Journal of Advances in Medical and Pharmaceutical Sciences

14(2): 1-12, 2017; Article no.JAMPS.34310
ISSN: 2394-1111

Saponin Fraction of *Gongronema latifolium* Reverses Dyslipidemia and Catalyzes Glucokinase in Lowering Blood Glucose Sugar in Streptozocin-Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author GOI designed the study and principally carried out the research work and wrote the manuscript. Author IAI carried out all Biochemical analysis and methodologies. Author OOE assisted in all laboratory research work and methodologies. Author BIAM contributed in developing and writing the manuscript. Author MUE directed the research work and contributed to key technical decisions. Generally, the conduct of the experiment and writing of the script was done by all the authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2017/34310
Editor(s):
(1) Nissar Darmani, Professor of Pharmacology, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, California, USA.
Reviewers:
(1) James Adams, University of Southern California, USA.
(2) Abdullahi M. Nuhu, Kaduna Polytechnic, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/20025

Original Research Article

Received 23rd May 2017
Accepted 28th June 2017
Published 13th July 2017

ABSTRACT

Objective: *Gongronema latifolium* (GL) is a bitter tasting leaf used in Nigeria as spice in food, and as herb for the treatment of diabetes, malaria and cardiovascular disease. Research work on the anti-diabetic activities of the whole leaf extract has been carried out, but none on the saponin fraction of the leaf. A preliminary study showed that saponin-rich ethyl acetate fraction (GSF) of GL was active as an anti-diabetic agent. The aim of this study was to evaluate the sugar-lowering and anti-lipidemic effects of ethyl acetate saponin fraction (GSF) of GL in Streptozotocin-induced diabetic Albino Wistar rats.

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Methods: Streptozotocin-induced diabetic rats were treated with 200 mg/kg bw and 400 mg/kg bw dose of saponin fraction of *Gongronema latifolium* twice daily for 21 days. The effects of GL fraction on fasting blood glucose (FBG), serum glucose, serum insulin, body weight, total cholesterol (TC), HDL-c, LDL-c, VLDL-c, triglyceride (TG) and C-peptides concentrations were determined.
Results: After 21 days treatment, GSF administration showed significantly lower FBG concentration compared to the diabetic control (DC) group. GSF increased serum insulin concentration and C-peptide levels dose-dependently. The fraction also decreased liver weight, liver cholesterol, plasma cholesterol, total cholesterol and triglyceride concentration dose-dependently. GSF 1 and 2 increased HDL-c and lowered the plasma concentration of LDL-c and VLDL-c. GSF significantly ($p<0.05$) increased body weights of diabetic animals time-dependently taken at 7 days interval.
Conclusion: The findings from this study suggest that GSF may provide alternative sugar-lowering effect in diabetes, and may have the potential to reverse dyslipidemia and improve body weight gain in type 2 diabetes conditions.

Keywords: Diabetes; Gongronema latifolium; saponin-fraction; lipid cholesterol; hypoglycaemic; hypolipidemic.

1. INTRODUCTION

Elevated blood glucose is a common feature in diabetes mellitus, usually detected by testing fasting blood glucose (FBG) concentration in patients. Scientific data from several studies have led to the conclusion that diabetes mellitus is a heterogeneous group of disorders characterized by persistent hyperglycemia, with attendant but persistent dyslipidemia in a majority of cases [1-4]. It is also said to be a heterogeneous metabolic disease which results from a complex and poorly understood interaction between genetic factors and lifestyle components [5]. It is further said to be multifactorial in presentation, difficult to manage and has no clear cut cure because of its complexity in presentation and associated diet needs of sufferers [1,2].

The disease is not only characterized by hyperglycaemia but also by a rise in atherogenic lipid profile, both of which greatly increase the risk of coronary heart disease, organ damage, muscle wastage and several metabolic diseases [2-4].

Across the globe, there is an estimated ten million people suffering from diabetes mellitus. The disease is said to be responsible for over 5% deaths around the globe annually [1]. In type 1 diabetes, there has been a characteristic but attendant damage to key organs including the liver, kidneys and the pancreas. In the liver, the hepatocites are usually lacerated and damaged during diabetes, freely releasing liver enzymes including AST, ALT and ASP into the general blood circulation. The kidney architecture is usually altered with attendant nephrotoxicity, and elevated sodium electrolyte concentration. In the case of the pancreas, the β-cells of the islets of langerhans may be destroyed, resulting in loss of insulin producing cells and insulin availability to clear sugar in the blood stream [3,4,6,7,8].

Diabetes mellitus is more common among Black populations, Hispanics and Asians. However, the black population account for over 60% of diabetic cases in the world [9].

