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Effect of *Gongronema latifolium* on lipid profile, oral glucose tolerance test and some hematological parameters in fructose-induced hyperglycemia in rats

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ABSTRACT

Gongronema latifolium (GL) has been used traditionally in the management of various ailments. The effects of GL on some haematological and biochemical parameters in fructose-induced hyperglycaemia were studied. Forty rats were randomly assigned to four groups of 10 rats each. Control was received normal rat chow, fructose + *G. latifolium* group was received 66% D-fructose mixed with 34% chow and crude leaf extract of GL daily. Fructose only group was received 66% D-fructose and the fourth group was received GL extract only respectively for 30 days. All animals were fed ad libitum and had free access to water. Oral blood glucose tolerance test was determined using 2 g/Kg in all groups of rats and blood samples were obtained by cardiac puncture for haematological and biochemical analyses. The blood glucose level was significantly raised in fructose-fed only group (140.6 ± 2.9 mg/dl) when compared to GL + fructose group (110.3 ±5.8 mg/dl) and control (88.1 ± 3.6 mg/dl). There was observed significant reductions in blood glucose and glucose tolerance following GL supplementation. The lipid profile values were significantly higher in fructose-fed group compared with other groups but these levels were significantly reduced following GL supplementation. The white blood cells (WBC) and platelets count in GL and fructose + GL group were significantly raised when compared with the control group. The red cell parameters were not significantly altered compared to the control group. The results show that the consumption of *G. latifolium* reduces hyperglycaemia and hyperlipidaemia hence the cardiovascular risk factors observed in diabetes mellitus.

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Introduction

Gongronema latifolium Benth (Asclepiadaceae) (figure 1) is a climbing perennial edible herbaceous non woody shrub commonly grown in Nigeria. Its common name is amaranth globe and is locally known as *utasi* by the Efiks, Ibibios and Quas, *utazi* by the Igbos and *arokeke* by the Yorubas (1). The plant is found in rainforest, deciduous and secondary forests, and also in mangrove. The leaves have simple opposite arrangement with round margin. The flowers are small, bisexual, regular with a pedicel that is 2-4mm long (2). The leafy vegetable can be propagated by its seed or by planting the cut softwood or root. In Eastern Nigeria, the leaves are used to prepare soup for lactating mothers to stimulate appetite and reduce post-partum contraction. *Gongronema latifolium* is traditionally used for a number of medicinal and nutritional purposes. *Gongronema latifolium* is used traditionally in the management and treatment of malaria, hypertension and diabetes mellitus (3).

Fructose is widely used as a sweetening substitute for glucose or sucrose in food processing (4) and has become a major constituent of modern diet. Fructose is employed in the preparation of desserts, condiments, and carbonated beverages. The consumption of large amounts of refined carbohydrates in food and beverage increases the risk of dyslipidaemia (5), obesity (6), insulin resistance (7), and heart disease (8). The high intake of added sugar, a prominent source of low-nutrient calories in processed or prepared foods and caloric beverages is a relatively new phenomenon (6). Studies have shown that normal rats fed with fructose enriched diet developed hypertension accompanied with metabolic abnormalities including hyperglycemia, insulin resistance, hyperinsulinemia and hypertriglyceridemia (9-10). Feeding of high fructose diet (HFD) can provide a type 2 diabetic dietary model associated with insulin resistance (11) and hypertriglyceridemia (12). Fructose overload is known to disturb glucose metabolism and glucose uptake pathways resulting in insulin resistance observed in both human and animal models (13).

Individual and synergistic anti-diabetic effects of *Gongronema latifolium* have been reported (14). Scientific studies have established the hypoglycaemic, cardio-protective, hypolipidaemic, anti-inflammatory and antioxidative effects of aqueous and ethanolic extracts of *G. latifolium* leaf (15,16).

