DOI: 10.2478/cipms-2019-0023 Curr. Issues Pharm. Med. Sci., Vol. 32, No. 3, Pages 125-129

Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: http://www.curipms.umlub.pl/

Hypolipidemic, hepatoprotective, nephroprotective and anti-lipid peroxidation properties of a methanol extract of *Paullinia pinnata* root-bark, in alloxan-induced hyperglycemic rats

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ARTICLE INFO

Received 02 October 2018
Accepted 16 January 2019

Keywords:
antioxidant,
Paullinia pinnata,
hepatoprotective,
nephroprotective,
lipid profile.

ABSTRACT

This study evaluated the hypolipidemic, hepatoprotective, nephroprotective and anti-lipid peroxidation properties of a methanol extract of *Paullinia pinnata* root-bark, in alloxan-induced hyperglycemic rats. The extract of *P. pinnata* root-bark was prepared using a cold maceration method with 80% methanol and concentrated at 40°C in hot air oven. The extract was administered once daily per os at 50, 100 and 200 mg/kg for 21 consecutive days. Distilled water (5 mL/kg) and glibenclamide (2 mg/kg) were used as the vehicle and reference standard, respectively. The serum lipid profile, markers of liver and kidney functions, antioxidant status (malondialdehyde level, superoxide dismutase and catalase activities), histopathological changes in liver and kidney were examined 24h after the last treatment on day 21. The extract reduced serum lipid profile, markers of liver and kidney functions of treated rats relative to vehicle-treated rats. The superoxide dismutase and catalase activities of the extract treated rats were also elevated relative to the vehicle-treated rats. The extract reversed liver and kidney injuries induced by alloxan in the treated rats. This study provides some basic information which suggest that *P. pinnata* could be effective in managing diabetic complications.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease that affects people of all cultures, ages and social class [1]. In 2010, the global prevalence of diabetes was 285 million and the 2030-projected prevalence is 439 million [2]. The diabetic condition is characterized by hyperglycemia and dyslipidemia. Hyperglycemia and dyslipidemia provoke elevated elaboration of reactive oxygen species such as superoxide anions, hydrogen peroxide and hydroxyl radical ions, and nonenzymatic glycation of proteins that predispose to oxidative stress [3]. Herein, oxidative stress is implicated in the development and complications of diabetes mellitus [4]. The main complications of diabetes are micro- and macro-vascular diseases – the major causes of hospitalization and mortality in diabetic patients [5,6]. The elimination of side effects or complication in the clinical management of diabetes mellitus is still a mirage in medical practice [7]. In recent times, there is increasing interest in the ethno-medical management of diabetes with natural products of plant origin. One of such plant is *Paullinia pinnata* – used especially in South Eastern Nigerian medicine [8,9].

Paullinia pinnata L., commonly called "Bread and Cheese", belongs to the family *Sapindaceae*. It is described as a woody or sub-woody plant and is common in the tropical secondary forest, along stream and the savanna belt [10]. The leaf and/or root infusion or decoction is used in

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the traditional cure of erectile dysfunction, malaria, diabetes, wounds, dysentery and many bacterial infections [11]. Anti-typhoid, free radical scavenging, antimicrobial, fibroblast stimulatory and vascular endothelial muscle relaxant effects were reported in investigations of the crude extract and of compounds isolated from *P. pinnata* [11-13]. Flavone glycoside [14], pinnatoside [13] and Lupane triterpenoid [12], moreover, have been isolated from *P. pinnata*. In addition, Okwudili and co-researchers [9], have reported the effects of a single dose of *P. pinnata* root-bark on fasting blood glucose (FBG) of alloxan-induced diabetes. There is, however, no information on the effects of the extract on the serum biochemical and histopathological changes of alloxan-induced diabetes. Thus, we evaluated the hypolipidemic, hepatoprotective, nephroprotective and anti-lipid peroxidation properties of methanol extract of *Paullinia pinnata* root-bark in alloxan-induced hyperglycemic rats.