The two most common forms of diabetes are type 1 diabetes (T1D), previously known as insulin-dependent diabetes (IDDM) and type 2 diabetes (T2D) previously known as non-insulin-dependent diabetes (NIDDM). Both are known to be caused by a combination of genetic and environmental risk factors. However, there are other rare forms of diabetes that are directly inherited. These include maturity onset diabetes in the young (MODY), and diabetes due to mutations in mitochondrial DNA (DDMMD) [2,10].

All forms of diabetes have very serious effects on health. In addition to the consequences of abnormal metabolism of glucose such as hyperglycemia as well as hyperlipidemia and dyslipidemia, and the glycosylation of proteins, there are a number of long-term complications associated with the disease [11,12,13]. These include cardiovascular, peripheral, vascular, ocular, neurologic and renal abnormalities, which are responsible for morbidity, disability and premature deaths in young adults [1,14,15]. The disease is also associated with renal failure in males and reproductive complications in females causing problems for both mothers and their children [16].

Although improved glycemic control and management may decrease the risk of developing these complications, diabetes remains a very significant cause of social, psychological and financial burdens in populations, families and governments worldwide [14,17].

Diabetes mellitus has risk factors including family genetics, overweight, high blood pressure, impaired glucose tolerance and impaired fasting glucose among others [14,17,18].

In Nigeria, the disease affects both males and females. However, the incidence of occurrence of the disease is more in the male populations than in females, and this may be associated with genetic factors, lifestyle and other environmental components. The disease is associated in genetically susceptible individuals, with excessive intake of alcoholic drinks, carbohydrates and fatty foods, as well as inactive lifestyle and general lack of exercise, resulting in excessive weight gain and perturbations in glucose metabolism [14,16].

The management of the disease includes reduction in calories from protein-energy intake, regular exercise and dieting, accompanied by the use of prescription drugs. However, ethno-medical practice had used the leaves of *Gongronema latifolium* among other herbs to manage diabetes, high blood sugar and cardiovascular complications long before the advent of civilization [4,6,19].

Therefore, this study was designed to evaluate the efficacy of the saponin-rich fraction of the leaf extract of *Gongronema latifolium* in lowering blood glucose sugar in laboratory animals, as well as the effect of the fraction on some serum lipid components, and the overall management of both hyperglycaemia and hyperlipidemia using laboratory rats as mammalian study models.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Insulin and streptozotocin were purchased from Sigma-Aldrich (St Louis, MO, USA). Glucose, cholesterol, lipid cholesterol and triglyceride test kits were purchased from Agappe Assay kits (Germany). All other solvents, chemicals and reagents used in this study were obtained commercially (BDH, UK), and were all of analytical grade. Lipid cholesterol components (VLVL, LDL, HDL, TG and TC) were assayed by standard analytical methods using Agappe Assay kits (Germany). Glass Column for Column chromatography and silica gel for reverse phase fractionation (Silane 343, pore size 0.15 µm) were purchased from Merck, Germany.

2.2 Source of Plant

Fresh leaves of *Gongronema latifolium* Benth (*Asclepiadaceae*), locally known as Utazzi in the Eastern Region of Nigeria, were harvested from a cultivated land at Assiga, in Yakurr Local Government Area of Cross River State of Nigeria. The plant was authenticated by Dr. M Eko, a Botanist in the Department of Botany, University of Calabar, Nigeria, and a voucher specimen (No. BT100 33C) was deposited at the Herbarium unit of the Department of Botany, University of Calabar.

2.3 Method for Extraction and Fractionation of Saponin-rich Fraction (GSF)

One (1) kg of fresh *Gongronema latifolium* leaves was weighed and washed with clean water. The washed leaves were chopped into pieces and air-dried in the laboratory, and homogenized using a Warring blender. The homogenized leaves were then macerated in 5 litres of ethanol (96%) in a rubber-stoppered quick-fit flat-bottomed flask containing anti-bump glass beads, heated over a water bath at 45±2.50°C for 30 minutes and then allowed to stand for 12 hrs. The mixture was filtered with a cheese cloth and then with Whatman no 4 filter paper. The filtrate was transferred into a 2 litre quick-fit flask and concentrated *in vacuo* to dryness using a rotary evaporator. The concentrated solution was allowed to dry completely in a dessicator, to afford 207 g of dry extract [20].

100 g of the dry extract was reconstituted in 30% MeOH and loaded to a pressurized glass column on silica gel support for column chromatography. Glass column chromatography was packed with silica gel in 30% MeOH (Silane 343, pore size 0.15 µm) for reverse phase fractionation of the crude extract to afford ethyl acetate saponin-rich fraction of *Gongronema latifolium* (GSF) [20].

