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Full Length Research Paper

Evaluation of hypoglycaemic activity of ethanol extract of *Gongronema latifolium* (Asclepiadaceae) leaves

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The mechanism of anti-diabetic activity of *Gongronema latifolium* was evaluated. The ethanol extract of the leaves of *G. latifolium* were fractionated using solvents of increasing polarity, namely n-hexane, chloroform, ethylacetate and ethanol. Phytochemical screening of the dried fractions were carried and then acute toxicity tests on mice carried out. The induction of diabetes mellitus was achieved with 150 mg/kg b.w for mild diabetes and 300 mg/kg b.w for the severe condition. The effects of the crude ethanol extract (CEE) and its fractions on alloxan-induced hyperglycaemia were monitored. The result obtained reveals that crude ethanol extract significantly and dose-dependently reduced hyperglycaemia. The fractions of the ethanol extract equally reduced hyperglycaemia but the level of reduction was affected by the phytochemical content. This suggests that an intact pancreas is required for the hypoglycaemic action which is the mechanism of action of the sulphonylureas.

Key words: Anti-diabetic, *Gongronema latifolium*, hyperglycaemia, hypoglycaemia, phytochemical screening.

INTRODUCTION

Diabetes mellitus is a prototype of chronic disease and one of the world's leading causes of death, illness and reduced quality of life in both industrialised and industrialising nations of the world. It is therefore a major public health problem because of the direct and indirect cost of its treatment and the elevated morbidity and mortality of the disease (Matthews and Matthews, 2011). The British Medical Association (BMA) dictionary defines diabetes mellitus as a disorder caused by insufficient or absent production of the hormone insulin by the pancreas or because the tissues are resistant to the effects. Recently, DeFronzo et al. (2013) described the main factors involved in the diabetes pathophysiology, calling them the ominous octet: reduced peripheral insulin resistance, characterized by: 1) increased lipolysis, 2) reduction of glucose uptake by muscle, liver and adipose tissue, 3) beta cell dysfunction, with relative decrease in insulin production; 4) increased hepatic gluconeogenesis, 5) reduction of the incretin effect (optimization of insulin and glucagon glucose -dependent secretion, reduced gastric emptying, and induced satiety by GLP-1 - glucagon like peptide-1) present in the intestine, 6) increased renal glucose reabsorption, 7) hyperglucagonemia, and 8) neurotransmitter dysfunction. Either ways,

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Abbreviations: CEE, Crude ethanol extract; **nHE,** n-hexane extract; **CLE,** chloroform extract; **EAE,** ethylacetate extract; **REE,** residual ethanol extract.

hyperglycaemia generated in the blood triggers a whole chain of homeostatic disorders resulting in derengagement of metabolic processes of carbohydrates, proteins and fats (DeFronzo et al., 2013). Clinical conditions of the vascular system, kidney, eye, nerves and skin affect all ages and imposes huge economic burden on families.

Pharmacological intervention in the disease has a primary objective, which is targeted towards reducing hyperglycaemia and minimizing macrovascular and microvascular complications of the disease. According to United Kingdom Perspective Study (UKPDS), intensive glycaemic control will reduce risk of microvascular complications in patients with type 2 diabetes. Reduction of microvascular complications with intensive treatment of hyperglycemia were also observed in patients with type 1 diabetes (DCCT, 1993). This involves the use of insulin or oral hypoglycaemic agents. Hypoglycemic agents act on different pathways of diabetes and can restore the glycemia in different ways, such as increasing pancreatic insulin secretion, increasing glucose uptake by muscle and fat tissue, inhibiting hepatic glucose production, stimulating the incretin effect, reducing the renal or intestinal glucose uptake and directly replacing insulin (Inzucchi et al., 2012; Shlafer and Marieb, 1989). Based on the fact that hyperglycemia is caused by multiple factors, and none of the available drugs act on all metabolic disorders of diabetes, often becomes necessary to combined two or more drugs therapy to achieve glycemic targets. In their observation, UKPDS noted that despite the introduction of new oral hypoglycaemic agents for the management of diabetes mellitus, they have not yet been able to control the blood glucose level satisfactorily or reduce the complications of end organ anomalies associated with the disease. Thus, the discovery of new drugs or agents able to reduce glycemia and minimize complications of this disease is needed. Traditional antidiabetic plant treatment provides an object lesson in the functionality of foods (DCCT, 1993).

