Trop J Nat Prod Res, May 2020; 4(6):233-236 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effect of *Pterocarpus santalinoides* Leaf Extract on Oral Glucose Tolerance Test in Normal and 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.*

ARTICLE INFO

Article history:
Received 19 May 2020
Revised 05 June 2020
Accepted 26 June 2020
Published online 02 July 2020

ABSTRACT

Hypoglycemic effect of *Pterocarpus santalinoides* has been scientifically reported but without any clue to its mechanism of action. To elucidate its mode of hypoglycemic activity, the effect of the plant on postprandial hyperglycemia was investigated. Dried, pulverized leaves (500 g) of *P. santalinoides* was macerated in 80% methanol (1.5 L), filtered and concentrated to dryness. Preliminary phytochemistry of the extract was done and its acute toxicity was evaluated following the up-and-down method. The effect of varying doses (50, 100 and 200 mg kg^{-1}, *per os*) of the extract on oral glucose tolerance test was assessed in normal and alloxan (160 mg kg^{-1}, i.p.)-induced diabetic rats. The positive and negative control groups received glibenclamide (2 mg kg^{-1}) and distilled water (5 mL kg^{-1}), respectively. Glycosides, alkaloids, flavonoids, saponins tannins and terpenes were found to be present in the extract. Its acute toxicity test recorded neither death nor sign of toxicity in the rats even at the maximum oral dose of 5000 mg kg^{-1}. In normal rats, 50, 100 and 200 mg kg^{-1} of the extract exhibited significant ($p < 0.05$) hypoglycemic activity by lowering blood glucose levels to 70.00±2.85, 69.33±3.29 and 68.17±3.34 mg dL^{-1}, respectively, 180 min post-glucose load (2 g kg^{-1}). In diabetic rats, the extract (100 and 200 mg kg^{-1}) significantly ($p < 0.05$) lowered blood glucose levels of treated rats when compared with the negative control group. The results show that *Pterocarpus santalinoides* leaf extract significantly lowers blood glucose levels in rats, via enhanced glucose utilization.

Keywords: *Pterocarpus santalinoides*, hyperglycemia, alloxan, glucose, postprandial.

Introduction

The use of plant materials for treating human and animal diseases dates prior to the advent of civilization.[1] Since then, interest in plant extracts for healing purposes has been on the increase, owing to their easy accessibility, cost-effectiveness and safety.[2] The World Health Organization (WHO) estimates that 80% of the population of developing countries (which constitute about 4 billion people) rely on traditional medicine for their primary healthcare needs.[3] It is on record that 85% of traditional medicine involves the use of plant materials for the treatment of diseases.[4] Also, about 25% of synthetic drugs prescribed globally originated from plants.[5] Today, researchers are combing this vegetation treasure hub for biologically active phytoconstituents, which are leads towards the discovery of novel drugs that will combat some chronic ailments, which seem to be defying currently available remedies.

One of such chronic ailments is diabetes mellitus – a metabolic disorder characterized by persistent hyperglycemia, resulting from insufficient insulin secretion or poor utilization by cells.[6,7] Currently, treatment of diabetes involves insulin therapy, oral hypoglycemic drugs (OHDs) and lifestyle intervention through exercise and modification of diet.[8] Side effects associated with the use of OHDs and insulin therapy such as severe hypoglycemia, weight gain and gastrointestinal disturbances.[7,9] have led to the search for new, potent and safer antidiabetic drugs.

Oral glucose tolerance test measures the ability of the body to utilize glucose.[10] It is a convenient means of screening substances, especially ethnobotanicals for antidiabetic potential.[11] *Pterocarpus santalinoides* is a culinary vegetable found in Nigeria and some other West African countries. Common names of the plant in some ethnic groups in Nigeria include: nturukpa (Igbo), gunduru (Hausa), gbengbe (Yoruba), nja (Efik), ikyarakwa or kereke (Tiv), maganchi (Nupe), okumeze (Edo) and piegwu or uturukpa (Igede).[12] Decoction of the leaves of *P. santalinoides* is used to relieve symptoms of diabetes, stomach ache, diarrhea.[13,14] Previous studies have reported hypoglycemic activity of the plant,[11,15] however, its mechanism of action is still unknown. This study seeks to investigate the effect of the methanol leaf extract of *Pterocarpus santalinoides* on postprandial hyperglycemia in rats, as a means of elucidating the possible mechanism of action of the plant.