The column was eluted first with 30% and 50% methanol (MeOH) to afford flavonoid-rich fractions 1 and 2 (which were stored away under refrigeration for a separate study). The column was then eluted with 70% MeOH and ethyl

acetate (EtoAc) solution respectively, to afford fractions that froth in water to form large and stable foam [20]. These fractions were combined as saponin-rich fraction for this study.

2.4 Albino Wistar Rats and Animal Treatment

Albino Wistar rats weighing 100-120 g were purchased from the animal house of the Department of Physiology, College of Medical Sciences, University of Calabar, Nigeria. The animals were housed in clean cages and maintained under standard laboratory conditions of 27±2.50°C and12 h light/dark cycle, and were allowed free access to standard diet and water *ad libitum.* The animals were acclimatized for 7 days in the animal house, Department of Biochemistry, University of Calabar, Nigeria, before the start of experiment.

2.5 Induction of Diabetes Using Streptozotocin

Thirty two (32) of the animals comprising of four groups (2, 3, 4 and 5) were induced with diabetes by a single intraperitoneal injection of freshly prepared solution of streptozotocin (Sigma, USA) (50 mg streptozotocin in 0.1 M cold sodium citrate buffer (pH 4.5)/ kg body weight of animal, after an overnight fast [21]. The animals were considered diabetic if the blood glucose values were >200 mg/dl on the third day, after streptozotocin administration and were selected for the experiment. This was estimated using a one touch glucometer [22].

2.6 Experimental Design

The experimental design (Table 1) consisted of forty (40) Albino Wistar rats of both sexes, divided into five groups of eight rats each (four diabetic and one non-diabetic group). The four diabetic groups were induced with diabetes by intraperitoneal (IP) injection using 50 mg/kg bw streptozotocin dissolved in 0.1 M cold sodium citrate buffer solution. Diabetes was confirmed after 3 days by the determination of fasting blood glucose (FBG), and if FBG values were >200 mg/dl [23]. Groups 1 and 2 served as non-diabetic and diabetic controls respectively and were given placebo treatment (0.20 ml 10% DMSO). Group 3 diabetic rats received 5 IU/kg bw of insulin subcutaneously per day, and groups 4 and 5 diabetic rats received 200 mg/kg bw and 400 mg/kg bw of saponin fraction of *Gongronema latifolium* orally twice daily for 28 days together with 0.20 ml 10% DMSO (placebo treatment). Group 3 animals were administered insulin intramuscularly twice daily throughout the experimental period, while the crude drug (GSF) was given by oral intubulation twice daily. At the end of 21days of treatment, food was withdrawn from the animals 12 hrs before anaesthesia and sacrifice. Animals were fasted overnight (12 hrs), but had free access to water. The rats were then anaesthetised using chloroform and sacrificed. Blood was obtained from the animals by cardiac puncture into sterile plain tubes. Serum was obtained from the blood by centrifugation at 3000 rpm for 10 minutes and then used for analysis of serum glucose, cholesterol and lipid profile [6,23].

2.7 Estimation of Fasting Blood Glucose (FBG), Serum Glucose and Plasma Insulin

Fasting blood glucose (FBG) was determined weekly during the 21 days experimentation period using a glucose oxidase kit and used according to the manufacturer's instructions. Serum glucose concentration was determined before and at the end of the treatment period. Plasma insulin concentration was determined using the iodine (I^{125}) Insulin Radioimmunoassay kit and following the manufacturer's instructions.

2.8 Estimation of Cholesterol Content and Other Lipid Components

Total cholesterol and triacylglycerol were determined in the blood serum according to the method of [24]. High density lipoprotein (HDL) cholesterol content was determined in the blood serum according to the method [25]. Low density lipoprotein (LDL) cholesterol content was calculated from the difference between the total cholesterol concentration (TC), the HDL cholesterol concentration and triacylglycerol content, according to the method of [25]. VLDL cholesterol content was obtained by calculation, based on the equation that,

VLDL cholesterol = triacylglycerol content x 5

2.9 LD_{50} of Plant Extract

The LD_{50} of *Gongronema latifolium* saponin fraction (GSF) was determined using the method described by [26]. The LD_{50} was determined to form a basis for dosage regimen of GSF and, employing sub-lethal doses of the GSF fraction.

Table 1. Experimental animal groupings and treatment plan

Group	Treatment	No of animals	Administration of drug per day
1	NC	8	0.20 ml of 10% DMSO
2	DC	8	0.20 ml of 10% DMSO
3	INS	8	5 IU/kg bw of animal
4	GSF	8	200 mg/kg bw of animal
5	GSF	8	400 mg/kg bw of animal

NC = normal control, DC = diabetic control, INS = insulin, GSF = Gongronema latifolium saponin fraction, bw = body weight

2.10 Statistical Analysis

Analysis of variance (ANOVA) was used in analyzing the data generated by this study. Results were expressed as means ± standard deviation. Values of $P<0.05$ were regarded as being significant.

