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Original Research Article

Assessment of the antidiabetic potential of *Pterocarpus santalinoides* extract in alloxan-induced diabetic rats

Kelechi G Madubuike[1]*, Aruh O Anaga[2], Isaac U Asuzu[2]
[1]Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, [2]Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Nigeria

For correspondence:* ***Email:* *drkaycee2002@yahoo.com;* ***Tel:*** *+234-8036689778*

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Abstract

Purpose: *To establish the pharmacological basis for the antidiabetic use of Pterocarpus santalinoides.*
Methods: *Alloxan-induced diabetic rats were given graded doses (50, 100 and 200 mg/kg) of P. santalinoides extract (PSE) for 21 days. The fasting blood glucose (FBG), body weight, in vivo antioxidant assay, and lipid profile were determined.*
Results: *Pterocarpus santalinoides extract at all doses tested caused significant ($p < 0.05$) reduction in FBG and significant ($p < 0.05$) increase in body weight of treated rats when compared with the control. There were significant ($p < 0.05$) increases in the activities of antioxidant enzymes and high-density lipoprotein, while the levels of total cholesterol, triglycerides and low-density lipoprotein were significantly ($p < 0.05$) reduced in PSE-treated rats.*
Conclusion: *These results demonstrate the significant antidiabetic activity of P. santalinoides extract in albino Wistar rats, thus suggesting its potential application for the management of diabetes in humans, Furthermore, the findings may explain its use in ethnomedicine as an antidiabetic regimen.*

Keywords: *Pterocarpus santalinoides, Diabetes, Lipoprotein, Antioxidant, Hypoglycemia*

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INTRODUCTION

Diabetes manifests as chronic hyperglycemia, owing to reduced secretion of insulin by the body, inefficient insulin action, or both [1]. Globally, there is steady rise in the incidence of diabetes and it is expected that in the year 2030, the statistics of persons with diabetes mellitus will rise to 366 million [2]. Diabetes has also been reported in animals, mainly dogs and cats [3]. The disease is usually characterized by persistent hyperglycemia, dyslipidemia and weight loss, as well as decline in antioxidant defense [4]. Currently, treatment of diabetes involves insulin therapy, administration of oral hypoglycemic drugs (OHDs) and modification of lifestyle, especially with respect to nutrition and exercise [4]. However, considerable side effects associated with the use of insulin and OHDs have led to increase in demand for organic recipes with antidiabetic potential which are perceived to be safer [5].

Pterocarpus santalinoides is a plant of immense value in Nigerian folkloric medicine. In most rural communities in Nigeria, decoction of leaves of *P.*

santalinoides is taken orally to relieve symptoms of diabetes and other ailments [5]. The present work investigated *Pterocarpus santalinoides* leaf extract for antidiabetic potential using an alloxan-induced diabetic albino rat model.

EXPERIMENTAL

Plant collection, identification and extraction

Leaves of *P. santalinoides* were harvested in Nsukka, Nigeria, and authenticated at the Bioresources Development and Conservation Programme (BDCP), Nsukka. A representative sample (with identification number MOUAU/VPP/2014/017) was kept in the Veterinary Physiology and Pharmacology departmental herbarium in Michael Okpara University of Agriculture, Umudike.

The harvested leaves were spread under shade (22 – 29 °C) to dry, and afterwards reduced to coarse powder using an electric blender. A total of 500 g of the sample was macerated in 80% methanol, with vigorous shaking six-hourly for 72 h. The residue was removed by filtration and the filtrate concentrated in a rotary evaporator and completely dried in an oven (40 °C). The yield was determined and the extract preserved in a refrigerator (4 °C) as *Pterocarpus santalinoides* extract (PSE) until needed [5].

Animals

Approximately 10-weeks old albino rats that were bred in the Laboratory Animal House belonging to the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, were utilized for the study. The animals weighed between 116. 6 ± 3. 02 g, and were kept in stainless steel cages. Standard pelleted feed (Vital feed®, Nigeria) and clean drinking water were served *ad libitum* to the rats. The experimental protocol was approved by the Institution's Research Ethics Committee (approval no. MOUAU/CVM/REC/19007), and the rats were handled in strict compliance with NIH Guidelines for Care and Use of Laboratory Animals [6].

Acute toxicity study

The up-and-down procedure was employed to determine the acute toxicity of PSE. Three (3) albino rats were orally dosed with 5000 mg/kg of PSE. The animals were allowed free access to feed and drinking water for 14 days during which they were monitored for evidence of toxicity and death [7].

