

1 **Saponin-rich Fraction of *Gongronema latifolium* reverses dyslipidemia and catalyzes**
2 **glucokinase in lowering blood glucose sugar in Streptozocin-Induced Diabetic Rats.**

3 ABSTRACT

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

11 Methods: Streptozotocin-induced diabetic rats were treated with 200mg/kg bw and 400mg/kg
12 bw dose of saponin-rich fraction of *Gongronema latifolium* twice daily for 21 days. He
13 effects of GL fraction on fasting blood glucose (FBG), serum glucose, serum insulin, body
14 weight, total cholesterol (TC), HDL-c, LDL-c, VLDL-c, triglyceride (TG) and C-peptides
15 concentrations were determined.

16 Results: After 21 days treatment, GSF administration showed significantly lower FBG
17 concentration compared to the diabetic control (DC) group. GSF increased serum insulin
18 concentration and C-peptide levels dose-dependently. The fraction also decreased liver
19 weight, liver cholesterol, plasma cholesterol, total cholesterol and triglyceride concentration
20 dose-dependently. GSF 1 and 2 increased HDL-c and lowered the plasma concentration of
21 LDL-c and VLDL-c. GSF significantly ($p<0.05$) increased body weights of diabetic animals
22 time-dependently taken at 7 days interval.

23 Conclusion: The findings from this study suggest that GSF may provide alternative sugar-
24 lowering effect in diabetes, and have the potential to reverse dyslipidemia and improve body
25 weight gain in type 2 diabetes.

26 **Key words:** Diabetes, *Gongronema latifolium*, saponin-fraction, lipid cholesterol,
27 hypoglycaemic, hypolipidemic.

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29

30 1. Introduction.

31 Elevated blood glucose is a common feature in diabetes mellitus, usually detected by testing
 32 fasting blood glucose (FBG) concentration in patients. Scientific data from several studies
 33 have led to the conclusion that diabetes mellitus is a heterogeneous group of disorders
 34 characterized by persistent hyperglycemia, with attendant but persistent dyslipidemia in a
 35 majority of cases (Alberti et. al, 1998; WHO, 2002; Sauders, 2007; Igile et al. 2015). It is also
 36 said to be a heterogeneous metabolic disease which results from a complex and poorly
 37 understood interaction between genetic factors and lifestyle components (Ahren et al. 1999).
 38 It is further said to be multifactorial in presentation, difficult to manage and has no clear cut
 39 cure because of its complexity in presentation and associated diet needs of sufferers (WHO,
 40 2002; Alberti et. al.1998). .

41 The disease is not only characterized by hyperglycaemia but also by a rise in atherogenic
 42 lipid profile, both of which greatly increase the risk of coronary heart disease, organ damage,
 43 muscle wastage and several metabolic diseases (WHO, 2002; Sauders, 2007; Igile et. al.
 44 2015).

45 Across the globe, there is an estimated ten million people suffering from diabetes mellitus.
 46 The disease is said to be responsible for over 5% deaths around the globe annually (Alberti
 47 et. al.1998). In type 1 diabetes, there has been a characteristic but attendant damage to key
 48 organs including the liver, kidneys and the pancreas. In the liver, the hepatocytes are usually
 49 lacerated and damaged during diabetes, freely releasing liver enzymes including AST, ALT
 50 and ASP into the general blood circulation. The kidney architecture is usually altered with
 51 attendant nephrotoxicity, and elevated sodium electrolyte concentration. In the case of the
 52 pancreas, the β -cells of the islets of langerhans may be destroyed, resulting in loss of insulin
 53 producing cells and insulin availability to clear sugar in the blood stream (Igile et. al. 2015;
 54 Edet et al. 2013; Akpaso et al. 2011; Komolafe et. al, 2009; Sauders, 2007).

55 Diabetes mellitus is more common among Black populations, Hispanics and Asians.
 56 However, the black population account for over 60% of diabetic cases in the world (Betterle
 57 et al, 1983).

