



Subacute effects of hydroethanolic extracts of the pulp of *Gambeya africana* on glucose plasmatic levels and oxidative stress markers in diabetic rats

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ABSTRACT

The management of hyperglycemia and oxidative stress is a key point in the control of diabetes and its complications. The work evaluated the hypoglycemic and antioxidant properties of pulp extracts of *Gambeya africana* fruits. The acute toxicity was conducted for 14 days at the dose of 2000 mg/kg via oral gavage. Diabetes was induced in rats by intravenous administration of streptozotocin (50 mg/kg). The effect of the extract on blood glucose levels of diabetic rats was monitored at 2h after administration; and on the 7th and 14th days of treatment (single dose of 400 mg/kg/day). After 2 weeks of treatment, the rats were sacrificed. The oxidative stress markers (Hydroperoxides, Malondialdehydes, Superoxide Dismutase, Catalase, and Total Antioxidant Capacity) and hepato/nephrotoxicity markers (Alanine Amino Transferase, urea, and creatinine) were determined. The hydroethanolic extract of *G. africana* fruits has a lethal dose upper than 2000 mg/kg. It significantly decreased glucose levels by 28.29% and 84.86% respectively after 2h and 14th days of treatment. The extract increased the antioxidant status and decrease oxidative stress, especially in the pancreas. This study suggests that the hydroethanolic pulp extract of *G. africana* fruits is a good hypoglycemic and antioxidant agent and could be a potential source of compounds for diabetes management.

1. Introduction

Diabetes has become one of the most dangerous diseases, with global prevalence reaching pandemic proportions by 2021, affecting 537 million adults [1,2]. It is a chronic metabolic disease caused by a multitude of environmental and genetic factors and characterised by chronic hyperglycemia [3]. Many studies have shown the depletion of the antioxidant system and the increase of prooxidants in diabetic patients [4–6]. The stress can exacerbate the microvascular complications of diabetes such as nephropathy and hepatopathy (with the release of hepatic enzymes) [7] and macrovascular complications such as cardiovascular diseases [8]. Hyperglycemia and oxidative stress are thus highly involved in the development of diabetes and its complications, presently considered as a major public health problem. The management of this disease could be achieved through the search for natural compounds with hypoglycaemic, antioxidant, and hypolipidemic properties, so as to make up for the synthetic antidiabetic drugs which have

shown side effects such as; gastrointestinal disturbances, hepatic disorders, and renal tumors [9]. Moreover, the search for compounds with several mechanisms of action can help solve the problem of poly-medication, which complicates the tolerance to certain drugs in patients, thus their efficacy. Food plants are rich in bioactive compounds, notably polyphenols which are known for their hypoglycaemic and antioxidant properties [10]. Several studies have shown that polyphenol-rich foods significantly correlated to a lower risk of diabetes and its complications [11,12]. Many antidiabetic mechanisms were attributed to polyphenols: inhibition of digestion and absorption of carbohydrates, stimulation of insulin secretion and glucose uptake, and inhibition of hepatic production of glucose [13,14].

These compounds are present in high amounts in fruits and several studies have been done in this regard. Fruits of *Irvingia wombolu* have shown antihyperglycaemic and antioxidant activities [15], while the fruits of Sapotaceae like *Chrysophyllum cainito* have shown antidiabetic properties [16]. *Gambeya africana* (Sapotaceae) is a plant cultivated for

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its delicious fruit, which is an excellent source of vitamin C, zinc, copper, and secondary metabolites including; tannins, alkaloids, and saponins [17]. In Cameroon, its fruits are used in the treatment of malaria, typhoid, and constipation [18]. The present study was conducted to evaluate the hypoglycaemic and antioxidant properties of the fruits' pulp extracts of *G. africana*.

2. Methodology

2.1. Reagents

All the chemicals used were of analytical grade and were purchased from Sigma Co., Louis, MO, USA.

2.2. Preparation of the extracts

Fruits of *G. africana* were harvested in December 2019 at Ondodo (East-Cameroon). They were identified at the national herbarium. After drying in an oven at 50 °C for 3 days, the pulp was separated from the kernel. The pulp (100 g) was ground and extracted by maceration for 48 h with 800 ml of solvent (water: ethanol; 1:1, v/v). The resulting supernatant was filtered using Whatman #1 filter paper (Whatman International Limited, Kent, England) using a funnel and concentrated to about 10% of the original volume by a rotavapor before drying in an oven at 50 °C. The hydroethanolic extracts were obtained and stored in desiccators.

