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Polyphenolic extract of *Paullinia pinnata* leaf exhibits antidiabetic, anthyperlipidaemic and antioxidant activities in alloxan-induced diabetic rats

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Abstract

Background: This study evaluated the antidiabetic, antihyperlipidaemic and antioxidant effects of polyphenolic extract of *Paullinia pinnata* leaf (PEPPL) in alloxan-induced diabetic rats.

Method: Diabetes was induced in Albino rats by intraperitoneal administration of alloxan monohydrate (120 mg/kg body weight (b. w.)). Male and female rats of average weight 150 ± 1.14 g were selected for the study. PEPPL at a dose of 25 and 50 mg/kg b. w. was administered single dose per day to alloxan-induced diabetic rats for a period of 14 days. The effects of PEPPL on blood glucose, hepatic hexokinase, pancreatic alpha amylase, hepatic glycogen, lipid peroxidation and antioxidant status were assessed in the diabetic rats.

Results: PEPPL elicited significant ($p<0.05$) reductions of blood glucose, lipid parameters except HDL-Cholesterol in which case was significantly increased alongside the antioxidant enzymes. The untreated diabetic rats showed significant ($p<0.05$) decrease in the activities of hepatic hexokinase, pancreatic alpha amylase and hepatic glycogen content. Hepatic glycogen content as well as hexokinase and pancreatic alpha amylase activities were significantly ($p<0.05$) restored.

Conclusions: The results revealed that polyphenolic extract of *P. pinnata* leaf offers promising antidiabetic and hypolipidaemic effects that may be attributed largely to its potent antioxidant ability.

Keywords: Diabetes; α-amylase; antioxidant; lipid profile; alloxan; glibenclamide; *Paullina pinnata* leaf; rats.

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Background

Diabetes mellitus is a metabolic disorder that is characterized by hyperglycaemia associated with impairment in insulin secretion and/or insulin action as well as aberrations in intermediary metabolism of carbohydrates, proteins and lipids [1, 2]. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with classical characteristic features such as blurring of vision, excessive thirst (polydypsia), excessive feeding (polyphagia), excessive urination (polyuria), and weight loss. In its most severe forms, ketoacidosis may be developed leading to stupor, coma and, in absence of effective treatment death ensues [3].

People with diabetes are at increased risk of developing cardiovascular, peripheral vascular and cerebrovascular disease [4, 5]. Hyperglycaemia and hyperlipidaemia are two important characters of diabetes mellitus, and excessive oxidative stress has been shown to be involved in the pathology and complications of diabetes mellitus [6, 7]. Many studies indicate the participation of free radicals in the pathogenesis of diabetes [8] and its complications [9, 10]. Free radicals are proficient enough in damaging cellular molecules, proteins, lipids and DNA, leading to alteration of cell functions [11]. Generally, the injurious effects of hyperglycaemia are separated into macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy).

Therapies used to treat patients with this disease are aimed at correcting one or more of the physiological abnormalities involved. Several hypoglycaemic drugs have been employed for the treatment of diabetes with different mechanism of actions. However, they have many setbacks, ranging from development of resistance and adverse effects to lack of responsiveness in large segment of patients population [12, 13].

Traditional medicine (herbal) is used for treatment of diabetes in both developing and developed countries where the cost of conventional medicines is a burden to the population [14]. Despite the introduction of hypoglycaemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Many medicinal plants have been found to be useful to successfully manage diabetes. One of the great advantages of medicinal plants is that these are readily available and have low side effects [15]. Many plants containing glycosides, alkaloids, terpenoids, flavonoids, carotenoids, *etc.* are frequently implicated as having antidiabetic effect [16]. Some of the reportedly important antidiabetic potential herbal plants sources include *Allium sativum*, *Aloe vera*, *Brassica juncea*, *Combretum micranthum*, *Elephantopus scaber*, *Hemidesmus indicus* [17], *Vernonia anthelmintica* [18], *Pterocarpus marsupus* [19], *Acacia ataxacantha* [20] *Eugenia floccose* [21], *Psidium guajava* [22] etc.

P. pinnata Linn belonging to the family of Sapindaceae is an African endemic climbing shrub, woody vine that inhabits the tropical rainforest zones of West Africa, stretching from Nigeria to Cameroon. Sapindaceae consists of about 136 genera and nearly 2000 species [23, 24]. Its leaves are compound with winged rachis. The fruits are red or dark pink when ripe [25-27]. Aqueous decoctions and powdered root of *P. pinnata* also called “sweet gum” in English are used in Nigeria (where it is called *ogbe-okuje* – “instant wound healer” by the Yoruba natives of the Southwestern region), Togo and Ghana as traditional medicine for treating sores, wounds, snake bites and other diseases such as erectile dysfunction, malaria, dysentery, menstrual pain and coughs [28-31]. Previous phytochemical investigations have shown the presence of alkaloid [32], triterpene, saponins [33], flavone glycoside [34], triterpenes, and steroids [35]. This study was thus aimed at investigating the antidiabetic, antihyperlipidaemic and antioxidant activities of polyphenolic extract of *Paullinia pinnata* leaf on alloxan-induced diabetic rats.

Methods

Plant material

Fresh leaves of *Paullinia pinnata* were obtained from Ijawaya Secondary School premises, along Oyo-Ogbomoso road, Oyo. The plant was identified and authenticated at the Herbarium of the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. A specimen with voucher number Herb/NIRPD/6404 was deposited for future references.

Experimental animals

Thirty (30) healthy albino rats (*Rattus norvegicus*) of both sexes weighing 150 ± 1.14 g were selected for the study. They were obtained from the animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria, and were maintained under standard laboratory conditions (12-hr light/dark cycle, 25 ± 2 °C). The rats were acclimatized for a week in the laboratory. They were fed with standard rodent diet and tap water *ad libitum.* The animals were handled according to the stated guidelines of Ethical Committee on the ethical use of animals in research.

