Hypoglycemic properties of ethanolic extracts of *Gongronema latifolium*, *Aloe perryi*, *Viscum album* and *Allium sativum* administered to alloxan-induced diabetic albino rats (*Rattus norvegicus*)

Ibegbulem CO¹ and Chikezie PC²

¹Department of Biochemistry, Federal University of Technology, Owerri, Nigeria
²Department of Biochemistry, Imo State University, Owerri, Nigeria

ABSTRACT: Background: Plants offer a wide range of natural compounds of medicinal values to humans and domestic animals. Objective: The ethanolic extracts of *Gongronema latifolium*, *Aloe perryi*, *Viscum album* (leaves) and *Allium sativum* (bulb) were investigated for their phytochemical/biochemical constituents and hypoglycemic properties. Materials and Methods: Hypoglycemia was induced in rats by a single dose (140 mg/kg) of intra-peritoneal injection of alloxan monohydrate in citrate buffer (pH 4.5). Suspensions of the ethanolic extracts were administered by intra-peritoneal injection at doses of 2 mg/kg every 16 h for 54 h. Collection of blood samples for estimation of fasting blood glucose (FBG) was carried out at regular time intervals of 0, 16, 32, 48 and 54 h, using the glucose oxidase method. Results: Phytochemical and biochemical screening showed the presence of saponins, tannins, flavonoids, proteins and carbohydrates in the four plant tissues under investigation. *A. sativum* and *G. latifolium* also tested positive for the presence of alkaloids. The capacities of the four ethanolic extracts to reduce FBG concentrations in treated rats at the 54 h were in the order: *A. perryi* > *G. latifolium* > *A. sativum* > *V. album*. Comparatively, at t = 16 h, FBG concentration of *V. album* treated rats was not significantly different (*p* > 0.05) from those of *G. latifolium* treated group. Likewise, FBG concentration of rats treated with *V. album* extract did not show a significant difference (*p* > 0.05) compared to the group administered with extract of *A. sativum*. Conclusion: The four plant extracts used in the present study exhibited approximately the same capacity to act as hypoglycemic agents in the treated rats and correlate with the therapeutic capacity of the standard drug, glimepiride.

KEYWORDS: Hypoglycemia, phytochemical, *A. perryi*, *G. latifolium*, *A. sativum*, *V. album*, glimepiride

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia and glycosuria produced by an absolute or relative insulin insufficiency (Insulin-Dependent Diabetes Mellitus (IDDM)) or insulin resistance by peripheral cells (Non-Insulin-Dependent Diabetes Mellitus (NIDDM)), or both.¹ The disease presents other metabolic and anatomic distortions and disturbances, including retinopathy, neuropathy, nephropathy, hyperlipidemia, hypercholesterolemia, ketosis, atherosclerotic coronary artery and peripheral atherosclerotic vascular diseases.²,³ The individual also experiences weight loss, pathologic changes in the eye, renal dysfunction and neuropathy.⁴,⁵ Oxidant-free radicals have been implicated in the pathogenesis of IDDM.⁶,⁷ In experimental animals, injection and subsequent metabolism of 2, 4, 5, 6-tetraoxypyrimidine (alloxan) induces specific DNA fragmentation in pancreatic islets and cell damage has been attributed to the production of toxic free radicals.⁸ Alloxan-treated animals are widely used as models for IDDM studies.⁹,¹⁰ The reason for the high sensitivity of β-cells to alloxan is not clear, although there are speculations on the connection between alloxan sensitivity and the incidence of IDDM.

Plants offer a wide range of natural compounds of medicinal values to humans and domestic animals. *Gongronema*
latifolium (commonly called ‘arokeke’ and ‘utazi’ in the South Western and South Eastern parts of Nigeria) is a tropical rainforest plant primarily used as a spice and vegetable in traditional folk medicine.\textsuperscript{[2,14]} G. latifolium has anti-plasmodial activity;\textsuperscript{[15]} this supports the traditional use of the leaf extract of the plant for local treatment of malaria. Reports have shown that G. latifolium has anti-licking activities and is effective in the treatment of sore gums, colic, dyspepsia and anti-helmintic.\textsuperscript{[16]} Aloe perryi belongs to the family of Liliaceae, and the use of A. vera for the alleviation of constipation has been documented in Wistar rats.\textsuperscript{[17]} There are no reports on the use of A. perryi in ameliorating DM in human. Another member of the Liliaceae family, Allium sativa (garlic), apart from its medicinal purposes, is also used as seasoning in Africa, Southern Europe and Asia. It is a natural source of selenium to the body for proper immune response, and acts as an antioxidant.\textsuperscript{[18]} Extracts from Viscum album (Mistletoe) are widely used as alternative cancer and cardiovascular disease therapies in Europe and have been recognised to induce apoptosis.\textsuperscript{[19–22]} The hypoglycemic and antioxidant activity of V. album extract has also been investigated.\textsuperscript{[21]}

