Consumption of *Gnetum Africanum* and Effects on Dislipidemia in Diabetic Rats

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Introduction:

This study is to investigate the potential antioxidant activities of *Gnetum africanum* (GnA) and effects on dyslipidemia in diabetic rat.

Methods:

Part of the study was to assess the concentration of secondary metabolite (total: phenolic, flavonoid, and proanthocyanidin, tannin) and the antioxidant activities in methanol (MeOH) and methanol-water (MeOH-H₂O) extract of GnA. In the other hand 28 males Wistar rats weighing 207-292gr were randomly divided to four groups of 7 each including: Normal (Group I: rat were fed basal diet), Control (Group II: rat received 50mg/kg b.w of GnA.), STZ (Group III diabetic rat), STZ+GnA (Group IV: diabetic rats received 50mg/kg b.w of GnA). Diabetes mellitus was experimentally induced by injection of 60mg/kg b.w. of STZ.

Results:

The methanol-water extract of GnA recorded highest amount of total: phenolic (75.21 mg GAE/100 mg extract), flavonoid (16.75 mg QE/100 mg extract), tannin (0.43 mg TAE/100 mg extract) and proanthocyanidin (6.38 mg APE/100 mg extract). The results suggested that the two extract exhibit important antioxidant activities (MeOH: IC₅₀: 13.19 ± 0.06; IAA: 3.788 and MeOH-H₂O:15.37 ± 0.01; IAA: 3.252). STZ-induced diabetes caused significant increase in blood glucose, triglyceride (TG) and total cholesterol (TC) in group III (p<0.05).7 days after consumption of GnA we observed a decrease of biochemical parameters (blood glucose, serum TG and TC) (p<0.05).

Conclusion:

These findings suggested that antioxidant activities of GnA and anti-hyperlipidemia properties thus help in preventing future complications of diabetes.

Keywords: *Gnetum africanum*; Diabe; Dislipidemia; Antioxidant

Introduction

The increased risk of cardiovascular disease in diabetic patients is due to early and extensive atherosclerosis and although the pathogenesis of this atherosclerosis is obviously multifactorial. Dyslipidemia is an important indicator of cardiovascular risk in diabetics [1]. Most of the therapeutic intervention data on dyslipidemia come from type 2 diabetic patients. Type 2 diabetic dyslipidemia is characterized by both quantitative and qualitative abnormalities of lipoproteins with, typically a moderate increase in triglycerides (TG), a variable reduction in the level of HDL-cholesterol (HDL-c) and an accumulation of residual lipoproteins enriched in cholesterol [2-4]. The treatment of the dyslipidemia of a diabetic is a key element in the overall management of the cardiovascular risk of these patients. A statin is the first-line treatment of choice [5-8]. However, despite the proven benefit of such statin therapy on the reduction of major cardiovascular events, there is often a residual risk with statin too high in diabetics.

Food plants are known to have a beneficial effect on the health of diabetics, in particular in the regulation of blood glucose and in the prevention of cardiovascular complications [9-20]. This beneficial power of plants is due in large part to the content of phenolic compounds and their antioxidant power. *Gnetum africanum* (GnA) is the most popularity plant food in Gabon and is gaining equal popularity in other African countries such as Cameroon, Nigeria, Congo and Angola [21]. The seed of GnA is oval in shape and small in size, about 0.5 cm in diameter. They are greenish in color when and randomly from a thick bush around the rain unripe and reddish when ripe (Figure 1). Different studies have shown that chronic GnA administration resulted in decreased plasma sodium in the rat and also had an anti-oxidant and protective effect on retinopathy of the diabetic subject [22,23].
Nowadays no studies have shown the incidence of GnA consumption on dyslipidemia in diabetic subjects. The aim of this study is to show whether chronic consumption of *Gnetum africanum* could prevent dyslipidemia in diabetic rat. On the one hand, it will be necessary to evaluate the content of phenolic compounds and the antioxidant activities of the plant. Subsequently demonstrate the anti-hyperglycaemic capacity of the plant and assess the impact on plasma lipid concentration in diabetic rats.