The active principles contained in medicinal herbs not only alleviate dysfunctions but also regulate life processes and prevent disease conditions. Phytochemical componenents such as flavonoids from *Pterospartum tridentum* (Middleton and Kandaswami, 1993), alkaloid, boldine from boldo *Peumus boldus molin* (Jang et al., 2000), and steroidal saponins from fenugreek *Trignolla foenum-graecum* (Sauvaire et al.,1996) have been shown to interfere with free radical production and cholesterol deposition as well as protect against diabetes. Others operate as insulin like substance (Li et al., 2005) or in pancreatic regeneration (Shanmugasundaram et al., 1990) in order to suppress blood glucose levels. These components are combined with complimentary components which give the plant as a whole a safety and efficiency much superior to that of its isolated pure active compound (Pamplona - Roger, 2001).

Gongronema latifolium (Utazi) is a Nigerian dietary vegetable attributed with many medicinal properties and listed among the medicinally important vegetables of South West Nigeria (Eleyimi, 2008). Both whole vegetable feeding (Eleyimi, 2008) and extracts of the leaf and stem have also been implicated in reduction of blood glucose levels (Akinnuga et al., 2011 and Eleyimi, 2008). However, the chemical components of the vegetable involved in reducing hyperglycaemia and their mechanism of blood glucose lowering have not been established (Akinnuga et al., 2011). This work is therefore aimed at identifying the phytochemical components associated with reduction of hyperglycaemia and the probable mechanism by which the blood glucose reduction is achieved.

MATERIALS AND METHODS

Plant materials

Fresh leaf samples of *G. latifolium* Benth Asclepiadaceae (Utazi in Igbo, aroeke in Yoruba) were purchased from vegetable markets in Nsukka, Nigeria. They were air dried under shade and pulverized. The sample was weighed and stored in airtight containers.

Extraction

Pulverized air-dried leaves (1 kg) of the *G. latifolium* were macerated in 5 L of 96% ethanol for 48 h and the Whatman No. 1 filtrate (CEE) was dried at 40°C and stored at -10°C for use. The CEE (75 g) was fractionated on silica using solvents of increasing polarity: n-hexane, chloroform, ethylacetate and ethanol. Each of the fractions was dried and stored for use. Phytochemical screening of crude ethanol extract and its fractions were carried out using standard biochemical methods of Harborne (1973).

Animals

Mice

Albino mice (21) weighing between (17 to 30 g) were purchased from Departments of Pharmacology and Toxicology animal house of University of Nigeria, Nsukka. They were housed in metal cages in the Department of Biochemistry animal house under standard conditions of 12 h light/dark cycles, fed with pelleted feed and water *ad libitum*.

Rats

About 80 inbred Wistar albino rats weighing (150 to 250 g) were purchased from the Faculty of Veterinary Medicine animal house of University of Nigeria, Nsukka. They were also housed in the Department of Biochemistry animal house in similar conditions as those of the mice. Ethical laws on the use of experimental animals were obeyed.

Acute toxicity test

The acute toxicity study of the crude ethanol extract was carried by Lorke (1983) method using mice. The first phase involved three

Table 1. Acute toxicity study of the crude extract.

Group	Dose (mg/kg)	Observation
1	10	No fatality
2	100	No fatality
3	1000	No fatality
4	1600	No fatality
5	2900	No fatality
6	5000	No fatality

Table 2. Phytochemical composition of the crude extract/sub-fractions and n-hexane fraction of *Gongronema latifolium.*

Group	Phytochemical component
Crude ethanol extract (CEE)	Resins, terpenoid, steroid, fats/oils, alkaloids, flavonoids, saponins, carbohydrates, reducing sugars, proteins, glycosides
n-hexane extract (nHE)	Resins, terpenoids, steroids, fats/oils
Chloroform extract (CLE)	Resins, terpenoids, steroids, fats/oils, alkaloids, flavonoids
Ethylacetate extract (EAE)	Resins, terpenoids steroids, fats/oils, flavonoids, glycosides
Residual ethanol extract	Resins, terpenoids, steroids, flavonoids, saponins, alkaloids, carbohydrates, glycosides, proteins, reducing sugars

groups of three mice each administered 10, 100 and 1000 (mg/kg b.w) solutions of the crude ethanol extract (CEE) intraperitonally. The animals were monitored for 24 h for fatalities and signs of toxicity. The second phase involved four groups of three mice each administered 1600, 2900 and 5000 (kg/b.w) or saline extract and observation for fatalities done.

