Antidiabetic activity of aqueous and methanol extract and fractions of Gongronema latifolium (Asclepiadaceae) leaves in Alloxan Diabetic Rats

P. A. Akah, S.U. Uzodinma and C.E. Okolo

ABSTRACT

Rural dwellers in Nigeria often resort to herbal remedy and dietary control in the treatment of diabetes mellitus (DM). This work was undertaken to provide the rationale for the use of the leaves of Gongronema latifolium as a traditional antidiabetic agent. Methanol extract (ME) of the leaves of G. latifolium was prepared by soxhlet extraction while the aqueous extract (AE) was prepared by cold maceration. The methanol extract was separated into fractions by column chromatography to yield methanol fraction (MF), n-hexane fraction (HF) and chloroform fraction (CF). The extract and the fractions were evaluated for antidiabetic effect in alloxan-induced diabetes in rats. The blood sugar levels were assayed as indices of diabetes. The phytochemical analyses of the extracts and fractions as well as the LD_{50} of the ME were determined. The results indicated that intraperitoneal injection of AE, ME, CF, HF, and MF, (200-800 mg/kg/day) exhibited a significant (P<0.05) anti-diabetic effect by ameliorating alloxan-induced increase in blood sugar. Antidiabetic potency of the extracts and fractions was in the order; MF > ME > AE > HF > CF. Phytochemical analysis of the extracts and fractions indicated high concentration of proteins, flavonoids, saponins, alkaloids, terpenoids, and steroids while tannins, reducing sugar and acidic compounds were absent. The LD_{50} of the methanol extract was calculated to be 900mg/kg. The results of this study lend credence to the use of G. latifolium in the management of diabetes mellitus.

Keywords: Diabetes mellitus, Gongronema latifolium, alloxan, blood sugar.

INTRODUCTION

There is a global increase in the prevalence of diabetes mellitus predominantly, related to life styles and the resulting surge in obesity (King et al., 1998). Diabetes mellitus is associated with long term complications such as retinopathy, neuropathy and angiopathy (Kristova et al., 2008). DM is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into account (Trivedi et al., 2004). It has been estimated that about 171 million people worldwide suffer from diabetes (Roglic et al. 2004), and the use of orthodox drugs in the management of DM has not improved the situation. Plants are well known in traditional medicine for their hypoglycaemic activities. Available literature indicates that there are more than 800 plants species showing hypoglycaemic activities (Rajagopal and Sasikala, 2008). There has been increasing demand for the use of plant products with antidiabetic activity due to low cost, easy availability and lesser side effects. Therefore, plants are being continuously explored for their possible effect as hypoglycaemic agents (Bailey and Day, 1989).
Gongronema latifolium, locally known as “utazi” (Igbo, Nigeria) has been used since olden times in Nigerian ethnomedicine for the management of diabetes mellitus (Ugochukwu et al., 2003). Gongronema latifolium (Asclepiadaceae) is an edible rainforest plant native to the South Eastern part of Nigeria, has been widely used in folk medicine as a spice and vegetable (Gamaniel and Akah, 1996, Morebise et al., 2002), and for maintaining healthy blood glucose levels. Earlier studies on the crude leaf extract of the plant (Ugochukwu et al., 2003) reported the hypoglycemic, hypolipidemic and antioxidative effects in diabetic rats. In this study we evaluated the leaf crude extracts of G. latifolium and their solvent fractions for hypoglycemic activity in alloxan diabetic rats in order to ascertain the fractions/phytoconstituents endowed with the hypoglycemic effect.

MATERIALS AND METHODS

Plant material

Fresh leaves of G. latifolium were collected in May, 2009 from Nsukka market, Enugu state, Nigeria and were botanically identified by Mr. A. Ozioko of Bioresources Development and Conservation Programme (BDCP), Nsukka Enugu state Nigeria. The fresh leaves were air dried under shade for 7 days and pulverized into coarse powder. About 1 kg of the powder was extracted with 2.5 L of methanol by cold maceration for 48 hours and filtered (Trease and Evans, 2002). The filtrate was dried in rotary evaporator to obtain the methanol extract (200 g). The ME was further fractionated, using chromatographic techniques, with the following solvents in the order of increasing polarity viz n-hexane, chloroform and methanol. The yields afforded n-hexane (HF; 43.3 g; 14.3% w/w), chloroform (CF; 94 g 31.3% w/w), and methanol (ME, 122g; 406% w/w) fractions.