Materials and Methods

Collection and identification of plant material

Fresh leaves of *P. santalinoides* were collected from Nsukka, Enugu State, Nigeria, in October, 2014. Identification and authentication of the plant material was done by Mr. A. O. Ozioko, a taxonomist with the Bioresources Development and Conservation Programme (BDCP), Nsukka. A voucher specimen (with identification number MOUAU/VPP/2014/017) has been deposited in the herbarium of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

*Corresponding author. E mail: drkaycee2002@yahoo.com; madubuike.kelechi@mouau.edu.ng
Tel: +2348036689778

Citation: Madubuike KG, Anaga AO, Asuzu IU. Effect of *Pterocarpus santalinoides* Leaf Extract on Oral Glucose Tolerance Test in Normal and Alloxan-Induced Diabetic Rats. Trop J Nat Prod Res. 2020; 4(6):233-236. doi.org/10.26538/tjnpr/v4i6.4

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Preparation of plant extract
The leaves were air-dried and pulverized. Five hundred grams (500 g) of the pulverized leaves were macerated in 1.5 L of 80% methanol for 72 hours, with intermittent shaking. The residue was removed by filtration and the filtrate concentrated in a rotary evaporator, then dried in hot air oven (40°C). The extract was stored in a refrigerator (4°C) and labelled as MLEPS (Methanol leaf extract of *Pterocarpus santalinoides*) until the time of use.[16]
Percentage yield was calculated using the formula:

$$\%\ yield = \frac{a}{b} \times 100$$

Where, a = weight of the dried extract and b = weight of dried pulverized plant material.

Preliminary phytochemical analysis of MLEPS
Procedures outline by Harborne[17] and Trease and Evans[18] were followed in carrying out a qualitative phytochemical analysis of the extract.

Animals
Mature male albino rats (100-200 g) bred in the Laboratory Animal House of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria were used for the study. The rats were housed in stainless steel cages. Pelleted feed (Vital®, Nigeria) and drinking water were given *ad libitum*. The experimental protocol was approved by the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike Research Ethics Committee (Approval No. MOUAU/CVM/REC/190010), and the rats were handled in strict compliance with NIH Guidelines for Care and Use of Laboratory Animals (Pub. No. 85-23, Revised, 1985).

Acute toxicity study of MLEPS
The up-and-down-procedure was employed for the acute toxicity of MLEPS. Six (6) adult albino rats were randomly assigned into two groups (Test and Control) of 3 rats per group. Rats in the test group were given 5000 mg kg^{-1} of MLEPS, orally, while the control group received distilled water (10 mL kg^{-1}) orally. The rats were allowed free access to feed and drinking water for 14 days during which they were monitored for death and acute toxicity signs.[19,20]

Induction of experimental diabetes
Following 18 h fast, the fasting blood glucose levels (FBG) of mature male albino rats were determined using auto-analyzer (Accu-Check Active® glucose kit), with blood collected from the rats via a tail snip.[21] Diabetes was then induced in the rats by a single intraperitoneal administration of alloxan monohydrate (160 mg kg^{-1}) prepared with normal saline. The diabetic profile of the rats was evaluated by measuring their FBG levels every other day, and on the sixth day rats with FBG ≥ 126 mg dl^{-1} were accepted to be diabetic and used for the study.[22]

Effect of MLEPS on OGTT in normoglycemic rats
This test was carried out in line with the method of Aslan *et al.*[23] Thirty male albino rats were fasted for 18 h and randomly assigned into 5 groups of 6 rats each. The fasting blood glucose (FBG) at 0 h was determined before treatment. Groups 1 and 2 received 5 mL kg^{-1} of distilled water and 2 mg kg^{-1} glibenclamide respectively, while groups 3, 4, and 5 were treated with 50, 100 and 200 mg kg^{-1} of MLEPS, respectively. All doses of the extract and the reference drug were administered via the oral route. Thirty minutes post-drug or extract treatment, all the rats received oral glucose load of 2 g kg^{-1}. Blood glucose levels of the rats were measured at 30, 60, 120 and 180 min, after glucose load.

Effect of MLEPS on OGTT in alloxan-induced diabetic rats
Oral glucose tolerance test was performed in alloxan-induced diabetic rats following the procedure described for normal rats.

Statistical analysis
Data obtained from the study were presented as mean ± S. E. M. and analyzed using One Way Analysis of Variance (ANOVA). Separation of the variant means was done by means of the Least Significance Difference (LSD) of the different groups. Probability values less than 0.05 were considered statistically significant.

Results and Discussion

Plant extraction
Following the drying of the filtrate in the hot air oven, the extract weighed 12.5 g, which represents 2.5% w/w of the pulverized plant material. The extract (MLEPS) was pasty and brownish.