Although a variety of drugs are available for the treatment and management of DM, herbal preparations are still prescribed widely as alternatives to synthetic ones (even when their biologically active compounds are unknown) because of their minimum side-effects and relatively low cost. Furthermore, for many centuries plants have been used medicinally for the treatment of diverse disorders/ailments and there are numerous documented claims of herbal remedies for DM. Almost five decades ago, Jain et al.\textsuperscript{[23]} posited that ingestion of Allium cepa (onion) and A. sativum (garlic) juice resulted in better utilization of glucose in rabbits. Recently, the applications of natural substances for the prevention, management and treatment of DM have been reported by several researchers and there are increasing search for herbal hypoglycemic agents.\textsuperscript{[21,23–25]} In view of this, it has been considered pertinent to investigate the hypoglycemic properties of various extracts of G. latifolium, A. perryi, V. album and A. sativum in alloxan induced diabetic Rattus norvegicus.

**MATERIALS AND METHODS**

**Collection of plant specimens**

Fresh samples of G. latifolium, A. perryi, V. album and A. sativum were harvested between October and November, 2011 from the Botanical Gardens of Imo State University and Federal University of Technology, Owerri, Nigeria. The plant specimens were identified and authenticated by Dr. F. N. Mbogwu at the Herbarium of the Department of Plant Science and Biotechnology, Imo State University, Owerri. A voucher specimen was deposited at the Herbarium for reference purposes.

**Preparation of extracts**

Plant samples were washed under a continuous stream of distilled water for 15 min and air-dried at room temperature for 5 h. The separate leaves were further dried for 5 h in an oven at 60°C and subsequently ground with a ceramic mortar and pestle. Twenty-five grams (25 g) of each pulverized specimen was suspended in 250 mL of ethanol/water mixture (1:2 v/v) in stoppered flasks and allowed to stand for 24 h. The suspensions were filtered with Whatman No. 24 filter papers. The filtrates were concentrated in a rotary evaporator at 50°C and dried in vacuum desiccators. The yield was calculated to be G. latifolium (3.4% w/w), A. perryi (3.1% w/w), V. album (2.2% w/w) and A. sativum (3.5% w/w). These extracts were finally suspended in phosphate buffered saline (PBS) solution (extract vehicle), osmotically equivalent to 100 g/l PBS (0.9 g NaCl, 17.0 Na₂HPO₄,2H₂O and 2.43 g NaH₂PO₄,2H₂O), and used in all the studies with doses expressed in milligram per kilogram body weight (mg/kg) of the animals.

**Phytochemical and biochemical study**

Phytochemical and biochemical screening was carried out for the presence of tannins, carbohydrates, flavonoids, saponin, alkaloids, glycosides and proteins as described by Ayoola et al.\textsuperscript{[26]}

**Experimental animals**

Wistar albino rats (8–10 weeks of age) of both genders and weighing 17–21 g of were obtained from an animal house at the University of Port Harcourt, Port Harcourt, Nigeria. Throughout the study, rats were fed with standard commercial feed (Ewu Feed Mills, Edo State, Nigeria) and water ad libitum, in well-ventilated stainless steel cages. After randomization on a weight basis, the rats were acclimatized for a period of 7 days at ambient temperatures of 25 ± 5°C, 30–55% of relative humidity and 12 h light/12 h darkness cycle. The handling of the animals was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

**Induction of diabetes**

Diabetes was induced in the rats as previously described by Mohini et al.\textsuperscript{[27]} A single dose (140 mg/kg) of alloxan monohydrate in citrate buffer (pH 4.5) was administered to the rats via intra-peritoneal injection. Hyperglycemia was confirmed 48 h after alloxan injection.\textsuperscript{[28]} Surviving
rats with FBG concentrations higher than 250 mg/dL were included in the study.[29,30]

**Study design and fasting blood glucose estimation**

Experimental animals were deprived of food and water for 16 h before the commencement of the feeding experiment. A total of twenty-eight (28) rats were divided into seven groups (n = 4) each as follows:

- **Group Control-Normal (Control-N):** The animals of this group were non-diabetic and received only PBS (1 ml/kg/16 h, i.p.) for 54 h.
- **Group Control-Diabetic (Control-D):** The animals of this group were diabetic and received PBS (1 ml/kg/16 h, i.p.) for 54 h.
- **Group T1 (D + V. album):** The animals of this group were diabetic and received V. album (2 mg/kg/16 h, i.p.) for 54 h.
- **Group T2 (D + A. sativum):** The animals of this group were diabetic and received A. sativum (2 mg/kg/16 h, i.p.) for 54 h.
- **Group T3 (D + G. latifolium):** The animals of this group were diabetic and received G. latifolium (2 mg/kg/16 h, i.p.) for 54 h.
- **Group T4 (D + A. perryi):** The animals of this group were diabetic and received A. perryi (2 mg/kg/16 h, i.p.) for 54 h.
- **Group T5 (D + Glimepiride):** The animals of this group were diabetic and received Glimepiride (0.09 mg/kg/16 h, i.p.) for 54 h.