Preparation of polyphenolic extract of Paullinia pinnata

Fresh leaves of *P. pinnata* were air-dried, made into powder using an electric blender and then sieved. The dried powdered leaf sample was soaked in absolute methanol in a chilled blender and immediately macerated at increasing speed for 3 minutes. The resultant sample was placed in the freezer for 15 minutes to chill and again macerated at increasing speed for 3 minutes. The sample was suspended in 80 % aqueous methanol and the cold maceration step described above was repeated twice. The resulting mixture was then filtered, the filtrate was concentrated using a rotatory evaporator under vacuum, and the concentrate was lyophilized and designated polyphenolic extract of *Paullina pinnata* leaf (PEPPL).

Polyphenolic assay

Total polyphenols was determined using Folin-Ciocalteu assay as described by Danilewicz [36]. Briefly, triplicate samples of 10 mg/mL of PEPPL extract were dissolved into methanol, and then mixed with 2.9 ml of 2% Na_2CO_3. The solution was left to stand for 2 minutes at room temperature before 0.1 ml of 0.2 N Folin-Ciocalteu reagent was added. The mixture was incubated for 30 minutes at room temperature after which its absorbance was spectrophotometrically read at 750 nm. Gallic acid (0.1 – 1.0 mg/mL in methanol) was used as a standard, and the total phenolic content of the extract was expressed in milligram gallic acid equivalents (mg gallic acid/g extract).

Induction of experimental diabetes and determination of blood glucose

Diabetes was induced by a single intraperitoneal administration of alloxan monohydrate (120 mg/kg) prepared in 5% normal saline solution (an average of 0.90 mL per specimen) after fasting the animals for 12 hours. Confirmation of diabetes was done 72 hours after alloxan injection by means of Accucheck Active glucometer and compatible blood glucose strips. Blood sample for the fasting blood glucose (FBG) determination was obtained through tail puncture, and animals with FBG of ≥ 250 mg/dl were considered diabetic.

Animal grouping and extract administration

Thirty albino rats of both sexes with an average weight of 150 ± 1.14 g were randomly divided into five groups (A, B, C, D and E) of six rats each and were treated as shown below.
Group A: Non-induced control rats administered 1ml of distilled water daily
Group B: Alloxan-induced non-treated rats administered 1ml of distilled water daily
Group C: Alloxan-induced rats treated with Glibenclamide at a dose of 5mg/kg
Group D: Alloxan-induced rats treated with 25mg/kg body weight of PEPPL
Group E: Alloxan-induced rats treated with 50mg/kg body weight of PEPPL

Preparation of serum and tissue homogenate

After fourteen days of treatment, the animals were anaesthetized with diethyl ether and sacrificed by simply incising the jugular vein, the blood samples were collected into clean, dry, plain sample tubes. Blood samples were allowed to stand at room temperature for 30mins to form clot after which they were centrifuged at 3000 xg for 10 mins. Using Pasteur pipette, the sera were collected into clean, dry sample bottles and kept frozen until required for analysis. Following sacrifice, the rats were immediately dissected in order to isolate the liver and pancreas. The isolated tissues were cleaned with cotton wool, weighed and immediately stored in ice cold 0.01M Tris buffer. 1g of the liver, cut with a clean scalpel was then subjected to homogenization using mortar and pestle in ice-cold 0.01M Tris buffer (1:5 $^{w}/_{v}$). The homogenate was kept frozen until required for analysis.

Determination of hepatic parameters and antioxidant analysis

Before concentration of metabolites and enzyme activities were determined, the tissue homogenates were appropriately diluted using 0.01 M Tris buffer.

Hepatic hexokinase was assayed using the method of Brandstrup et al. [37]. The reaction mixture in a total volume of 5.3 ml contained the following: 1 ml of glucose (5 mM) solution, 0.5 ml of adenosine triphosphate (72 mM) solution, 0.1 ml of magnesium chloride (50 mM) solution, 0.4 ml of potassium dihydrogen phosphate (12.5 mM), 0.4 ml of potassium chloride (100 mM), 0.4 ml of sodium fluoride (500 mM) and 2.5 ml of Tris-HCl buffer (10 mM, pH 8.0). The mixture was pre-incubated at 37 °C for 5 minutes. The reaction was initiated by the addition of 2 ml of tissue homogenate. 1ml of the reaction mixture was immediately transferred to a tube containing 1 ml of 10% trichloroethanoic acid that was considered as zero time. A second aliquot was taken and deproteinised after 30 minutes incubation at 37 °C. The protein precipitate was removed by centrifugation and the residual glucose in the supernatant was estimated by the method of Trinder et al. [38].

Methods already described were employed in the determination pancreatic alpha amylase activity [39], hepatic glycogen concentration [40], serum

cholesterol level [41], serum and hepatic triglycerides [38], serum high density lipoproteins (HDL) [42], serum low and very low density lipoproteins (LDL and VLDL) [43], superoxide dismutase (SOD) [44], catalase [45], reduced glutathione (GSH) [46], and concentration of malondialdehyde (MDA) [47]. The Atherogenic Index (AI) was calculated by: log (TG/HDL-C), while Coronary Heart Disease Index (CHDI) was determined by: TC/HDL-C

Statistical analysis

Statistical Analysis was performed using GraphPad Prism version 6. The data were analysed by one-way analysis of variance (ANOVA) followed by Tukey Post-hoc test. All data are expressed as the mean of four replicates $\pm$ standard deviation of mean (S.D). Values were considered statistically significant at $p < 0.05$.

Results

The Total polyphenolic content of P. pinnata Leaf

The cold extraction process afforded a yield of 80 mg of *P. pinnata* / g of sample (8 % $^{w}/_{w}$). Total polyphenolic assay of PEPPL showed that the extract contains 150 mg gallic acid equivalent / g of extract.