Preliminary phytochemical analysis of MLEPS
The result revealed that MLEPS contains the following phytoconstituents: glycosides, alkaloids, flavonoids, saponins tannins and terpenes. Resins and polyuronoides were absent (Table 1).
These phytoconstituents present in MLEPS have been reported as bioactive antidiabetic principles,[24] some of them acting through the regeneration of damaged pancreatic islets and stimulation of calcium and glucose uptake.[25,26] It is likely that some of these phytoconstituents are responsible for the hypoglycemic activity exhibited by MLEPS in this study.

Acute toxicity
After 14 days observation period neither mortality nor any other sign of toxicity was recorded in the rats. The rats were active and fed normally. This is an indication that the extract was well tolerated by the rats. It also implies that the oral LD_{50} of MLEPS in rats is greater than 5000 mg kg^{-1}.[20,22]

Effect of MLEPS on OGTT in normoglycemic and alloxan-induced hyperglycemic rats
The effect of MLEPS on OGTT in normoglycemic rats is presented in Figure 1. The extract exhibited significant ($p < 0.05$) time-dependent decrease in the blood glucose levels of the treated rats when compared with the negative control. At 30 min post glucose load, there were sharp increases in the blood glucose levels of all the groups, which corresponds with the absorption of the administered glucose. Only the glibeclamide-treated group and the group treated with 200 mg kg^{-1} of MLEPS recorded significantly ($p < 0.05$) lower values when compared with the negative control. Between 60 and 120 min post glucose load, all doses of MLEPS and the reference drug recorded a sharp reduction of the blood glucose levels of the treated rats when compared with the control.
In the alloxan-induced diabetic rats, 200 mg kg^{-1} of MLEPS and glibenclamide (2 mg kg^{-1}) significantly ($p < 0.05$) reduced the blood glucose levels of the treated groups when compared with the negative control, at 60, 120 and 180 min post glucose loading (Table 2). Significance in the blood glucose decreasing effect of MLEPS was not observed at 50 and 100 mg kg^{-1}. These results indicate that MLEPS (200 mg kg^{-1}) effectively enhanced glycemic control in both normal and alloxan-induced diabetic rats.
Alloxan (2,4,5,6-tetraoxypyrimidine) monohydrate acts by selective destruction of beta cells of the pancreas through the generation of reactive oxygen species.[27] Partial or total destruction of the pancreatic beta cells results in insufficient or non-production of insulin, the major hormone responsible for glucose metabolism. This leads to persistent hyperglycemia, which is a cardinal sign of diabetes mellitus.[28] In this study, the FBG ranged between 327.2 ± 10.9 and 388.8 ± 18.6 mg dL^{-1}(Table 2) in the alloxan-treated rats which is an indication that experimental diabetes was achieved in the rats.
The oral glucose tolerance test (OGTT) evaluates the efficiency of the body to metabolize glucose.[27] The ability of MLEPS to significantly reduce postprandial hyperglycemia in both oral glucose tolerance tests suggests that *P. santalinoides* could enhance glucose utilization both

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

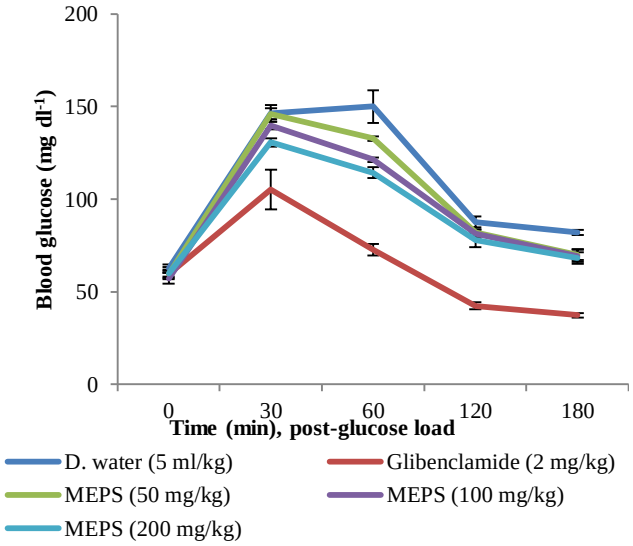

Figure 1: Effect of MLEPS on oral glucose tolerance in normoglycemic rats

Table 1: Phytochemical screening of MLEPS

Phytoconstituent	Inference
Glycosides	+
Alkaloids	+
Flavonoids	+
Saponins	+
Resins	-
Tannins	+
Polyuronoides	-

+ present; - absent

in normal and diabetic animals. In diabetic patients, postprandial hyperglycemia is implicated in the development of microvascular and macrovascular complications of the disease.[29] The extract, can therefore, be potentially useful in managing postprandial rise in blood glucose, thereby minimizing the risk of vascular complications of diabetes.