Also 1 kg of the powder was extracted with distilled water (100 ml) by cold maceration for 48 hours. The filtrate was freeze dried to obtain the aqueous extract (AE, 122 g, 12.2%). The fractions and extracts were subjected to phytochemical screening using standard methods (Harborne, 1988).

**Acute toxicity test** (LD₅₀)

The oral acute toxicity of the methanol extract (ME) was determined in mice as described by Lorke (1983).

**Induction of diabetes mellitus**

Alloxan monohydrate was used to induce diabetes in rats. Diabetes was induced by injecting a dose of 120 mg/kg of alloxan monohydrate intraperitoneally (Kannur et al., 2006). The alloxanized rats were kept for 7 days with free access to food and water. The rats were fasted on the 8th day for 12 hours and their blood glucose levels were determined using One Touch Glucometer (Lifescan, Johnson & Johnson, California, USA). Rats with glucose levels above 120 mg/dl were used for the study.

**Treatment protocol**

The diabetic rats were randomly divided into five groups (n = 6/groups). Group 1, 2 and 3 received 200, 400, and 800 mg/kg of aqueous extract respectively. Group 4 received 2 mg/kg glybenclamide while group 5 received 2 ml/kg normal saline. Same procedures were performed using the methanol extract at same doses. The effect of the extracts on normoglycemic rats was also investigated. In another series of experiment, MF, and HF (400 mg/kg) were administered to diabetic rats (n=6)

**Analysis of blood sugar levels.**

Blood samples were collected from the tail vein after overnight fast at the intervals of 0, 2, 4, 8, 16, and 32 hrs. The blood glucose level in the samples was estimated using One Touch Glucometer (Lifescan, Johnson & Johnson, California).

**Statistical analysis**

Data obtained were analyzed using One Way Analysis of Variance (ANOVA) (SPSS Version 14) software and expressed as mean ± SEM. Differences between means were regarded significant at P < 0.05 (LSD post hoc test).

**RESULTS**

**Phytochemical tests**

Phytochemical screening of the extracts and fractions of G. latifolium showed the presence of various chemical constituents. Saponins, proteins, carbohydrates, resins, alkaloids, flavonoids, terpenoids, and steroids are conspicuously present in large amount in the crude extract. (Table 1).

**Acute toxicity test**

Death was recorded in 1000 mg/kg dose therefore a geometric mean of the dose where death occurred and the dose...
preceding the recorded death was calculated and the LD$_{50}$ was found to be 0.9 g/kg.

<table>
<thead>
<tr>
<th>Table 1: Results of Phytochemical Analysis.</th>
<th>Crude Extract</th>
<th>Methanol Fraction</th>
<th>Chloroform Fraction</th>
<th>n-Hexane Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saponins</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
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<tr>
<td>2. Proteins</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
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<tr>
<td>3. Tannins</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>4. Carbohydrates</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
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<tr>
<td>5. Reducing Sugars</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6. Resins</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>7. Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>8. Alkaloids</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>9. Glycosides</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10. Terpenoids</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11. Steroids</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>12. Fats and oils</td>
<td>+</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>13. Acidic compounds</td>
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**Effect of the aqueous extract on glycemic and non-glycemic rats**

The effect of aqueous extract on the blood sugar level of diabetic rats was presented in Fig 1. There was a gradual decrease in the blood sugar level from 0 hour to 32 hours. The decrease seems to be significant (p<0.05) in the 800 mg/kg dose. There was no significant decrease in the blood sugar level of normoglycaemic rats treated with *G. latifolium* aqueous extract.