Effect of PEPPL on fasting blood glucose level and body weight ratio of alloxan induced diabetic rats

Figure 1 shows the effects of polyphenolic extract of *P. pinnata* leaf on fasting blood glucose level of alloxan-induced diabetic rats. There was continuous increase in the fasting blood glucose (FBG) level of alloxan-induced non-treated rats from the first day of treatment till the fourteenth day. Oral administration of 25 and 50 mg/kg body weight polyphenolic extract of *P. pinnata* leaf caused reduction in the FBG level, comparable to those of animals treated with glibenclamide.

Figure 2 represents the percentage of selected organ to body weight ratio (liver, kidney and pancreas) of alloxan induced diabetic rats treated with PEPPL. There was only a significant decrease in the weight of the pancreas relative to body weight in alloxan-induced group when compared with the non-induced control. Rats treated with 25mg/kg body weight of the PEPPL and the standard drug (glibenclamide) caused significant increase ($p<0.05$) in the pancreas to body weight ratio when compared with the alloxan-induced non-treated rats. However, no significantly ($p>0.05$) different from the ratios of the non-induced control in the liver and kidney

Effects of polyphenolic extract of P. pinnata leaf on glycogen concentration in alloxan-induced diabetic rats

Figure 3 represents the hepatic glycogen concentrations in diabetic rats orally administered PEPPL for 14 days. There was a significant decrease ($p < 0.05$) in hepatic glycogen concentrations of alloxan-induced non-treated animals compared to the non-induced control. Administration of 25 mg/kg bw PEPPL as well as glibenclamide significantly raised ($p < 0.05$) glycogen concentrations when compared to the diabetic but untreated group.

Effects of Polyphenolic extract of Paullinia pinnata Leaf on Carbohydrate metabolizing Enzymes of alloxan-induced diabetic rats.

Figure 4 and 5 present the effects of PEPPL on carbohydrate-metabolizing enzymes (hepatic hexokinase and pancreatic alpha amylase) of alloxan-induced diabetic rats respectively. Treatment of alloxan-induced rats with PEPPL brought about a significant increase ($p < 0.05$) in the hepatic hexokinase when compared with the alloxan-induced non-treated rats. The activity of the enzyme achieved was not significantly different from that achieved by glibenclamide.

Also, there was a significant ($p < 0.05$) reduction in the activity of pancreatic alpha amylase of alloxan-induced rats when compared with non-induced control. Oral administration of PEPPL caused significant ($p < 0.05$) elevation of pancreatic alpha amylase when compared with alloxan-induced non-treated rats.

Effects of polyphenolic extract of Paullinia pinnata leaf on lipid profiles of alloxan-induced diabetic rats

Figures 6, 7 and 8 present the effects of PEPPL on lipid profile of alloxan-induced diabetic rats treated with PEPPL. The level of serum total cholesterol (TC) and triglycerides (TAG) were significantly higher in alloxan-induced rats when compared with values in non-induced control. The levels of serum TC and TAG in hyperglycaemic rats treated with 25 mg/kg b.w. PEPPL were not significantly different ($p <0.05$) compared with values in non-induced control. These values in animals treated with 25 mg/kg b.w. as well as 50 mg/kg b.w. PEPPL were comparable to those of animals treated with the glibenclamide.

A significant increase ($p <0.05$) in the serum level of LDL and significant decrease in HDL level were observed in alloxan-induced rats when compared to non-induced control. Level of LDL was significantly ($p <0.05$) reduced and the level of HDL was significantly higher in the serum of alloxan-induced rats that received 25 mg/kg, 50 mg/kg bw

PPP and standard drug (glibenclamide) when compared to alloxan-induced non-treated control.

There was significant ($p < 0.05$) increase in the level of atherogenic and coronary heart disease indices in the serum of alloxan-induced rats when compared to non-induced control. The level of atherogenic and coronary heart disease indices was significantly ($p < 0.05$) lower in the serum of alloxan-induced rats that received 25 mg/kg, 50 mg/kg bw PPP and standard drug (glibenclamide) when compared to alloxan-induced non-treated control.

Effects of polyphenolic extract of Paullinia pinnata leaf on the antioxidant status of alloxan-induced diabetic rats

Figure 9 depicts the liver and serum activity of superoxide dismutase (SOD) of alloxan-induced diabetic rats treated with the PEPPL. There was a significant ($p < 0.05$) decrease in the activity of SOD in serum and liver of alloxan-induced rats when compared to non-induced control. The activity of SOD increases significantly ($p < 0.05$) in the serum and liver of alloxan-induced rats that were treated with PEPPL and standard drug. 25 mg/kg bw administration produced a better SOD activity normalization effect than 50 mg/kg bw.

Figures 10 and 11 show the hepatic activities of catalase (CAT) and reduced glutathione of alloxan-induced diabetic rats administered PEPPL. The hepatic activity of CAT was significantly ($p < 0.05$) lower in alloxan-induced untreated rats when compared with recorded values in non-induced control. However, oral administration of 25 mg/kg bw and 50 mg/kg bw PEPPL restored the activity to levels not significantly ($p < 0.05$) different from the non-induced control rats. Treatment with PEPPL brought about catalase activity restoration very much better than the standard drug glibenclamide.

The hepatic concentration of reduced glutathione (GSH) in alloxan-induced rats decreased significantly ($p < 0.05$) when compared to recorded values in non-induced control. Treatment of hyperglycamic rats with 25 mg/kg and 50 mg/kg bw PEPPL caused significant ($p < 0.05$) increase in the hepatic concentration of reduced glutathione normalizing the concentration to values that are not significantly different from that recorded for non-induced control rats as well as those treated with glibenclamide.

Figure 12 shows the hepatic and serum levels of malondialdehyde (MDA) in alloxan-induced diabetic rats treated with PEPPL. There was a significant ($p < 0.05$) increase in the level of malondialdehyde in the liver and serum of alloxan-induced non-treated control when compared to values of non-induced control. Treatment with PEPPL restored both the hepatic and serum MDA levels to normal values not significantly ($p < 0.05$) different from that of the non-induced control animals and glibenclamide-treated diabetic rats.