The hypoglycemic action of MLEPS may be mediated through reduction of intestinal glucose absorption, suppression of renal glucose re-absorption and/or gluconeogenesis inhibition which is highly activated by 16 h fasting before glucose load.[30] It is also possible that in the diabetic rats, MLEPS mimicked the reference drug (glibenclamide) by lowering blood glucose through enhanced insulin secretion by the surviving beta cells of the pancreas.[31] A major drawback of glibenclamide and other sulfonylureas is their tendency to cause severe hypoglycemia, after treatment as observed in this study, however, *Pterocarpus santalinoides* extract at the doses tested did not reduce the blood glucose level of the rats below the normal range (70-110 mg dL^{-1}),[32] 2 hours after glucose load (Figure 1).

Conclusion

The results of this study showed that methanol leaf extract of *Pterocarpus santalinoides* exerts significant reduction of postprandial hyperglycemia in normal and alloxan-induced diabetic rats. This, therefore, substantiates the folkloric use of the plant in managing diabetes mellitus.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 2: Effect of MEPS on oral glucose tolerance in alloxan – induced hyperglycemic rats

Groups/Treatment	Blood glucose levels (mg/dl)				
	0 min	**30 min**	**60 min**	**120 min**	**180 min**
1. Distilled water (5ml kg^{-1})	327.2 ± 10.9	523.0 ± 12.3	558.0 ± 18.1	527.2 ± 14.1	516 ± 16.5
2. Glibenclamide (2mg kg^{-1})	373.6 ± 10.8	502.6 ± 10.5	465.8 ± 18.2*	335.8 ± 18.2*	242 ± 16.5*
3. MEPS (50 mg kg^{-1})	336.6 ± 19.9	518.8 ± 13.9	543.4 ± 15.9	479.4 ± 17.6	452 ± 21.7
4. MEPS (100 mg kg^{-1})	388.8 ± 18.6	554.2 ± 15.1	567.2 ± 10.4	540.8 ± 12.2	437.2 ± 20.2*
5. MEPS (200 mg kg^{-1})	332.4 ± 19.9	484.2 ± 10.9	475.4 ± 12.1*	420.4 ± 15.8*	345.6 ± 17.8*

* $p < 0.05$ when compared with negative control

Acknowledgements

The authors are grateful to Mr. A. O. Ozioko of the Bioresources Development and Conservation Programme (BDCP), Nsukka for authenticating the plant specimen.