MATERIALS AND METHODS

Collection of plant material and extraction. The root-bark of *Paullinia pinnata* was harvested from Inyi Enugu-Ezike, Enugu state, Nigeria and authenticated by Mr. A.O. Ozioko. The plant material was processed, extracted using a cold maceration method with 80% methanol and concentrated in hot air oven at 40°C as reported by Okwudili *et al*. [9].

Experimental animals. Thirty male Wistar rats (100-115 g) were used for the study. The animals were housed in cages (6 per cage) in a well-ventilated room at ambient temperature (25-27°C). They were served feed and tap water *ad libitum* except when fasted is recommended, as reported by Okwudili *et al*. [9], in line with the published guidelines of the National Research Council [15]. The experimental protocol was approved by the institutional ethic committee.

Design of the experiment. Diabetes was induced in the rats by injecting alloxan monohydrate (160 mg/kg) intraperitoneally to overnight (16 h) fasted rats as described by Okwudili *et al*. [9]. Thirty alloxan-induced hyperglycemic rats were randomly divided into 5 groups (l-V; n = 6). The rats were treated orally once daily for 21 consecutive days as follows:

- Group I distilled water (vehicle), 5 ml/kg
- Group II glibenclamide (GLB), 2 mg/kg
- Group III-V received 50, 100 and 200 mg/kg of *Paullinia pinnata* extract (PPE), respectively.

After the treatment on day 21, the rats were fasted for 16 hours and blood was collected via the median canthus for serum sample preparation. Thereafter, they were sacrificed via cervical dislocation, followed by the excision of liver and kidney, both of which were preserved in 10% formal saline. Also, 0.50 g liver was collected for the preparation of 10% homogenate in phosphate buffer saline (pH 7.0).

Serum Biochemical analysis. The serum after separation from the blood cells was used for estimation of total cholesterol, triglycerides, high density lipoproteins cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), albumin, creatinine and urea levels with the aid of a commercially available diagnostic kit (Randox Diagnostic, United Kingdom). Serum low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald equation [16]:

$$\text{LDL-C (mg/dl)} = \text{Total cholesterol} - (\text{Triglycerides}/5) - \text{HDL-C}.$$

***In vivo* antioxidant status.** The supernatant of the liver homogenate after separation with centrifuge at 1000 rpm was used in the evaluation of lipid peroxidation, catalase and superoxide dismutase activities as described by Draper and Hadley [17], Aebi [18] and Xin *et al*. [19], respectively

Histopathology. The liver and kidney tissues preserved in 10% formal saline were processed and stained with haematoxylin and eosin for histopathological examination under digital light microscope (×400 magnification) as described by Donkor *et al*. [20].

Data analysis. The obtained data were statistically evaluated using one-way-analysis of variance (ANOVA), followed by Least Significant Difference (LSD) test with SPSS software. The mean values were considered significant at $p < 0.05$.

RESULTS

Effects of PPE on the lipid profile. The serum total cholesterol, triglyceride, VLDL-C and LDL-C levels were diminished ($p < 0.05$) in the GLB and PPE (all doses used) treated groups relative to the vehicle-treated group. PPE (50 mg/kg) produced a elevated ($p < 0.05$) serum HDL-C levels in the treated group relative to GLB- and vehicle-treated groups (Table 1)

Table 1: Effects of PPE on the lipid profile

Group (n = 6)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)
Vehicle, 5 ml/kg	94.64± 5.19	189.94± 23.26	20.28± 3.18	37.99± 4.65	36.72± 0.72
GLB, 2 mg/kg	40.86± 2.19*	153.12± 16.44	15.48± 4.41	30.63± 3.29	5.25± 1.07*
PPE, 50 mg/kg	59.34± 4.16*	60.07± 14.48*	37.93± 2.67*	12.01± 2.90*	10.18± 2.67*
PPE, 100 mg/kg	59.38± 2.41*	81.67± 9.11*	22.52± 2.24	16.33± 1.82*	20.53± 4.35*
PPE, 200 mg/kg	39.42± 2.07*	65.33± 18.18*	18.76± 2.43	13.07± 3.64*	7.67± 2.66*