Anti-diabetic study

The method of Abdel-Hassan et al. (2001) was used to determine the effect of crude ethanol extract on diabetes mellitus on wistar albino rats weighing between (150 to 250 g). After an overnight fast, diabetes mellitus was induced by intraperitoneal administration of aqueous solution of alloxan monohydrate (Sigma-Aldrich, USA) (150 mg/kg b.w) to five groups of five rats each with an uninduced sixth group. Diabetes was confirmed after 72 h of blood glucose level above 300 mg/dl from blood collected from the tail (Al-Hader et al., 1994). Varied doses of the crude ethanol (50, 100 and 300 mg/kg b.w.) fixed dose of the standard drug (100 mg/kg) and the control were administered orally to the appropriate groups and blood glucose levels were monitored at intervals of 0, 1, 3, 6, 12 and 24 h. The percentage reduction in blood glucose level was computed for each dose of extract and the standard drug.

A second set of six groups of five rats each and a control (uninduced) were also treated with the same dose of the alloxan (150 mg/kg b.w). This time a fixed dose of the four fractions of the ethanol extract, the control and standard drug were administered orally according to the procedure described above.

A third set of five groups of five rats each and a sixth group (uninduced) were administered with higher dose of alloxan (300 mg/kg b.w) according to procedure described above. Fixed doses of crude and four fractions (300 mg/kg b.w) were administered orally and blood glucose levels monitored at those hours.

Statistical analysis

Data from the study was analysed by SPSS version18 using one way analysis of variance and subjected to Fischer LSD post HOC. Results were expressed as mean ± SEM. Differences between means were considered significant at $p<0.05$.

RESULTS

The result in Table 1 reveals that none of the mice died even at highest doses of the extract (2900 and 5000 mg/kg). Therefore, the extract is safe and non-toxic for use. These fractions were known to exhibit pharmacologically active phytochemicals namely flavonoids, saponins, alkaloids, glycosides, steroids, terpenoids, fats, oils and resins to name a few. The fractions of the crude varied in their composition of phytochemicals as shown in Table 2.

The result from Table 3 shows that the glibenclamide significantly ($p < 0.05$) reduced mean blood glucose level (85%). Similarly and dose-dependently, the ethanol extract inhibited blood glucose level by 75.3, 80.4 and 83.2% at 50, 100 and 300 mg/kg doses, respectively. However reduction in blood glucose level was more consistent over the period of time with 100 mg/kg extract than the other test doses. In addition, the standard drug gave the highest blood glucose level inhibition (85.2) than the highest dose of extract (83.2).

Table 3. Effect of the crude ethanol extract of *Gongronema latifolium* leaves on 150 mg/kg b.w. alloxan-induced diabetes mellitus.

Treatment group	Dose	Mean blood glucose concentration (mg/100 ml)/Time (h)						
		0	1	3	6	12	24	% Reduction after 24 h
Normoglycaemic	5 ml	124.25 ± 5.5[a]	114.50 ± 8.0[a]	88.25 ± 20.8[a]	83.50 ± 6.8[a]	77.00 ± 3.7[a]	59.25 ± 4.5[a]	52.30
Diabetic (CEE)-treated	50 mg	365.00 ± 23.4	344.25 ± 551.1	309.50 ± 45.6	261.75 ± 82.6	245.00 ± 88.9	90.00 ± 10.1	75.30
Diabetic (CEE)-treated	100 mg	435.50 ± 54.3	406.00 ± 74.3	330.50 ± 42.3	315.00 ± 56.9	148.25 ± 22.1	85.25 ± 6.2[a]	80.40
Diabetic (CEE)-treated	300 mg	427.00 ± 62.2	455.50 ± 53.50	434.75 ± 58.6	470.50 ± 54.9	434.75 ± 70.4	71.75 ± 9.9[a]	83.20
Diabetic-Untreated: Tween/solution	5 ml	356.25 ± 23.67	429.50 ± 21.9	363.50 ± 27.1	384.00 ± 19.0	341.25 ± 22.5	270.00 ± 9.2	24.2
Diabetic- treated glbenclamide	100 mg	460.75 ± 23.6	257.50 ± 22.4[a]	200.25 ± 10.6[x]	156.50[x] ± 24.5	118.25 ± 28.6[x]	68.25± 9.2[a]	85.2

Values presented as mean ± SEM, [x] P<0.05 [a]P<0.001 against negative control.