**Methods**

Plant fresh materials: fresh plant sample of *Gnetum africanum* was obtained from market and identified by the botanists of the national herbarium. Plant was divided in two sets (a set for biology study and a set for phytochemical study). Preparation of extracts for polyphenols measure and antioxidant activity: the fresh plant was dried in the open air and reduced to powder by use of a clean electric blender. The powders were kept at room temperature until required. Air-dried powdered leaves (20 g) of GnA were separately extracted with 480 ml of methanol-water (MeOH-H$_2$O, 80/20, v/v) and 620 ml of methanol (MeOH, 100%) by maceration for 48 h. The choice of water was to mimic the traditional method of preparing crude plant extracts and that of methanol was to enhance the extraction of more constituents. Extracts were filtered and dried under reduced pressure at 40° C. Extracts were stored in freezer at -20°C until further tests.

Figure 1: Photo of *Gnetum africanum*

Determination of total polyphenol content: the Folin-Ciocalteu method was used to measure total amount of polyphenol content [24]. Aliquots of 0.25 ml of leaf extracts (1 mg/ml) were mixed with 1.25 ml Folin–Ciocalteu reagent (0.2 N diluted in MeOH). A reagent blank using MeOH instead of sample was prepared. After 5 min incubation at room temperature, 1 ml sodium carbonate solution (7.5%) was added. Samples were incubated at room temperature for 1 h and the absorbance was measured at 765 nm versus the prepared blank. All tests were carried out in triplicate and polyphenol content was expressed as mg of gallic acid equivalents (GAE) per 100 mg of leaf extract using the following linear equation based on the calibration curve: $Y = 0.0887 X + 0.0028$ with $R^2 = 0.9926$. Where $Y$ stands for the absorbance and $X$ is the concentration as gallic acid equivalents (μg/ml).

Determination of proanthocyanidin content: proanthocyanidins (PAs) were quantified with the hydrolysis test of proanthocyanidins in a hot acid-alcohol medium into anthocyanidins. This method allows taking into account all the units of flavans-3-ols constituting the polymers [25]. The heating step destroys the anthocyanidins pigments generated by flavan-4-ols and eliminates part of the chlorophyll pigments. The routine assay was performed by mixing 0.16 ml (1 mg/ml) of the extract with 2.33 ml of 30% HCl-butanol solution (v/v). The mixture was put in tightly closed tube and vortexed for 1 min. Subsequently, the tube was heated at 100°C for 2 h and after cooling, the absorbances were read at 550 nm. Apple procyanidins (DP ≈ 7.4) treated as aforementioned were used as a standard. Results were expressed as apple procyanidins equivalent (APE) using the following linear equation based on the calibration curve: $Y = 0.0006 X + 0.0024$ with $R^2 = 0.9869$. Where $Y$ stands for the absorbance and $X$ is the concentration as apple procyanidins equivalents (μg/ml).

Determination of flavonoid content: total flavonoid contents were determined by the aluminum chloride (AlCl$_3$) colorimetric assay method, using quercetin as a standard [26]. The total flavonoid contents were expressed as quercetin equivalents in milligrams per gram sample using the following linear equation based on the calibration curve: $Y = 0.0017 X - 0.0038$ with $R^2 = 0.9926$. Where $Y$ stands for the absorbance and $X$ is the concentration as quercetin equivalents (μg/ml) Determination of tannins content: the reference method of European community was used to measure total amount of tannins using the following linear equation based on the calibration curve: $Y = 0.0006 X + 0.0024$ with $R^2 = 0.9869$. Where $Y$ stands for the absorbance and $X$ is the concentration as apple procyanidins equivalents (μg/ml).

**Antioxidant activity index:** the Antioxidant Activity Index (AAI) was assessed according to the method described by [27]. This method is based on the DPPH radical test. Briefly, the working reagent was prepared by dissolving 10 mg of DPPH in 100 mL ethanol. Graded concentrations of extracts ranging from 12.5 to 200 μg/mL obtained by two-fold dilutions were prepared and 100 μL of each dilution were mixed with 100 μL of the working solution of DPPH. Absorbencies were measured at 517 nm after 15 min incubation at room temperature in the dark. Ascorbic acid (Vitamin C) and Butylated Hydroxyanisole (BHA) were used as references. The ability to scavenge DPPH radical was calculated by the following equation:
The IC₅₀ (concentration providing 50% inhibition) of extracts and standards was determined using regression curves in the linear range of concentrations. The AAI was then calculated as follows:

$$\text{AAI} = \frac{[\text{DPPH}]}{\text{IC}_{\text{A}}} = \frac{(\mu g.mL^{-1})}{(\mu g.mL^{-1})}.$$ 

[DPPH] is the final concentration of DPPH.