Figure 1 Leaf of Gongronema latifolium collected from a stem on December 12, 2018

Gongronema latifolium has antioxidant activity (17, 18). Some bioactive phytochemicals found in *G. latifolium* which may contribute to its anti-diabetic property include β-sistosterol, lupenyl esters, pregnane ester, glycosides, essential oils and saponins (19, 20). Effects of *Gongronema latifolium* on haematological indices of alloxan induced diabetic rats have been reported (21) but there appears to be little or no scientific data of some biochemical parameters of fructose induced hyperglycemia such as glucose tolerance. The glucose tolerance test is used to determine how quickly glucose is cleared from the blood. The glucose tolerance test is commonly used to test for hyperglycemia and impaired pancreatic function (22). Hence, this study was designed to assess the effect of ethanolic extract of *Gongronema latifolium* on glucose tolerance, haematological indices and lipid profile of fructose induced hyperglycemia fed Wistar rats.

Materials and methods

Drugs and chemicals

Lipid profile kits for total cholesterol, triglyceride and high density lipoprotein cholesterol kits were purchased from Randox reagent (UK). D-Fructose was obtained from Sigma Chemical Co (St. Louis, USA).

Preparation of plant materials

Fresh leaves of *Gongronema latifolium* where obtained from Adiabo in Odukpani Local Government Area of Cross River State Nigeria. The plant was identified and deposited at the herbarium in Department of Botany, University of Calabar, Nigeria (Voucher number: GLB 4612). The leaves were picked, washed in clean water, dried in a shade and crushed to fine powder using manual grinder. The ground leaf powder (600 g) was soaked in Ethanol (95% v/v BDH) for 5 days. It was then filtered with Whatman No 1 filter paper. The filtrate was dried in an oven at 40°C. This yielded a gummy paste which was stored at 4 ºC till subsequent use.

Experimental animals

Forty Wistar rats (weighing between 80-120 g) were used for the study and grouped into four groups of ten rats each. They were obtained from the animal house of the Department of Physiology, University of Calabar, Calabar, Nigeria. Ethical approval for the study was obtained from the Faculty of Basic Medical Science Animal Research Ethics Committee, University of Calabar (Approval No: 019PY20317). The animals were kept in plastic cages at room temperature of 28±2°C with 12h light/dark cycle.

Group 1 (control) was received normal chow and drinking water. Group 2 was received ethanol extract of leaves of *Gongronema latifolium* orally at a dose of 200 mg/kg body weight based on previous study (23) and normal chow. Group 3 was received 66% fructose mixed with 34% chow and crude leaf extract of *Gongronema latifolium,* orally at a dose of 200 mg/kg body weight. Group 4 was received 66% fructose mixed with 34% chow and tap water. Hyperglycaemia was induced following a previously reported method (24). The rats were treated with the extract daily for 30 days.

Weight determination

The total body weight of rats was measured using a digital weighing balance, before and after the experimental period and recorded as Initial (IBW) and Final Body Weight (FBW), respectively. The mean body weight for each group of rats was measured from total weights. Weight changes were expressed as final body weight-initial body weight/initial body weight ×100.

Blood glucose level and oral glucose tolerance test (OGTT)

Blood glucose level was determined by the glucose oxidase method. Blood glucose concentrations were determined from a drop of blood obtained by pricking the tail using a glucometer (Accu-check Active, Roche Diagnostics, Mannheim, Germany). Oral glucose tolerance test (OGTT) was conducted in each rat after 12 h fast at the end of the experimental period (30 days). Each animal was administered with oral glucose solution at a dose of 2 g/kg body weight and blood samples were collected from the tail at 0 min (before glucose administration), 30, 60 and 120 min after glucose load. The blood glucose concentration was determined as described previously. From the blood glucose concentration data of the OGTT, the area under the curve (AUC) was calculated for each group using the equation:

$AUC=(t_1-t_0)/2(C_0+C_1)+(t_2-t_1)/2(C_1+C_2)+(t_3-t_2)/2(C_3+C_2)$…(where t = time and C = concentration of glucose)(25). The % difference in AUC was calculated as $((AUC_{test} - AUC_{control}) / AUC_{control}) \times 100$.

