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# Fasting blood sugar and clinical biochemistry profiles of diabetic rats treated with methanol leaf extract of *Gnetum africanum* Welw.

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*Gnetum africanum* Welw. is widely used in West Africa for the treatment of diverse diseases including diabetes and as food. However, scientific information on its antidiabetic activity using dose-response tests and sub-acute anti-diabetic study is scarce. This was studied using alloxan induced diabetic rats. Dose response test (n = 6) was conducted using different doses of the extract (400, 800, 1600 mg/kg); distilled water and glibenclamide were administered to the negative and positive controls, respectively. A 21-day sub-acute antidiabetic study was used to assess the effect of different doses (200, 400 and 800 mg/kg) of the extract on fasting blood sugar (FBS) and clinical biochemistry (n = 10). *Gnetum africanum* methanol leaf extract produced significant dose and time-dependent reductions in FBS. The highest reduction was observed six hours post treatment in rats treated with 1,600 mg/kg of the extract ($p < 0.0001$). The extract also elicited significant ($p < 0.01$; $p < 0.001$) reductions in FBS in the sub-acute study. The extract significantly ($p < 0.05$; $p < 0.01$) reduced the cholesterol, triglycerides, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) of diabetic rats. Serum liver enzymes, urea and creatinine were significantly ($p < 0.05$) reduced in treated diabetic rats. These results validate the indigenous use of *Gnetum africanum* in treatment of diabetes.

**Keywords:** African joint fir, Diabetes, Body weights, Dyslipidaemia, Liver enzymes

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Diabetes mellitus is a serious, chronic disease that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood sugar, or glucose), or when the body cannot effectively use the insulin it produces, leading to hyperglycaemia[1]. WHO estimates that globally, 422 million adults aged over 18 yrs were living with diabetes in 2014. This represents a three-fold increase from 108 million, three decades ago, between 1980 and 2014[1]. Conditions like polyuria, polydipsia, neurotoxicity, ketoacidosis and polyphagia are related to hyperglycaemia and glycosuria[2]. Consequently, most anti-diabetic therapies are targeted towards reduction in blood glucose levels using diverse mechanisms. Unfortunately though, their effectiveness has been greatly hampered by the side effects they elicit, including alterations in blood lipid profile[2,3]. Thiazolidinediones have been shown to alter lipid profiles in patients with Type 2 diabetes. As a monotherapy, rosiglitazone is associated with increases in total low density lipoprotein and high density lipoprotein cholesterol levels and either no change or increases in triglycerides. Patients treated with pioglitazone have displayed mean decreases in triglyceride levels, mean increases in HDL cholesterol levels and no consistent mean changes in LDL and total cholesterol levels[4]. Also, TZDs increase serum AST and are thus contraindicated in liver disease patients[3].

*Gnetum africanum* Welw. (African jointfir) is an edible plant widely in West Africa used as a vegetable. In Nigeria, it is called *afang* (*Efik*/ *Ibibio*) *Ukazi*, *Okazi* (*Igbo*), *Yala* (*Ogoja*), *Ajaabaje*; *Ajakotale* (*yoruba*), in Cameroon it is known as *Eru*, *Okok*, *mfumbua* or *fumbua* and *Koko* in Angola, Gabon, Central African Republic[5]. *G. africanum* has been used in Nigeria in the treatment of piles and high blood pressure[6]. Also, Nigerians use it in the treatment of an enlarged spleen, sore throats and as a cathartic[5]. In Ubangi (DR Congo), it is used treat to nausea and considered as an antidote to arrow poison made from *Periploca nigrescens* afzel[5,7]. In Cameroon, the leaves are chewed to mitigate the effects of drunkenness and also taken as an enema against constipation and to ease childbirth. They are also used to treat diabetes, boils, and fungal infection in

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the fingers[7]. *Gnetum africanum* leaves have great culinary value in West Africa. They are eaten raw, shredded and used in preparing soups and stews[5]. It is a good source of protein and has been noted as an anti-inflammatory, anticarcinogenic and antioxidant[8]. It has also been reported to have anti-diabetic usage in folkloric medicine[7], and is widely used by people of southern Nigeria as food and therapy for diabetics. The aim of this study is to scientifically study the effects of this plant on glycaemic and clinical biochemistry profiles of alloxan induced diabetic rats. This will provide a lead for the development of new anti-diabetic drugs that are relatively free from adverse effects.