2.3. Animals and treatment

2.3.1. Animals

Adult male Wistar albino rats of 8 weeks old, weighing 150–180 g were obtained from the animal house of the Department of Biochemistry, University of Yaounde I, Cameroon. The animals were acclimatized in the experimental animal room for 7 days with a 12 h light and 12h dark cycle before the start of experimentation. Standard feed and water were provided *ad libitum* to all experimental animals.

2.3.2. Oral acute toxicity study

The acute oral toxicity study was sanctioned is conducted in compliance with Organization for Economic Co-operation and Development, guideline 423, which stipulates the use of only three animals per group (Paragraph 23) [19,20]. The animals were fasted overnight (~12 h) and weighed. Two groups of 3 rats each were used. One of these groups was treated with the extract (test group) and the second (control group) was administered with the vehicle (water). A test dose of the extract was calculated concerning the bodyweight of every fasted animal and administered via oral gavage at 2000 mg/kg of body weight. The animals were regularly and individually observed for behavioral changes (motricity, weakness, aggressivity audition and pain sensibility) and general toxicity signs (bodyweight loss, change in skin, diarrhea, rate of respiration, coma, and death) after dosing for the first 24 h, with special attention being given during the first 4 h. Thereafter, observation was continued daily for a total of 14 days [21].

2.3.3. Experimental induction of diabetes

The schedules and procedures were performed in the experimental animal house of the Laboratory of Biochemistry of the University of Yaounde I, Cameroon. The study was approved by an institutional animal ethical committee. In accordance with Al-Shamaony et al. [22], diabetes was induced by intraperitoneal administration of Streptozotocin (50 mg/kg BW) dissolved in freshly prepared 0.01 M citrate buffer pH4.5. After 48 h rats with marked hyperglycemia (fasting blood glucose ≥ 200 mg/dL) were selected and used for the study.

2.3.4. Experimental design on diabetic rats

In the experiment, a total of 20 rats (15 diabetic rats, 5 normal rats)

were used. The rats were randomly assigned into four groups of five rats each after the induction of diabetes. One normal control group (only water); one diabetic control group (only water) and three other groups receiving respectively Tolbutamide (400 mg/kg in water), hydroethanolic extract of pulp (400 mg/kg) in the water of *G. africana*. The blood glucose levels of all rats was recorded at the beginning, 2h, 7th days, and 14th days. The Trinder glucose activity test [23], using glucose oxidase was used to monitor the blood glucose using a test strip in tail vein blood. After 14 days of treatment, the 12 h-fasted animals were sacrificed by cervical decapitation under anesthesia. Blood was withdrawn with Ethylene Diamine Tetra Acetate tubes and centrifuged at 3000 rpm for 10 min to obtain the plasma which was stored at –20 °C for the measurement of biochemical parameters. The liver and pancreas were collected, washed with saline solution, and homogenate prepared in sodium chloride (0.9%). The supernatant was stored at –20 °C until analysis. Erythrocyte suspension was made by washing blood residue three times with sodium chloride and followed by lyses with water and stored in the freezer at –20 °C for further uses.

2.4. Oxidative stress markers

The prooxidant system was evaluated by the determination of hydroperoxides and Malondialdehyde (MDA) lipid peroxidation markers. The antioxidant system was evaluated by determining thiols protein levels and activities of superoxide dismutase and catalase.

2.4.1. Hydroperoxide levels

Hydroperoxides were determined in plasma and homogenates of organs following the protocols described by Jiang et al. [24].

2.4.2. Malondialdehyde levels

MDA levels were determined in plasma and homogenates of organs following the protocol described by Yagi et al. [25] and it was exploited without any modification. The principle of the method is as follows; carbonylated molecules like malondialdehyde generated from the decomposition of hydroperoxides, react with thiobarbituric acid to form a pink chromophore with absorbance at 532 nm.

2.4.3. Superoxide dismutase (SOD) activity

The activity of SOD was evaluated according to the method described by Misra and Fridovich [26] based on the inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazan. The color formed at the end of the reaction can be measured at 560 nm.

2.4.4. Catalase (CAT) activity

The activity of CAT was evaluated according to the method described by Sinha [27] based on the fact that H₂O₂ remaining after the action of CAT combines with potassium dichromate to form an unstable blue-green complex which is later decolorized into a green complex on heating which absorbs light at a wavelength of 570 nm.

2.4.5. Thiols protein levels

Thiols protein levels were measured in all prepared biological samples according to the method described by Ellman [28] which is based on the fact that thiols carried by proteins are measured by following the evolution of the concentration of 2-nitro-5-thiobenzoate which is a yellow complex formed by the reduction of 5,5-Dithio-bis-(2-nitrobenzoate) (Ellman's reagent) at 415 nm.