Effect of PSE on fasting blood glucose, body weight, antioxidant lipid profile of alloxan-induced diabetic rats

Experimental diabetes (fasting blood glucose (FBG) ≥ 126 mg/dL) was achieved in 50 rats with single intraperitoneal injection of 160 mg/kg alloxan monohydrate after 18 h fast [4]. The diabetic rats were randomly assigned to 10 groups (n = 10) and were given the following treatment: group 1 served as negative control and received 5 ml/kg of distilled water. Rats in group 2 received 2 mg/kg glibenclamide (the reference drug) while groups 2, 3 and 4 rats were treated with 100, 200 and 400 mg/kg of PSE respectively. All animals were treated orally for 21 consecutive days. The fasting blood glucose levels and the body weights of the rats were measured before the treatment and subsequently, on days 1, 7, 14 and 21 after treatment. On day 21, blood samples were obtained for serum biochemical analyses by inserting capillary tubes into the retro-orbital plexus of the rats' media canthus.

Determination of fasting blood glucose (FBG)

The FBG of the rats were determined using an auto analyzer (Accu Check Active®) glucose kit using blood samples collected from the tail vein after a tail snip [4].

Assessment of body weight of rats

The body weights of the rats were measured weekly using a digital weighing balance.

In vivo antioxidant assay

Catalase assay was based on the method of Aebi [8]. Hydrogen peroxide (H_2O_2) decomposition rate is decreased by catalase, hence H_2O_2 (0.1 mL) was reacted with the catalase in 10 µL of the test serum and the decomposition rate of H_2O_2 was measured at 240 nm, using a spectrophotometer. Lipid peroxidation was assessed by determining the level of malondialdehyde (MDA) that reacted with thiobarbituric acid to form a red or pink colored complex which was read photometrically at 532 nm [9].

Evaluation of lipid profile

The manufacturer's specifications contained in Randox® test kit were followed to determine the concentrations of total cholesterol, triglyceride (TG), high density lipoproteins (HDL) and low density lipoproteins (LDL) in the rats' serum

samples. Very low-density lipoprotein (VLDL) was calculated as 20 % of TG [10].

Statistical analysis

One-way analysis of variance was used to analyze the data obtained from this study with the aid SPSS software. Separation of the variance means was done by the least significance difference of the various groups. Values of $p < 0.05$ were accepted as statistically significant.

RESULTS

Extraction yield and acute toxicity

The extraction of *P. santalinoides* with hydromethanol yielded 2.7 % (w/w). The extract was pasty, and brownish in color and it had a sweet smell. In the acute toxicity test, neither mortality nor any other sign of toxicity was recorded within the 14-days observation period.

Effect of PSE on fasting blood glucose levels

Figure 1 shows the effect of PSE on the fasting blood glucose of alloxan-induced diabetic rats following 21 days treatment. The results showed that the extract, at all doses tested caused significant ($p < 0.05$) dose- and time-dependent decreases in FBG of rats on days 1, 7, 14 and 21, when compared to the control. The antihyperglycaemic effect of PSE at the dose of 200 mg/kg on day 21 (76.8%) was comparable to that of 2 mg/kg glibenclamide (79.2%).

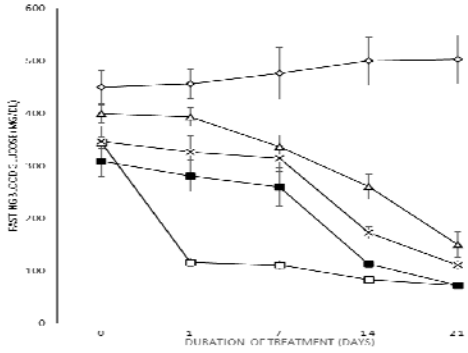

Figure 1: Effect of PSE on the FBG of alloxan-induced diabetic rats treated for 21 days. ***Key:*** -◊- = Distilled water (5 ml/kg), -□- = glibenclamide (2 mg/kg), -Δ- = PSE (50 mg/kg), -×- = PSE (100 mg/kg) and ¯▪¯ = PSE (200 mg/kg)

Effect of PSE on body weight

The results of the effect of 21-day treatment of alloxan-induced diabetic rats with PSE is presented in Figure 2. The varying doses (50, 100 and 200 mg/kg) of PSE and the reference drug (2 mg/kg glibenclamide) exhibited significant ($p < 0.05$) increase in the body weight of rats on day 21 when compared with the control.