3. RESULTS

3.1 Effect of GSF on Body Weight Changes

Table 2 shows the effect of saponin-rich fraction of *Gongronema latifolium* (GSF) on body weights of animals when compared to the diabetic control group over a period of 21 days of drug administration. The saponin-rich fraction (GSF) of GL treated groups showed a gradual but significant increase in body weight gain during the 3 weeks period of treatment. The effect was similar to the result produced by insulin which was used as the reference drug.

3.2 Effect of GSF on Serum Glucose Concentration of Diabetic Animals

Table 3 shows the effect of GSF on serum glucose concentration. After 3 days of diabetes induction, the blood sugar level rose significantly ($p>0.05$) above normoglycemic level (see groups DC, INS and GSF1 and GSF2), when compared to the normal control (NC) group. Over a period of 21 days, the glucose levels in the treatment groups decreased significantly ($p<0.05$), when compared to the diabetic control group (DC) that was not treated. The % reduction in blood glucose concentration was 26.43% (DC group), 57.81% (INS group) and 54.28% and 58.28% respectively for GSF groups 1 and 2. Blood glucose level in NC group remained steady throughout the 28-day period of experimentation.

3.3 Effect of GSF on Fasting Blood Glucose (FBG) of Diabetic Animals

Table 4 shows the effect of saponin-rich fraction of *Gongronema latifolium* on fasting blood glucose (FBG), plasma insulin concentration and c-peptide level in experimental laboratory animals. Induction of diabetes completely disrupted C-peptides in DC group, when compared to NC group, INS and GSF treated groups.

3.4 Effect of GSF on Liver Weight, Liver Cholesterol, Plasma and Total Cholesterol Levels of Diabetic Rats

Table 5 shows the effect of the saponin fraction of *Gongronema latifolium* leaf on liver weight,

Table 2. Body weight profile of animal groups treated with Saponin-fraction of *Gongronema latifolium* extract

Groups	No. of animals	Initial body weight (g)	Body weight at day 7 (g)	Body weight at day 14 (g)	Body weight at day 21 (g)	Change in body weight (g)
NC	8	105.55±2.28	115.95±2.50	128.92±3.0	135.55±2.75	+46.24±7.8
DC	8	94.22±3.25	94.05±2.40*	90.44±4.05*	86.50±2.42*	-4.45±2.69^
INS	8	93.85±2.40	99.91±1.45[a]	122.77±2.21[a]	127.05±2.15	+28.06±0.63*
GSF1	8	93.95±2.50	98.92±2.22[b]	118.71±2.09[b]	122.75±2.84	+22.95±0.5*
GSF2	8	93.79±2.41	101.00±3.05[c]	121.40±2.11	125±1.97	+25.66±1.5

Values are expressed as mean±SEM (n = 8). NC = Normal control rats administered 10% DMSO; DC = Diabetic control rats administerd 10% DMSO; INS = Diabetic rats administered reference drug insulin (5I U/kg bw of animal); GSF1 = Diabetic rats administered Gongronema latifolium saponin fraction (200 mg/kg bw of animal); GSF2 = Diabetic rats administered Gongronema latifolium saponin fraction (400 mg/kg bw of animal).
**Significantly different from NC at p<0.05*
a and b = Significantly differentt from DC at p<0.05

Table 3. Effect of saponin-rich fraction of *Gongronema latifolium* on serum glucose levels in albino wistar rats

Sample	Initial glucose (mg/dL)	Final glucose (after 28 days) (mg/dL)	Reduction (%)
NC	120.00±5.0	118.00±6.50	1.67
DC	292.00±21.05	214.67±14.42*	26.48*
INS	301.0±6.34	127.33±0.36*[a]	57.81*[a]
GSF1	304.0±30.54	138.67±16.62*[b]	54.2*[b]
GSF2	302.2±22.25	125.77±17.20*[b]	58.28*[b]

Values are expressed as mean±SEM (n = 8). NC = Normal control rats administered 10% DMSO; DC = Diabetic control rats administerd 10% DMSO; INS = Diabetic rats administered reference drug insulin (5I U/kg bw of animal); GSF1 = Diabetic rats administered Gongronema latifolium saponin fraction (200 mg/kg bw of animal); GSF2 = Diabetic rats administered Gongronema latifolium saponin fraction (400 mg/kg bw of animal).
**Significantly different from NC at p<0.05*
a and b = Significantly differentt from DC at p<0.05

liver cholesterol and plasma and total cholesterol concentrations. Treatment significantly (p<0.05) decreased liver weight, liver cholesterol, plasma cholesterol and overall total cholesterol in treatment groups when compared to DC group. The slightly elevated levels of the parameters tested in normal control (NC) group, may be due to lipid sources from the rat chow diet.

