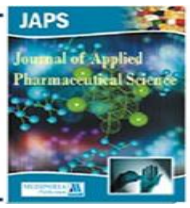
Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 15-11-2011
Revised on: 19:11:2011
Accepted on: 22-11-2011

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

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

P. A. Akah, C.E. Okolo
Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. Nigeria

S.U. Uzodinma
Department of Clinical Pharmacy, Nnamdi Azikiwe University, Awka, Nigeria

For Correspondence
P. A. Akah
Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. Nigeria

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 *Gogronema 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 lead 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 activity (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).

There is a high prevalence of utilization of alternative medicines for the treatment of diabetes mellitus in many regions of the world (Akah *et al.,* 2002). In Africa, some of the plants commonly used for the treatment of diabetes include, *Bridelia ferruginea* (Iwu, 1980), *Dioscorea dumentorum* (Undie and Akubue, 1986, Iwu *et al.*, 1990) and *Vernonia amygdalina* (Akah and Okafor, 1992). There are experimental evidence for the hypoglycemic effects of some medicinal plants, at least in experimental model of diabetes (Akah and Okafor, 1992, Akah *et al*., 2002). Bitter principles from the fruist of *Momordica charantia* (Sharma *et al.,* 1960, Chatterjee, I963) and leaves of *Vernonia amygdalina* (Akah and Okafor, 1992) have been associated with hypoglycemic effects.

One plant, *Gongronema latifolium*, locally knows 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) 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 Evens 2002). The filtrate was dried in rotatory 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).

Animals

Adult albino rats of both sex (110-200 g) bred in the Laboratory Animal House of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria were used for the study. The animals were maintained under standard laboratory conditions and had free access to standard pellets (Top feeds PLC, Nigeria) and water. On transfer to the laboratory, animals were allowed two weeks of acclimatization before the commencement of the experiment. All animal experiments were in compliance with National Institute of Health Guide for care and use of laboratory Animals (Pub No. 85-23 revised), and approved by the University Ethical Committee on the use of Laboratory Animals.

Acute toxicity test (LD_{50})

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 intraperitonially (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 8^{th} 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 1: Results of Phytochemical Analysis.

S. No	Constituent	Crude Extract	Methanol Fraction	Chloroform Fraction	n-hexane Fraction
1.	Saponins	+ + +	+ + +	-	-
2.	Proteins	+ + +	+ + + +	-	-
3.	Tannins	-	-	-	-
4.	Carbohydrates	+ + +	+ + +	-	-
5.	Reducing Sugars	-	-	-	-
6.	Resins	+ + +	+	+ + +	+ + + +
7.	Flavonoids	+ +	+	+ +	-
8.	Alkaloids	+ + +	+ +	+ + +	-
9.	Glycosides	+ + +	+ + +	-	-
10.	Terperoids	+ + +	+ +	-	+ + + +
11.	Steroids	+ + +	+ +	-	+ + + +
12.	Fats and oils	+	-	-	+ +
13.	Acidic compounds	-	-	-	-

(-) => Not Present . (+) => Present in small concentration. (++) => Present in moderately high concentration. (+++) => Present in high concentration. (++++) => Abundantly Present.

Effect of the aqueous extract on glycemic and non-glycemic rats

The effect of aqueous extract on the blood sugar level of glycemic rats is 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 normaglycaemic rats treated with *G. latifolium* aqueous extract.

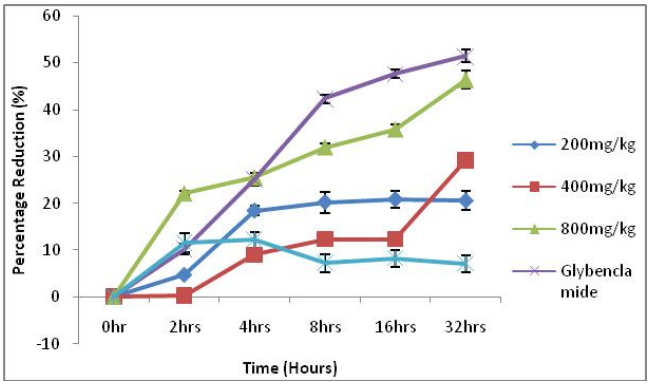


Fig 1: Effects of aqueous extract of *G. Latifolium* on diabetic rats.