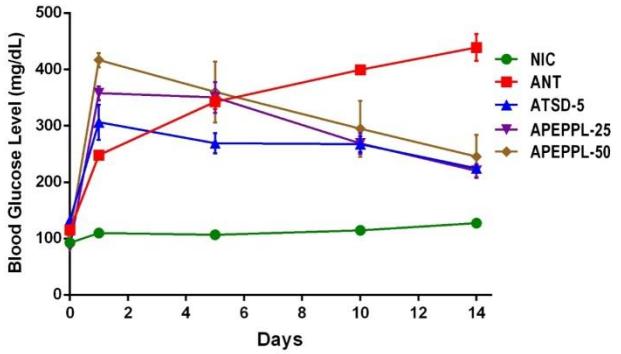

Figure 1. Effect of polyphenolic extract of *Paullinia pinnata* leaf on the fasting blood glucose level of alloxan-induced diabetic rats

Values are expressed as mean of four replicates ± SEM; values with different superscripts are significantly different ($p < 0.05$). NIC = non-induced control; ANT = alloxan-induced non-treated control; ATSD-5 = alloxan+5mg/kg b.wt. glibenclamide; APEPPL-25 = alloxan+25mg/kg b.wt. PEPPL; APEPPL-50 = alloxan+50mg/kg b.wt PEPPL

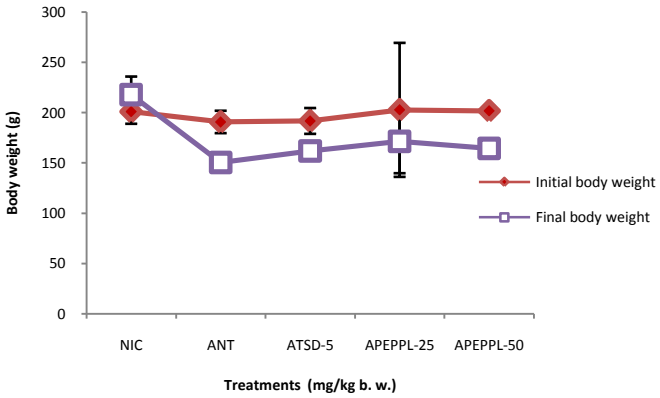

Figure 2: Effect of Administration of Polyphenolic extract of *Paullinia pinnata* Leaf on body weight of Alloxan-Induced Diabetic Rats

Values are expressed as mean of four replicates ± SEM; values with different superscripts are significantly different ($p < 0.05$). NIC = non-induced control; ANT = alloxan-induced non-treated control; ATSD-5 = alloxan+5mg/kg b.wt. glibenclamide; APEPPL-25 = alloxan+25mg/kg b.wt. PEPPL; APEPPL-50 = alloxan+50mg/kg b.wt PEPPL

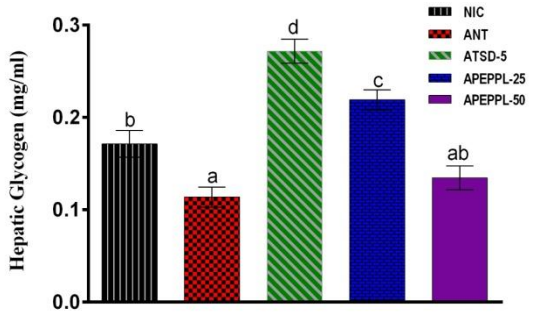

Figure 3. Effects of polyphenolic extract of *Paullinia pinnata* leaf on hepatic glycogen of alloxan-induced diabetic rats.

Values are expressed as mean of four replicates ± SD; values with different superscripts are significantly different ($p < 0.05$). NIC = non-induced control; ANT = alloxan-induced non-treated control; ATSD-5 = alloxan+5mg/kg b.wt. glibenclamide; APEPPL-25 = alloxan+25mg/kg b.wt. PEPPL; APEPPL-50 = alloxan+50mg/kg b.wt APEPPL

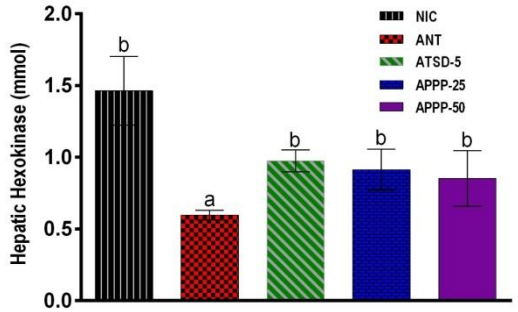

Figure 4. Hepatic hexokinase activity of alloxan-induced diabetic rats treated with polyphenolic extracts of *Paullinia pinnata*

Values are expressed as mean of four replicates ± SD; values with different superscripts are significantly different ($p < 0.05$). NIC = non-induced control; ANT = alloxan-induced non-treated control; ATSD-5 = alloxan+5mg/kg b.wt. glibenclamide; APEPPL-25 = alloxan+25mg/kg b.wt. PEPPL; APEPPL-50 = alloxan+50mg/kg b.wt PEPPL

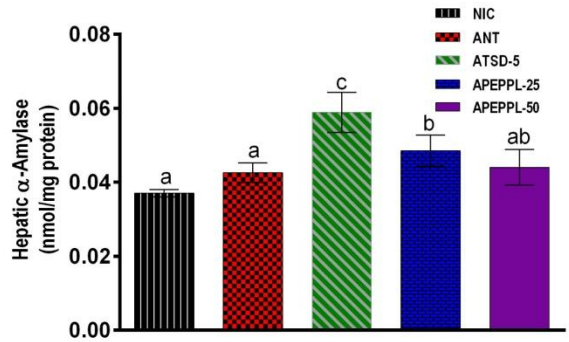

Figure 5. Activity of pancreatic alpha amylase in alloxan-induced diabetic rats treated with polyphenolic extracts of *Paullinia pinnata*