2.5. Statistical analysis

All data were expressed as mean values \pm standard deviation. Significant differences among the groups were determined by the test of Kruskal Wallis followed by the post hoc of Bonferonni using the IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA). Statistical significance was considered at $p < 0.05$.

3. Results

3.1. Acute toxicity

The oral administration of the hydroethanolic extract of the pulp of *G. africana* at 2000 mg/kg induced no abnormal signs of toxicity (Table 1). After administration of the unique dose of the extract, all animals presented similar behavior, any sign of toxicity, and no mortality was registered after 14 days. The extract seems to be safe at a dose level of 2000 mg/kg, and the Letal Dose₅₀ was considered to be >2000 mg/kg.

3.2. Hypoglycemic activity

The hypoglycemic activity of the extract is presented in Table 2. There was a significant reduction ($p < 0.05$) in blood glucose levels after acute treatment of diabetic rats compared to the diabetic control. The hydroethanolic extract of the fruit of *G. africana* showed a reduction of 28.29% after 2 h against 1.5% for the diabetic non-treated rats. The tolbutamide showed the best reduction (48.32%) compared to the extract. The subacute treatment also significantly decrease ($p < 0.05$) blood glucose levels compared to the diabetic control. The group was treated with the hydroethanolic extract of the fruit of *G. africana* showed a reduction of 84.86% after 14 days against 1.20% for the diabetic non-treated group. The extract showed the best activity compared to Tolbutamide (77.23%).

3.3. Antioxidant activity

The effect of the extract on the lipoperoxidation markers is shown in Table 3. The diabetic non-treated group have a high level of hydroperoxides and MDA ($p < 0.05$) compared to the normal group in the pancreas. There was no significant difference between the diabetic treated groups and the diabetic control group in the different compartments concerning the levels of hydroperoxides. The diabetic treated group with the hydroethanolic extract of the fruit of *G. africana* has a significantly low levels of MDA in the pancreas compared to the diabetic control group (1.44 vs 2.06 μM).

The effect of the extract on the enzymatic markers of the antioxidant status is shown in Table 4. The diabetic non-treated group have a high activity of SOD in the pancreas ($p < 0.05$) compared to the normal group. The diabetic group treated with the hydroethanolic extract of the fruit of *G. africana* has a significantly low activity of SOD in the pancreas compared to the diabetic control group (1.8 vs 2.17 UI/mg proteins). There was no significant difference in the other compartments. The diabetic non-treated group has a high activity of Catalase in the pancreas and liver ($p < 0.05$) compared to the normal group. The diabetic group treated with the hydroethanolic extract of the fruit of *G. africana* has a significant high activity of Catalase in the pancreas (4.06 vs 3.56 mmol de H_2O_2 cons/min/g proteins) and liver (3.49 vs 3.2 mmol de H_2O_2 cons/min/g proteins) compared to the diabetic control group.

Table 1

General appearance and behavioral observations of acute toxicity study.

Observations	Control Group	Test Group
Motricity	Normal	Normal
Weakness	Not present	Not present
Aggressivity	Not present	Not present
Audition	Normal	Normal
Pain sensibility	Normal	Normal
Body weight	Not loss	Not loss
Change in skin	Not effect	Not effect
Diarrhea	Not present	Not present
Rate of respiration	Normal	Normal
Coma	Not present	Not present
Death	Alive	Alive

Table 2

Effect of the extracts on the glucose levels (mg/dl) after 14 days of treatment.

Groups	Glycemia at t = 0 day	Glycemia at t = 2 h	Glycemia at t = 7 days	Glycemia at t = 14 days
Normal Control	74.33 \pm 7.72 (0)	79.70 \pm 4.80 (-2.71)	72.21 \pm 5.44 (-2.81)	73.67 \pm 4.99 (-0.25)
Diabetic Control	460 \pm 116.63 (0)	453.1 \pm 140.89 (-1.50)	447.03 \pm 63.68 (-2.83)	454.48 \pm 58.64 (-1.20)
Diabetic + 400 mg/kg Hydro-ethanolic Extract of <i>G. africana</i>	454.4 \pm 108.60 (0)	326.2 \pm 182.84 (-28.29) ^a	84.7 \pm 7.40 (-81.36) ^a	68.79 \pm 11.47 (-84.86) ^a
Diabetic + 400 mg/kg Tolbutamide	429 \pm 111.11 (0)	238 \pm 42.44 (-48.32) ^a	169.83 \pm 14.73 (-54.38) ^a	97.67 \pm 8.81 (-77.23) ^a

Values are expressed as mean \pm SD (n = 5) and values in brackets are the percentages of reduction of glycemia.