## Methodology

### *Plant collection and extraction*

*Gnetum africanum* Welw. leaves were collected between October and November 2014 from its natural habitat in Orba, Nsukka, Enugu State. The plant sample was identified by a plant taxonomist and a voucher specimen catalogued MOUAU/VPP/2014/017 was deposited in the herbarium of the department of veterinary physiology and pharmacology. The plant materials were dried under mild sunlight. They were pulverized into coarse powder of about 1 mm in diameter. Plant material (2 kg) was extracted by cold maceration method in 80 % methanol for 48 h with intermittent shaking at 2 h intervals after which they were filtered with Whatman No. 1 filter paper. The filtrate was then concentrated *in vacuo* using rotary evaporator connected to a cold water circulator and a pressure pump at 40 $^{o}$C and 210 milibar. The extracts were stored in a refrigerator as *Gnetum africanum* extract (GAE). Percentage yield was calculated:

% Yield= b/a x 100/1

Where, a = weight of original plant material used for extraction and b = weight of the recovered extract.

### *Experimental animals*

Mature male albino rats weighing 110-180 g and bred in the Laboratory Animal Units of the Faculties of Veterinary Medicine and Pharmaceutical Sciences, University of Nigeria, Nsukka were used for the experiments. They were housed in an environment of normal ambient temperature and the lighting period was about 12 h daily and relative humidity of 40-60 %. The rats were kept in stainless steel cages, supplied with clean drinking water and fed *ad libitum* with standard commercial pelleted feed (Vital feed, Nigeria). Ethical conditions governing the conduct of experiments with life animals were strictly followed[9,10].

### Screening of extract for anti-diabetic activity

### *Induction of experimental diabetes*

Diabetes was induced in 80 male albino Wistar using standard procedure[11]. The rats were fasted for 18 h after which their fasting blood sugar levels were determined with blood obtained from the rats' tail vein using an autoanalyzer (Accu Check Advantage II$^{(R)}$ glucose kit). Diabetes was then induced in the rats by a single intra-peritoneal administration of alloxan monohydrate at 160 mg/kg. The fasting blood sugar levels (FBS) of the rats were checked every other day. On the sixth day diabetes was established in the rats with FBS of ≥ 120 mg/dL.

### *Dose-response antidiabetic study*

Thirty alloxan-induced diabetic male rats were used for the study. The rats were randomly divided into 5 groups consisting of 6 rats per group and treated as follows: group 1 rats received distilled water (10 mL/kg) which served as negative control group. Group 2 rats were given glibenclamide (2 mg/kg) and served as positive control, while groups 3, 4 and 5 served as treatment groups and were given 400, 800 and 1600 mg/kg of *G. africanum* extract by gastric gavage. The doses used were selected after a series of preliminary testing using lower doses. The FBS of all the rats were measured at 0, 1, 3 and 6 h post treatment using an auto analyzer (Accu Check Advantage II® ) glucose kit. The blood samples were collected from the tail vein after a snip.

### *Sub-acute antidiabetic study*

Effect of 21-days oral administration of GAE on body weights, FBS and clinical biochemistry, in alloxan-induced diabetic was studied. Fifty (50) male diabetic albino rats were randomly divided into 5 groups of 10 rats per group. Group 1 (negative control) received distilled water 10 mL/kg. Group 2 (positive control) received 2 mg/kg glibenclamide while groups 3, 4 and 5 rats were administered 200, 400 and 800 mg/kg GAE, respectively through gastric gavage. The drugs or extract and distilled water were administered to the rats for 21 days. Body weights and FBS levels of the rats were measured on days 1, 7, 14 and 2[12]. On day 21 being the last day of the treatment, blood samples were collected into dry sample bottles, allowed to stand for 30 min and centrifuged at 2,500 rpm for 15 min. Serum was harvested for clinical biochemistry tests including lipid profile, liver and kidney function tests.