Table 4. Effect of CEE and fractions on blood glucose level at 300 mg/kg alloxan-induced diabetes in rats.

Treatment group	Dose (mg/kg)	Mean blood glucose concentration (mg/100 ml)/Time (h)						
		0	1	3	6	12	24	% Reduction
Normoglycaemic	5 ml	124.25 ± 5.5	114.50 ± 8.9	88.25 ± 20.8	83.50 ± 6.8	77.00 ± 3.7	59.25 ± 4.5	52.3
Diabetic CEE- treated	300	513.00 ± 40.0	419.0 ± 49.0	412.00 ± 36	433.0 ± 22.0	401.00 ± 25.0	418.00 ± 26.00	18.52
Diabetic n-HE- treated	300	519.00 ± 12.0	494.00 ± 23.0	523.0 ± 69.0	523.0 ± 53	515.0 ± 46	495.00± 39.0	4.6
Diabetic CLE- treated	300	390.00 ± 40.0	347.50 ± 35.0	371.00 ± 36.0	424.0 ± 41.0	370.0 ± 28.0	432.00 ± 84.0	5.13
Diabetic EAE- treated	300	468.00 ± 52	517.0 ± 32.0	599.0 ± 0.7	600.0 ± 0.0	520.0 ± 32.0	562.00 ± 23.0	6.30
Diabetic REE- treated	300	464.00 ± 33	491.00 ± 65.0	524.0 ± 38.0	536.0 ± 25.0	545.0 ± 28	502.00 ± 8.0	7.89
Diabetic- untreated	300	593.800 ± 55.0	592.0 ± 8.0	600.0 ± 0.0	600.0 ± 5.0	592.0 ± 8.0	556.17 ± 47	6.33
Diabetic treated glibenclamide	100	374.00 ± 33.0	297.0 ± 6.0	218.0 ± 24.0	152.0 ± 19.0	113.0 ± 9.6	77.0 ± 2.0	79.4

No significant difference of means between treated groups.

Effect of CEE and its fractions on blood glucose level

The mean blood glucose level is recorded in Table 4. The result shows that the extracts and glibenclamide significantly (p<0.05) reduced blood glucose with respect to the untreated. The percentage blood glucose levels of REE, EAE, CLE and n-HE were 77.9, 76.4, 72.1 and 56.1%, respectively. However, glibenclamide gave higher reduction of blood glucose level (83.47%).

Effect of crude and its fractions at severe induction of diabetes

The mean blood glucose level of crude ethanol extract and its fractions at severe induction of diabetes is shown in Table 4. There was no significant reduction of blood glucose level by the extracts but glibenclamide still showed reduction in blood glucose level.

DISCUSSION

The results from this work show that *G. latifolium*

leaf extract contained important phytochemicals such as terpenoids, steroids, flavonoid, alkaloids and saponins in the other and glycosides associated with anti-diabetic activities. Many studies have implicated these fiber, alkaloids, flavonoids such as quercitin, kaempferol and caffeoyl glucoside as well as saponins and glycosides have all been demonstrated to inhibit hyperglycaemia in animal models (Shimizu et al., 2001 and Abdel- Hassan et al., 2001). Therefore, it can be adduced that the presence of such phytochemicals in the *G. latifolium* leaf extract might account for its hypoglycaemic activity.

The crude extract (CEE) in this study evoked a significant ($P<0.05$) and dose-dependent reduction in blood glucose level.

The effect of the phytochemicals in blood glucose reduction is demonstrated in the result obtained by the fractions of the crude extract. It was also observed that the extracts were inadequate to bring down the blood glucose level when alloxan-induction was severe at 300 mg/kg b.w (Table 4). Alloxan attacks pancreas depleting the beta cells responsible for releasing insulin, the hormone responsible for glucose metabolism (Shimizu et al., 2001). The ineffectiveness of the fractions, therefore, suggests that the extracts might have induced reduction in hyperglycaemia by stimulating the pancreatic beta cells to release insulin, and so in the case of severe damage to the pancreas their potency was lost. This mechanism corresponds to the function of sulphonylureas as oral hypoglycaemic agent (Shlafer and Marieb, 1989). Another member of the Asclepiadaceae family as *G. latifolium*, namely, *Gymnema sylvestre* with potent hypoglycaemic activity has been reported to operate by the same mechanism (Shanmugansundaram et al., 1990). These reporters also implicated saponins in the hypoglycaemic activity of the medicinal plant. In a previous work Shlafer and Marieb (1989), reported that components of the vegetable inhibited alpha glucosidase activity, which is another mode of action of oral hypoglycaemic drugs.