**Effect of the fraction on glycemic rats**

The effects of methanol fraction (MF), chloroform fraction (CF), n-hexane fraction (HF) and glibenclamide on the blood glucose level of diabetic rats are presented in Fig 4. The MF recorded a marked decrease in the blood glucose levels from the 2nd hour till 32nd hour (P< 0.05). The n-hexane fraction and chloroform fraction showed non-significant (P < 0.01) effect on 2nd to 16th hour after treatment but a significant (p < 0.05) on the 32nd hour.

**Effect of methanol extract on glycemic and non-glycemic rats**

The anti-hyperglycemic effect of the methanolic extract of the leaves of *G. latifolium* (200, 400, 800 mg/kg) and glibenclamide (2 mg/kg) on blood glucose levels of diabetic and non-diabetic rats are shown in Fig 2 and 3. The extract exhibited effect in a dose dependent manner. The 800 mg/kg of methanol extract showed a significant (p < 0.05) decrease in the blood sugar level while the other doses (200 and 400 mg/kg) showed no significant effect (p>0.05). In non-diabetic rats, the reduction in the blood glucose levels by the methanol extract was not significant (p ≥ 0.05) in the diabetic rats. The percentage reduction in blood sugar was significant (p ≥ 0.05) at 8th, 16th and 32nd hour after treatment with 800 mg/kg of *G. latifolium*. 

![Fig 1: Effects of aqueous extract of *G. Latifolium* on diabetic rats.](image1)

![Fig 2: Effects of methanol extract of *G. Latifolium* on non-diabetic rats.](image2)

![Fig 3: Effects of methanol extract of *G. Latifolium* on diabetic rats.](image3)

![Fig 4: Effect of fractions of *G. Latifolium* on diabetic rats.](image4)
DISCUSSION

Diabetes mellitus is a fastest growing metabolic disease in the world, and as the knowledge of the multi factorial/heterogeneous nature of the disease increases so does the need for more challenging and appropriate therapies. Diabetes mellitus is one of the most common chronic diseases and is associated with hyper lipidemia and co-morbidities such as obesity and hypertension (Bierman et al., 1975). In order to establish the scientific basis for the utility of G. latifolium in the treatment of diabetes, evaluation of the hypoglycemic activity of the methanolic extract and solvent fractions on normal and diabetic rats was carried out.

The results of the present study indicated that G. latifolium leaf extracts and fractions reduced the glucose level in alloxan diabetic rats. Alloxan is known to induce free radical production and cause tissue injury, and the pancreas is especially susceptible to the action of alloxan induced free radical damage. The ME exhibited significant (p < 0.05) antihyperglycemic activity in alloxan – induced hyperglycemia without causing hypoglycemia (Fig 2). It has been suggested that regeneration of islet beta cell following destruction by alloxan may be the primary mechanism of the recovery of alloxan-injected rats following drug administration (Gorray et al., 1986). Therefore, G. latifolium could be inducing pancreatic cell regeneration. Similar effects in streptozotocin-treated diabetic animals were reported for “Pancreas Tonic” (Rao et al., 1986). The phytochemical result showed that MF was very rich in terpenoids, saponins, flavonoids, glycosides and carbohydrate. Literature showed that saponins and flavonoids are good antidiabetic metabolites (Sharma et al., 2010). Other fractions (CF and HF) lacked these metabolites and this may account for their non-significant (P ≥ 0.05) antidiabetic effect. Alkaloids, glycosides, carbohydrate, bitter principles and saponins have similarly been implicated in the antidiabetic activities of plant (Reher et al., 1991; Sikarwar and Patil, 2010). The non- significant (p> 0.05) effect on normoglycemic rats suggest that unlike insulin and other common hypoglycemic agent (Khan and Shechter, 1991) overdose of the extract may not result in hypoglycemia. The extract appears relatively safe judging from the acute toxicity test. The result of this present study therefore justifies the local use of G. latifolium leaves in the treatment of diabetes mellitus.

REFERENCES