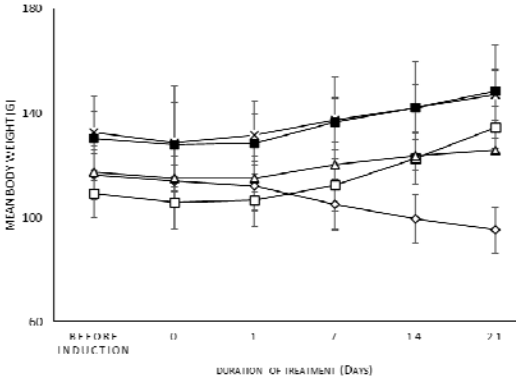

Figure 2: Effect of PSE on body weight of alloxan-induced diabetic rats. ***Key:*** -◊- = Distilled water (5 ml/kg), -□- = glibenclamide (2 mg/kg), -Δ- = MEPS (50 mg/kg), -×- = MEPS (100 mg/kg) and ¯▪¯ = MEPS (200 mg/kg)

Antioxidant effect of PSE in alloxan-induced diabetic rats

The results showed significant ($p < 0.05$) reduction in level of MDA in alloxan-induced diabetic rats after 21 days of treatment with varying doses of PSE. when compared with the control. Catalase activity of the diabetic rats was significantly ($p < 0.05$) increased by PSE (100 and 200 mg/kg) and glibenclamide (2 mg/kg), when compared with the control. In the study, the antioxidant effect of PSE in alloxan-induced diabetic rats was dose-dependent (Figure 3).

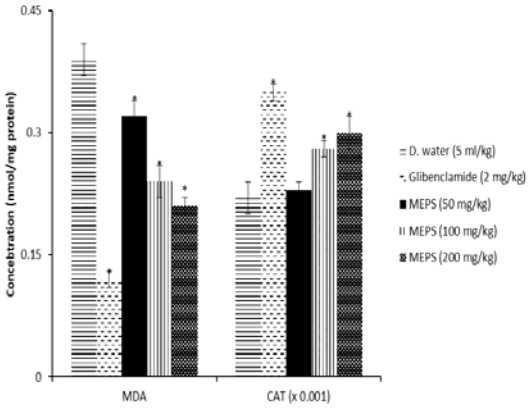

Figure 3: Effect of PSE on MDA and catalase levels of alloxan-induced diabetic rats treated for 21 days. *$P < 0.05$ compared with distilled water (5 ml/kg) group

Table 1: Effect of PSE on the lipid profile of alloxan-induced diabetic rats treated for 21 days

Group/ treatment	TC (mg/dL)	TG (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)
Distilled water (5 ml/kg)	94.27 ± 0.96	107.87 ± 4.86	47.73 ± 0.97	21.07 ± 0.61	23.97 ± 0.08
Glibenclamide (2mg/kg)	66.40 ± 0.70*	78.60 ± 3.43*	19.50 ± 1.32*	35.50 ± 0.92*	11.40 ± 0.50*
MEPS (50 mg/kg)	89.00 ± 1.56*	89.57 ± 0.32*	36.53 ± 1.89*	33.20 ± 1.19*	19.20 ± 0.40*
MEPS (100 mg/kg)	78.33 ± 2.19*	87.00 ± 0.57*	26.30 ± 0.87*	35.7 0± 1.40*	16.33 ± 0.06*
MEPS (200 mg/kg)	77.50 ± 0.32*	68.20 ± 2.90*	27.03 ± 0.80*	35.30 ± 0.83*	15.17 ± 0.31*

*$P < 0.05$, compared with the control

Effect of PSE on lipid profile

Table 1 represents the lipid profile of alloxan-induced diabetic rats after 21 days treatment with different doses of PSE. The result revealed that PSE (50, 100 and 200 mg/kg) and glibenclamide (2 mg/kg) significantly ($p < 0.05$) decreased the concentrations of serum total cholesterol, triglyceride, low density lipoprotein and very low-density lipoproteins levels in the treated rats, when compared with the control group. High-density lipoprotein level was significantly increased by the same doses of the extract and the reference drug in the treated groups, when compared with its level in the rats of the control group.