58 The two most common forms of diabetes are type 1 diabetes (**T1D**), previously known as
 59 insulin-dependent diabetes (**IDDM**) and type 2 diabetes (**T2D**) previously known as non-

60 insulin-dependent diabetes (**NIDDM**). Both are known to be caused by a combination of
61 genetic and environmental risk factors. However, there are other rare forms of diabetes that
62 are directly inherited. These include maturity onset diabetes in the young (**MODY**), and
63 diabetes due to mutations in mitochondrial DNA (DDMMD) (WHO, 2002; Akan, 2006).

64 All forms of diabetes have very serious effects on health. In addition to the consequences of
65 abnormal metabolism of glucose such as hyperglycemia as well as hyperlipidemia and
66 dyslipidemia, and the glycosylation of proteins, there are a number of long-term
67 complications associated with the disease (Ebong et. al. 2006 & 2008; Atangwho et al, 2007).
68 These include cardiovascular, peripheral, vascular, ocular, neurologic and renal
69 abnormalities, which are responsible for morbidity, disability and premature deaths in young
70 adults (WHO, 2014; Odey et al, 2014, Igile et al. 2015). The disease is also associated with
71 renal failure in males and reproductive complications in females causing problems for both
72 mothers and their children (Smith et al 2014).

73 Although improved glycemic control and management may decrease the risk of developing
74 these complications, diabetes remains a very significant cause of social, psychological and
75 financial burdens in populations, families and governments worldwide (Steinberg, 2005;
76 WHO 2014) .

77 Diabetes mellitus has risk factors including family genetics, overweight, high blood pressure,
78 impaired glucose tolerance and impaired fasting glucose among others (Steinber, 2005;
79 Lewis, 2006; WHO 2014) .

80 In Nigeria, the disease affects both males and females. However, the incidence of occurrence
81 of the disease is more in the male populations than in females, and this may be associated
82 with genetic factors, lifestyle and other environmental components. The disease is associated
83 in genetically susceptible individuals, with excessive intake of alcoholic drinks,
84 carbohydrates and fatty foods, as well as inactive lifestyle and general lack of exercise,
85 resulting in excessive weight gain and perturbations in glucose metabolism (WHO 2014;
86 Smith et. al. 2014).

87 The management of the disease includes reduction in calories from protein-energy intake,
88 regular exercise and dieting, accompanied by the use of prescription drugs. However, ethno-
89 medical practice had used the leaves of *Gongronema latifolium* among other herbs to manage

90 diabetes, high blood sugar and cardiovascular complications long before the advent of
91 civilization (Edet et al. 2013; Efiogon et al. 2014; Igile et al. 2015).

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

97 **2.Materials and Methods:**

98 **2.1 Chemicals and Reagents**

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

107 **2.2 Source of Plant**

108 Fresh leaves of *Gongronema latifolium* were harvested from a cultivated land at Assiga, in
109 Yakurr Local Government Area of Cross River State of Nigeria. The plant was authenticated
110 by Dr. M Eko, a Botanist in the Department of Botany, University of Calabar, Nigeria, and a
111 voucher specimen was deposited at the Herbarium unit of the Department of Botany,
112 University of Calabar. .

113

114 **2.3 Method for Extraction and fractionation of Saponin-rich fraction (GSF)**

115 One (1) kg of fresh *Gongronema latifolium* leaves was weighed and washed with clean water.
116 The washed leaves were chopped into pieces and air-dried in the laboratory, and
117 homogenized using a Waring blender. The homogenized leaves were then macerated in 3
118 liters of ethanol (96%) in a rubber-stoppered quickfit flat-bottomed flask containing anti-
119 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 quickfit flask and concentrated *in vacuo* to dryness using a rotary evaporator. The concentrated solution was allowed to dry completely in a dessicator, to afford 207g of dry extract.

100g 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 0.15µm) for reverse phase fractionation of the crude extract to afford ethyl acetate saponin-rich fraction of *Gongronema latifolium* (GSF).

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. These fractions were combined as saponin-rich fraction for this study.

2.4 Albino Wistar Rats and animal treatment

Albino Wistar rats weighing 100-120g 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 and 12h 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) (50mg streptozotocin in 0.1M cold sodium citrate buffer (pH 4.5)/kg body weight of animal, after an overnight fast (Ghoraishian, 2006; Rao and Naidu, 2010). The animals were considered diabetic if the blood glucose values were >200mg/dl on the third day, after streptozotocin administration and were selected for the experiment. This was estimated using a one touch glucometer (Lifescan Inc; 1995, Milpas, California, USA).