3.5 Effect of GSF on Lipid Components of Diabetic Rats

Table 6 shows the effect of the saponin-rich fraction of *Gongronema latifolium* on plasma cholesterol and lipids and the %AAI (% Anti Atherogenic Index) ratio of the lipid cholesterol profile. The HDL-cholesterol lipids were high in the NC and treated groups when compared to the DC group. The LDL-cholesterol concentrations showed a significant decrease (p<0.05) in treatment groups when compared to the DC group. AAI may be expressed as the ratio of HDL-C to Total cholesterol (TC). It may be expressed as a ratio or as percentage.

The effect of GSF on lipid cholesterol and triglyceride profile, when compared to the NC, DC and insulin treated groups and the %AAI ratio are shown in Table 5. There was a significant decrease (p<0.05) in %AAI values of treatment groups when compared to values obtained for DC and NC groups respectively (Table 6).

4. DISCUSSION

Saponins are secondary metabolic products in plants which characteristically froth and form stable foam in water. Saponins have long been implicated as anti-diabetic agents, and substances that elicit hypoglycaemia, hypolipidemia and hypocholesterolemia, thus alleviating diabetic conditions in patients. The ethyl acetate fraction of *Gongronema latifolium* (GSF) frothed in water to form large stable foam, thus confirming it to be saponin-rich. This property qualified the fraction to be used for the present study, as saponins have been reported to lower blood sugar and plasma

Table 4. Effect of Saponin-rich fraction of *Gongronema latifolium* on Fasting Blood Glucose (FBG), Plasma Insulin and C-peptide levels in Albino Wistar Rats

Sample	FBG (mg/dL)	Insulin (U/ml)	C-peptide (ng/L)
NC	25.00±0.50	3.18±0.52	0.05±0.02
DC	177.33±6.75*	1.47±0.45*	0.00±0.00*
INS	62.05±4.22*[a]	2.21±0.35*[a]	0.04±0.02*[a]
GSF1	84.0±5.12*[b]	2.25±0.41	0.05±0.01*[b]
GSF2	77.4±6.07*[b]	2.79±0.25*[b]	0.07±0.02*[b]

Values are expressed as mean±SEM (n = 8). NC = Normal control rats administered 10% DMSO; DC = Diabetic control rats administerd 10% DMSO; INS = Diabetic rats administered reference drug insulin (5 IU/kg bw of animal); GSF1 = Diabetic rats administered Gongronema latifolium saponin fraction (200 mg/kg bw of animal); GSF2 = Diabetic rats administered Gongronema latifolium saponin fraction (400 mg/kg bw of animal).
**Significantly different from NC at p<0.05*
a and b = Significantly differentt from DC at p<0.05

Table 5. Effect of Saponin-rich fraction of *Gongronema latifolium* on Liver weight, Liver cholesterol and plasma cholesterol levels in Rats

Group	Liver weight (g)	Liver chol (mg/dl)	Plasma chol (mg/dl)	Total chol (mg/dl)
NC	1.82 0±0.11	4.55± 0.05	32.44± 5.50	115.95±4.59
DC	1.94 ±0.09*	6.97±0.07*	89.71± 6.55*	254.40±29.84*
NS	1.75± 0.12[a]	2.94± 0.08 [a]	24.86±4.90 [a]	114.59±6.04 [a]
GSF 1	1.78 ±0.10[b]	3.25 ±0.09 [b]	26.92±6.25 [b]	118.07± 5.74 [b]
GSF 2	1.72±0.12[c]	2.77±0.11[c]	22.92±3.33[c]	113.34±4.52[c]

Values are expressed as mean±SEM (n=8). NC = Normal control rats administered 10% DMSO; DC = Diabetic control rats administerd 10% DMSO; INS = Diabetic rats administered reference drug insulin (5 IU/kg bw of animal); GSF1 = Diabetic rats administered Gongronema latifolium saponin fraction (200 mg/kg bw of animal); GSF2 = Diabetic rats administered Gongronema latifolium saponin fraction (400 mg/kg bw of animal).
**Significantly different from NC at p<0.05*
a, b and c = significantly differentt from DC at p<0.05

Table 6. Effect of Saponin-rich fraction of *Gongronema latifolium* on serum lipid components