*$p < 0.05$ relative to the vehicle-treated group. GLB – glibenclamide, PPE – *Paullinia pinnata* extract, HDL-C – high density lipoproteins cholesterol, VLDL-C – very low density lipoproteins cholesterol, LDL-C – high density lipoproteins cholesterol

Effect of PPE on liver function markers. The PPE (all doses) treatment produced no change ($p > 0.05$) in activity of ALP relative to the vehicle-treated group, while GLB treatment decreased ($p < 0.05$) the serum activity of ALP relative to the vehicle-treated group (Table 2). All doses of PPE and GLB reduced ($p < 0.05$) AST and ALT activities of treated groups relative to the vehicle-treated group. Again, PPE (50 mg/kg) and GLB diminished ($p < 0.05$) total protein levels of the treated group relative to the vehicle-treated group. Albumin levels of all doses of PPE- and GLB-treated groups were not significant ($p > 0.05$) when related to the vehicle-treated group (Table 2).

Table 2. Effects of PPE on liver function markers

Group (n = 6)	ALP (IU/l)	AST (IU/l)	ALT (IU/l)	Total protein (g/dl)	Albumin (g/dl)
Vehicle, 5 ml/kg	73.29± 9.16	145.00± 1.35	63.50± 4.91	6.54± 0.33	3.01± 0.12
GLB, 2 mg/kg	37.65± 1.10 *	114.00± 6.42*	16.00± 1.79*	7.38± 0.55*	3.70± 0.26
PPE, 50 mg/kg	61.78± 7.52	73.00± 13.65*	19.67± 2.38*	5.48± 0.32*	3.18± 0.04
PPE, 100 mg/kg	61.75± 12.6	92.67± 26.22*	19.00± 1.73*	6.37± 0.44	3.71± 0.20
PPE, 200 mg/kg	70.53 ± 11.22	105.00± 17.61*	27.67± 8.40*	5.97± 0.14	3.18± 0.12

*$p < 0.05$ relative to the vehicle-treated group, GLB – glibenclamide, PPE – *Paullinia pinnata* extract, ALP – alkaline phosphatase, AST – aspartate aminotransferase, ALT – alanine aminotransferase

Effects of PPE on the kidney function markers. The PPE (all doses used) and GLB decreased ($p < 0.05$) the serum creatinine and urea levels of treated rats relative to the vehicle-treated rats. The lowest reductions in serum creatinine and urea levels were recorded at 50 mg/kg dose of PPE (Table 3).

Table 3. Effects of PPE on the kidney function markers

Group (n = 6)	Urea (mg/dl)	Creatinine (mg/dl)
Vehicle, 5 ml/kg	93.70±12.25	0.69±0.04
GLB, 2 mg/kg	36.53±5.61*	0.43±0.10*
PPE, 50 mg/kg	47.50±8.70*	0.53±0.01*
PPE, 100 mg/kg	48.95±6.29*	0.62±0.06*
PPE, 200 mg/kg	55.93±12.73*	0.64±0.01*

*$p < 0.05$ relative to the vehicle-treated group, GLB – glibenclamide, PPE – Paullinia pinnata extract