We considered criteria of according to which plant extracts show poor antioxidant activity when AAI < 0.5, moderate antioxidant activity when AAI between 0.5 and 1.0, strong antioxidant activity when AAI between 1.0 and 2.0, and very strong when AAI > 2.0.

Preparation of *Gnetum africanum* aqueous extracts for biology study: the extract was then filtered through Whatman No.1 filter paper and the residue was discarded. The resultant filtrate was then evaporated to dryness and stored in capped bottles at 4°C until use. The desired amount of extract was dissolved in water to make 50 mg/ml of stock solutions for lower and higher doses respectively.

Animals: twenty-eight males Wistar rats weighing 207-292 g (IFFA-CREDO, France) were used for this experiment. The animals were born in the laboratory from twenty litters, culled to 7 pups per litter to ensure normal body growth. The animals were housed in standard cages under controlled temperature conditions (22±1°C). Food and water were available ad libitum throughout the experiment. From birth, the rats were kept on a reversed 12:12 light–dark cycle (dark period 08:00–20:00). All experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (no. 85-23, revised 1996), the recommendations of the European Community Council for the Ethical Treatment of Animals (no. 86/609/EEC) and the regulations of the University of Masuku. All efforts were made to minimize animal suffering.

Experimental induction of diabetes: the seven rats were treated with normal pellet diet and the rest were given as per the grouping. The animals were rendered diabetic by a single subcutaneous injection of STZ (55 mg/kg b.w.) [16,17]. STZ (Sigma USA) at a dose of 55 mg/kg was prepared in cold citrate buffer (pH 5) and administered. The STZ-injected animals exhibited hyperglycemia after 72 h. Blood samples were taken by tail vein puncture and fasting blood glucose levels were monitored using glucometer (ACCU-CHEK) [28]. Rats with fasting blood glucose level ≥200mg/dl were considered diabetic and were used in the study. The diabetic rats were randomly divided into four groups each consisting of seven rats and the study was continued up to 7 days.

Experimental Design

- **Group I (Normal):** rat group were fed basal diet throughout the experiment
- **Group II (Control):** rat group treated with an oral dose of GnA 50mg/kg
- **Group III (STZ):** STZ-induced diabetic rats
- **Group IV (STZ+GnA):** STZ-induced diabetic rats treated with an oral dose of GnA 50mg/kg

Determination of glycosuria: the determination of the presence of glucose in the urine was done with commercially available urinary strips (ACON). To do this, 50μl of urine in the summer deposited at the place indicated on the strip. After 1 minute of migration. The intensity of the staining is characteristic of the concentration of glucose in the urine Biochemical estimations: at the end of the experimental period, the rats were deprived of food overnight and sacrificed by cervical decapitation. The blood samples were collected on ethylenediaminetetraacetic acid containing tubes and serum was separated immediately. Serum lipid profiles were measured by clinical chemistry analyzer (COBAS C111). Statistical analysis: data were statistically evaluated by one-way ANOVA, followed by Dunnett’s multiple comparison tests. The values were considered significant when P<0.05.

Results

The contents of total phenolic, total flavonoids, total tannins and total proanthocyanidins of extracts from GnA are presented in Table 1. The methanol-water extract of GnA recorded highest amount of total phenolic (75.21 mg GAE/100 mg extract), total flavonoid (14.02 mg QE/100 mg extract) and proanthocyanidin (6.21 mg APE/100 mg extract) contents. However, the methanol extract also recorded slightly more amounts of total tannins (0.43 mg TAE/100 mg extract) and total proanthocyanidin (6.38 mg APE/100 mg extract) contents.