Collection of blood samples

After the OGTT, each rat was sacrificed following anaesthesia and blood collected by cardiac puncture. Each blood collected was divided into two, half emptied into EDTA plain bottles and allowed to stand for two hours to clot. Serum was obtained from clotted blood by centrifugation using a bench top centrifugation at 3000 g for 10 minutes. The resulting serum was stored at – 20°C till further use. The remaining half of the whole blood samples collected were put in EDTA tubes and used for determination of the levels of haemoglobin (Hb), haematocrit, red blood cell (RBC), white blood cell (WBC) and platelet counts. All haematological analyses were carried out within 6 hours of sample collection.

Determination of lipid profile

The serum level of triglycerides was determined by colorimetric enzymatic test glycerol-phosphate oxidase method (26). The level of total cholesterol in the serum was determined by the use of monoreagent enzymatic cholesterol colorimetric test method of Siedel *et al*. (27). The serum level of high-density lipoproteins cholesterol (HDL-chol) was determined by the enzymatic colorimetric method (28). The equation of Friedewald *et al.* (28) was used to calculate the value of LDL-cholesterol from the measured total cholesterol, HDL-cholesterol and triglyceride (TG). The equation is as follows: LDL-cholesterol = Total cholesterol – TG/2.2 – HDL- cholesterol.

Determination of haematological parameters

The blood parameters namely red blood cell (RBC) count, packed cell volume (PCV), haemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and platelet count were obtained using a fully automatic blood cell counter (Model PCE 210, Japan).

Statistical analysis

Data are presented as mean ± standard error of mean (SEM). Data was analysed using One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad Prisms software version 6 for Windows, GraphPad Software (San Diego California USA). A p value of 0.05 was considered significant.

Results

Blood glucose level

Table 1 shows the result of daily oral administration of extract of leaves of *Gongronema latifolium* for 30 days in fructose-induced hyperglycemia Wistar rats. The result of the animals treated with only extract of leaves of *Gongronema latifolium* showed significantly (P < 0.05) reduced blood glucose level compared with untreated animals. Significant (P < 0.05) reduction in the concentration of blood glucose was also observed in the group that received Fructose + *Gongronema latifolium* leaves extract compared to the group treated with fructose only.

Table 1 Blood glucose level in normal and fructose-fed rats treated with *Gongronema latifolium* extracts

Group	Initial (mg/dl)	After feeding (mg/dl)
Group 1 (Control)	96 ± 3.6	88.1 ± 3.6
Group 2 (Fructose + GL)	103 ± 5.6	110.3 ±5.8*
Group 3 (Fructose only)	101 ± 4.7	140.6 ± 2.9*+
Group 4 (GL only)	105 ± 3.0	81.3 ± 5.0

* = P < 0.05 compared with initial level; + = P < 0.05 compared with groups 1 and 2 (n = 6)

Body weight

The initial and final body weight of animals in the control and experimental groups are presented in Table 2. The result shows that there was no significant difference in the initial body weight of rats in all the groups. At the end of the feeding period all animals in all groups had a significant weight gain. The final body weight was comparable for all groups. However, the percentage weight gain was 100%, for the control group, 74% for fructose + *Gongronema latifolium* group, fructose only (83.2%) and GL only (82.8%). At the end of the feeding period, the lowest percentage weight gain was observed in the Fructose + *Gongronema latifolium* group which was significantly (P < 0.05) reduced compared with control and the other groups. The reduced gain in body weight may be attributed to the retarding effect of *Gongronema latifolium* on growth parameters and loss in muscle and adipose tissue resulting from breakdown of tissue protein and fatty acids.

Table 2 Changes in body weight of normal and fructose-fed rats treated with *Gongronema latifolium* extracts

Group	Initial	Final	Change (%)
Control	100 ±3.6	200 ± 5.2	100
GL only	105 ± 4.0	192 ± 4.2	82.8
Fructose + GL	103 ± 4.6	180 ± 5.8	74.7
Fructose only	101 ± 4.6	185 ± 5.9	83.2

Results are mean ± SEM (n = 6)

Glucose tolerance test

The result of oral glucose tolerance test is presented in figure 2. The area under curve (AUC) was 9860 minute.mg/dl for control and 12124 minute.mg/dl for GL only group. It was 14729 minute.mg/dl for fructose + GL group and 19072 minute.mg/dl in fructose fed group. The area under curve in fructose fed and *Gongronema latifolium* + fructose fed group were significantly ($P < 0.01$) decreased when compared with *G. latifolium* group. The percentage increase of AUC in fructose fed-group over control was 93.4% while it was 49% in *G. latifolium* +fructose fed group and 22% in the *G. latifolium* group only.