^a Values are statistically significant at $p < 0.05$ compared to the diabetic control rats.

The effect of the extracts on the non-enzymatic marker of the antioxidant status (Thiol proteins) is shown in Table 5. The diabetic non-treated group have low level of thiol proteins in the plasma and liver ($p < 0.05$) compared to the normal group. The diabetic group treated with the hydroethanolic extract of the fruit of *G. africana* has a significant high level of thiol proteins in the plasma (141.37 vs 113.23 μM) and liver (120.96 vs 81.02 μM) compared to the control diabetic group.

The hydroethanolic extract of the fruit of *G. africana* showed the best antioxidant activity compared to Tolbutamide.

4. Discussion

Diabetes is a metabolic disorder characterised by chronic hyperglycaemia. A high blood glucose levels can bind to compounds like haemoglobin through glycation and lead to the production of reactive oxygen species which are at the origin of oxidative stress [29]. Hyperglycaemia and oxidative stress are therapeutic targets in the management of diabetes and its complications. From which the present study had as aim to evaluate the hypoglycaemic and antioxidant properties of the fruits' pulp extracts of *G. africana*.

Despite the large use of the extract plants in traditional medicine, there is a lack of studies related to their toxicity. No signs of toxicity were observed for each extract and the LD₅₀ > 2000 mg/kg. So this extract is considered safe and low toxic. Olagunju and Adeneye [30] already noted that the compounds which have LD₅₀ > 1000 mg/kg could be considered safe and low toxic.

Streptozotocin is a chemical substance used as an experimental model to induce hyperglycaemia. Indeed, selectively destroying the beta cells of the pancreas, it decreases the synthesis of insulin resulting in hyperglycaemia [31]. After the induced hyperglycaemia in rats by streptozotocin, the hydroethanolic extract of *G. africana* fruits brought about a decrease in an acute (2h after administration) and sub-acute (14 days of treatment), effects that were comparable to that of Tolbutamide (Tables 2 and 4). This extract could act through the same mechanism as Tolbutamide which is an antidiabetic drug from the class of sulfonylurea. The sulfonylureas block the ATP dependent potassium pump thereby enabling calcium entry and subsequently the release of insulin, which will then stimulate the binding and utilization of glucose by cells [32], which could explain the drop in glycaemia in the different test groups. This hypoglycaemic effect could as well be explained by the presence of polyphenols in this extract which could simultaneously inhibit the digestion and absorption of carbohydrates. Indeed, polyphenols are known is powerful inhibitors of digestive enzymes or intestinal glucose transporters [33]. Moreover, it can promote the regeneration of beta cells thanks to their antioxidant properties [34].

Table 3
Effect of the extracts on the prooxidant status markers.

Groups	Hydroperoxides (μM)			MDA (μM)		
	Plasma	Liver	Pancreas	Plasma	Liver	Pancreas
Normal Control	34.1 \pm 2.17	7.12 \pm 0.6	6.55 \pm 0.8*	0.87 \pm 0.01	1.01 \pm 0.06	1.21 \pm 0.12*
Diabetic Control	41.39 \pm 2.47	8.79 \pm 0.35	8.86 \pm 0.5	1.08 \pm 0.02	1.34 \pm 0.03	2.06 \pm 0.02
Diabetic + 400 mg/kg Hydroethanolic of <i>G. africana</i>	38.93 \pm 1.31	7.57 \pm 0.50	7.18 \pm 1.0	1.09 \pm 0.05	1.33 \pm 0.06	1.44 \pm 0.05*
Diabetic +400 mg/kg Tolbutamide	35.05 \pm 3.63	8.21 \pm 1.12	7.83 \pm 0.6	1.06 \pm 0.06	1.3 \pm 0.11	1.71 \pm 0.09

Values are expressed as mean \pm SD (n = 5). MDA: Malonedialdehyde. * Values are statistically significant at p < 0.05 compared to the diabetic control rats.

Table 4
Effect of the extracts on the enzymatic markers of the antioxidant status.