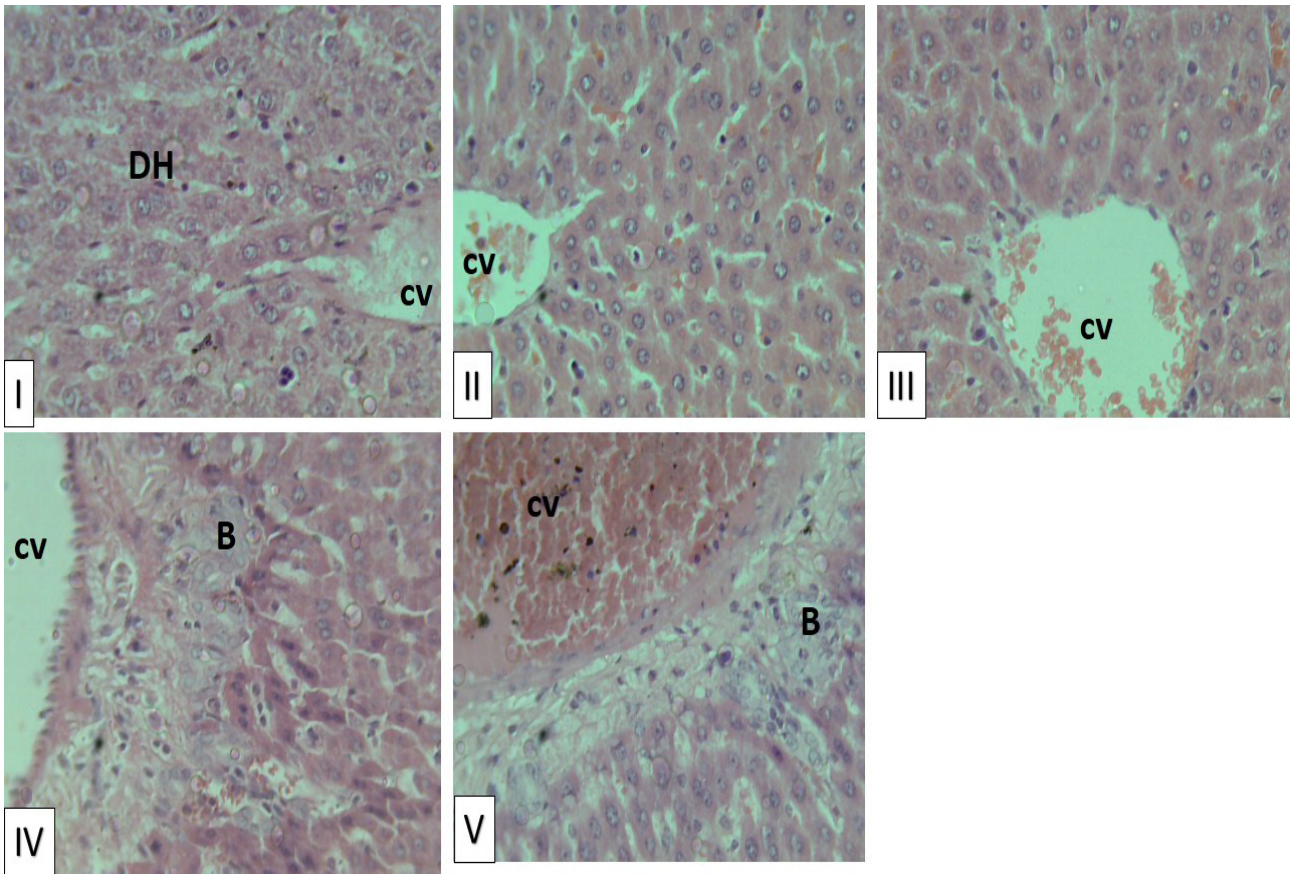
Effects of PPE on the antioxidant markers. All doses of PPE used and GLB diminished ($p < 0.05$) the malondialdehyde levels in the treated rats when related to the vehicle-treated rats (Table 4). The PPE elevated ($p < 0.05$) the activities of superoxide dismutase and catalase in the treated rats relative to the vehicle-treated rats. The antioxidant activities of PPE were comparable with the effects of GLB (Table 4).

Table 4: Effects of PPE on the antioxidant markers

Group (n = 6)	Malondialdehyde (µmole/g protein)	Catalase (µmole/g protein)	Superoxide dismutase (IU/g protein)
Vehicle, 5 ml/kg	0.13±0.00	0.05±0.01	2.72±0.07
GLB, 2 mg/kg	0.11±0.02*	0.33±0.00*	4.83±0.27*
PPE, 50 mg/kg	0.02±0.00*	0.54±0.01*	5.33±0.68*
PPE, 100 mg/kg	0.09±0.02*	0.10±0.24*	4.66±0.41*
PPE, 200 mg/kg	0.11±0.01*	0.09±0.04*	3.88±0.92*