Values are expressed as mean of four replicates ± SD; values with different superscripts are significantly different ($p < 0.05$). NIC = non-induced control; ANT = alloxan-induced non-treated control; ATSD-5 = alloxan+5mg/kg b.wt. glibenclamide; APEPPL-25 = alloxan+25mg/kg b.wt. PEPPL; APEPPL-50 = alloxan+50mg/kg b.wt PEPPL

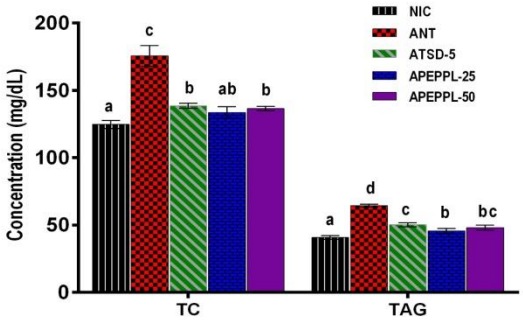

Figure 6. Levels of serum total cholesterol and triglycerides of alloxan-induced diabetic rats treated with polyphenolic extracts of *Paullinia pinnata*

Values are expressed as mean of four replicates ± SD; values with different superscripts are significantly different ($p < 0.05$). NIC = non-induced control; ANT = alloxan-induced non-treated control; ATSD-5 = alloxan+5mg/kg b.wt. glibenclamide; APEPPL-25 = alloxan+25mg/kg b.wt. PEPPL; APEPPL-50 = alloxan+50mg/kg b.wt PEPPL

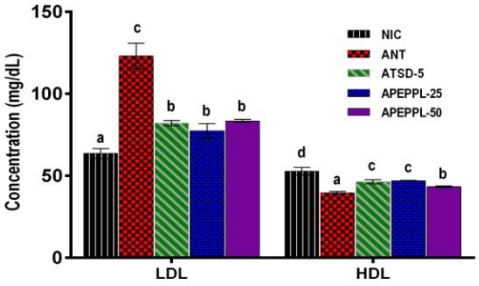

Figure 7. Levels of serum LDL and HDL of alloxan-induced diabetic rats treated with polyphenolic extracts of *Paullinia pinnata*

Values are expressed as mean of four replicates ± SD; values with different superscripts are significantly different ($p < 0.05$). NIC = non-induced control; ANT = alloxan-induced non-treated control; ATSD-5 = alloxan+5mg/kg b.wt. glibenclamide; APEPPL-25 = alloxan+25mg/kg b.wt. PEPPL; APEPPL-50 = alloxan+50mg/kg b.wt PEPPL

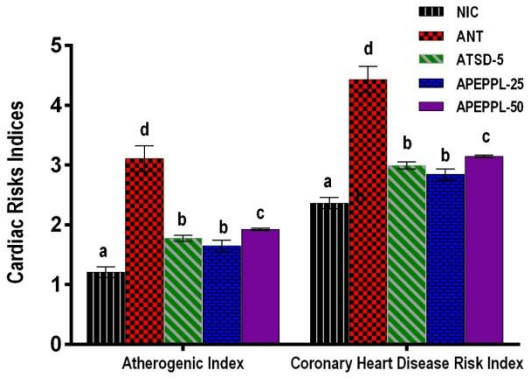

Figure 8. Atherogenic and coronary heart disease risk indices of alloxan-induced diabetic rats treated with polyphenolic extracts of *Paullinia pinnata*

Values are expressed as mean of four replicates ± SD; values with different superscripts are significantly different ($p < 0.05$). NIC = non-induced control; ANT = alloxan-induced non-treated control; ATSD-5 = alloxan+5mg/kg b.wt. glibenclamide; APEPPL-25 = alloxan+25mg/kg b.wt. PEPPL; APEPPL-50 = alloxan+50mg/kg b.wt PEPPL

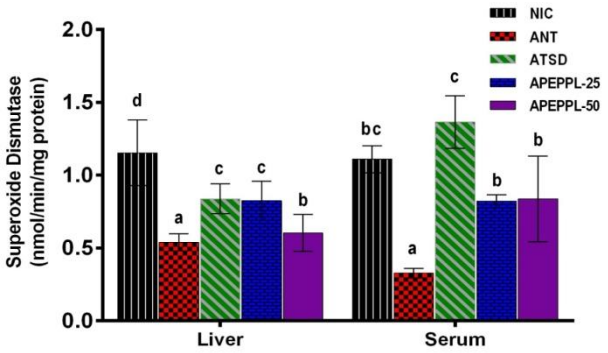

Figure 9. Hepatic and serum activities of superoxide dismutase of alloxan-induced diabetic rats treated with polyphenolic extracts of *Paullinia pinnata*

Values are expressed as mean of four replicates ± SD; values with different superscripts are significantly different ($p < 0.05$). NIC = non-induced control; ANT = alloxan-induced non-treated control; ATSD-5 = alloxan+5mg/kg b.wt. glibenclamide; APEPPL-25 = alloxan+25mg/kg b.wt. PEPPL; APEPPL-50 = alloxan+50mg/kg b.wt PEPPL

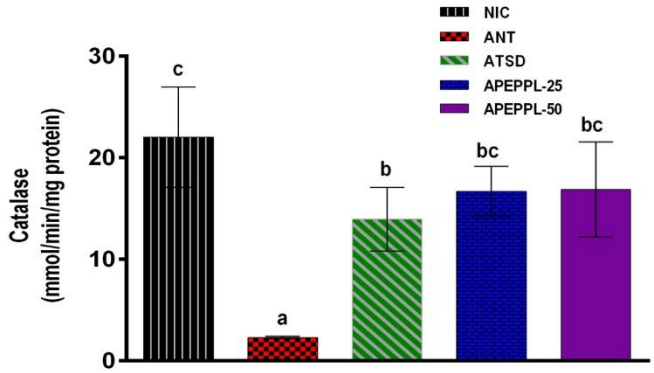

Figure 10. Hepatic activity of catalase of alloxan-induced diabetic rats treated with polyphenolic extracts of *Paullinia pinnata*

