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The Effect of *Gongronema latifolium* Leaf Extract on Blood Biochemical Assay in Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author EGS designed the study, wrote the protocol and the first draft of the manuscript, coordinates and interprets the data. Author EUI managed the literature searches, analyses of the study and the experimentation of analysis while author ADO performed the experimental process and identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Gongronema latifolium (GL) is used extensively in the indigenous system of medicine as an anti-diabetic agent. Hypoglycemic and hypolipidemic activities of *Gongronema latifolium* extract in Type 2 diabetic rat models were evaluated using standard analytical methods while Blood was collected on every 3 days through the rat's tail vein for glucose estimation using One Touch Glucometer. Blood glucose in the diabetic animals decreased significantly *(P*=.05) from initial by 66.34% upon treatment with *Gongronema latifolium*. Diabetes induction caused significant increases (*P*=.05) in total cholesterol (TC) with 54.42% and low density lipoprotein (LDL) with 55.0% compared to the

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normal control (NC), while treatment with extracts of *Gongronema latifolium* significantly decreased (*P*=.05) these by (58.70% TC) and (71.70% LDL) respectively. Also the amino transferases (ALT and AST) parameters activities which increased by 66.83% and 72.87% in the diabetic control (DC) rats became reduced upon treatment with *Gongronema latifolium*. Thus the plant extract was capable of ameliorating hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats and could be a potential source for isolation of new orally active agent(s) for anti-diabetic therapy.

Keywords: Aminotransferases; blood glucose; diabetes mellitus; Gongronema latifolium; hyperglycemia; hyperlipidemia.

1. INTRODUCTION

Diabetes mellitus is a chronic, debilitating and often deadly disease. It is a condition that arises when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin produced. Diabetes is a metabolic disorder of multiple aetiology, characterized by chronic hyperglycaemia with disturbances of carbohydrates, fat and protein metabolism owing to overproduction and / or underutilization of glucose [1]. The important metabolism affected by diabetes is the lipid metabolism which has the potential to develop atherosclerosis and cardiovascular complications [1]. In 2003, the International Diabetes Federation estimated that approximately 194 million rise to 333 million, amounting to 6.3% of the world's population living with diabetes and about two-third of these people lived in developing countries [2]. The prevalence of type 2 diabetes is rising at an alarming rate throughout the world, due to increases in life expectancy, obesity and sedentary lifestyles and it is destined to become one of the world's most important and costly diseases. Of particular cause for concern is the dramatic rise of type 2 diabetes in children and adolescents. In type 2 diabetes mellitus lipid abnormalities are almost the rule. Typical finding are elevation of total and VLDL cholesterol, triglyceride concentration, lowering of HDL cholesterol and a predominance of small, dense LDL particles [3].

The African continent counts approximately 13.6 million people with diabetes. The Africa Region of IDF, which mainly includes sub-Saharan Africa, counts approximately 7 million people with diabetes. Estimates for the region for 2025 are likely to double and reach 15 million. Whereas Nigeria has the highest number of people with diabetes (with approximately 1,218,000 people affected), Reunion has the highest diabetes prevalence in the African Region (13.1%). Nigeria also has the highest number of people with impaired glucose tolerance with an estimated 3, 85 million people [2].

Gongronema latifolium Benth Hook, (*Asclepiadaceae*) is an herbaceous shrub, with yellow flowers and a stem that yields characteristic milky exudates when cut. It is commonly grown in gardens in Calabar, Cross River State, Nigeria. It is locally called "utasi" by the Efiks, Ibibio and Quas; "utazi" by the Igbos and "arokeke" by the Yorubas in Nigeria. It is a rain forest plant which has been traditionally used in the South-Eastern part of Nigeria for the management of diseases such as diabetes, high blood pressure and others [4].

Diabetes is the fourth leading cause of death in most developed countries. Each year, over three million deaths worldwide are attributable to diabetes-related causes. Thus in a complex disease like diabetes mellitus, where little is talked about in aspects of prevention and cure, but rather management, there is an increased focus on plants in the search for appropriate hypoglycemic / hypolipidemic agents. Firstly, because the traditional medicine practitioners reported that natural products may be better treatments than currently used conventional drugs. Secondly the plants by secondary metabolic means contain a variety of herbal and non-herbal ingredients that are thought to act on a variety of targets by various modes and mechanisms - given the multi – factorial pathogenicity of the disorders. According to [5], herbal therapies have pharmacological principles within themselves that work together in a dynamic way to produce therapeutic efficacy with minimum side effects. The working hypothesis is 'GL extract may provide the efficacy in the protection against dyslipidemia, hyperglyceamia and hepatotoxicity in diabetes'. It is in this light that this work was designed to investigate the antidiabetic efficacy of GL extract employed traditionally in the management of diabetes in Asia and the African sub-Region.