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 ($p \geq 0.05$) in the diabetic rats. The percentage reduction in blood sugar was significant ($p \geq 0.05$) at 8th, 16th and 32nd hour after treatment with 800 mg/kg of *G. latifolium*.

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.

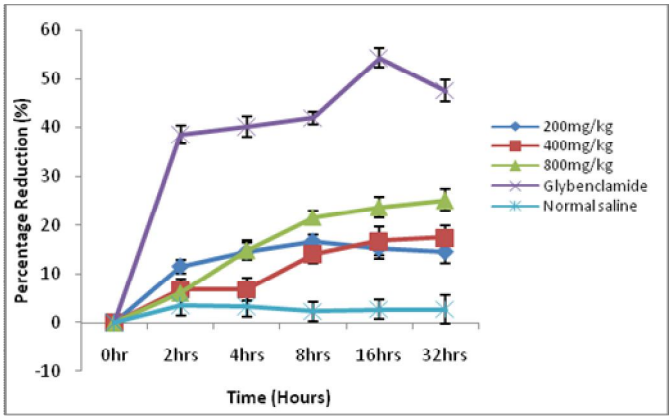


Fig 2: Effects of methanol extract of *G. Latifolium* on non-diabetic rats.

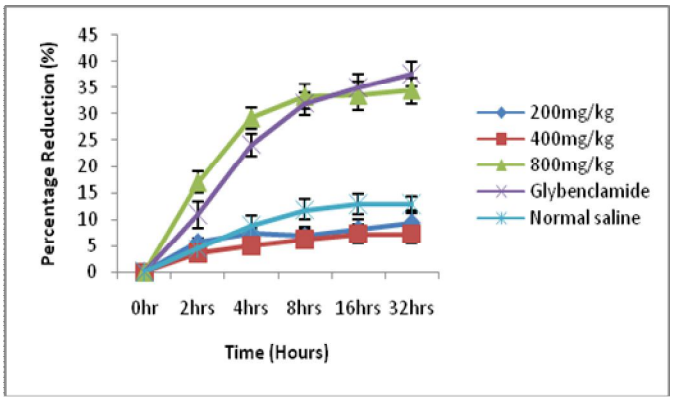


Fig 3: Effects of methanol extract of *G. Latifolium* on diabetic rats.

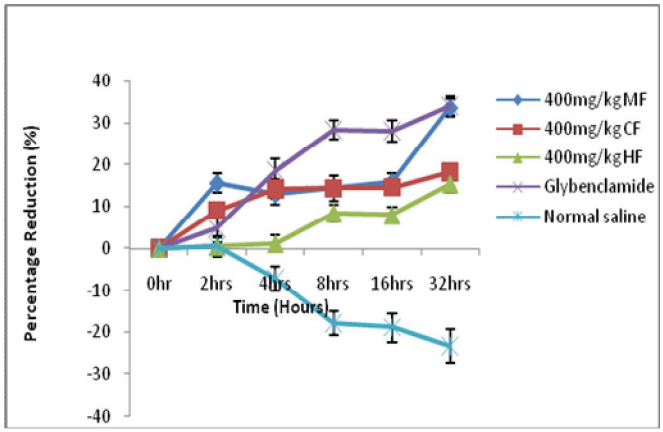


Fig 4: Effect of fractions of *G. Latifolium* on diabetic rats.

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 ($P \geq 0.05$) 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

Akah P. A and Okafor C. L. Blood sugar lowering effect of *Vernonia amygdalina* in an experimental rabbit model. Phytotherapy Res. 1992; 6:171-173.

Akah P. A, Okoli C. O, Nwafor S. V. Phytotherapy in the management of diabetes mellitus. J. Natural Remedies. 2002; 2: 1-10

Bailey C.J. and Day C. Traditional plants medicine as treatment for diabetes. Diabetes Care. 1989; 12:553-564.

Bierman E.L., Amaral J.A.P. and Balknap B.H. Hyperlipidemia and Diabetes mellitus. Diabetes. 1975; 25: 509-515.