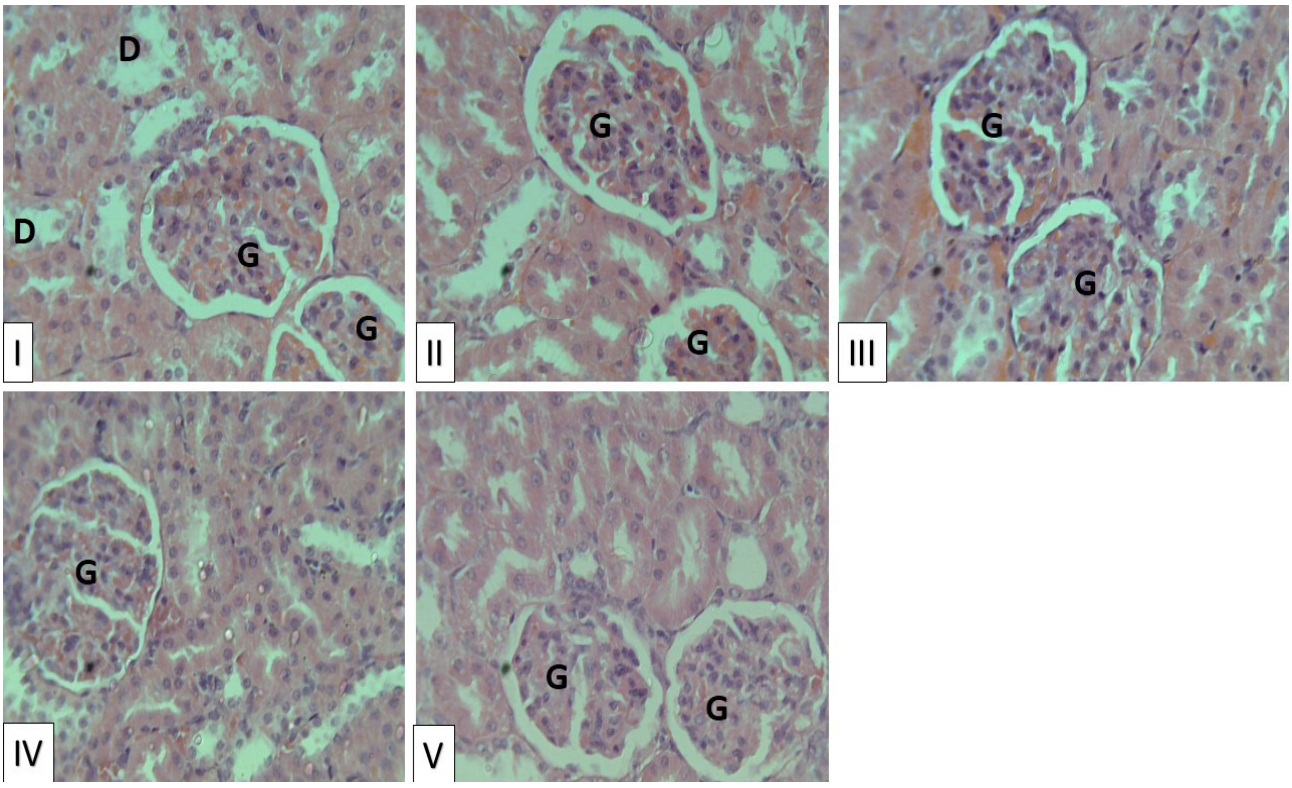
*$p < 0.05$ relative to the vehicle-treated group, GLB – glibenclamide, PPE – *Paullinia pinnata* extract

The effects of PPE on the histopathological lesions induced by alloxan in the liver and kidney. The PPE (50 mg/kg) and GLB (showing normal hepatocyte) reversed degeneration and necrosis of hepatocytes induced by alloxan in the treated rats. The slides of PPE (100 and 200 mg/kg) treated rats showed areas of periportal fibrosis and bile duct proliferation with area of normal hepatocyte (Fig. 1). All doses of PPE (showed normal kidney section) reversed the tubular degeneration and necrosis of kidney induced by alloxan in the treated rats (Fig. 2).