2. MATERIALS AND METHODS

2.1 Plant Materials and Extraction

Fresh matured *Gongronema latifolium* leaves were collected from a cultivated land at Ibiaku Itam, Nigeria in March, 2012. They were authenticated by Dr. E. G. Amanke in the Department of Botany, University of Calabar, Nigeria and voucher specimen deposited in the Department of Botany herbarium, University of Calabar. The wet method of extraction according to [6] was adopted for the preparation of the ethanol extract.

2.2 Acute Toxicity Study

2.2.1 Animals

The acute toxic study was used to determine a safe dose for *GL.* Swiss Albino mice (20-25 g) of both sexes obtained from the Department of Pharmacology and Toxicology, University of Uyo, Nigeria were used for the experiment after a14 day acclimatization. Animals of the same sex were kept together in clean plastic cages under standard conditions; (temperature 28±2ºC, relative humidity 50±5%) and maintained in a 12 h light/dark cycle. "All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as ethical guide for care and use of laboratory animals of the Faculty of Pharmacy, University of Uyo, Nigeria. All experiments were examined and approved by the appropriate ethics committee.

2.2.2 Methods

Lethal dose (LD_{50}) determination was conducted using the method of [7]. The evaluation was done in two phases. In phase one, four groups of six mice each, were treated with 2000, 4000, 6000 and 8000 mg extract b.w orally respectively. The mice were observed for clinical signs , symptoms of toxicity and death within 24 h. Based on the results of phase one, another four groups of six (6) fresh mice per group were each treated with 1000, 2000, 3000 and 4000 mg extract/kg b.w intraperitoneally (ip) respectively in the second phase. Clinical signs and symptoms of toxic effects and mortality were then observed for 24h. The LD_{50} was then calculated according to [7] using the formulae;

$$LD_{50} = DM - \frac{\Sigma(Z \times d)}{n}$$

Where:

Dm = The largest dose which kills all animals.
z = Mean of dead animals between 2 successive groups
d = The constant factor between 2 successive doses.
n = Number of animals in each group.
Σ = The sum of (z × d)

2.3 Experimental Animals / Induction of Diabetes

Adult male Wistar rats (200-250 g) obtained from the animal house of the Department of Biochemistry, University of Calabar, Nigeria, were used for the experiment. They were housed in polypropylene cages at room temperature under standard conditions (temperature 28±2ºC, relative humidity 50±5%) and maintained in a 12 h light/dark cycle. The animals were allowed to acclimatize for two weeks. Diabetes was induced in thirty fasted overnight rats, by a single intraperitoneal injection of freshly prepared solution of streptozotocin; 50 mgkg-1b.w. in 0.1M cold sodium citrate buffer pH 4.5. The animals that were considered as being diabetic had their blood glucose values to be >200 mg/dL on the third day after streptozotocin injection.

2.4 Chemical / Reagents

Assay kits used for the biochemical assays of Lipid profile- Triglycerides (TG), Total cholesterol (TC), High density lipoprotein (HDL), Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) were obtained from Randox Laboratories Ltd., Admore Diamond Road, Crumlin, Co., Antrim, United Kingdom, Qt 94QY: glucose and Alpha amylase were from Agape Diagnostics LTD. Streptozotocin was from Sigma, USA.