The outcome of this study validates the use of *G. latifolium* as a medicinal plant therapy for diabetes mellitus. The components of the vegetable require an intact pancreas for maximum activity, possibly to increase insulin production, which is the mode of operation of the sulphonylureas.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Abdel-Hassan IA, Abdel-Barry JA, Mohammed ST (2001). The hypoglycaemic and anti hyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. J. Ethnopharmacol. 71:325-330.

Akinnuga AM, Bamidele O, Ekechi P, Adeniyi OS (2011). Effects of an ethanolic leaf extract of *Gongronema latifolium* on some haematological parameters in rats. Afr. J. Biomed. Res. 14(2):153-156.

Al-Hader AA, Hasan ZA, Agel MB (1994). Hypoglycemic and insulin release inhibitory effect of *Rosemorinus officialis*. J. Ethnopharmacol. 43:217-222.

DeFronzo RA, Eldor R, Abdul-Ghani M (2013). Pathophysiologic approach to therapy in patients with newly diagnosed type 2 diabetes. Diabetes Care 36:S127-S138.

Diabetes Control and Complications Trial Research Group (DCCT) (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Eng J Med.* 329:977-86.

Eleyimi AF (2008).Chemical composition and anti-bacterial activity of *Gongronema latifolium*. J. Zhejian Univ. Sci. B. 8(5): 352-358

Harborne JB (1973). Phytochemical methods. A guide to modern techniques of plant analysis 2nd ed., Chapman and Hall. USA. pp. 33-80

Inzucchi SE, Bergenstal RM, Buse JB et al. (2012). Management of hyperglycaemia in type 2 diabetes: a patient-centered approach. Position statment of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia 55:1577-96.

Jang YJ, Song JH, Shin YK, Han ES, Lee CS (2000). Protective effect of boldine on oxidative mitochondrial damage in streptozotocin - induced diabetic rats. Pharmacol. Res. 42(4):361 - 371.

Li Y, Kim J, Liu X, Himmeldirik K, Ren Y, Wagner TE and Chen X (2005). Natural anti-diabetic compound 1, 2, 3, 4, 6 - penta-o galloyl - D - pyranose binds to insulin receptor and activates, insulin - mediated glucose transport signalling pathway. Biochem. Biophys. Res. Commun. 336:430-437.

Lorke D (1983). A new approach to practical acute toxicity testing. Arch. Toxicol. 54:251-281.

Matthews DR, Matthews PC (2011). Banting Memorial Lecture 2010. Type 2 diabetes as an 'infectious' disease: is this the Black Death of the 21st century? Diabet. Med. 28:2-9.

Middleton E, Kandaswami I (1993). The Impact of plant flavonoids on mammalian biology: implication for immunity, inflammation and cancer in the flavonoids: Advances in Research Since 1986 ed. J.B. Harborne, champman and Hall, London. pp. 620 - 652.

Pamplona - Roger GD (2001). Encyclopedia of medicinal plants. Education and Health Library (1) pp. 1 - 398 ed.

Sauvaire Y, Baissac Y, Leconte O, Petite P, Ribes G (1996). Steroid saponins from fenugreek and some of their biological properties in *Saponins Used in Food and Agriculture* eds. Water and Yamasaki, Plenum Press, N.Y. pp. 37-46.

Shanmugasundaram ERB, Gopinath KI, Shanmugasundaram RK and Rajendram VM (1990). Possible regeneration of the islets of langerhans in streptozotocin - diabetic rats given Gymnema sylvestre leaf extracts. J. Ethnopharmacol. 58:39-41.

Shimizu K, Ozeki M, Lino A, Nakajyo S, Urakawa N, Atsuchi M (2001). Structure activity relationships of triterpenoid derivates extracted from *Gymnema inodurum* leaves on glucose absorption. Japan J. Pharmacol. 86:223-229.

Shlafer M, Marieb E (1989). The Nurse, Pharmacology and Drug Therapy. Addison Wesley, Menlopark, California.