Comparison of Total phenolic content (TPC), Total flavonoid content (TFC) Total Tannins Content (TTC) and Total Proanthocyanidins Content (TPA) of leaves in two solvents

<table>
<thead>
<tr>
<th>Plant</th>
<th>Extracts</th>
<th>TPC (mg GAE/100 mg)</th>
<th>TFC (mg QE/100 mg)</th>
<th>TTC (mg TAE/100 mg)</th>
<th>TPA (mg APE/100 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gnetum africamum</em></td>
<td>MeOH</td>
<td>69.66±0.2</td>
<td>14.02±0.06</td>
<td>0</td>
<td>6.21±0.01</td>
</tr>
<tr>
<td></td>
<td>MeOH-H₂O</td>
<td>75.21±0.09</td>
<td>16.75±0.03</td>
<td>0.43±0.02</td>
<td>6.38±0.002</td>
</tr>
</tbody>
</table>

AAI = [(DPPH) (µg.mL⁻¹)] / IC₅₀ (µg.mL⁻¹). [DPPH] is the final concentration of DPPH.

The IC₅₀ and IAA values of the two solvents extracts of GnA are shown in Table 2. The result suggested that the methanol extract exhibit highest activity (IC₅₀ 13.19 μg/ml) relatively to methanol extract which also gave better result (IC₅₀ 15.37 μg/ml). As it can...
To assess the impact of the consumption of GnA on taking weight in the diabetic subject, the body weight of rats was taken (Figure 2). All our results show that there is no difference (p>0.05) between the normal group and control. By contrast, 7 days after induction of diabetes a decrease in body weight was observed (p<0.05) in both groups STZ (-12.41%) and STZ+GnA (-8.55%) compared to the normal group. The comparison between the STZ and STZ+GnA group shows that the consumption of GnA is associated with an increase (+4.29%) of body mass in diabetic rats (p <0.05).

In order to evaluate the incidence of GnA consumption on the urine glucose content, glycosuria was evaluated (Table 3). The results show a 50% decrease (p<0.05) of glycosuria in STZ+GnA.

Table 3 shows the impact of GnA consumption on blood glucose, total protein, triglycerides (TG) and total cholesterol. The results show that the induction of diabetes by STZ is associated with an increase (+210%) of blood glucose in STZ group compared to the normal group (p<0.05). Consumption of GnA after 7 days (STZ+GnA) resulted in a decrease (-56%) of the glucose levels compared to STZ group. There is also a hypertriglyceridemia (Table 3) in diabetic rats (STZ) which decreases after 7 days of consumption of GnA (STZ+GnA). Similarly, consumption of GnA induces a reduction (-31.74%) of plasma triglycerides in the control group compared to the normal group.
to Normal. 7 Days after the induction of diabetes we observed increase (+78%) of total cholesterol (p<0.05) in diabetic rats (STZ) compared to the Normal and Control. By against the consumption of GnA induced a decrease (p<0.05) in total cholesterol in diabetic rats (STZ+GnA)

**Discussion**

The aim of this study was to show the hypolipidemic effect of a food plant, Gnetum africanum, in a rat made diabetic by streptozotocin injection. Our results show a loss of body mass associated with hyperglycemia and hyperlipidemia of Wistar rats rendered diabetic by intraperitoneal injection of STZ. In Wistar rats treated with streptozotocin, weight loss was associated with hyperglycemia and hyperlipidemia (triglycerides and total cholesterol) [29]. Diabetes is often associated with dyslipidemia, a main risk factor of cardiovascular diseases. Therefore, the levels of serum triglyceride and cholesterol are usually elevated in diabetic patients [1-9].

In our study, GnA could decrease plasma triglyceride level and attenuate dyslipidemia. The hypolipidemic action of plants is in agreement with earlier studies [30-33]. This beneficial effect on glycemic status is expected to happen as antihyperglycemic effects of plants has been confirmed with repeated studies. Antihyperglycemic effect of plants is achieved by different mechanisms including decreasing glucose absorption from intestine, enhancing insulin secretion from beta cells, increasing glucose uptake by tissues, inhibiting glucose production in liver, and increasing pancreatic tissue regeneration and/or presence of insulin-like agents in plants [34-41]. More phytochemicals results show the presence of phenolic compounds associated with antioxidant activities. It is well established that phenolic compounds have a hypoglycemic and anti-hyperlipidemic effects. It is well established that the flavonoids lower the blood glucose and reduces plasma cholesterol [42-46]. These results show that Gnetum africanum could be used as an alternative treatment for diabetic lipid syndrome. This alternative could help developing countries or access to basic drugs is relatively expensive.

**Conflicts of Interest**

There are no conflicts of interest

**Acknowledgments**

The authors say thanks to the management of International Medical Centre of Franceville for their technical assistance throughout the period of the research.

**References**

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