2.5 Experimental Design

Eighteen (18) male rats that were diabetic were then included in the study and were divided into three (3) groups of six (6) animals each while the forth group was the non diabetic(NC). The experimental groupings were as follows: GL = Diabetic rats treated orally with 200 mg/kg of *Gongronema latifolium* twice daily, Insulin = diabetic rats treated subcutaneously with 5 units/kg.b.w of insulin, DC = diabetic control; diabetic rats given distilled water as placebo, NC = non-diabetic rats also given placebo treatment,

the rats were then kept for the next 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycemia. Blood was collected on every 3 days through the rat's tail vein for glucose estimation using One Touch Glucometer; the experimental period was 21 days. At the end of the experimental period which was twenty one days, food was withdrawn from the rats and they were fasted overnight but had free access to water. They were then euthanized under chloroform vapor and sacrificed. Immediately, blood samples were collected for sera preparation by cardiac puncture into sterile plain tubes. Serum samples were separated from the clot by centrifugation at 3,000 rpm for 10 minutes using bench top centrifuge (MSE Minor, England) and stored frozen until needed for analysis. All analysis was completed within 24 hours of sample collection.

2.6 Biochemical Assays

The concentration of the respective parameters; TG, TC and HDL were read directly using AJ 122 chemistry analyser (spectrophotometer) China, whereas the concentration of VLDL was extrapolated by dividing the respective concentration of TG by 5 while LDL-cholesterol was estimated using the method by Friedewald (1972) that LDL-C = TC -(HDL-C) – VLDL. [8], AST and ALT were determined using Reitman and Frankel [9]. Glucose concentrations in the blood were determined by the use of One Touch Glucometer (Lifescan, Inc., 1995 Milpitas, Califonia 95035, USA). Quantitative determination of glucose and Alpha amylase in serum using Agape Diagnostics LTD, ($CNPG_3$ Methodology) based on [10].

2.7 Statistical Analysis

The results were analyzed by one-way ANOVA, using SPSS statistical package. All data were expressed as Mean±SEM and difference between groups considered significant at P = .05.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Acute toxicity studies

When the ethanolic extract of *GL* was administered orally in mice, all the animals remained alive and did not manifest any significant physical sign of toxicity at doses as high as 8000 mg/kg and its LD_{50} was estimated to be >500 mg/kg. Thus it was concluded that *GL* leaf extract orally administered to mice was safe as no drug-related toxicity was detected even at the highest dose investigated over the 24hour observation period. Intraperitoneal administration produces no noticeable neurological or behavioral effects within the six hour observation. However, 100% lethality at 2000 mg/kg and 0% lethality at 1000 mg/kg were recorded after 24 hours, hence intraperitoneally; the LD_{50} was calculated to be 1500 mg/kg.

3.1.2 Effect of treatment on blood and serum glucose levels

This effect is shown on Table 1. The streptozotocin induced diabetes significantly (P=.05) increases the blood glucose level of DC group to 406.67±27.68 mg/dl while its final concentration of 333.00±23.08 mg/dl was significantly higher than GL (169.67±16.62 mg/dl), INSULIN (77.33±10.06 mg/dl), and NC (73.16±2.06 mg/dl) final concentrations. There was a significantly decreased (P=.05) levels of the blood glucose by 66.34, and 86.62 percent respectively in *GL* and insulin groups. The serum glucose level in the DC group (11.92±0.80 mmo/dl) was significantly (P=0.05) higher compared to NC (4.64± 0.20 mmol/L), while *GL* showed a rather significant decrease (P=0.05) in the serum glucose (9.42±0.92 mmol/L, compared with the DC (Table 1).

3.1.3 Effect of treatment on serum lipid profile

Table 2 shows the changes in serum lipid concentration following a 21-day treatment period. Serum TC and LDL concentration was decreased in all the diabetic treated groups except the insulin group that showed a significant increase when compared with DC. These decrease were however non significant, compared to NC but were significant in comparison with DC. Also, GL showed a significant decrease when compared to the insulin group. The GL and insulin groups caused a non significant difference in TG and LDL in comparison with NC. TG in the serum of DC and GL indicated a non significant decrease compared to NC. There was a significant increase in TG of GL when compared with insulin group. Serum VLDL concentration showed a non significant decrease in GL group compared with NC.

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3.1.5 Effect of treatment on some serum enzymes

The effect of some serum enzymes are as shown on Table 3. Serum aminotransferase activities (AST and ALT) were raised significantly (*P*=.05) by 3.19 and 3.01 fold respectively in the DC group relative to NC group. GL and insulin groups caused a significant reduction in AST and ALT relative to the DC group. Alpha-amylase in the DC group (254.14±45.41U/L) increased significantly compared to NC (166.59±26.65U/L). Treatment with the extract caused a significant decrease in the level of the enzyme (171.37±9.97U/L) compared to insulin treated rats (261.19±36.52U/L), indicating protection of the pancreas. It is observed that the enzyme level in the GL (261.19±36.52U/L) was rather significantly increased (*P*=.05) in comparison with NC.

