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Antidiabetic effect of *Picralima nitida* aqueous seed extract in experimental rabbit model

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Abstract

Objective: The blood sugar lowering effect of *P. nitida* aqueous seed extract was investigated on normoglycaemic and hyperglycaemic rabbits. Materials and methods: The hypoglycaemic effect of *P. nitida* was evaluated using alloxan (80 mg/kg body weight, i.p) induced hyperglycaemic rabbits. The extracts potency was compared with standard drug, tolbutamide and distilled water. The LD_{50} was determined using mice. Results: A dose of 648 mg/kg body weight of extract caused maximum lowering of blood sugar levels in both normal and alloxanized rabbits. The mean fasting blood sugar in the normoglycaemic rabbit was reduced by 19.46% within 3 h, while in alloxanized rabbits blood sugar level was reduced by 75.5% within 6 h. The LD_{50} of the extract in mice was 1601.2 ± 60.5 mg/kg body weight when given i.p. Conclusion: *P. nitida*, though a crude drug, exhibited a faster onset of action and more persistent in hyperglycaemic situation than tolbutamide standard controls. This qualifies it to be used in ethnomedical diabetic management.

Keyword: *Picralima nitida*, Antidiabetic activity, Alloxan.

1. Introduction

A large number of medicinal plants are used in ethnomedicine globally as palliative therapy of diabetes dellitus like *Dioscorea dumentorum,* [1, 2] *Bridelia ferruginea* [3], *Allium sativum* [4], *Vernonia amygdalia* [5] and *Anacardium occidentale* [6].

Picralima nitida (Apocynaceae) Staph is a deciduous tree of about 20m in height with dense crown and widely distributed in the tropical rainforest of Africa [7, 8]. The fruit is broadly abovoid, smooth and glabrous measuring about 15cm long and 10cm in diameter. Each fruit contains three flattened seeds embedded in pulp[8]. The seeds of *P. nitida* are used as quinine substitute in ethnomedical treatment of malaria [9]. It has also been reported to have curative effect in respiratory infections and as enema in Ghana [10].

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Almost all parts of the plant are used in the treatment of ailments; the root bark, seeds and leaves are used in the treatment of all types of fever, as antitussive, for wound healing, as aphrodisiac [11], trypanosomiasis treatment and as local anaesthetic comparable to cocaine [12]. Some of its alkaloids posses antibacterial and analgesic properties [13], central nervous system depressant effect as well as intestinal smooth muscles spasmogenic activity [14].

It is widely used plant in West Africa for different purposes but very little is known or said about the effect on blood sugar level to the best of our knowledge. For this purpose, this study was designed to evaluate the blood sugar lowering effect of the commonly used aqueous extract of *P. nitida* seed using experimental animals.

2. Materials and methods

2.1 Plant materials

The pods of *P. nitida* were collected through a herbalist at Orlu, Imo State Nigeria and identified by Dr. (Mrs.) S.I. Inya-Agha of Department of Pharmacognosy, University of Nigeria, Nsukka in 1999. A voucher sample of the pod was deposited in the Departmental Herbarium.

2.2 Preparation of the aqueous extract

The pods were broken open, the seeds were obtained and air-dried for 2 weeks. The seeds were ground to fine. About 150 g of the powdered seed was macerated in 350 ml of distilled water at room temperature for 24 h with intermittent shaking. The material was filtered and freeze - dried to solid residue (21.6%). The extract was chemically tested for the presence of different chemical constituents using standard methods [15].

2.3 Animals

Wistar albino mice (20 - 41g) bred in the Department of Pharmacology Animal Unit of the University and local strain of adult rabbits (0.61-0.81kg) were used in the experiments. The animals were kept under room temperature with access to water and food for 1 week before the commencement of experiments.

2.4 Acute toxicity test

The LD_{50} of the extract was determined in mice ip using the method of Tainter and Miller [16].

2.5 Determination of blood sugar lowering effect of extract

2.5.1 Using normoglycaemic rabbits

The animals were fasted for 12 h, but were allowed access to water before and while the experiment lasted. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the marginal ear vein, blood sugar level were determined by O-toluidine method [17]. Animals having blood sugar concentrations of 84-102mg% after the 12 h fasting were grouped into three of five animals per group.

