Evaluation of the Anti-diabetic and Antioxidant Activities of the Methanol Leaf Extract of *Berlinia grandiflora*

O.J. Ode, C.O. Nwaehujor and C.F. Nwinyi

Hypoglycemic and anti-oxidant activities of the methanol leaf extract of *Berlinia grandiflora* were studied in streptozotocin-induced diabetic rats to scientifically prove its folkloric use. Osmotic fragility test and phytochemical analysis of the extract were investigated. Hyperglycemia was induced in rats by injection of 50 mg kg\(^{-1}\) (i.p.) of freshly prepared streptozotocin. Three doses of the extract (50, 100 and 250 mg kg\(^{-1}\)) per os were used with glibenclamide (2 mg kg\(^{-1}\)) as reference drug and a negative control. Fasting blood glucose was monitored at 1, 3 and 6 h. Anti-oxidant activity was studied using the DPPH and FRAP spectrophotometric assays. Ascorbic acid was used as standard. Acute toxicity studies revealed the extract was safe at 500 mg kg\(^{-1}\). Treatment of streptozotocin-induced hyperglycemic rats using the crude extract of *B. grandiflora* reduced the fasting blood glucose levels significantly \((p<0.01)\) in a dose dependent manner. DPPH and FRAP results were comparable to Ascorbic acid. The crude extract did not disrupt erythrocyte cell membranes. Phytochemical analysis revealed presence of tannins, alkaloids, flavonoids and glycocides. This suggests that *B. grandiflora* possesses anti-diabetic, anti-oxidant and membrane stabilizing activities. The extract could be a potential source of novel anti-diabetic and anti-oxidant agents.

Key words: Anti-diabetic, anti-oxidant, streptozotocin, glibenclamide, DPPH, FRAP, *Berlinia grandiflora*, osmotic fragility
INTRODUCTION

Diabetes mellitus is a major cause of severe health complications and premature deaths in many developing countries. Presently, it has been estimated that at least 150 million people in the world have diabetes (Sharma et al., 2007). It is even predicted that by 2025, up to 300 million people will be affected (Amos et al., 1997). Type 2 diabetes which is the commonest form of diabetes is a metabolic disorder of multiple origins characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism resulting in defects in insulin secretion, insulin action or both. It is becoming epidemic in developing countries (WHO, 1985). Oxidative stress-induced tissue damage with Reactive Oxygen Species (ROS) is implicated as a cause and consequence of a variety of disorders including diabetes, coronary heart disease and cancer (Knight and McCafferty, 1996).

The prolonged effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure and/or neuropathy with risk of foot ulcers, amputation, charcot joints and features of autonomic dysfunction, including sexual dysfunction (Harris et al., 1987; Jayalalake et al., 1993). Diabetic patients are at increased risk of cardiovascular, peripheral and cerebro-vascular diseases. The possible symptoms include thirst, polyuria, blurred vision and weight loss but in severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death (Alberti and Zimmet, 1998).

Conventional treatment regimens for diabetes include diet control and exercise, insulin replacement therapy, oral hypoglycemic agents and inhibitors of α-glycosidase enzyme (Sabos et al., 1999). Bioactive anti-diabetic principles were isolated from plants employed in folk medicine. Panaxana A and B from the roots of Panax ginseng Mey. (Araliaceae), ephedrins A, B, C, D and E from the aerial parts of Ephedra distachya L. (Ephedraceae), moran A from the root barks of Morus alba L. and β-sitosterol from the green beans of Coffee arabica L. (Rubiaceae) are some of the documented examples (Ivorn et al., 1989).

Berlinia grandiflora (Leguminosae) is a popular plant commonly employed as remedy to treat diverse health problems in Nigerian traditional medical practice including diabetes. The plant is widespread from Nigeria, Guinea and Mali in the west to Central African Republic and Democratic Republic of Congo in the south (Keii, 1989). Berlinia grandiflora is synonymous with Berlinia acuminata Sol. ex. Hook. and Berlinia heudeolotiana Baill (Irvine, 1961). A decoction of leafy twigs was equally used as febrifuge, cholagogue, purgative and anti-emetic. Leaf decoctions are taken as a tonic (Mackinder and Harris, 2006).

The present study on the anti-diabetic and antioxidant activities of B. grandiflora leaves becomes rational in view of the need to explore for more effective drugs necessary for treatment of diabetes and also provide scientific proof to the folkloric claim.