Values are expressed as mean of four replicates ± SD; values with different superscripts are significantly different ($p < 0.05$). NIC = non-induced control; ANT = alloxan-induced non-treated control; ATSD-5 = alloxan+5mg/kg b.wt. glibenclamide; APEPPL-25 = alloxan+25mg/kg b.wt. PEPPL; APEPPL-50 = alloxan+50mg/kg b.wt PEPPL

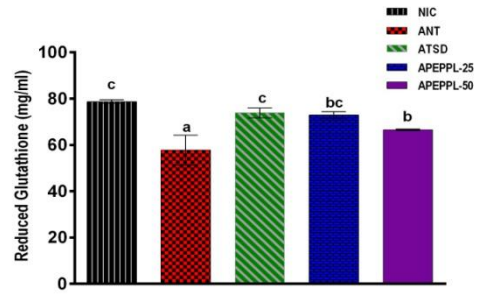

Figure 11. Concentration of reduced glutathione in liver of alloxan-induced diabetic rats treated with polyphenolic extracts of *Paullinia pinnata*

Values are expressed as mean of four replicates ± SD; values with different superscripts are significantly different ($p < 0.05$). NIC = non-induced control; ANT = alloxan-induced non-treated control; ATSD-5 = alloxan+5mg/kg b.wt. glibenclamide; APEPPL-25 = alloxan+25mg/kg b.wt. PEPPL; APEPPL-50 = alloxan+50mg/kg b.wt PEPPL

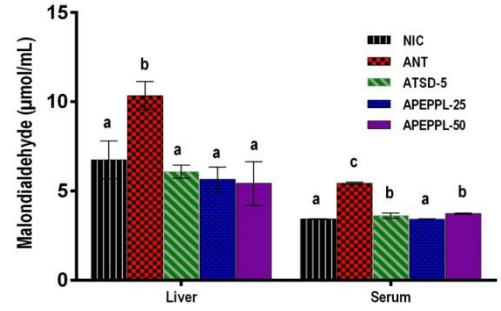

Figure 12. Hepatic and serum malondialdehyde concentrations of alloxan-induced diabetic rats treated with polyphenolic extracts of *Paullinia pinnata*

Values are expressed as mean of four replicates ± SD; values with different superscripts are significantly different ($p < 0.05$). NIC = non-induced control; ANT = alloxan-induced non-treated control; ATSD-5 = alloxan+5mg/kg b.wt. glibenclamide; APEPPL-25 = alloxan+25mg/kg b.wt. PEPPL; APEPPL-50 = alloxan+50mg/kg b.wt PEPPL

Discussion

Plants are used to treat many ailments. Medicinal plants used to treat hypoglycaemic or hyperglycaemic conditions are of considerable interest for ethnobotanical community as they are recognized to contain valuable medicinal properties in different parts of the plant and a number of plants have shown varying degree of hypoglycaemic and anti-hyperglycaemic activity [48]. The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents [49]. Several species of medicinal plants are used in the treatment of diabetes mellitus, a disease affecting large number of people world-wide.

Polyphenolic compounds are ubiquitous in foods of plant origin, and thus they constitute an integral part of the human diet [50]. Interest in polyphenols has greatly increased recently because these phytochemicals are known to suppress rates of degenerative processes such as cardiovascular disorders and cancer [50-53]. Some of these potential health benefits of polyphenolic substances have been related to the action of these compounds as antioxidants, free radical scavengers, quenchers of singlet and triplet oxygen and inhibitors of peroxidation [54]. As a group, phenolic compounds have been found to be strong antioxidants against free radicals and other reactive oxygen species, the major cause of many chronic human diseases [55, 56].

The major chemical substances in polyphenolic extract of *P. pinnata* were catechic tannins and flavonoids. The analysis of polar extracts demonstrated that these tannins were of a catechic type with a high degree of polymerization. Flavonoids and tannins are phenolic compounds and plant phenolics are also a major group of compounds that act as primary antioxidants or free radical scavengers [57-59]. Tannins and saponins are also found to be effective antioxidants, antimicrobial, and anti-carcinogenic agents [60].

In the preliminary study, acute oral administration of *Paullinia pinnata* to rats indicated that the plant is toxic at the dose of 100 mg/kg body weight and above. It thus showed that this plant might not be safe for medicinal use at high dose.

Glycemic Studies

In this study significant hyperglycaemia was achieved within 72 hours after alloxan (120 mg/kg body weight intraperitoneal) injection. This is in agreement with the summation of Lenzen [61]. Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemia [62]. Alloxan induces damage and death of pancreatic islet-cells in several experimental animal models, thus causing diabetes mellitus and decreasing the secretion of insulin. The cytotoxic action of this diabetogenic agent is mediated by reactive oxygen species, Alloxan and the product of its reduction, dial uric acid; establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Therefore, highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of ß-cells.

Observations in this study correlates with the previous research findings, in that the blood glucose levels significantly increased in alloxan-induced non-treated diabetic rats when compared with non-induced control. The alloxan-induced rats treated with 25 mg/kg and 50 mg/kg b. wt polyphenolic extract of *P. pinnata* leaf showed significant ($p < 0.05$) reduction in their fasting blood glucose on the day 14 of the experiment. The reduction in fasting blood level was not compared to normal values but the glucose level could be reduced to values close to that of non-induced control when the numbers of days for extract administration is increased. The lower dose 25 mg/kg body weight PEPPL was the most effective dosage reducing the fasting blood glucose.

The blood glucose data obtained clearly indicate that polyphenolic extract from *Paullinia pinnaa* produced significant antidiabetic effects in alloxan-induced diabetic rats. The results obtained were similar to those obtained by Marrif et al. [63] as well as Twaij and Al-Badr [64]. It is possible that the plant may reverse the catabolic features of insulin deficiency, decrease the release of glucagons or increase that of insulin, stimulate directly glycolsis in peripheral tissues, increase glucose removal from blood or reduce glucose absorption from the gastrointestinal tract [63].