References

1. Ernst E, Schmidt K, Wider B. Complementary and alternative medicine research in Britain. Compl Ther Clin Pract. 2005; 11:17-20.
2. Madubuike KG, Yusuf NO, Robinson ES. Evaluation of the *in vitro* and *in vivo* antioxidant potentials of *Jatropha tanjorensis* methanolic leaf extract. IJPPR. 2015; 7(4):1-5.
3. Shizha E and Charema J. Health and wellness in South Africa: Incorporating indigenous and western healing practices. Int J Psychol Counsel. 2011; 3(9):167-175.
4. Anitha M, Sakthidevi G, Muthukumarasamy S, Mohan VR. Effect of *Cynoglossum zeylanicum* (Vehl ex Hornem) Thunb . Ex Lehm on oral Glucose Tolerance in rats. J Appl Pharm Sci. 2012; 2(11):075-078.
5. Yue-Zhong S. Recent natural products-based drug development: A pharmacological industry perspective. J Nat Prod. 1998; 61:1053-1071.
6. Turner R, Hansson P, Lancet S. Issues of diabetes. BMJ. 2000; 351:1755-1762.
7. Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale's Pharmacology. (6th ed.). Philadelphia: Churchill Livingstone/Elsevier; 2007. 402-408 p.
8. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. Ten years follow-up of intensive glucose control in type 2 diabetes. New Engl J Med. 2008; 359:1577-1589.
9. Harvey RA, Champe PC. Pharmacology. (4th ed.). (2009). New Delhi: Wolters Kluwer Pvt. Ltd; 2009. 285 – 297 p.
10. Prince PS, Menon VP, Pari L. Hypoglycaemic activity of *Syzigium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats. J Ethnopharmacol. 1998; 61:1-7.
11. Onoja SO and Anaga AO. Evaluation of the antidiabetic and antioxidant potentials of methanolic leaf extract of *Helianthus annuus* L. on alloxan-induced hyperglycemic rats. Comp Clin Pathol. 2014; 23:1565-1573.
12. Odeh IC and Tor-Ayiin TA. Phytochemical and antimicrobial evaluation of leaf extracts of *Pterocarpus santalinoides*. Eur J Med Plants. 2014; 4:105-115.
13. Aba PE and Udechukwu IR. Comparative hypoglycemic potentials and phytochemical profiles of 12 common leafy culinary vegetables consumed in Nsukka, Southeastern Nigeria. J Basic Clin Physiol Pharmacol. 2018; 29(4):313-320
14. Nworu CS, Akah PA, Nwachukwu JO, Asogwa C. Antidiarrhoeal activity of *Pterocarpus santalinoides* L'Herit ex DC leaf extract. J Compl Integr Med. 2009; 6:1553-1560.
15. Okwuosa CN, Unekwe PC, Achukwu PU, Udeani TKC, Ogidi UH. U. H. Glucose and triglyceride lowering activity of *Pterocarpus santalinoides* leaf extracts against dexamethasone resistance hyperlipidaemia and insulin resistance in rats. Afr J Biotechnol. 2010; 10(46):9415-9420.
16. Madubuike KG, Ezeja MI, Ezeigbo II. Antidiarrhoeal activity of *Pterocarpus santalinoides* leaf extract in mice. Cont J Ani Vet Res. 2012; 4:1-6.
17. Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis. (2nd ed.). London: Chapman and Hall; 1991. 1-31, 84-86, 222-236 p.
18. Evans WC. Trease and Evans Pharmacognosy. (16th ed.) London: WB Saunders Co.; 2009. 135-147 p.
19. OECD. Organization for Economic Co-operation and Development guidelines for the testing of chemicals 425. Acute toxicity – up-and-down-procedure (UDP). [Online]. 2008 [Cited 2018 July 17]. Available from: http://www.oecd.org.
20. Enegide C, David A, Fidelis SA. A new method for determining acute toxicity in animal models. Toxicol Int. 2013; 20(3):224-226.
21. Ezeigbo II and Asuzu IU. Anti-diabetic activities of the methanol leaf extracts of *Hymenocardia acida* in alloxan-induced diabetic rats. AJTCAM. 2012; 9(2):204-209.
22. Ezeja MI, Anaga AO, Asuzu IU. Anti-diabetic, anti-lipidemic and antioxidant activities of *Gouania longipetala* methanol leaf extract in alloxan-induced diabetic rats. Pharmacol Biol. 2015; 53:605-614.
23. Vinson J, Su X, Zubik L, Bose P. Phenol antioxidant quantity and quality in foods fruit. J Agric Food Chem. 2001; 49(11):5375-5321.
24. Tiwari AK and Madhusudana RJ. Diabetes mellitus and multiple therapeutic approaches of Phytochemicals: present status and future prospects. Current Sci. 2002; 83:30-38.
25. Saidu AN, Obiokpa FI, Olukotun IO. Phytochemical screening and hypoglycemic effect of methanolic fruit pulp extract of *Cucumis sativus* in alloxan induced diabetic rats. J Med Plant Res. 2014; 8 (39):1173-1178.
26. Aslan M, Delrioman OD, Orhan N, Sezik E, Yesilada E. *In vivo* antidiabetic and antioxidant potential of *Helichrysm plicatum* in Streptozotocin-induced diabetic rats. J Ethnopharmacol. 2007; 109:54-59.
27. Omeh YN, Onoja SO, Ezeja MI, Okwor PO. Sub-acute antidiabetic and *in vivo* antioxidant effects of methanolic extract of *Bridelia micrantha* leaf on alloxan-induced hyperglycaemic rats. J Compl Integr Med. 2014; 11(2):99-105.
28. Federiuk IF, Casey HM, Quinn MJ, Wood MD, Ward WK. Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: Route of administration, pitfalls and insulin treatment. Comp Med. 2004; 54(3):252-257.
29. Ceriello A. Postprandial Hyperglycemia and Diabetes Complications. Is It Time To Treat? Diabetes. 2005; 54(1):1-7.
30. Oliveira HC, dos Sontos MP, Grigulo R, Lima LL, Martins DTO, Lima JCS, Stoppiglia LF. Activity of *Vataira macrocarpa* extract in rats. J Ethnopharmacol. 2003; 115:515-519.
31. Oguanobi NI, Chijioke CP, Ghasi SI. Effects of aqueous leaf extract of *Ocimum gratissimum* on oral tolerant test in type – 2 model diabetic rats. AJPP. 2012; 6(9):630-635.
32. Wang Z, Yang Y, Xiang X, Zhu Y, Men J, He M. Estimation of the normal range of blood glucose in rats. Wei Sheng Yan Jui. 2010; 39(2):133-137.