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 50mg/kg bw streptozotocin dissolved in 0.1M cold sodium citrate buffer solution. Diabetes was confirmed after 3 days by the determination of fasting blood glucose (FBG), and if FBG values were >200mg/dl. Groups 1 and 2 served as non-diabetic and diabetic controls respectively and were given placebo treatment (0.20ml 10% DMSO). Group 3 diabetic rats received 5IU/kg bw of insulin subcutaneously per day, and groups 4 and 5 diabetic rats received 200mg/kg bw and 400mg/kg bw of saponin fraction of *Gongronema latifolium* orally twice daily for 28 days together with 0.20ml 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 12hrs before anaesthesia and sacrifice. Animals were fasted overnight (12hrs), 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.

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	5IU/kg bw of animal
4	GSF	8	200mg/kg bw of animal
5	GSF	8	400mg/kg bw of animal

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

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 was determined in the blood serum according to the method of Rudel and Morris (1973). Triacylglycerol content in the serum was carried out according to the method of Chattergen and Shinde, (2008). High density lipoprotein (HDL) cholesterol content was determined in the blood serum according to the method of Friedewald et. al (1972). 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 Friedewald et. al (1972). VLDL cholesterol content was obtained by calculation, based on the equation that,

$$\text{VLDL cholesterol} = \text{triacylglycerol content} \times 5.$$

2.9 LD₅₀ of Plant Extract

The LD₅₀ of *Gongronema latifolium* saponin fraction (GSF) was determined using the method described by Lorke (1983). The LD₅₀ was determined to form a basis for dosage regimen of GSF and, employing sub-lethal doses of the GSF fraction.

2.10 Statistical Analysis

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

3.0 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, 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 ratio of the lipid cholesterol profile. The HDL-cholesterol lipids were high in the NC and treated groups when compared to the DC group.

231 The LDL-cholesterol concentrations showed a significant decrease ($p<0.05$) in treatment
232 groups when compared to the DC group.

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

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

Groups	No. of animals	Initial body weight (g)	Body weight at day7 (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*
GSF 1	8	93.95±2.50	98.92±2.22 ^b	118.71±2.09 ^b	122.75±2.84	+22.95±0.5*
GSF 2	8	93.79±2.41	101±3.05 ^c	121.40±2.11	125±1.97	+25.66±1.5

239 Values are expressed as mean±SEM (n=8). NC = normal control rats administered 10% DMSO; DC =
240 diabetic control rats administered 10% DMSO; INS = diabetic rats administered reference drug insulin
241 (5IU/kg bw of animal); GSF1 = diabetic rats administered *Gongronema latifolium* saponin fraction
242 (200mg/kg bw of animal); GSF2 = diabetic rats administered *Gongronema latifolium* saponin fraction
243 (400mg/kg bw of animal). *Significantly different from NC at $p<0.05$
244
245 a and b = significantly different from DC at $p<0.05$
246
247

248 **Table 3: Effect of Saponin-rich fraction of *Gongronema latifolium* on**
249 **Serum Glucose levels in Albino Wistar Rats.**
250

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*	26.43*
INS	301.0±6.34	127.33±0.36 ^{*a}	57.81 ^{*a}
GSF 1	304.0±30.54	138.67±16.62 ^{*b}	54.2 ^{*b}

GSF 2	302.2±22.25	125.77±17.20 ^{*b}	58.28 ^{*b}
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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 (5IU/kg bw of animal); GSF1 = diabetic rats administered *Gongronema latifolium* saponin fraction (200mg/kg bw of animal); GSF2 = diabetic rats administered *Gongronema latifolium* saponin fraction (400mg/kg bw of animal). *Significantly different from NC at p<0.05

a and b = significantly different from DC at p<0.05

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}
GSF 1	84.0±5.12 ^{*b}	2.25±0.41	0.05±0.01 ^{*b}
GSF 2	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 administered 10% DMSO; INS = diabetic rats administered reference drug insulin (5IU/kg bw of animal); GSF1 = diabetic rats administered *Gongronema latifolium* saponin fraction (200mg/kg bw of animal); GSF2 = diabetic rats administered *Gongronema latifolium* saponin fraction (400mg/kg bw of animal). *Significantly different from NC at p<0.05

a and b = significantly different 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*
INS	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 administered 10% DMSO; INS = diabetic rats administered reference drug insulin (5IU/kg bw of animal); GSF1 = diabetic rats administered *Gongronema latifolium* saponin fraction