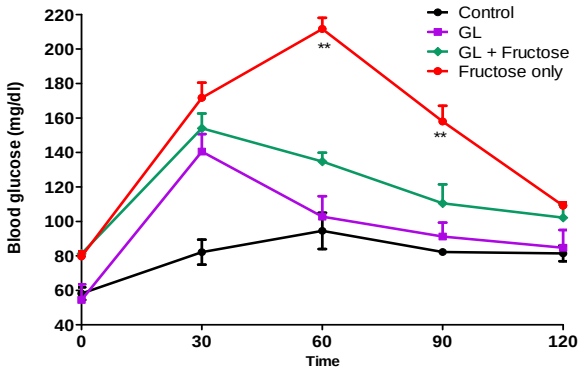

Figure 2 Oral glucose tolerance test in normal and hyperglycemic rats treated with *Gongronema latifolium* extracts ** = $P < 0.01$ compared with fructose only group (n = 6)

Lipid profile

The mean serum lipid profile is presented in Table 3. There was a significant increase in the serum levels of total cholesterol, TG, LDL-cholesterol and a significant ($p < 0.01$) decrease in the HDL-cholesterol of the rats in the fructose-fed only group when compared to the normal control group. However, GL administration caused a significant ($p < 0.01$) decrease in the serum levels of total cholesterol, triglyceride, LDL-cholesterol and a significant ($p < 0.01$) increase in the HDL-cholesterol when compared to the fructose-fed only group. GL did not alter the lipid profile adversely when compared with the control.

Haematological indices

The mean haematological indices in rats fed on fructose and *Gongronema latifolium* is presented in Table 4. The WBC count was 5.4 ± 0.4 x 10^9cells/L for control; 8.4 ± 0.8 for fructose with *Gongronema latifolium* group; 6.4 ± 0.7 for fructose only; and 9.3 ± 0.9 for *Gongronema latifolium* only group. All the groups showed increasing trend. The WBC count in the Fructose + *Gongronema latifolium* group and *Gongronema latifolium* treated groups were significantly ($P < 0.01$) higher than control group. The mean RBC count in rats fed on fructose and *Gongronema latifolium* in the control group is 7.9 ± 0.7 × 10^{12} cells/L; 8.9 ± 0.1 ×10^{12} cells/L for fructose with *Gongronema latifolium* group; 7.9 ± 0.9 × 10^{12} cells/L for fructose only; and 9.4 ± 0.4×10^{12} cells/L for *Gongronema latifolium* only group. The RBC values in the test groups were not too different from that of control group.

The mean Hb values in rats fed on fructose and *Gongronema latifolium* is as presented in table 3, in which the haematological indices is recorded for control 15.7 ± 0.82 g/dl; 15.2 ± 0.12g/dl for fructose with *Gongronema latifolium* group; 14.9 ± 0.40 g/dl for fructose only; and 15.4 ± 0.71 g/dl for *Gongronema latifolium* only group. There was no significant difference in Hb values between the experimental groups and control. There was no significant change in the PCV levels of rats fed on fructose and *Gongronema latifolium* in all the test groups when compared with the control. The results for rats fed on fructose and *Gongronema latifolium* show that there was no significant change in the concentrations of platelets, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) when compared with the control group.

