins[15]. Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC[16]. In this study, the RBC membrane lipid peroxide levels in diabetic rats were not measured. However, the red blood cells parameters such as Hb, MCHC, MCH, PCV, MCV and RCDW were studied to investigate the beneficial effect of *A. africana* extract on the anaemic status of the
diabetic rats. The levels of RBC, Hb, haematocrit, LUC and MCHC in the diabetic animals were drastically reduced which may be attributed to the infections on the normal body systems. This observation agrees with report of Baskar et al[17] who reported antihyperglycemic activity of aqueous root extract of Rubia cordifolia in streptozotocin–induced diabetic rats. The alterations of these parameters are well known to cause anaemic condition in man[18]. Following plant extract administration, the level of RBC and its related indices were appreciably improved especially at 200 mg/kg. This gives an indication that the plant extract may contain some phytochemicals that can stimulate the formation or secretion of erythropoietin in the stem cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells[19]. The stimulation of this hormone enhances rapid synthesis of RBC which is supported by the improved level of MCH and MCHC[20]. These parameters are used mathematically to define the concentration of haemoglobin and to suggest the restoration of oxygen carrying capacity of the blood. Though, the action mechanism of this plant is not investigated in this study. However, it may be attributed to the ability of plant extract to lower lipid peroxidation level that causes haemolysis of erythrocytes[21]. Previous study on this plant revealed the presence of flavonoids, proanthocyanidins, tannins, phenols and flavonols in this plant. These compounds have been reported to possess strong antioxidant capacity[7], therefore, could inhibit peroxidation of polyunsaturated fatty acids in the cell membrane and haemolysis of red blood cells in the diabetic animals reported by Torell and Faure et al[22, 23, 24].

Streptozotocin is a well known chemical that suppresses the immune system by damaging WBC and certain organs in the body[24]. The intraperitoneal injection of streptozotocin into rats significantly reduced the WBC count and its differentials such as basophils, monocytes, eosinophils, lymphocytes and neutrophils. The reduction of these parameters could be linked to suppression of leucocytes from the bone marrow which may account for poor defensive mechanisms against infection[25]. Consequently, they might have effects on the immune system and phagocytic activity of the animals[22]. The white blood counts and its related indices were significantly restored to near normal after plant extract administration at both doses. The presence of some phytochemicals with ability to stimulate the production of white blood count in the extract could be responsible for the observed result in the treated rats[10]. The extract at both dosages significantly improved the levels of WBC, monocytes, lymphocytes, eosinophils and neutrophils as compared with glibenclamide treated group. However, the extract did not have any significant effect on basophils in this study.

Platelet aggregation ability has been shown in diabetic patient with long term poor glycaemic control due to lack or deficiency of insulin[5]. Platelets known as thrombocytes help to mediate blood clotting, which is a meshwork of fibrin fibres. The fibres adhere to any vascular opening and thus prevent further blood clot. It plays a crucial role in reducing blood loss and repairing of vascular injury[26]. The reduction of platelets levels in diabetic rats induced with streptozotocin was confirmed in this study in relation to the normal control rats. Long term reduction of this parameter may result in internal and external haemorrhage and finally leads to death. However, after plant extract administration, the level of platelet was improved markedly especially at the dose of 100 mg/kg while that of 200 mg/kg did not have strong effect as compared with diabetic untreated rats. This effect indicated the ability of the plant extract to stimulate the biosynthesis of clotting factors[27] due to the presence of active compounds that might help to precipitate blood coagulation or clotting, especially during severe bleeding or haemorrhage[28].

The feed and water intake of the diabetic rats were significantly increased as compared with the normal rats. These symptoms are well known markers of type 2 diabetes in both human and animal models which are direct consequence of insulin deficiency[30]. The feed intake was significantly reduced after administration of A. africana. The dose of 100 mg/kg showed higher activity than 200 mg/kg which was also reflected in water intake. The water intake of diabetic animals was significantly higher than the diabetic treated rats. The dose of 100 mg/kg was significantly lower than the group treated with 200 mg/kg. These results were similar to the report of Kim et al[31] who demonstrated the effect of Morus alba in controlling the desire for food and water intake under diabetic condition. A significant decrease in the body weights (28–33 g) of diabetic animals was observed 10 days after induction of streptozotocin into the animals. The loss in the body weight of the diseased animals agrees with the finding of Oyedemi et al[29] who observed similar effect on diabetic animals induced with streptozotocin. This reduction has been linked to degradation of structural proteins and muscle wasting. Oral administration of plant extract at both doses was able to improve the body weight of the animals. The result indicated that extract of A. africana possessed the ability of managing glucose level as well as controlling muscle wasting and induced adipogenesis[32].

In conclusion, from the data obtained in the present study we can conclude that the aqueous bark extract of A. africana possesses antihyperglycemic properties. In addition, the extract could prevent various complications of diabetes as well as improving some haematological parameters. Further experimental investigation is also needed to exploit its relevant therapeutic effect to substantiate its ethnomedical usage.

Conflict of interest statement
We declare that we have no conflict of interest.

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