Due to the association of obesity with diabetes mellitus, weight control is an important aspect of diabetes management. The loss in weight of the diabetic rats has also been shown in some studies in which it was observed that during diabetes mellitus, the blood sugar increases and results in lack of sugar in the cells; forcing, the cells to use amino acids and fatty acids as a source of energy which eventually leads to the reduction of proteins and fats in the body which causes body weight loss. Poor glycaemic control usually results in weight loss. The results (Figure 2) showed that all the animals used loss weight during the study. The result showed that at both dose, polyphenolic extract from *Paullinia pinnata* showed a significant decrease in body weight. It is also important to note that oral administration of the extracts did not promote the natural growth process of these animals with diabetes.

Hepatic hexokinase activity

One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate [65, 66]. Treatment of diabetic rats with glibenclamide and polyphenolic extract of *P. pinnata* showed an increased hexokinase activity, The mechanism(s) of action of *P. pinnata* is not yet known exactly, but from its effect on this glycolytic enzyme, its mechanism of action might be by increasing the flux of glucose into glycolytic pathway in an attempt to reduce high blood glucose concentration. This is also confirmed by the significant decreased hepatic glucose level in diabetic rats treated with extract when compared to alloxan-induced non-treated rats

In this study, there was also an obvious significant reduction in the level of hepatic pancreatic alpha amylase in the alloxan-induced treated rats when compared with the non-induced control, the reduction was returned to almost normal after the oral administration of polyphenolic extract of *P. pinnata* leaf and glibenclamide for 14 days of the experiment. The result shows that the extract has a stimulant effect on this enzyme and probably has the same effect on other pancreatic hydrolases.

Hepatic Glycogen

Glycogen is the primary intracellular storable form of glucose in various tissues and its level in such tissues especially the liver is a direct reflection of insulin activity [67]. In this study, there was significant reduction in the glycogen content in the liver of

alloxan-induced non-treated rats are in accordance with the finding of Ahmed et al. [68] and significant increase in hepatic glycogen of alloxan-induced rats upon oral administration of polyphenolic extract of *Paullinia pinnata* leaf, which is comparable to that of glibenclamide, thus confirming its insulin potentiating action to a marked extent. This may be due to the activation of glycogen synthase system and inhibition of glycogen phosphorylase by the extract [69]. It may also be due to decreased enzymatic activities of hexokinase and phosphofructokinase resulting in depletion of liver and muscle glycogen [48].

Therefore, the significant increase in the hepatic glycogen concentration (Figure 3) of the alloxan-induced rats treated with the polyphenolic extract of *P. pinnata* (25 mg/kg b.wt and 50 mg/kg b.w.) support the likelihood that the extract contains chemical substances that may serve as insulin analogue which may promote the conversion of the inactive form of glycogen synthetase to the active form and the conversion of the active form of glycogen phosphorylase to its inactive form. Any of these effects will enhance conversion of blood glucose into glycogen.

This is in accordance with the finding of El-Shenawy and Abdel-Nabi [70], Rawi et al. [71] and Lalhlenmawia *et al.* [72] who attributed the increase in liver glycogen of diabetics treated with different plants extracts and glibenclamide to the increased insulin response.

Serum Lipid Profile

Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both [73]. Beside hyperglycaemia, several other factors like hyperlipidaemia and enhanced oxidative stress play a major role in diabetic pathogenesis [74]. Insulin affects many sites of mammalian lipid metabolism.

Diabetic-induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose due to underutilization of glucose [75, 76]. The lack of insulin and elevations of the counter-regulatory hormones lead to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissue [77, 78]. The fatty acids from adipose tissues are mobilized for energy purpose and excess fatty acids are accumulated in the liver, which are converted to triglyceride [79]. The marked hyperlipidaemia that characterizes the diabetic state may therefore be regarded as a consequence of unlimited actions of lipolytic hormones on the fat depots [80]. Lowering of serum lipid levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease in diabetes [80].

In this study, oral administration of polyphenolic extract of *P. pinnata* at 25 mg/kg and 50 mg/kg body weight in alloxan-induced rats exerted significant ($p < 0.05$) reduction in the level of TC, TAG, LDL, VLDL and significant ($p < 0.05$) elevation in HDL level are in accordance with the findings of Mendez and Balderas [81] and Mitra et al. [82]. However, significant decrease in serum lipid profile levels observed on treatment with the polyphenolic leaf extract of *Paullinia pinnata* may presumably be mediated by a control of lipid metabolism by some of the phytochemicals present in the plant. Many nutritional factors such as saponins and tannins have been reported to contribute to the ability of herbs to improve dyslipidemia [76, 78].

Preliminary phytochemical screening of the extract revealed the presence of polyphenolic compounds (such as tannins and flavonoids) among other saponin. This may be responsible for the lipid-lowering effect of *P. pinnata* on plasma lipid. Saponins are known anti-nutritional factors, which lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its faecal excretion [76, 78, 83]. Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol [78]. Hence, saponins have been reported to have hypocholesterolic effect [83].

Kumarappan et al. [84] reported that administration of polyphenolic compounds to alloxan-induced diabetic rats reduced hyperlipidaemia and attributed this to a reduction in the activity of hepatic HMG-CoA reductase, which is the first committed enzymatic step of cholesterol synthesis. This lowers elevated LDL cholesterol levels, resulting in a substantial reduction in coronary events and deaths from coronary heart disease (CHD) that occurs in diabetics [85]. Thus, the observed hypolipidaemic effect of *P. pinnata* can be therefore, linked to the synergistic actions of phytochemicals like saponins and polyphenolic compounds contained in the plant extract. It is reported that the derangement of glucose, fat and protein metabolism during diabetes, results into the development of hyperlipidaemia [86, 87].