3.2 Discussion

In acute toxicity study, a scale proposed by Lorke [11] roughly classifies substances according to their LD_{50} as follows: Very toxic (LD_{50} < 1.0 mg/kg), toxic (LD_{50} up to 10.0 mg/kg), less toxic (LD_{50} up to 100.0 mg/kg) and only slightly toxic (up to 1000.0 mg/kg). Substances with LD_{50} values greater than 5,000 mg/kg are practically non-toxic. Based on this proposal, the intraperitoneal LD_{50} of 1500 mg/kg show that the *G. latifolium* extract is slightly toxic. The high oral LD_{50} (> 5000 mg/kg) obtained suggested that the extracts are practically non-toxic through this route and are therefore safe in the rats and in its traditional use orally for treatment of the diseases they are indicated for. According to the OECD protocol GL leaf extract may be classified as non toxic since the limited dose of an acute toxicity is generally considered to be 5.0 g/kg bw [12,13].

Streptozocin-induced hyperglycaemia has been described as a useful experimental model to study the activity of antidiabetic agents [14]. Streptozocin selectively destroyed the pancreatic insulin secreting β cells, leaving less active cell resulting in a diabetic state [15]; which was the observation in DC of this study. The present results indicate significant increase in blood and hepatic glucose levels in diabetic rats. The administration of *Gongronema latifolium* actually reduces the blood glucose levels in diabetic rats. This suggests that the plant has hypoglycemic activities. This study confirms the earlier findings that *GL* leaf extract has anti diabetic effect [4]. Three weeks of daily treatment of ethanolic extract of *Gongronema latifolium* led to a fall in blood sugar levels, with the effect reaching maximum after 15 days of treatment and remains constant up to the third week; this was similar to the work of Rao and Naidu [16]

The serum glucose was significantly increased in the DC but the intervention with the extract tends to ameliorate this effect. Reductions in blood and serum glucose in the treated diabetic rats observed in this study are in accordance with the reports of two researches [5,17]. The presence of flavonoids and phenolics compounds in the extract may be responsible for this observation and the possible mechanism though not investigated in this study may be attributed to the ability of the extract to potentiate insulin secretion from pancreatic beta cells or sensitizing insulin receptors [18].

During diabetes, the levels of serum lipids (cholesterol, free fatty acids and phospholipids) are usually elevated. The marked hyperlipaemia that characterizes the diabetic state is a consequence of the uninhibited actions of lipolytic hormones on the fat depots [19,20]. Accelerated, β-oxidation steming from increased fat mobilization (a consequence of insulin deprivation) generates a high concentration of acetyl CoA in the liver and is channelled to cholesterol and triacylglycerol synthesis given that fatty acid synthesis is impaired. These are usually exported to blood as VLDL where they

Table 1. Effect of treatment on blood and serum glucose levels of diabetic and non-diabetic rats

Fasting blood glucose levels				
Group	**Initial (mg/dl)**	**Final (mg/dl)**	**%Change**	**Serum glucose (mmol/L)**
GL	504.00±30.54*,a,b	169.67±16.62*,a,b	66.34*,a,b	9.42±0.92*,b
INSULIN	578.00±6.34*	77.33±10.06*	86.62*	3.97±0.66*
DC	406.67±27.68*,b	333.00±23.08* ,b	18.12*,b	11.92±0.80*,b
NC	73.59±2.21	73.16±2.06	0.58	4.64±0.20

**P<0.05 vs NC; a = P<0.05 vs DC; b = P<0.05 vs insulin; mean ± SE, n = 6, DC = diabetic control, NC = non-diabetic control*

Table 2. Effect of treatment on serum lipid profile

GROUP	TG (mg/dl)	TC(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
GL	66.20±7.57	105.07±5.74a, b	60.20±2.94*,a,b	58.10±5.36*,a, b	13.24±1.51
INSULIN	82.86±9.91	448.59±36.04*	66.36±0.15*	398.80±38.17*, a	16.57±1.98
DC	75.23±14.56	254.40±29.84*	64.22±2.33*	205.23±31.01*	15.05±2.91
NC	76.41±9.65	115.95±14.59	8.26±1.04	92.40±15.06	15.28±1.93