MATERIALS AND METHODS

Animals: Adult male out-bred albino wistar rats weighing 150-175 g were purchased from the Laboratory Animal Facility of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. Animals were kept in clean stainless steel wire mesh cages, maintained at normal temperature and natural daylight/night conditions. The rats were allowed 7 days to acclimatize with free access to standard commercial feed pellets (Vital feed®, Nigeria) and clean drinking water. The laboratory animals were used in accordance with laboratory practice regulation and the principle of laboratory animal care as documented by Zimmermann (1983).

Chemicals, reagents, drugs and equipment: Analytical grade chemicals and reagents, freshly prepared solutions and standard drugs were used for all experiments. Acetate buffer 300 mm pH 3.6, lead acetate, potassium dihydrogen phosphate (KH₂PO₄), dipotassium hydrogen phosphate (K₂HPO₄), L-aspartic acid, α-ketoglutaric acid, sodium pyruvate, hydrochloric acid, sodium hydroxide, sodium chloride, sodium bicarbonate, Fehling's I and II reagents, Dragendorff's reagent, Meyer's reagent, Wagner's reagent, Morish's reagent, streptozotocin (Sigma-Aldrich, Germany), glibenclamide (Roche, USA), ascorbic acid, methanol, DPPH (Sigma-Aldrich, Germany), Ferric III chloride-hexahydrate-2,4,6-tripryridyl triazine (TPTZ) obtained from Fluka, Switzerland were used in the studies. Vortex, model Genje 2 (Fisher Scientifique, New Jersey, USA), rotary evaporator (Buchi, Switzerland), water bath, refrigerator, spectrophotometer (Spectrumlab 752S, B. Bran scientific instrument company, England), weighing balance (Mettler), mortar and pestle, hot air oven (Gallenkamp, UK) and laboratory mill were used in the experiments.

Plant collection: Fresh leaves of Berlinia grandiflora were collected in September, 2009 from Nsukka local government area, Enugu state of Nigeria. The plant leaves were authenticated by Mr A.O. Ozioko of the Department
of Botany, University of Nigeria, Nsukka. A voucher sample was kept in the departmental herbarium (UNN/BD-4123.012). The leaves were subsequently dried at room temperature on top of a laboratory bench and later pulverized into coarse powder using a grinding mill.

**Extraction of the plant material:** One thousand g of *Berlinita grandiflora* dried leaves was extracted by cold maceration in 80% methanol with intermittent shaken at regular intervals of 3 h for 72 h. The extract was filtered and concentrated using vacuum rotary evaporator at 40°C. The percentage yield of the extract was subsequently determined.

**Acute toxicity:** Twenty-five adult albino Wistar rats (140-160 g) of both sexes were randomly assigned to five groups (A-E) consisting of 5 rats per group. Group A, B, C, D and E received 10, 50, 100, 250 and 500 mg kg⁻¹, respectively of the crude extract. All treatments were orally by stomach intubation. The rats were allowed access to feed and water *ad libitum* and were then observed for a period of 48 h for signs of toxicity and death.

**Experimental design:** Adult male albino wistar rats weighing between 120-150 g were identified and randomly allocated to 5 separate groups (I-V) comprising 5 rats in a group. All rats were fasted overnight before the administration of streptozotocin (STZ) obtained from Sigma Aldrich, Germany. Freshly prepared STZ dissolved in 0.1 M citrate buffer and pH 4.5 was given by a single intraperitoneal injection at a dose of 50 mg kg⁻¹. Control rats were injected with citrate buffer only. After 3 days post administration of alloxan, the fasting blood glucose of each rat was measured. A blood glucose level of 9 mmol L⁻¹ and above was taken as positive for diabetes. Treatment procedures were then instituted. Group I (negative control) received buffer (10 mL kg⁻¹), group II (positive control) was given glibenclamide (2 mg kg⁻¹), group III received *B. grandiflora* extract (50 mg kg⁻¹), group IV received *B. grandiflora* extract (100 mg kg⁻¹), while group V was given *B. grandiflora* extract (250 mg kg⁻¹). Treatments were administered by gastric intubation orally. At 1, 3 and 6 h post-treatments, blood sample was collected from individual rat tail vein under mild anesthesia. The fasting blood sugar level was measured using the Accu-chek Advantage II glucometer. Percentage reductions in fasting blood glucose level in all the experimental groups were calculated using the formula:

\[
\text{Percentage decrease in blood glucose level} = \left(1 - \frac{\text{Fasting glucose level before treatment}}{\text{Fasting glucose level after treatment}}\right) \times 100
\]