Blood samples were drawn from the tip of the tails of the rats at regular time intervals of 0, 16, 32, 48 and 54 h for FBG estimation. FBG was estimated by glucose oxidase method according to the Randox® kit manufacturer’s procedure (Randox® Laboratories Ltd. Ardmore, United Kingdom).

**Statistical analysis**

The results were expressed as mean ± SEM, and statistically analyzed by one way ANOVA followed by Dunnett test, with level of significance set at p < 0.05.

**RESULTS**

Phytochemical and chemical screenings showed the presence of tannins, saponins, flavonoids, proteins and carbohydrates in the four plant tissues under investigation. A. sativum and G. latifolium tested positive for the presence of alkaloids.

The FBG concentrations (mg/dl) of the seven (7) groups of rats within the experimental time (t) = 54 h are presented in Figure 1. The FBG concentrations of the Control-N and Control-D groups showed low variability. The FBG concentration of Control-N group ranged between 96.5 ± 0.54 and 97.5 ± 0.71 mg/dl (54 ≥ t ≥ 0; p > 0.05), whereas Control-D group was between 232.5 ± 1.26 and 233.5 ± 1.43 mg/dl (54 ≥ t ≥ 0; p > 0.05). As an overview of the results, all the treated rats (Groups T1-T5) exhibited reductions in FBG concentrations as the experimental time progressed, while the Group Control-D showed little change. Comparatively, the standard drug (Glimepiride) exhibited the highest capacity to reduce FBG concentrations in treated rats (Group T5: D + Glimepiride).

The capacities of the four ethanolic extracts to reduce FBG concentrations in treated rats by 54 h of the experiment were in the order: A. perryi > G. latifolium > A. sativum > V. album (Table 1). Indeed, the A. perryi extract caused the reduction of FBG concentration from 240 ± 1.11 mg/dl (t = 0 h) to 189 ± 1.02 mg/dl at t = 54 h, representing 22.50% reduction in FBG concentration. The administration of V. album extract to rats caused a FBG reduction of 15.55 ± 0.92% at t = 54 h (Table 1). The FBG concentrations of the V. album, A. sativum and A. perryi treated groups, as well as for the standard control group (D + Glimepiride) showed significant differences (p < 0.005) between each preceding time intervals of treatment when t > 16 h. In contrast, G. latifolium extracts induced no significant (p > 0.005) reduction in FBG concentration between the 32 and 48 h. Generally, within the experimental time range (54 ≥ t ≥ 0) h, the four extracts caused a progressive reduction in FBG concentrations but did not exhibit the capacity to return FBG concentrations to the normal levels observed in Control N animals (96.8 ± 0.57 mg/dl) in the rats during the experimental period.

At t = 16 h, the FBG concentration in D + V. album rats was not significantly different (p > 0.05) from those of the D + G. latifolium group. Likewise, the FBG concentration

![Figure 1. Comparative effect of ethanolic extracts of V. album, A. sativum, G. latifolium A. perryi and Glimepiride on FBG in alloxan-induced diabetic rats. Percentages of FBG concentration are mean of n ± SEM of four (4) blood samples.](image)
of rats treated with extract \( V. \) \( \text{album} \) did not show a significant difference \((p > 0.05)\) compared to the group administered with extract of \( A. \) \( \text{sativum} \) (Table 1).

**DISCUSSION**

The results of the present study supports previous reports on the hypoglycemic properties of \( V. \) \( \text{album} \) leaf extracts,\[^{[21,31]}\] \( E. \) \( \text{floccosa} \) Bedd leaf extracts,\[^{[32]}\] \( R. \) \( \text{ellipticus} \) fruit extracts,\[^{[33]}\] \( D. \) \( \text{peregrina} \) leaf extracts,\[^{[35]}\] \( V. \) \( \text{rosea} \) whole plant extracts,\[^{[24]}\] \( T. \) \( \text{catappa} \) Linn fruit extracts\[^{[33]}\] and onion and garlic extracts.\[^{[34]}\]

A target-based therapeutic approach towards diabetes mellitus treatment using medicinal plants has been extensively discussed.\[^{[19]}\] These previous studies suggest that the active principles of these plant extracts exerted therapeutic benefits by either mimicking the physiologic actions of insulin and/or facilitating insulin secretion.