Chatterjee, K. P. On the presence of an antidiabetic principles of *Momordica charantia*. India J. Physiol. Pharmacol. 1963;7: 240-245.

Gamaniel K. S and Akah P. A. Analysis of the gastrointestinal relaxing effect of the stem extract of *Gongronema latifolium*. Phytomedicine. 1996; 2:293-296.

Gorray, K.C., Baskin D. Brodsky J. and Fujimota W.Y. Responses of Pancreatic B-cell to alloxan and streptozotocin in the guinea pig. Pancreas. 1986;1 (2): 130-138.

Harborne J.B. Phytochemical methods. Chapman and Hall. London. (1989) 166-226.

Iwu, M. M. Antidiabetic properties of *Bridelia ferruginea* leaves. Planta Med. 1980;39: 247.

Iwm, M. M, Okunji, C. O, Akah, P. A, Tempesta, M. S and Corley, D. G. Dioscoretine: the hypoglycemic principle of *Dioscorea dumentorium*. Planta Med. 1990;56: 119-120

Kannur D. M., Mukkeri V.I and Akki P. Antidiabetic activity of *Caesalpinia bonducella* seed extracts in rats. Fitoterapia. 2006; 77: 546-549.

Khan C.R. and Shedter. Insulin, Oral hypoglycaemic agents in Goodman and Gilman's The Pharmacological Basis of Therapeutics. 8th ed. Pergamon Press. New York. (1991) 1462-1495.

King H., Aubert R.E. and Herman W.H. Global burden of diabetes. Prevalence, numerical estimates and Projection. Diabetes Care. 1998; 21: 1414-1431.

Kristova V, Liskoya S, Sotnikova, S, Vojtko R. Kurtansky A. SUlodexide improves endothelial dysfunction in streptozocin induced diabetes in rats. Physiol. Res. 2008: 491-494

Lorke D. A new Approach to Practical Acute Toxicity Testing. Arch. Toxicol. 1983; 54: 275-287.

Morebise O, Fafunso M. A., Makinde J.M., Olagide O.A and Awe E.O. Anti-inflammatory property of the leaves of *Gongronema latifolium*. Phytother. Res. 2002;16:75-77

Rajapogal K and Sasikala S. Anti-hyperglycaemic and anti-hyperlipidaemic effects of *Nymphaca stellate* in alloxan induced diabetic rats. Singapore Med. J. 2008; 49:137-141

Rao R.M. Salem F.A. and Gleason-Jordan I. Antidiabetic effects of a dietry supplementary "Pancreas Tonic". J. Nat Med. Assoc. 1998; 90 (10): 614-618.

Reher. G., Slijepcevic M and Krans L. Hypoglycaemic activity of triterpenes and tannins from *Sarcopterium spinosum* and two sanguisorba species. Planta Med. 1991; 57:57-58.

Roglic G, Wild S, Green A, Sicree R and King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27 (5) 1047-1053.

Sikarwar M.S. and Patil M.B. Antidiabetic activity of *Crateva nurvala* stem bark extracts in alloxan-induced diabetic rats. J. Pharm. Bioallied. Sci. 2010; 2:18-21.

Sharma, V. N, Sogani, R. K and Arora, R. O. Some observations on the hypoglycemic activity of *Momordica charantia*. Indian J. Med. Res. 1960; 48: 471-475.

Sherma R.D., Sarkhar D.K. and Hazra M.B. Toxicological evaluation of fenugreek seeds: a long term feeding experiment in diabetic patients. Phytother. Res. 2010; 10: 519-520.

Trivedi, N.A. Majumder B. Bhatt J.B. Hermavathi K.G. Effects of Shilajit on blood glucose and lipid profile in alloxan-induced diabetic rats. Indian J. Pharmacol. 2004; 36: 373-376

Ugochukwu N. H, Babady N.E, Cobourne M and Gasses S.R. The effect of *Gongronema latifolium* extract on serum lipid profile and oxidative stress in hepatocyte of diabetic rats. J. Biosci. 2003; 28 (1): 1 – 5.

Undie, A.S and Akubue P. I. Pharmacological evaluation of *Dioscorea dumentorium* tuber used in traditional antidiabetic therapy. J. Ethnopharmacol. 1986; 15: 133-144.