Group	TG(mg/dl)	HDL-Chol (mg/dl)	VLDL- Chol (mg/dl)	LDL-Chol (mg/dl)	%AAI
NC	76.41±9.65	68.26 ±1.50	92.40 ± 15.25	15.28 ± 2.00	58.36
DC	82.23± 9.56*	64.22 ±2.33*	205.29 ± 31.00*	16.45 ± 2.50*	26.16*
NS	72.86± 9.91*[a]	66.36 ± 0.50*[a]	398.80 ± 38.20*[a]	14.57 ± 2.00*[a]	14.79*[a]
GSF 1	66.20± 7.75*[b]	66.52 ± 2.94*[b]	258.10 ± 5.35*[b]	14.85 ± 1.50*[b]	16.58*[ab]
GSF 2	64.09± 2.25*[c]	67.55 ± 1.05*[c]	229.45 ± 10.05*[c]	14.45 ± 0.22*[a]	14.82*[c]

Values are expressed as mean ± SEM (n = 8). NC = Normal control rats administered 10% DMSO; DC = Diabetic control rats administered 10% DMSO; INS = Diabetic rats administered reference drug insulin (5 IU/kg bw of animal); GSF1 = Diabetic rats administered Gongronema latifolium saponin fraction (200 mg/kg bw of animal);

cholesterol, and reverse dyslipidemia in man and experimental animals, as well as for the management and treatment of diabetes and its complications.

In potential drug screening, the determination of LD_{50} (i.e the dose which has proved to be lethal, causing death to 50% of test group of animals), is usually an initial step in the assessment and evaluation of the toxic or medicinal potential of a substance. It is an initial assessment of toxic manifestations (providing information on health hazards likely to arise from short-term exposure to novel drugs), and this is one of the initial screening experiments performed with all crude drugs and potential drugs, aimed at ensuring safe use of the final product. In this study, the LD_{50} of the saponin-rich fraction of *Gongronema latifolium* was determined to be 2500 mg/kg body weight, in accordance with the [26].

Several studies have shown that diabetes is indeed a heterogeneous disease characterized by hyperglycemia and atherogenic lipid profile [1]. Type 1 diabetes is sometimes accompanied by damage to key organs of the body including the liver, kidney and the pancreas. Severe hyperglycemia accompanied by hyperlipidemia and hypercholesterolemia is observed as a result of the destruction of the insulin producing pancreatic Islets β-cells of Langerhans and hepatic damage [8]. Damage to key organs has also been observed in experimental animal models upon induction of diabetes using either streptozotocin or higher doses of alloxan [6,7].

In diabetes condition, there is usually observed high plasma lipid cholesterol concentrations, dyslipidemia and hyperglycemia. Dyslipidemia is always associated with diabetic milletus which is a risk factor for some endocrine-related diseases including cardiovascular disease. It is thought that this occurs due to deposition of plaque along artery walls caused by crystals of LDL-c, leading to blockage of arterial passage and atherosclerosis [23]. Disproportionate perturbations are usually observed in dyslipidemia due to slow blood sugar uptake and gluconeogenesis, depending on the extent of diabetic condition. Human diets worldwide are rich in lipids, fats and oils which are precursor substances to LDL, VLDL, IDL and HDL-cholesterol, as well as triglycerides and total cholesterol. High concentrations of these lipoproteins, except HDL-c have been associated

with cardiovascular disease. In this study, there was elevated LDL, VLDL, total cholesterol and triglyceride concentration when the animals were induced with diabetes. Under this condition, HDL concentration decreased [5]. However, when the animals were treated with extract of saponin-rich fraction of *Gongronema latifoliumi*, there was a significant decrease ($p<0.05$) in TC, LDL, VLDL and triglyceride concentrations, while the HDL-C concentration was elevated. The results were consistent with those of earlier studies [2,11,12,13,19,27].

Hyperlipidemia are disorders of the rates of synthesis or clearance of lipoproteins from the bloodstream, biochemically detected by measuring plasma triacylglycerol and total cholesterol [17,28]. Hyperlipidemia may be classified on the basis of which class of lipoprotein is cleared. The different types of classification is on the basis of accumulation of chylomicrons, elevated low density lipoprotein (LDL) concentration, abnormalities of Apo-E or increased very low density lipoprotein (VLDL) level due to obesity, overweight or diabetes [29]. Hyperlipidemia therefore is a potent risk factor for atherosclerosis and coronary heart disease which presents in substantial proportion of young adults [17]. According to data from National Health and Nutrition Examination Survey (NHANES), between 11.7% of adults aged 20-39 and 41.2% of adult aged 40-64 have elevated low density lipoprotein cholesterol (LDL-C) level [30]. Studies on adults with familiar hypercholesterolemia have shown that, cardiovascular risk is increased early among those with high low density lipoprotein cholesterol (HLDL) level [31]. Similarly, adults with extremely low LDL-C level conferred by genetic polymorphisms have significantly lower than average risk of cardiovascular disease [32]. Populations consuming *Gongronema latifolium* may likely have lower risk of cardiovascular disease as demonstrated in this study.