**Determination of osmotic fragility:** The effect of the methanol extract of *B. grandiflora* on erythrocyte membrane stability as determined by mean corpuscular fragility was studied using the method of Parpart *et al.* (1947) as modified by Elekwa *et al.* (2003). A 10 g L⁻¹ solution was made from 100 g L⁻¹ NaCl stock solution at pH 7.4 with 150 mM phosphate. Dilutions equivalent to 9.0, 7.0, 6.0, 5.0, 4.0, 3.0, 2.0 and 1.0 g L⁻¹ NaCl in 4.5 mL each were prepared. A given volume (0.5 mL) of the extract at graded concentrations (0.2, 0.6 and 1.0 mg mL⁻¹) respectively was separately added to 4.5 mL of the hypotonic solutions to make a final volume of 5 mL. The control and the different concentrations of the extract had the same NaCl concentrations while 9.0 g L⁻¹ NaCl was used as the blank. A fixed volume (0.05 mL) of blood sample was added to 5 mL of the various hypotonic solutions and immediately mixed by inverting severally. The tubes were allowed to stand for 30 min at room temperature. The contents were remixed and centrifuged at 1500 rpm for 5 min. The absorbance of the supernatant was read at 540 nm. The tests were carried out in triplicates and the mean absorbance for each sample recorded. The Mean Corpuscular Fragility (MCF), which is the concentration of saline causing 50% haemolysis of the erythrocytes, was obtained from a plot of percentage lysis against NaCl concentration (g L⁻¹).

**Phytochemical analysis:** The crude methanol extract of *B. grandiflora* was tested for the presence of alkaloids, flavonoids, tannins, terpenes and saponins using the method of Trease and Evans (1983). Equal volume of distilled water in a separate test tube served as the control for each of the tests.

**Evaluation of antioxidant capacity using the 1, 1-diphenyl-
2-pierylhydrazyl radical (DPPH) spectrophotometric assay:** The free radical scavenging activity of extracts was analysed by the DPPH assay following a standard method (Mensor *et al.*, 2001). A given volume (2 mL) of the extract at varying concentrations ranging from 10-400 μg mL⁻¹ each was mixed with 1 mL of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 min of incubation in the dark at room temperature. The experiment was done in triplicate. The percentage antioxidant activity was calculated as follows:

\[
\text{% Antioxidant Activity (AA)} = \left(1 - \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}}{\text{Abs}_{\text{control}}}\right) \times 100
\]

Methanol (1.0 mL) plus 2.0 mL of the extract was used as the blank while 1.0 mL of the 0.5 mM DPPH
solution plus 2.0 mL of methanol was used as the negative control. Ascorbic acid (vitamin C) was used as reference standard.

**Ferric reducing/antioxidant power (FRAP) assay:** The total antioxidant potential of sample was determined using a Ferric Reducing Ability of Plasma (FRAP) assay of Benzie and Strain (1996) as a measure of “antioxidant power”. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe³⁺-tripyridyltriazine compound from colorless oxidized Fe²⁺ form by the action of electron donating antioxidants. Standard curve was prepared using different concentrations (100-1000 μmol L⁻¹) of FeSO₄.₇H₂O. All solutions were used on the day of preparation. In the FRAP assay the antioxidant efficiency of the extract under the test was calculated with reference to the reaction signal given by an Fe²⁺ solution of known concentration, this representing a one-electron exchange reaction. Ascorbic acid was measured within 1 h after preparation. The sample to be analyzed was first adequately diluted to fit within the linearity range. All determinations were performed in triplicate.

Calculations were made by a calibration curve:

\[ \text{FRAP value of sample (μM)} = \text{Absorbance at 593 nm} \times \text{FRAP value of std (1000 μM)} \]

**Statistical analysis:** All data were expressed as Mean±SEM. Data were analyzed using one way Analysis of Variance (ANOVA) at 5% level of significance. Least significant difference was used to detect the difference among the treatment groups.

**RESULTS**

**Description of the extract:** The extract of *Berlinia grandiflora* leaves was brown in colour and gave a yield of 14.5% w/w dry extract.