### *Lipid profile*

Total cholesterol was evaluated using enzymatic colorimetric chod-pad test method[13], with Quimica

Applicada test kit. Triglycerides (TG) was also determined spectrophotometrically[13]. High density lipoprotein (HDL was evaluated as described in Quimica Clinica Applicada test kit. Low density lipoprotein (LDL) was determined as the difference between total cholesterol and cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethylene-glycol monomethyl ether[13]. Very low density lipoprotein was calculated as VLDL = 0.2 x TG (where TG is total glycerides).

***Liver function tests***

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were analysed[14] respectively using Randox kits (Randox Laboratories, United Kingdom); serum albumin was estimated[14]; total bilirubin was determined colorimetrically[15] while total protein was assayed by the direct Biuret method[14].

***Kidney function tests***

Kidney function was evaluated by the estimation of serum urea and creatinine[13], using Randox kits (Randox Laboratories, UK).

## Statistical analysis

Data generated were subjected to one-way analysis of variance (ANOVA), means were compared using the least significant difference method and differences in means were considered significant at $p < 0.05$.

## Results

**Extraction of *Gnetum africanum***

The methanol leaf extract of *Gnetum africanum* was dark green in colour, and an oily consistency. The per cent yield was 10.3 % w/w.

**Dose-response antidiabetic study**

There were dose and time dependent reductions in FBS of diabetic rats treated with *Gnetum africanum* extract (Table 1). At the 6$^{th}$ h, the mean FBS of rats given GAE (800 mg/kg) was 53.00±7.73 mg/dL. This was significantly ($p < 0.0001$) lower than that of the distilled water treated rats which was 259.20 ± 34.07 mg/dL and represents an 85 % reduction in FBS, and was better than glibenclamide, which produced 83 % reduction in FBS.

## Sub-acute antidiabetic study

***Fasting blood sugar (FBS)***

There was a decrease in mean FBS of rats treated with the different doses of GAE in all the weeks. GAE caused dose-dependent percentage reduction in FBS at day 21. Fasting blood sugar level was reduced by 66.22 %, 71.50 % and 73.56 % at 200, 400 and 800 mg/kg respectively as against 65.54 % by the reference drug, glibenclamide (Table 2).

***Effect of G. africanum on body weights of alloxan induced diabetic rats***

Table 3 shows the changes in body weights of alloxan induced diabetic rats in the control group and treated groups for 21 days. In the distilled water

Table 1 — Dose-response effect of *Gnetum africanum* on FBS of alloxan induced diabetic rats

| Group | Treatment mg/kg | FBS (mg/dL) | | | | % reduction | | |
|---|---|---|---|---|---|---|---|---|
| | | 0h | 1h | 3h | 6h | 1h | 3h | 6h |
| 1 | Distilled water (10 mL/kg) | 237.60±27.11 | 284.2±22.26 | 264.4±24.90 | 259.20±34.07 | -19 | -11 | -9 |
| 2 | Glibenclamide 2 | 359.60±15.89 | 265.2±28.36 | 123.20±30.74$^{**}$ | 60.40±3.14$^{****}$ | 26 | 65 | 83 |
| 3 | GAE (400 ) | 248.20±39.91 | 205.00±34.46 | 139.40±39.93$^{*}$ | 91.20±28.10$^{**}$ | 17 | 43 | 63 |
| 4 | GAE (800) | 343.40±42.41 | 157.00±7.75$^{**}$ | 73.2±10.82$^{***}$ | 55.20±3.65$^{****}$ | 54 | 78 | 83 |
| 5 | GAE (1600) | 373.60±34.00 | 227.00±44.89 | 186.40±40.66 | 53.00±7.73$^{****}$ | 39 | 50 | 85 |

Data presented as mean ± SE *$p < 0.05$, **$p < 0.01$, ***$p < .001$, ****$p < 0.0001$ when compared to the negative control.