Hypercholesterolemia is a condition of excessively high plasma cholesterol level [33]. According to recent studies, hypercholesterolemia is considered to be a well established risk factor for coronary heart disease with elevated low density lipoprotein being the mechanism underlying it, and with a high risk cholesterol serum level above 160mg/dl [33]. In this study, the saponin-rich fraction of *Gongronema latifolium* completely reversed all forms of dyslipidemia caused by STZ-induced diabetes.

Primary hypercholesterolemia is in general believed to be the result of reduced activity of LDL receptors [34]. Total cholesterol can be broken down into a diagnostic lipoprotein profile including high density lipoprotein (HDL), intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL), chylomicron remnants and triglycerides [33]. High density lipoprotein is considered to be beneficial as high levels of HDL-c have been correlated with reduced risk of negative cardiovascular event [35,36]. On the other hand, elevated level of low density lipoprotein cholesterol and triglycerides are considered detrimental to health as their elevated concentration in plasma has been correlated with poor cardiovascular outcomes [35]. Also, intermediate density lipoprotein, very low density lipoprotein and chylomicron remnants have been reported to potentially play an active role in peripheral vascular and coronary artery disease development [36]. The significant reduction of these types of lipoproteins caused by treatment of diabetic rats with extract of saponin-fraction *Gongronema latifolium,* strongly confirms the claims of populations using the plant in Nigeria for the treatment of diabetes [37,38].

High concentrations of plasma cholesterol has been shown to interfere and alter vascular structures and function as it builds within the lining of the vascular walls, thus interfering with endothelial function and leading to lesion and plaques [17,28,37,38]. Therefore elevation of hypercholesterolemia in diabetes may be associated with endothelial dysfunction and dislipidemia [37,38].

Thus, the effect of the saponin-fraction of the leaves of *Gongronema latifolium* on dyslipidemia (hyperlipidemia and hypercholesterolemia), predisposed by artificially induced diabetes, using streptozotocin, was evaluated in laboratory animals, and the treatment was shown to be effective in reversing hyperglycemia and dyslipidemia. The results were consistent with earlier results using other plant extracts as alternative to otherwise very expensive and unaffordable drugs [4,12].

This study showed that, induction of diabetes caused a significant ($P<0.05$) increase in blood glucose concentration in groups DC (292.00 ± 21.05 mg/dL), INS (301.0 ± 6.34 mg/dL) and GSF 1 and 2 groups (304.0 ± 30.54 mg/dL and 302.2 ± 22.25 mg/dL respectively), in addition to dyslipidemia. These values and conditions were found to be consistent with previous results using streptozotocin as diabetes inducing

agent [39]. The induction of diabetes completely disrupted C-peptide concentration (0.00±0.00 ng/L). However, upon treatment with saponin-rich fraction of *Gongronema latifolium* (using two concentrations 200 mg/kg and 400 mg.kg bw) for 28 days, the blood glucose decreased significantly ($p<0.05$) to 138.67±16.62 mg/dL for GSF 1 group, 125.77±17.20 mg/dL for GSF 2 group, and 127.33±0.36 mg/dL for insulin treated group, when compared on the other hand to the DC group (214.67±14.42 mg/dL), and to the normal control group (118.00±6.50 mg/dL) (Table 2). The reduction of blood glucose (96%) by *Gongronema latifolium* is consistent with the results obtained by [6], and compares favourably well with the reduction (86%) by insulin and NC, during the 28 days treatment. On the other hand the diabetic control (DC), recorded slight decrease in blood glucose (Table 2), suggesting that the decrease observed in the GSF treated group is attributable to the hypoglycemic efficacy of the saponin-rich fraction of *Gongronema latifolium*. Treatment also restored C-peptide concentration to (0.07±0.01 ng/L) for GSF and (0.04±0.02 ng/L) for insulin, when compared to the C-peptide concentration (0.00±0.00 ng/L) for DC group. This confirms the claims of ethno-medical practitioners that aqueous extracts of *Gongronema latifolium* is effective in the management and treatment of all forms of diabetic conditions and its complications.