In this study, all doses of the plant extract used produced a significant beneficial effect on serum lipid profile in alloxan-induced diabetic rats. About 30% of blood cholesterol is carried in the form of HDL-C. Significant lowering of total cholesterol and rise in HDL-C is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions [82, 88-90]. HDL-C function to remove cholesterol atheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease [83, 91]. Therefore, the observed increase in

the serum HDL-C level on administration of both doses of the extract in alloxan-induced diabetic rats, indicates that the extract have HDL-C boosting effect.

More so, the stabilization of serum triglyceride and cholesterol levels in rats by the plant extract may be attributed to glucose utilization and hence depressed mobilization of fat [80, 92]. This implies that the plant extract may be useful in reducing the complications of hyperlipidemia and hypercholesterolemia which often coexist in diabetics [93]. The study also revealed that administration of the extract at both doses significantly lowered the serum LDL-C in alloxan-induced diabetic rats with the 25 mg/kg body weight of the extract being the most effective. Studies have shown that chronic insulin deficiency as observed in alloxan-induced diabetes in experimental animals is associated with diminished levels of LDL-C receptors. This results to an increase in LDL particles and consequently increases serum level of LDL-C [79].

The abnormalities in lipid metabolism lead to alteration not only in the levels of serum lipids but also lipoproteins that in turn play an important role in the occurrence of premature and severe atherosclerosis which affects patients with diabetes Ravi et al., [93b]. Thus, measurement of these parameters is necessary to prevent cardiac complications in diabetic conditions [94].

Lipid Peroxidation

Lipid peroxidation refers to the oxidative degradation of lipid that impairs cell membrane functions resulting in cell damage and leading to several pathologies and cytotoxicity [95]. The lipid peroxidation process has been implicated in a variety of disease conditions [96]. The role of free radicals in etiopathogenesis of diabetes mellitus is well known [97]. Sato et al. [98] were the first to report the increased level of lipid peroxidation in the plasma of diabetic patients. Previous study has revealed an increase lipid peroxidation in the plasma of diabetic rats [99].

The increases in the levels of LPO due to the effects of diabetes are shown in Figure 12. The results obtained showed that lipids of the alloxan-induced non-treated rats are vulnerable to peroxidation due to the increased oxidative stress during diabetes. LPO plays an important role in aging, atherosclerosis and in a number of diabetic complications [100, 101]. As diabetes and its complications are associated with free radical medicated cellular damage [102], herbal hypoglycaemic agents are administered to diabetic rats to assess their antioxidant potential. In this study, polyphenolic extract of *P. pinnata* and glibenclamide effected significant reduction in the level of liver and TBARS when compared to alloxan-induced non-treated control.

Antioxidant Enzymes

Antioxidant enzymes are critical part of cellular protection against reactive oxygen species and ultimately oxidative stress. Oxidative stress is determined by the balance between the generation of ROS such as superoxide anion (O_2) and the antioxidant defence systems such as superoxide dismutase (SOD). Antioxidants enzymes involved in the elimination of ROS include SOD, CAT and GSH, respectively. In this study, there was significant decrease in the activity of SOD in the liver and serum of alloxan-induced non-treated rats when compared with non-induced control as shown in Figure 9. This indicates a decrease in the antioxidant defense system. However, treatment with polyphenolic extract of *P. pinnata* at 25mg/kg and 50mg/kg body weight of rats increased the activity of the SOD in the liver and serum. Since oxidative stress contributes significantly to the pathophysiology of diabetes [103], substances that suppress oxidative stress might be therapeutically beneficial. Studies have shown that exogenously administered antioxidants have protective effects on diabetes, thus providing insight into the relationship between free radicals and diabetes [103-105].

Catalase is an enzymic antioxidant widely distributed in all animal tissues and the highest activity is found in the red blood cells and liver. Catalase decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [106]. Therefore, a reduction in the activity of the enzyme may result in a number of deleterious effects due to the accumulation of hydrogen peroxide. In this study, significant ($p < 0.05$) decrease in the level of catalase in the liver of alloxan-induced non-treated rats was observed. Treatment with extract and standard drug glibenclamide significantly maintained the catalase activity close to normal.

Reduced glutathione (GSH) is a major non-protein thiol in living organisms which plays a central role in coordinating the body's antioxidant defence processes. Perturbation of GSH status of a biological system has been reported to lead to serious consequences [107]. Decline in GSH concentration in the serum of alloxan-induced non-treated rats, and its subsequent return towards near normally in plant extracts treated rats reveal the antioxidant effect of *P. pinnata*. Explanations of the possible mechanism underlying the antioxidant properties of this drug include the prevention of GSH depletion and destruction of free radicals [108]. These two factors could be attributed to the antioxidant properties of *P. pinnata.*

Conclusions

The results obtained from this study showed that oral administration of polyphenolic extract of *P. Pinnata* leaf has significant glycemic control, exhibiting

glucose lowering effect in alloxan-induced hyperglycaemic rats; In addition, it also has beneficial anti-dyslipidemic and antioxidant properties.

Abbreviations

PEPPL: Polyphenolic Extract of *Paullinia pinnata* Leaf; HDL-C: High Density Lipoprotein Cholesterol; FBG: Fasting Blood Glucose; LDL-C: Low Density Lipoprotein Cholesterol; VLDL: Very low Density Lipoproteins; TC: Total Cholesterol; SOD: Superoxide Dismutase; CAT: Catalase; GSH: Reduced Glutathione; MDA: Malondialdehyde; TAG: Triglycerides; CHD: Coronary Heart Disease; LPO: Lipid Peroxidation; ROS: Reactive Oxygen Species; TBARS: Thiobarbituric Acid Reactive Substances; HMG-CoA : 3-Hydroxy-3-Methylglutaryl Coenzyme-A

Authors' Contribution

MON designed, supervised the experiments and edited the manuscript; MOS participated in the supervision and guide the analysis and interpretation of results; AFO carried out the experimental work and analysed the data; AAY developed the manuscript and review the presentation of results.

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Conflict of interest

The authors declare that they have no conflict of interest.

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