Discussion

The result in this study shows that after consumption of *Gongronema latifolium* in fructose-treated group, there was a significant reduction in serum glucose level, glucose tolerance test, serum lipid profile and haematological indices. High fructose diet has been associated with elevation of plasma glucose, insulin and triglycerides in animal models. Experimental diabetic diet containing higher levels of fructose contribute to a metabolic disturbance resulting in hyperglycemia and hyperlipidemia (30). A study had demonstrated the relationship between high fructose diet with elevation of plasma glucose and triglycerides in animal models (31). The result of reduced blood glucose parameter recorded in the experimental animals indicates that *G. latifolium* leaves may possess hypoglycemic or anti-diabetic properties. This is in agreement with previous studies that reported that aqueous and ethanolic *G. latifolium* extracts had hypoglycemic properties in animal model through the activation of some glucose metabolic enzymes in the liver (32). Therefore, the observed hypoglycemic effects of *G. latifolium* could be beneficial in preventing diabetes mellitus.

The oral glucose tolerance test (OGTT) was done at the end of the experimental period. The glucose tolerance ability of Fructose +GL was better than the Fructose only group and the glucose level was found to be relatively reduced as well. This could probably be due to the effect of phytochemicals present in the plant leaves of *G. latifolium*. These phytochemicals present are several types of alkaloids, flavonoids, total phenolic compound, lignan, terpenes, sterol, allicin, hydroxycinnamic acids, saponin and carotenoid (33) and phytols, alkaloids and saponins that have

Table 3 Lipid profile in normal and fructose-fed rats treated with *Gongronema latifolium* extract

Parameters	Control	GL only	GL + Fructose	Fructose
Total cholesterol	2.2±0.07	2.0±0.08	2.9±0.14**	3.5±0.12**a
Triglyceride	1.4±0.06	1.2±0.05	2.1±0.05**	2.6±0.07**a
HDL-Cholesterol	1.8±0.05	1.6±0.05	1.6±0.08	0.99±0.04**a
LDL-Cholesterol	1.9±0.06	2.1±0.05	2.9.0±0.05**a	3.8±0.05**

* = $P < 0.05$ vs. control, ** = $P < 0.01$ vs. control; a = $P < 0.01$ vs GL + Fructose fed group

Table 4 Haematological indices in control and experimental groups treated with fructose and G. latifolium leaf extract

Parameter	Control	Fructose + *GL*	Fructose	*GL* only
WBC ($\times 10^9$ cells/L)	5.4 ± 0.4	8.4 ± 0.8*	6.4 ± 0.7	9.3 ± 0.9*
RBC ($\times 10^{12}$ cells/L)	7.9 ± 0.7	8.9 ± 0.1	7.8 ± 0.9	9.4 ± 0.4
HB (g/dl)	15.7 ± 0.8	15.2 ± 0.1	14.9 ± 0.4	15.4 ± 0.7
PCV (%)	51.7 ± 1.9	49.9 ± 0.6	48.5 ± 0.8	48.1 ± 2.4
Platelets ($\times 10^9$ cells/L)	552 ± 16.8	680 ± 15.8**	637 ± 15.0**	730 ± 16.9**
MCV (cell/L)	55.1 ± 1.3	55.2 ± 0.9	55.7 ± 0.9	51.2 ± 1.1
MCHC (g/cell)	31.1 ± 0.4	30.8 ± 0.2	30.6 ± 0.2	32.1 ± 0.4

antihyperglycaemic effect (34). Also, it is known that these phytochemical constituents can stimulate insulin actions on the beta cells of the pancreas. The phytochemical such as polyphenol present in *G. latifolium* has been reported to possess antidiabetic activity (35). Polyphenols is known to attenuate hyperglycaemia, lipidaemia and alleviate oxidative stress (36). The reductions in blood glucose level due to *Gongronema latifolium* extract administration may be attributed to the ability of the plant extract to alter the inhibitory activity of fructose on glucokinase, the glucose sensor of the beta cells (37). The significant reduction in the blood glucose level after treatment with *Gongronema latifolium* observed in this study agrees with previous reports in rats (32, 38-39). Therefore, it is credible to suggest that this antidiabetic activity may be solely dependent on the activity of *Gongronema latifolium* extract.