The biochemical mechanism by which the sapoinin-rich fraction of *Gongronema latifolium* and other saponin-rich plant extracts lower hyperglycemia and reverse dyslipidemia has not been fully explained and elucidated. In liver parenchyma cells and β-cells of the pancreas, the enzyme glucokinase or hexokinase D is the predominant enzyme responsible for the phosphorylation of glucose in glycolysis. In β-cells, glucokinase functions as the glucose sensor, determining the threshold of insulin secretion. In the liver, the enzyme facilitates glucose phosphorylation during hyperglycemia. Since hexokinase D serves as a glucose sensor in neurons of the hypothalamus, playing a key role in the adrenergic response to hypoglycemia, it appears saponins serve as catalyst and activator of the hexokinase enzyme, to sense the presence of sugar and at the same time phosphorylate the sugar to glucose-6-phosphate, a committed step in glycolysis and sugar clearance in the blood. Thus saponin-rich fraction of *Gongronema latifolium* may catalyze epinephrine and glucokinase enzyme to function when the intracellular concentration of glucose in the hepatocyte is elevated such as during diabetes or during the brief period following the consumption of a carbohydrate-rich meal, when high levels of glucose are delivered to the liver via the portal vein. Also in diabetes, especially type 1, there is damage to the liver, pancreas and the kidney. Saponins function to ameliorate these damaged organs, and confer hepatoprotection and nephroprotection to the organs, as well as help to anti-oxidatively repair damaged pancreatic β-cells. They also anti-oxidatively help to sequester and mop up free radicals generated by STZ during diabetes induction.

The catalysis of glucokinase by the saponin-rich fraction of *Gongronema latifolium* in lowering blood glucose sugar, may also proceed via the inflammatory response pathway involving the release of complement or cytokines in response to diabetic inflammation, and on phagocytic cellular response-dependent on the function of polymorphonuclear leucocytes and monocytes which sometimes occur in diabetes. The inflammatory response process is non-specific, requires changes in the permeability of cell membranes and depends on humoral factors such as acute-phase response reactants (complement or cytokines). The acute-phase response is an innate or natural immune system, which is phylogenetically older than the so-called acquired or adaptive immunity, and provides a rapid first-line defence mechanism against infection, stress and inflammation, based on non-lymphoid tissue components.

The induction of diabetes also caused a significant ($P<0.05$) increase in total liver cholesterol (254.40±29.84 mg/dl) and plasma cholesterol (89.71± 6.55 mg/dl), corresponding to 54.42% and 39% increase respectively, with attendant increase in liver weight (1.94±0.09 g)(Table 4). Upon treatment with saponin-rich fraction of *Gongronema latifolium,* a significant ($p<0.05$) reduction in liver weight (1.78±0.10 g), liver cholesterol (3.25±0.09 mg/dl), total cholesterol (118.07±5.74 mg/dl), and plasma cholesterol (26.92±6.25 mg/dl) were observed, when compared to the NC and DC groups respectively (Table 4). A similar trend had been reported in none diabetic mice fed with diets supplemented with leaves of *Vernonia amygdalina* [20].

There was also a significant (P<0.05) increase in LDL (16.45±2.50 mg/dl), VLDL (205.29± 31.00 mg/dl), and triglycerides (82.23±9.56 mg/dl), while the HDL concentration (64.22 ±2.33 mg/dl) was low upon induction of diabetes, compared to animals in the normal control group (Table 5). Upon treatment with saponin-rich fraction of *Gongronema latifolium the observed* dyslipidemias were normalized. The lipid cholesterol values were reversed. There were significant (p<0.05) decreases in LDL concentration (14.25±1.50 mg/dl) and triglyceride concentration (66.20±7.75 mg/dl). There was an increase in HDL concentration (66.52±2.94 mg/dl) when compared to the diabetic control (Table 5). The VLDL concentration was not affected and remained the same. The results obtained for GSF compared favourably well with the results obtained for the standard insulin drug.

5. CONCLUSION

The study confirmed the hypolipidemic, hypoglycaemic and hypocholesterolemic effects of saponin-rich fraction of *Gongronema latifolium* in laboratory animal models. The authors suggest that, the saponin-rich fraction of *Gongronema latifolium* probably modified the hydrophilic-hydrophobic interface between the lipid cholesterol and aqueous fraction of plasma, in favour of hypolipidemia and hypocholesterolemia. The restoration of C-peptide concentration to normal level (0.05mg/dL) may be attributable to the anti-oxidative effects of the saponin constituents in the saponin-rich fraction of the plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

SUGGESTION FOR FURTHER RESEARCH

It is suggested by this study that research into the biochemical mechanism by which plants or saponin-rich fractions of plants elicit sugar-lowering effects and reverses dyslipidemia be thoroughly investigated, as this may aid the treatment and management of this global disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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