Table 2 — Effect of sub-acute administration of *Gnetum africanum* extract on the FBS of alloxan-induced diabetic rats

| Group | Treatment (mg/kg) | FBS(mg/dL) | | | | |
|---|---|---|---|---|---|---|
| | | Day 1 | Day 7 | Day 14 | Day 21 | % Reduction at Day 21 |
| 1 | Distilled water (10 mL/kg) | 214.40±22.65 | 211.60±20.89 | 215.00±22.49 | 214.40±22.65 | 1.39 |
| 2 | Glibenclamide (2) | 92.00±3.83$^{**}$ | 81.25±1.31$^{***}$ | 86.25±8.76$^{***}$ | 92.00±3.83$^{**}$ | 65.54 |
| 3 | GAE (200) | 103.75±40.16$^{**}$ | 73.00±2.34$^{***}$ | 82.00±8.83$^{***}$ | 103.75±40.16$^{**}$ | 66.22 |
| 4 | GAE (400) | 233.00±32.67 | 91.00±9.23$^{**}$ | 68.50±7.23$^{***}$ | 66.33±7.48$^{***}$ | 71.50 |
| 5 | GAE (800) | 298.16±57.44 | 109.50±21.98$^{**}$ | 109.57±32.47$^{***}$ | 78.83±10.63$^{***}$ | 73.56 |

Data presented as mean ± SE. **$p < 0.01$; ***$p < 0.001$ when compared to the negative control

treated group, there was a decrease in body weights from day 0 to day 7 which represents a percentage weight loss of 2.65 ± 3.84 %. However, both the positive control and the rats treated with *G. Africanum* had weight gains the same period. On day 21, rats treated with 200 mg/kg of GAE showed the best weight gain of 55.54 ± 15.30 % ($p < 0.05$).

***Effect of G. africanum extract on lipid profile of alloxan–induced diabetic rats***

The serum concentrations of total cholesterol, high density lipoproteins (HDL), triglycerides, very low density lipoproteins (VLDL) and low density (LDL) of control and experimental groups of alloxan induced diabetic rats, after a 21-day period, are presented in Table 4**.**

GAE (200 mg/kg), as well as the reference drug, glibenclamide produced significant ($p < 0.01$) reduction in cholesterol level of rats when compared with the negative control group. The extract (800 mg/kg) reduced triglyceride level of diabetic rats ($p < 0.01$). Glibenclamide caused similar reduction which was not significant. Serum levels of VLDL and LDL were significantly ($p < 0.05$; $p < 0.01$) reduced by different doses of GAE as well as by the reference drug. There were no significant differences in HDL of control and GAE-treated diabetic rats ($p < 0.05$).

***Effect of G. africanum on liver function tests of alloxan-induced diabetic rats***

Results of the liver function tests are presented in Table 5. The extract did not bring about increases in any of the liver enzymes studied. Rather, there were reductions in serum alkaline phosphatase enzyme in rats treated with GAE at all doses and glibenclamide. However, this was only significant in rats treated at the dose of 800 mg/kg. GAE at all doses tested also caused reduction in serum aspartate transaminase enzyme when compared with the negative control ($p < 0.05$).

Table 3 — Effect of *Gnetum africanum* on body weights and percentage weight gain of alloxan induced diabetic rats