(200mg/kg bw of animal); GSF2 = diabetic rats administered *Gongronema latifolium* saponin fraction (400mg/kg bw of animal). *Significantly different from NC at $p<0.05$
a, b and c = significantly different 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 (mg/dL)	VLDL (mg/dL)	LDL (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*
INS	72.86± 9.91* ^a	66.36 ± 0.50* ^a	398.80 ± 38.20* ^a	14.57 ± 2.00* ^a	14.79* ^a
GSF	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 (5IU/kg bw of animal); GSF1 = diabetic rats administered *Gongronema latifolium* saponin fraction (200mg/kg bw of animal); GSF2 = diabetic rats administered *Gongronema latifolium* saponin fraction (400mg/kg bw of animal). *Significantly different from NC at $p<0.05$
a, b and c = significantly different from DC at $p<0.05$

4.0 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 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 LD₅₀ (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 LD₅₀ of the saponin-rich fraction of *Gongronema latifolium* was determined to be 2500mg/kg body weight, in accordance with the Lorke method (1983).

Several studies have shown that diabetes is indeed a heterogeneous disease characterized by hyperglycemia and atherogenic lipid profile (Alberti et. al.1998). 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 (Komolafe et. al, 2009). Damage to key organs has also been observed in experimental animal models upon induction of diabetes using either streptozotocin or higher doses of alloxan (Akpaso et al. 2011; Edet et al. 2013).

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 (Aguila and Manderim-de-Lacerda, 2003). 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 (Ahren et al 1999). However, when the animals were treated with extract of saponin-rich fraction of *Gongronema latifolium*, 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 (Ebong et. al. 2006 & 2008; Atangwho et al, 2007; Efiong et. al. 2013; Iwara et. al. 2015, Igile et. al. 2015).

Hyperlipidemia are disorders of the rates of synthesis or clearance of lipoproteins from the bloodstream, biochemically detected by measuring plasma triacylglycerol and total cholesterol (Steinberg 2005; 2006). 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 (Havel *et al.*, 1995). Hyperlipidemia therefore is a potent risk factor for atherosclerosis and coronary heart disease which presents in substantial proportion of young adults (Steinberg, 2005). 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 (James *et al.* 2014). Studies on adults with familiar hypercholesterolemia have shown that, cardiovascular risk is increased early among those with high low density lipoprotein cholesterol (HDL) level (Hopkins 2003). Similarly, adults with extremely low LDL-C level conferred by genetic polymorphisms have significantly lower than average risk of cardiovascular disease (Sniderman *et al.*, 2005). 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 (American Heart Association, 2007). 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 (The Expert Panel, 1988). 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 (Brown & Goldstein 2006). 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 (American Heart Association, 2007). High density lipoprotein is considered to be beneficial as high levels of HDL-c have been correlated with reduced risk of negative cardiovascular event (Segrest et al 1999 & 2002). 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 (Segrest et al. 1999). 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 (Segrest, 2002). 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 (Kannel et al 1979; Snidermann et al. 1991).

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 (Kannel et al 1979; Snidermann et al. 1991; Steinberg 2005; 2006). Therefore elevation of hypercholesterolemia in diabetes may be associated with endothelial dysfunction and dislipidemia (Kannel et al 1979; Snidermann et al. 1991).

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 (Ebong et al 2008, Igile et al. 2015).

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 (Andrade-Cetto and Wiedenfeld H. 2001). 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 200mg/kg and 400mg/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 Edet et al (2013), 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 $(0.07 \pm 0.01 \text{ ng/L})$ for GSF and $(0.04 \pm 0.02 \text{ ng/L})$ for insulin, when compared to the C-peptide concentration $(0.00 \pm 0.00 \text{ ng/L})$ 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 saponin-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* (Igile et. al. 1995).

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.

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.

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.

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