**P=0.05 vs NC; a = P=0.05 vs DC; b = P=0.05 vs insulin; mean ± SE, n = 6, DC = diabetic control, NC = non-diabetic control*

Table 3. Effect of treatment on some serum enzymes in diabetic and non-diabetic rats

GROUP	AST(U/L)	ALT(U/L)	Alpha-amylase (U/L)
GL	41.33±2.01*, a, b	18.27±5.01*, a, b	261.19±36.52a
INSULIN	73.33±2.11*	46.67±1.72*, a	347.78±26.83*
DC	70.67*±0.42*	69.33±18.09*	254.14±45.41*
NC	19.17±2.52	23.00±4.02	166.59±28.65

**P=0.05 vs NC; a = P=0.05 vs DC; b = P=0.05 vs insulin; mean ± SE, n = 6, DC = diabetic control, NC = non-diabetic control*

also meet decreased utilization as a result of the inhibition of degradation-hormone sensitive lipoprotein lipase, by lack of insulin. These accumulate in blood to constitute hypercholesterolemia and triacylglycerolemia of diabetes as evident in this study. Induction of diabetes in this study caused significant increase in TC, LDL and HDL compared to NC but the intervention with GL however reduced these indices, this was in consonance with the works of these scientists [21,22,23]. The hypolipidaemic effect of GL can be explained as a consequence of reduction in blood glucose. This observation indicates the tendency towards fine hormonal regulation or control following extract treatment, as this diabetogenic hormonal interaction play a fine role in glucose control.

Alanine aminotransferase, (ALT), aspartate aminotransferase (AST), are present in the hepatic and biliary cells and these enzymes are usually released from the hepatocytes and leak into circulation causing increase in their serum levels under hepatocellular injury or inflammation of the biliary tract cells resulting predominantly in an elevation of the alkaline phosphatase levels [24]. The plant GL is reported to posses some phytochemicals like flavonoids, tannins and saponins that may play some roles in the hypolipidaemic effect of some plants [25,26,27]. These chemicals tend to act in capillaries and help in recovery of vascularization of the pancreas [28,29].

Diabetes induction with STZ is observed to be associated with increased serum enzyme activities [30]. Hence the elevated levels of the enzymes in DC; ALT and AST generally, was attributed to injury caused to the hepatocydtes by streptozotocin which now affects the normal functions of the liver, since these enzymes are indisputably, markers of liver injury as stated earlier, they are localized in the cytoplasm under normal conditions and are released into the circulation under abnormal conditions (e.g. cellular damage) [31,32]. The increased levels of

ALT and AST in diabetic control rats (DC) observed in this study was a clear indication of a kind of injury or the other, caused by streptozotocin (STZ) toxicity (STZ: Safe Working Practices Information page) [33]. However, treatment for 21 days with extracts of GL reduced the activities of these enzymes indicating the hepatoprotective effect of extract of GL which also have been reported by [34,4]. Pancreatic α-amylase is a key enzyme in the digestive system that catalyses the initial step in hydrolysis of starch to maltose and finally to absorbable glucose. Degradation of dietary starch leads to elevated postprandial hyperglycaemia. Retardation of starch hydrolysis by inhibition of pancreatic α- amylase is one of therapeutic approaches for the control of postprandial hyperglycaemia in pre-diabetes, diabetes and obesity [35]. GL is seen to markedly inhibit pancreatic α-amylase in this study which is in accordance with the following reports [36,5,37]. These results suggest that GL may be potentially useful to control postprandial hyperglycaemia in patients with type 2 diabetes through inhibition of pancreatic α-amylase. It has been shown that the pancreatic α-amylase inhibitory activity of flavonols and flavones (constituents of *G. latifolium*) is associated with hydrogen bonds between the hydroxyl groups of the polyphenol ligands and the catalytic residues of the binding site, leading to formation of a conjugated pi-system that stabilizes the interaction with the active site [38]. Thus, flavones and flavones reported in GL may interact with protein via hydroxyl groups to form polar groups of hydrogen bonds in amino acid residues using covalent and/or non-covalent interaction.

4. CONCLUSION

The findings of this work clearly show that GL posses hypoglycemic and hypolipidemic activities thereby ameliorating the effects of hyperglycemia, and lipid abnormalities in diabetes mellitus.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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