| | | Mean Body Weights (g) | | | | | % weight gain | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group | Treatment mg/kg per os | Before Induction | Day 0 | Day 7 | Day 14 | Day 21 | Day 0 | Day 7 | Day 14 | Day 21 |
| 1 | Distilled water (10mL/kg) | 121.90 ±8.08 | 122.72 ±6.88 | 117.30 ±4.64 | 124.83 ±7.62 | 134.40 ±4.23 | 1.07 ±1.90 | 2.65 ±3.84 | 2.84 ±3.60 | 5.81 ±5.41 |
| 2 | Glibenclamide (2mg/kg) | 131.80 ±8.16 | 130.25 ±2.05 | 151.75 ±2.05 | 147.33 ±7.17 | 155.67 ±11.29 | 16.20 ±6.49 | 16.20 ±6.49 | 11.87 ±11.56 | 18.68 ±16.10 |
| 3 | GAE 200 (mg/kg) | 116.68 ±9.60 | 116.58 ±10.74 | 131.75 ±20.14 | 162.33 ±18.22 | 175.67 ±21.46 | 20.10 ±13.82 | 20.10 ±13.82 | 43.80 ±12.57* | 55.54 ±15.30* |
| 4 | GAE 400 (mg/kg) | 133.10 ±11.61 | 129.10 ±11.75 | 144.00 ±11.47 | 148.40 ±11.47 | 155.60 ±11.07 | 8.72 ±2.59 | 8.72 ±2.59 | 12.00 ±2.22 | 17.94 ±4.68 |
| 5 | GAE 800 (mg/kg) | 116.35 ±4.25 | 119.98 ±5.39 | 139.67 ±8.31 | 142.00 ±6.58 | 151.00 ±7.00 | 3.01 ±1.69 | 19.76 ±4.28 | 21.90 ±2.33 | 29.6 1±2.28 |

Data presented as SE±mean, *p< 0.05 significance when compared to the negative control.

Table 4 — Effect of *Gnetum africanum* extract on lipid profile of alloxan – induced diabetic rats

| Group | Treatment mg/kg per os | Total cholesterol(mg/dL) | HDL (mg/dL) | Triglycerides (mg/dL) | VLDL (mg/dL) | LDL (mg/dL) |
|---|---|---|---|---|---|---|
| 1 | Distilled water 10mL/kg | 108.10±3.41 | 62.86± 3.34 | 147.60±7.00 | 30.50±1.22 | 30.50±4.85 |
| 2 | Glibenclamide 2 | 91.40±1.61** | 54.46± 0.71 | 82.19±10.78 | 18.00±2.14* | 19.90±3.47 |
| 3 | GAE 200 | 90.30±1.81** | 58.71± 2.48 | 118.38±1.00 | 21.68±1.01* | 13.20±1.00** |
| 4 | GAE 400 | 96.71±6.89 | 55.38± 2.87 | 121.95±27.66 | 24.40±5.53 | 18.60±6.04 |
| 5 | GAE 800 | 96.60±2.80 | 55.53± 2.55 | 81.17±1.15** | 16.10±0.50** | 26.30±3.10 |

Data presented as SE±mean, *$p < 0.05$, **$p < 0.01$ significance when compared to the negative control.

Table 5 — Effect of *G. africanum* on liver function tests of alloxan induced diabetic rats.

| Group | Treatment mg/kg per os | ALP (IU/L) | AST (IU/L) | ALT IU/L) | Albumin (g/dL) | Bilirubin (g/dL) | Total protein (g/dL) |
|---|---|---|---|---|---|---|---|
| 1 | Distilled water 10 mL/kg | 49.78±5.17 | 46.37±7.69 | 22.00±12.38 | 4.35±0.27 | 0.17±0.04 | 6.47±0.21 |
| 2 | Glibenclamide 2 | 40.62±4.22 | 38.65±4.47 | 18.32±2.35 | 3.64±0.33 | 0.11±0.01 | 6.15±0.17 |
| 3 | GAE 200 | 41.48±5.84 | 22.15±3.45* | 17.25±1.52 | 4.51±0.28 | 0.19±0.81 | 6.73±0.29 |
| 4 | GAE 400 | 39.42±3.37 | 34.75±7.87 | 17.00±2.07 | 3.99±0.28 | 0.21±0.11 | 6.79±0.15 |
| 5 | GAE 800 | 32.97±5.05* | 47.65±10.08 | 22.00±4.94 | 4.21±0.13 | 0.24±0.17 | 6.83±0.22 |

Data presented as SE±mean, *$p < 0.05$ significance when compared to the negative control.