**Acute toxicity study of the extract of *Berlinia grandiflora* leaves in rats:** The leaf extract induced no overt toxicity or death in the experimental rats within the dose range (10-500 mg kg⁻¹) used. However, the highest test dose (500 mg kg⁻¹) of the extract caused transient dullness that lasted for 30 min after treatment. Preliminary tests showed that doses above 500 mg kg⁻¹ were toxic with predominant central nervous signs in rats.

**Phytochemical analysis:** Phytochemical tests revealed the presence of tannins, alkaloids, flavonoids and glycosides in the methanol leaf extract of *B. grandiflora*.

**Anti-diabetic activity of *Berlinia grandiflora* leaf extract:** The graded doses (50, 100 and 250 mg kg⁻¹) of the extract and glibenclamide (2 mg kg⁻¹) significantly (p<0.01) induced progressive decreases in the mean fasting blood glucose levels of rats compared to untreated (control) values at 1, 2 and 3 h post treatments (Table 1). At 1 h after treatment, glibenclamide produced 69% reduction in the mean fasting blood glucose levels, both 50 and 100 mg kg⁻¹ of the extract lowered 52% while 250 mg kg⁻¹ of the extract induced 62% reduction in the glucose values. The lowering effect of glibenclamide (2 mg kg⁻¹) on the rat glucose values was not significantly (p>0.05) different from that of the extract at 250 mg kg⁻¹. At 3 h, treatments caused similar decreases in the mean fasting blood glucose levels (glibenclamide = 76%; 50 mg kg⁻¹ extract = 55%; 100 mg kg⁻¹ extract = 56%; 250 mg kg⁻¹ extract = 64%). At 6 h post treatments, glibenclamide (2 mg kg⁻¹) and the highest test dose (250 mg kg⁻¹) of the extract had comparable effects in lowering the fasting blood glucose levels of the experimental rats relative to the effects of the extract at 50 and 100 mg kg⁻¹.

**Osmotic fragility test:** The Median corpuscular Fragility (MCF) values for erythrocytes induced by the methanol extract of *B. grandiflora* is presented in Table 2. There was no significant (p>0.05) variations in the MCF values of the different concentrations of the extract compared with the control.

### Table 1: Mean fasting blood glucose level of diabetic rats treated with buffer (10 mL/kg), glibenclamide (2 mg kg⁻¹) and *B. grandiflora* leaf extract (50, 100, 250 mg kg⁻¹)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Buffer (10 mL kg⁻¹)</th>
<th>Glibenclamide (2 mg kg⁻¹)</th>
<th><em>B. grandiflora</em> extract (50 mg kg⁻¹)</th>
<th><em>B. grandiflora</em> extract (100 mg kg⁻¹)</th>
<th><em>B. grandiflora</em> extract (250 mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>4.24±0.3</td>
<td>4.60±0.6</td>
<td>4.54±0.2</td>
<td>4.60±0.4</td>
<td>4.44±0.5</td>
</tr>
<tr>
<td>02</td>
<td>20.51±3.4</td>
<td>19.51±4.0</td>
<td>17.51±3.7</td>
<td>16.42±4.1</td>
<td>18.38±4.3</td>
</tr>
<tr>
<td>06</td>
<td>19.29±3.2</td>
<td>6.12±2.0 (69%)</td>
<td>8.45±3.0 (52%)</td>
<td>7.89±2.1 (55%)</td>
<td>7.01±1.3 (62%)</td>
</tr>
<tr>
<td>03</td>
<td>19.21±4.1</td>
<td>4.74±1.7 (76%)</td>
<td>7.89±2.1 (55%)</td>
<td>7.20±2.5 (55%)</td>
<td>6.59±2.5 (64%)</td>
</tr>
<tr>
<td>06</td>
<td>18.51±3.5</td>
<td>3.51±1.1 (82%)</td>
<td>9.57±4.1 (45%)</td>
<td>6.61±2.1 (60%)</td>
<td>3.68±1.0 (80%)</td>
</tr>
</tbody>
</table>

00 h: Normal blood glucose level before injection of alloxan. 0 h: Fasting blood glucose level after challenge with alloxan, at 1h, 3h and 6h indicate significant (p<0.05, p<0.01) differences between treatment groups on one hand and when compared to control (untreated) group on the other hand, respectively.
Table 2: Effects of *B. grandiflora* extract on osmotic fragility

<table>
<thead>
<tr>
<th>Concentration of extract (mg mL(^{-1}))</th>
<th>MCT expressed as [NaCl] (g L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 (Control)</td>
<td>3.5 ±1.40</td>
</tr>
<tr>
<td>0.2</td>
<td>3.3 ±2.70</td>
</tr>
<tr>
<td>0.6</td>
<td>4.0 ±4.4</td>
</tr>
<tr>
<td>1.0</td>
<td>4.2 ±3.6</td>
</tr>
</tbody>
</table>

There was no significant (p>0.05) variations in the MCT values of the different concentrations of extract when compared with the control.