Groups	SOD (UI/mg proteins)			Catalase (mmol de H ₂ O ₂ cons/min/g proteins)		
	Hemolysate	Liver	Pancreas	Hemolysate	Liver	Pancreas
Normal Control	1.01 \pm 0.13	1.95 \pm 0.09	1.36 \pm 0.11*	2.69 \pm 0.21	3.5 \pm 0.21*	4.12 \pm 0.32*
Diabetic Control	1.27 \pm 0.12	2.47 \pm 0.09	2.17 \pm 0.29	3.45 \pm 0.26	3.2 \pm 0.22	3.56 \pm 0.24
Diabetic + 400 mg/kg Hydroethanolic of <i>G. africana</i>	1.22 \pm 0.15	2.3 \pm 0.24	1.8 \pm 0.25*	2.78 \pm 0.21	3.49 \pm 0.23*	4.06 \pm 0.24*
Diabetic +400 mg/kg Tolbutamide	1.23 \pm 0.11	2.24 \pm 0.24	1.9 \pm 0.15	2.75 \pm 0.15	3.4 \pm 0.21*	3.7 \pm 0.28

Values are expressed as mean \pm SD (n = 5). SOD: Superoxide Dismutase. * Values are statistically significant at p < 0.05 compared to the diabetic control rats.

Table 5
Effect of the extracts on the non-enzymatic marker of the antioxidant status (Thiol proteins).

Groups	Thiol proteins (μM)		
	Plasma	Liver	Pancreas
Normal Control	161.3 \pm 12.62*	126.12 \pm 11.54*	85.26 \pm 4.2
Diabetic Control	113.23 \pm 16.13	81.02 \pm 9.40	95.44 \pm 1.09
Diabetic + 400 mg/kg Hydroethanolic of <i>G. africana</i>	141.37 \pm 7.11*	120.96 \pm 15.02*	91.91 \pm 5.25
Diabetic +400 mg/kg Tolbutamide	132.21 \pm 6.34*	112.54 \pm 11.12*	87.74 \pm 8.32

Values are expressed as mean \pm SD (n = 5). * Values are statistically significant at p < 0.05 compared to the diabetic control rats.

Omoboyowa et al. [35] and Hedge et al. [16] had also demonstrated the hypoglycemic properties of certain fruits belonging to the Sapotaceae family but on an experimental model of hyperglycemia induced by alloxan. While Ajayi et al. [36] had demonstrated the hypoglycemic properties of the *Chrysophyllum albidum* fruits on an experimental model of hyperglycemia induced by streptozotocin in 14 days.

Streptozotocin is a glucose analogue which preferentially accumulates in the beta cells of the pancreas, it ensures toxicity by degrading DNA leading to the production of reactive oxygen species and thus oxidative stress [37]. The stress which is at the origin of an increase in the production of pro-oxidants and defects in the antioxidant system, plays a crucial role in the development of diabetes and its complications [38]. The principal consequence of stress at the cellular level is lip-oxidation characterised by the formation of primary products like hydroperoxides and secondary products like MDA, which are harmful to the body [39]. Enzymatic antioxidants namely; SOD, catalase and Glutathione peroxidase play an important role in cellular defence against oxidative damage. SOD enables the dismutation of superoxide anion to hydrogen peroxide which is further decomposed by catalase to yield water and oxygen [40]. At the pancreatic level, the extract increased the activity of catalase while decreasing that of SOD (Table 4). This could be explained by the fact that after the action of streptozotocin, there is a direct production of hydroperoxides from the reaction catalysed by SOD and the substrate of catalase. This shows that there was more necessity in activating SOD compared to catalase. The extract could be acting by boosting the endogenous antioxidant status, namely catalase leading to the degradation of hydroperoxides, which is

characterised by a decrease in MDA level in the pancreas (Table 3). The extract equally boosted the endogenous antioxidant status at the hepatic and plasma level; however, no significant difference was observed as what concerns markers of the pro-oxidant status in these compartments. This could be due to the fact that, streptozotocin being specific to the pancreas, the oxidative damage was a high in this compartment compared to the others. Bahadoran et al. [41] had already shown the polyphenols' ability to boost the endogenous antioxidant status by activating catalase and Glutathione peroxidase and reducing the level of oxidative stress. These results are in accordance of works of Ajayi et al. [36] who demonstrated the benefits effects of *Chrysophyllum albidum* fruits on MDA, SOD, CAT and thiol proteins in an experimental model of hyperglycemia induced by streptozotocin.

5. Conclusion

The present study shows that the hydroethanolic pulp extract of the *G. africana* fruit possesses antioxidant and hypoglycemic activities. One of the limitations of this study is that the time frame of the experiment did not allow for an assessment of the effect of the fruit on the cardiovascular complications of diabetes. It is encouraging enough to warrant further studies on the constituents responsible of these activities, their mechanism of action and to establish it's therapeutic in the management of diabetes and it's complications.

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Authors' contributions

Takuissu, Ambamba and Nanhah carried out the study. Takuissu wrote the manuscript. Mandob, Ngoumen and Fonkoua reviewed. Ngondi contributed to conception, design and analysis of data. All authors have read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

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