An earlier report\[^{[31]}\] stated that \( V. \) \( \text{album} \) contains water-soluble and heat-resistant natural product(s) that enhance the release of insulin in hyperglycemic streptozotocin-induced diabetic rats. Although their report could not establish whether the \( \beta \)-amyrin, tyramine, quercitin, syringin and flavoyedorinin A and B components of the leaf extract was responsible for its hypoglycemic property, they noted that the hypoglycemic action of \( V. \) \( \text{album} \) extract was not mediated by lectins. In the present study, phytochemical screening of \( V. \) \( \text{album} \) extract showed the presence of flavonoids. Flavonoids are potent hypoglycemic agents.\[^{[16–38]}\] Therefore, the presence of flavonoids in the \( V. \) \( \text{album} \) extract was responsible for its hypoglycemic activity, possibly by the stimulation of insulin secretion.\[^{[31]}\] In another study, flavonoids were found to possess antioxidant activity as shown by promoting increased activity of superoxide dismutase (SOD) and decreased plasma malondialdehyde (MDA) concentrations in diabetic rats.\[^{[17]}\] Thus, the presence of flavonoids may also act to antagonize the generation of free radicals by alloxan and the associated pathophysiology of the diabetic state.

The hypoglycemic property of \( A. \) \( \text{sativum} \) extract reported here are in accordance with previous reports.\[^{[39,40]}\] Ayodhya et al\[^{[39]}\] states that the hypoglycemic activity of the ether extract of \( A. \) \( \text{sativum} \) was due to increased insulin-like activity, while Chauha et al\[^{[41]}\] suggested that oral administration of Ethanolic extract of \( A. \) \( \text{sativum} \), facilitated by its Allicin content, acted by stimulating insulin secretion from pancreatic \( \beta \) cells. The phytochemical contents of the Ethanolic extract of \( A. \) \( \text{sativum} \) in our study revealed the presence of varieties of plant natural agents found to possess hypoglycemic properties. Worthy of note are the tannins, flavonoids and alkaloids.\[^{[42,43]}\] In another study, flavonoids were found to be more effective in reducing oxidative stress, lipid peroxidation and increased reduced glutathione/oxidized glutathione (GSH/GSSG) ratio, thus confirming the ethnopharmacological use of \( G. \) \( \text{latifolium} \) in ameliorating the oxidative stress associated with diabetes. Rajasekaran et al\[^{[47]}\] reported the presence of several hypoglycemic-activity possessing elements in the gel of \( A. \) \( \text{perryi} \). They also showed that streptozotocin-induced diabetic rats treated with the ash of \( A. \) \( \text{perryi} \) resulted in hypoglycemic action. Thus, the presence of various inorganic trace elements in the gel appeared to account for the hypoglycemic nature of the plant.

In summary, the four plant extracts used in the present study exhibited approximately the same hypoglycemic capacity in the treated rats. There was also an extent of correlation with the therapeutic capacity of the standard drug, glimepiride.

---

**Table 1: Reduction in levels FBG concentrations in the presence of ethanolic extracts of \( V. \) \( \text{album} \), \( A. \) \( \text{sativum} \), \( G. \) \( \text{latifolium} \), \( A. \) \( \text{perryi} \) and glimepiride in alloxan-induced diabetic rats**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>( D + V. ) ( \text{album} )</th>
<th>( D + A. ) ( \text{sativum} )</th>
<th>( D + G. ) ( \text{latifolium} )</th>
<th>( D + A. ) ( \text{perryi} )</th>
<th>Glimepiride</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>5.46 ± 1.08</td>
<td>2.87 ± 1.23</td>
<td>5.49 ± 1.20</td>
<td>3.33 ± 1.09</td>
<td>11.81 ± 0.88</td>
</tr>
<tr>
<td>32</td>
<td>8.41 ± 0.99</td>
<td>6.03 ± 0.96</td>
<td>8.44 ± 0.89</td>
<td>6.25 ± 0.95</td>
<td>16.46 ± 1.11</td>
</tr>
<tr>
<td>48</td>
<td>10.93 ± 0.89</td>
<td>10.60 ± 1.00</td>
<td>8.44 ± 0.99</td>
<td>11.67 ± 1.07</td>
<td>28.69 ± 0.89</td>
</tr>
<tr>
<td>54</td>
<td>15.55 ± 0.92</td>
<td>17.67 ± 0.78</td>
<td>20.25 ± 0.92</td>
<td>22.50 ± 1.02</td>
<td>33.76 ± 0.99</td>
</tr>
</tbody>
</table>

Percentage reductions in the values of FBG concentration are mean of n ± SEM of four (4) blood samples.
REFERENCES