DISCUSSION

The absence of mortality or any other sign of toxicity 14 days after administration of PSE (5000 mg/kg) in the rats shows that the extract was tolerated by the rats. It also implies that the LD_{50} of PSE following oral administration in rats is greater than 5000 mg/kg [7]. Elevation of the fasting blood glucose (FBG) using alloxan monohydrate is a standard method of inducing experimental diabetes. Alloxan selectively destroys the insulin-producing islet cells of the pancreas resulting in FBG that is persistently higher than the normal value [4].

The antihyperglycemic activity of PSE was evidenced by its ability to significantly reduce FBG of treated rats when compared with the control. It is well established that glibenclamide (reference drug) produces hypoglycaemia by increasing insulin secretion by the pancreas [1]. In this study the antihyperglycemic effect of the PSE (200 mg/kg) was comparable to that of glibenclamide (2 mg/kg) on the 21st of the study. It is therefore possible that *P. santalinoides* mediates its antidiabetic action by enhancing insulin secretion by the existing residual beta cells of the islet.

Reduction in body weight (due to wasting of skeletal and adipose tissues) is a common feature in diabetes patients with poor glycaemic control. Hence, weight assessment is a vital tool used in diabetic study to monitor severity of the disease and/or response to treatment [11]. In the control group, continuous weight loss was observed in the diabetic rats throughout the experimental period (21 days). However, there was weight recovery and progressive weight gain in the extract-treated rats which could have resulted from effective glycaemic control by PSE. This finding agrees with the results of Kamtchouing *et al*, [11] in which sub-acute treatment of diabetic rats with plant extracts caused significant weight gain, against reduction in body weight in the untreated diabetic rats.

Oxidative stress, which depicts excessive production of reactive oxygen species (ROS) has been implicated in the pathogenesis of diabetes mellitus [12]. Alloxan monohydrate used to induce diabetes in this study is reported to mediate its activity through increased production of ROS which helps to destroy the beta-cells [13]. It is known that living organisms are rich in antioxidants. These antioxidants scavenge free radicals thereby preventing living cells from oxidative damage [14].

Results of previous studies support the use of antioxidant-rich plant extracts to mitigate the impact of oxidative stress in the treatment of diabetic patients [14]. In addition, it is documented that a positive correlation exists between natural antioxidants consumption and reduction in the incidence of diabetes [15]. The extract in this study caused significant increase in catalase (an antioxidant enzyme) as well as significant reduction in the level of MDA, which is a biochemical marker of lipid peroxidation especially in conditions of increased oxidative

stress [14]. These results indicate the ability of PSE to enhance physiological antioxidant defense system which is usually suppressed in cases of diabetes.

Deficiency or resistance to insulin seen in diabetes mellitus affects key enzymes and pathways in lipid metabolism, resulting in abnormalities in the levels of serum lipids. This condition known as diabetic dyslipidemia comprises elevated total cholesterol, triglycerides and low-density lipoprotein (LDL) as well as decreased high-density lipoprotein (HDL) particles [16]. The composition of lipid particles in diabetic dyslipidaemia has been proposed to be more atherogenic than other types of dyslipidemia and is thought to be the main cause of coronary heart disease in patients with diabetes [17].

In the present study, there were significant increases in the serum levels of, triglycerides, total cholesterol and LDL. in the diabetic control group, which were reduced in the PSE and glibenclamide-treated groups after 21 days treatment. Conversely, the serum level of HDL was significantly increased in the extract-treated groups when compared with the negative control group. These results show that the extract may inhibit the pathway of endogenous cholesterol synthesis and stimulates the biosynthesis of hepatic LDL receptors which play the role of taking up LDL into the liver, resulting in decreased LDL/HDL ratio in the PSE- treated rats [18]. The effect of PSE on the lipid profile of the diabetic rats suggests that PSE could help to prevent or slow down the development of atherosclerosis which is a major complication of diabetes.

CONCLUSION

The methanol leaf extract of *Pterocarpus santalinoides* possesses significant antidiabetic potential as well as hypolipidaemic and antioxidant effects. The extract may also protect the liver against diabetes-induced hepatotoxicity. These findings justify the folkloric use of *P. santalinoides* for the treatment of diabetes mellitus.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Isaac U. Asuzu conceived and designed the study. Aruh O. Anaga collected and analysed the data while Kelechi G. Madubuike wrote the manuscript. All authors read and approved the manuscript for publication.

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