Legend: I – vehicle 5 mL/kg; II – glibenclamide 2 mg/kg; III – PPE 50 mg/kg; IV – PPE 100 mg/kg; V – PPE 200 mg/kg
CV shows central vein; B shows bile duct, DH shows degenerated hepatocyte

Figure 1: Effects of PPE on liver (×400; H & E) section of alloxan-induced hyperglycemic rats



Legend: I – vehicle, 5 mL/kg; II – glibenclamide, 2 mg/kg; III – PPE, 50 mg/kg; IV – PPE, 100 mg/kg; V – PPE, 200 mg/kg; "G" shows the glomerulus; "D" shows area of degeneration

Figure 2. Effects of PPE on kidney (×400; H & E) section of alloxan-induced hyperglycemic rats

DISCUSSION

The study evaluated the effects of *P. pinnata* on the lipid profile and antioxidant status, as well as the hepatoprotective and nephroprotective potentials in alloxan-induced hyperglycemic rats. The methanol extract of *P. pinnata* exhibited hypolipidemic, antioxidant, hepatoprotective and nephroprotective activities in the treated rats. The aforementioned pharmacological activities of *P. pinnata* were mediated by the phytoconstituents [14]. The doses used here were informed by the report of a previous study [9]. The lower doses of the extract produced better effects and this could be associated with receptor site saturation and inhibition. Of note, the dose-response curve of drugs are usually sigmoid in shape due to receptor site saturation and inhibition at higher doses [21].

The extract elicited *in vivo* antioxidant activity that was in line with the report of Jimoh *et al*. [22]. This suggests that PPE has the potential to mop up free radicals and stimulate antioxidant enzyme activity [13]. This also indicates that PPE inhibited the spontaneous generation of free radicals and cell membrane damage [23].

The extract produced hypolipidemic effects and corroborated the report of Asgary *et al*. [24] and Sharmin *et al*. [25] on pomegranate juice and *Momordica charantia*, respectively. The mechanism of the hypolipidemic activity was not elucidated but could be linked to the stimulation of LDL-C reductase, increased bile acid production and/or excretion, or inhibition of 3-hydroxy-3- methyl glutarylcoenzyme A reductase (HMGR) activities [26]. The hypolipidemic effect may be mediated by β-sitosterol and β-sitosterol-3-D-glucoside which have been isolated from *Paullinia pinnata* [12]. The hypocholesterolemic properties of PPE suggest that it could be effective in the management of hyperlipidemia and cardiovascular diseases. Herein, every 1% reduction in serum cholesterol is associated with 2% drop in the risk of heart disease [26].

The reduced ($p < 0.05$) activities of ALP, AST and ALT, as well as diminished urea and creatinine levels in the PPE and GLB treated rats relative to distilled water treated rats suggest that PPE exhibited hepatoprotective and nephroprotective properties. The extract reversed liver and kidney damage induced by alloxan in the treated rats. The hepatoprotective activity of PPE was more at 50 mg/kg dose. The hepatoprotective and nephroprotective activities of PPE could be linked to its antioxidant property [9,22]. Antioxidants act as membrane stabilizer via the inhibition of lipid peroxidation, the product of which (malondialdehyde) induces cell membrane damage [23]. Alloxan-induced diabetes and cellular toxicity is linked to the release of free radicals. Alloxan destroys pancreatic islet and other tissues through the generation of free radicals during the redox cycling of dialuric acid – a reduction product of alloxan [27]. Past research indicates that agents that mop-up free radicals have the potential to protect against the cytotoxic effects of alloxan [27].

In conclusion, the methanol extract of *P. pinnata* exhibited hypolipidemic and antioxidant effects and also reversed hepatic and kidney damage induced by alloxan in the treated rats. Our work, hence, provides some basic information that suggests that *P. pinnata* extract (at low dose) could be used in the management of diabetic complications.

ACKNOWLEDGEMENT

We are grateful to Mr A.O. Ozioko for the plant authentication.

ANIMAL RIGHTS STATEMENT

The Animal Ethical Committee approved the protocols.

CONFLICT OF INTERESTS STATEMENT

We declare no conflict of interests.

FINANCIAL DISCLOSURE STATEMENT

This study was not sponsored.

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