Absorbed fructose is metabolized rapidly by the liver. The exposure of the liver to large quantities of fructose leads to high stimulation of lipogenesis and triglyceride accumulation resulting in insulin resistance. When other carbohydrates are used, fructose continuously enter related pathways to produce glucose and promote the over production of triglyceride.

These negative effects of fructose metabolism have summoned it usage on diabetes mellitus induction in animal models (24). In the present study, fructose-fed only group showed significantly increased serum lipid profiles except HDL when compared with the control rats. However, treatment with *G. latifolium* extracts significantly reduced the total cholesterol, triglyceride and low density lipoprotein when compared to the fructose-fed only rats. Similarly, the high density lipoprotein cholesterol (HDL-cholesterol) which was reduced in the fructose-fed only rats was raised in the groups administered the *G. latifolium* extracts only. The observed reduction in the serum lipid levels except HDL-cholesterol compared with the control indicates that *G. latifolium* leaves could have hypolipidemic properties and this is consistent with previous studies that reported hypolipidaemic property of *G. latifolium* extracts in animal model (32, 40).

The result of this work showed weight gain in all animals in all groups but the lowest percentage weight gain was observed in the Fructose + *Gongronema latifolium* group which was significantly reduced compared with control and the other groups. This reduced gain in body weight may be attributed to the retarding effect of *Gongronema latifolium* on growth

parameters and loss in muscle and adipose tissue resulting from breakdown of tissue protein and fatty acids.

The result of this study shows significant increase in the white blood cell and platelet count of the experimental rats when compared to the control. White blood cells have been known to increase in cases of infections or assault to organs and tissues and as response to incoming xenobiotics or foreign bodies to the system. The observed increase in WBC count in this study is as a normal physiological response to the presence of the extract in the system. This collaborated with a previous study (3). Alternatively, the increase in the level of WBC may be attributed to glycoside content in the extract (21). Glycoside has anti-inflammatory property and vital effect on inflammatory processes of some pathological states such as bacterial infection, malaria and liver diseases (41).

Platelet play a role in blood clotting and this study showed a significant increase in the platelet count in all the test groups when compared to the control. This increase in platelet counts suggests that the active principles present in the leaf extract has the capacity to stimulate platelet synthesis. Interestingly, some haematological parameters as shown in this study, RBC, HB, MCV, and MCHC remain relatively unperturbed by the plant extract in all the treated groups and are in agreement with previous reports (3, 42). The findings in this study showed that consumption of *G. latifolium* leaves did not adversely affect the erythropoietic activity or haem synthesis.

This study has some limitations. The research focused did not use a positive control such as metformin or glibenclamide which are known hypoglycaemic agents used in diabetic condition due to the design of the study. However, it has been reported that the reduction of blood glucose by GL was similar compared to glibenclamide (43). Secondly, the level of oxidative stress and antioxidant enzymes were not measured. However, previous studies have shown that the leaf extract of *G. latifolium* has antioxidant property and reduces oxidative stress by elevating the levels of antioxidant enzymes such as GSH and SOD in diabetic and normoglycaemic conditions (23, 41, 44). Since hyperglycaemia was induced by fructose in this study, it is most likely that the antioxidants present in the extract could exert its beneficial effect in reducing hyperglycaemia and oxidative stress. Another limitation is that histology of some related organs and inflammatory markers such as adiponectin, interleukins and tissue necrotic factor α were not determined. However the anti-inflammatory property of the plant has been documented (20) and our previous histological study on the heart, kidney and aorta showed the protection of these organs by GL (44). There is need for further research on the effect of GL on inflammatory markers and possible signalling pathways in fructose-induced hyperglycaemia.

It is concluded that *G. latifolium* leaves extract reduced serum glucose and decrease oral glucose tolerance test, total cholesterol, triglyceride, LDL-cholesterol levels with increased HDL-cholesterol. The present study indicates that *G. latifolium* leaves has hypoglycaemic and hypolipidaemic potential and raises leucocytes and platelet counts probably due to the phytochemicals present in the leaves making it beneficial for the management/prevention of fructose-induced hyperglycaemia.

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Conflict of interest

The authors declare no conflict of interest.

Financial disclosure

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