Table 6 — Effect of *G. africanum* on kidney function tests of alloxan – induced diabetic rats

| Group | Treatment (mg/kg) per os | Creatinine (mg/dL) | Urea (mg/dL) |
|---|---|---|---|
| 1 | Distilled water 10 mL/kg | 1.20±0.02 | 53.47±5.05 |
| 2 | Glibenclamide 2 | 1.02±0.06 | 38.36±2.35* |
| 3 | GAE 200 | 1.00±0.05 | 36.86±4.17* |
| 4 | GAE 400 | 0.83±0.09* | 43.36±4.2 |
| 5 | GAE 800 | 0.69±0.13* | 36.01±2.26* |

Data presented as SE±mean, n=10, *p< 0.05 significance when compared to the negative control.

***Kidney function tests***

Table 6 shows the effect of GAE on serum creatinine and urea levels of alloxan- induced diabetic rats. There were no increase in serum creatinine and urea of rats treated with different doses of GAE. However, there were reduction in serum creatinine level of treated rats from 1.20 ± 0.02 mg/dL in the distilled water treated groups to 0.69 ± 0.13 mg/dL at the dose of 800 mg/kg ($p < 0.05$). Also, GAE caused reductions in serum urea level from 53.47 ± 5.05 mg/dL to 36.01 ± 2.26 mg/dL at 800 mg/kg ($p < 0.05$).

## Discussion

Reduction in FBS seen in both the dose response and sub-acute studies validate the folkloric use of *G. africanum* in the treatment of diabetes[7]. It is widely accepted that reduction in blood glucose level, in conjunction with a careful regulation of glucose homeostasis is the most important strategy taken in the treatment and management of diabetes mellitus. Apart from its report as an ethnomedical plant used for treatment of diabetes[7], data on scientific studies and validation of the anti-diabetic activity of *Gnetum africanum* are scarce. Several authors reported other activities including antibacterial[16,17] antifungal and antioxidant[18,19,20]. Also, diabetic rats given GAE at different doses had higher body weights and better percentage weight gain than the distilled water treated group. It has been observed that unexplained weight loss due to the breakdown of triglyceride stores, is one of the classic symptoms of diabetes mellitus[21]. Thus, reductions in FBS observed in the extract treated rats spared them from the weight loss associated with diabetes. The extract prevented the onset of dyslipidaemia that often accompany diabetes. Diabetic dyslipidaemia, characterized by low HDL, increased triglycerides and high postprandial lipaemia, is a risk factor for cardiovascular disease in type 2 diabetes mellitus[29]. Thus, *Gnetum africanum* can be assumed to possess cardioprotective effect and this can explain its use in management of high blood pressure as well[6]. Alloxan monohydrate, is known to elicit increase in serum liver enzyme markers in diabetic rats[23]. Consequently, the ability of GAE to bring about reductions in alloxan-induced elevation of serum AST and ALP is an indication of its possible hepatoprotective activity. This finding is in agreement with a previous study which demonstrated that *G. africanum* has hepatoprotective activity in paracetamol-induced liver damage[24]. The extract brought about reductions in serum creatinine and urea in alloxan induced diabetic rats. Alloxan is also known to induce changes in the kidney, especially abnormalities of the glomeruli, which were ameliorated by insulin treatment[25]. The kidney maintains optimum chemical composition of body fluid by acidification of urine and removal of metabolic wastes such as urea, uric acid, and creatinine. During renal diseases, including diabetic nephropathy, the concentration of these metabolites increases in blood[26]. The reductions in serum creatinine and urea of treated rats therefore suggest an amelioration of diabetic nephropathy seen in alloxan induced diabetic rats.

In conclusion, *G africanum* elicited dose and time dependent reductions in FBS of alloxan induced diabetic rats and improved body weights of rats. It is anti-dyslipidaemic, and has beneficial effects on the diabetic liver and kidneys, hence can be used treatment of diabetes in persons with impaired hepatic and renal functions.

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