**Ferric reducing/antioxidant power assay (FRAP):** The ferric reducing ability of plasma, a measure of the antioxidant ability, showed that the methanolic extract of *B. grandiflora* leaves produced a dose dependent antioxidant effect. At 10 μg mL\(^{-1}\), the mean antioxidant power (FRAP value) was 1.1 μM. This increased to 1.6 μM at 100 μg mL\(^{-1}\) and then 1.7 μM at 400 μg mL\(^{-1}\) (Fig. 2). Ascorbic acid has a standard FRAP value of 2.0 μM at 1000 μg mL\(^{-1}\).

**DISCUSSION**

*Berlinia grandiflora* methanol leaf extract was tolerated by experimental rats up to 500 mg kg\(^{-1}\). Preliminary studies revealed that the extract doses above 500 mg kg\(^{-1}\) were neurotoxic in rats. Graded doses (50, 100 and 250 mg kg\(^{-1}\)) of the extract demonstrated potent hypoglycemic effects in diabetic rats following treatments at 1, 3 and 6 h after induction of diabetes. The extract exhibited maximal anti-diabetic effect at 250 mg kg\(^{-1}\) and 6 h post treatment. There was an almost equipotent hypoglycemic effect with glibenclamide at 2 mg kg\(^{-1}\) (82%) (Table 1).

The extract induced no changes in the median corpuscular fragility values for erythrocytes different from the control (Table 2) thus, it did not therefore cause disruptive effect on rat erythrocytes and was able to stabilize and protect the cell membranes from oxidative damage. The methanol leaf extract of *B. grandiflora* also demonstrated significant antioxidant activities as revealed by the DPPH and FRAP spectrophotometric assays. In the DPPH assay, the crude extract produced a significant (p<0.05) concentration-dependent increase in antioxidant activity when compared with ascorbic acid. The antioxidant activity was comparable to ascorbic acid at the highest concentration (400 μg mL\(^{-1}\)). The extract produced 71.7% while ascorbic acid had 80.0% antioxidant activity at 400 μg mL\(^{-1}\) (Fig. 1). The principle of the FRAP assay is based on the reduction of a ferric-tripyridyl trizine complex to its ferrous form in the presence of antioxidants. The FRAP values of *B. grandiflora* extract at 10, 50, 100, 200 and 400 μg mL\(^{-1}\) were remarkably high compared to ascorbic acid which has a FRAP value of 2 μM at 1000 μg mL\(^{-1}\). There was no significant (p>0.05) variations between the FRAP values of the extract at the lowest (10 μg mL\(^{-1}\)) and highest concentration (400 μg mL\(^{-1}\)) (Fig. 2). The potent hypoglycemic effect of the extract which was maximally displayed at 250 mg kg\(^{-1}\) coupled with high antioxidant potential as revealed in the DPPH and FRAP spectrophotometric assays suggest that *B. grandiflora* leaves contain bioactive anti-diabetic compounds.
Patients with type 2 diabetes mellitus are not dependent on exogenous insulin but may eventually become dependent because the pancreatic islet β-cells fail to compensate for insulin resistance (Kopelman and Hitman, 1998). The role of reactive oxygen species in diverse disease pathologies including diabetes is highly acknowledged. Antioxidants block the process of oxidation by neutralizing free radicals as well as reducing the rate of chain initiation. The initiating radicals are scavenged and destroyed before oxidation sets in motion. Antioxidants do not completely get rid of free radicals in the body but they retard or minimize the damage caused (Trouillas et al., 2003). The body is therefore in need of a steady source of antioxidants.

CONCLUSION

In conclusion, this investigation revealed that the crude methanol extract of B. grandiflora leaves possesses appreciable anti-diabetic and antioxidant activities. The plant material could be a potential source for novel anti-diabetic and anti-oxidant agents.

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REFERENCES