The group A received 648mg/kg extract (maximum effective dose observed from preliminary work), B received 500mg/kg tolbutamide, and C received 3ml/kg Distilled water. The tolbutamide group and distilled water group were the respective control groups. All administered drugs were through intraperitoneal route.

2.5.2 Using hyperglycaemic rabbits

In alloxanized group, normal adult rabbits having blood sugar levels of 84 - 102mg% after the 12 h fasting were used. The animals were injected intravenously with 80mg/kg body weight of alloxan monohydrate (Sigma, USA), freshly prepared in distilled water. The animals were fed for 7 days.

On the day 8, the survivors were fasted for 12 h and their blood sugar levels determined by

O-toluidine method as above. Only animals with blood sugar level above 300 mg% were used for the experiment. The diabetic rabbits were divided into 3 groups of five animals each and treated as above but in day 9.

2.5.3. Collection of blood and estimation of blood sugar

At fixed time interval (0, 1, 3, 6, 9h) after treatment, blood samples were withdrawn from the marginal ear vein of the animals and their blood sugar levels were expressed as mg% ± SEM and the student's *t* - test was used to test the significance of difference between treated groups and distilled water control, and between the blood sugar levels at 0 h and at various time intervals in each treated group with P=0.05.

3. Results

3.1 Chemical Constituents of P.nitida extract

The aqueous extract of the seed gave positive chemical reactions for glycosides, saponins, tannins, alkaloids, proteins, and carbohydrates.

3.2 Acute toxicity test

Administered intraperitoneally, the LD_{50} of the extract in mice was 1601.2 ± 60.5 mg/kg.

3.3 Hypoglycaemic effect of P. nitida extract

The maximum reduction in blood sugar occurred at a dose of 648mg/kg of both normal and alloxanized rabbits. This extract dose significantly ($P < 0.05$) lowered the blood sugar level in fasted normal rabbit from a mean value of 99.2 ± 5.2 mg% (0h) to 78.4 ± 3.4 mg% (3h) (Table 1) while beyond that hour, the blood sugar level rose continually. The effect of tolbutamide at 500mg/kg followed almost the same fashion, with a maximum reduction at 6h. Normal distilled water caused no significant change in the blood sugar level.

The effects of the extract, tolbutamide and distilled water on the blood sugar levels of alloxanized rabbits are shown in Table 2. The extract reduced the mean fasting blood sugar levels from 404.3 mg% in 0 h to 99.0 mg% at 6 h while tolbutamide caused blood sugar levels reduction from 337.5 mg% in 0 h to 104.7 mg% in 3 h. The percentage reductions in blood sugar levels produced by the extract in the fasted normal and alloxanized diabetic rabbits were 19.46% and 75.5% respectively.

4. Discussion

The pharmacological investigations of the extract of *P. nitida* showed that the plant extract caused significant reduction in the blood sugar levels in hyperglycaemic rabbits. In the alloxan-induced diabetic rabbits, the extracts produced marked reduction in blood sugar level

Table 1
Effects of *P. nitida* aqueous seed extract, tolbutamide and distilled water on mean fasting blood sugar of rabbits

Drug	Doses (mg/kg)	Fasting blood sugar (mg%)					Percentage Max. Reduction
		0 h	1 h	3 h	6 h	9 h	
Extract	648	99.2 ± 3.5	89.7 ± 1.5	78.4 ± 2.6*	84.4 ± 0.9	87.8 ± 2.3	19.45
Tolbutamide	500	84.4 ± 2.0	78.4 ± 3.1	65.8 ± 1.7	60.1 ± 2.2*	72.4 ± 1.2	28.85
Distilled water	3 ml/kg	87.8 ± 2.3	97.2 ± 1.0	93.4 ± 2.0	88.5 ± 2.7	88.7 ± 1.8	--

n = 5. Values are expressed as mean ± SEM; * $P < 0.05$ *vs* respective control;

Table 2.
Effects of *P. nitida* seed extract, tolbutamide and distilled water on mean fasting blood sugar of alloxanized rabbits

Drug (mg/kg)	Doses	Fasting blood sugar (mg%)					Percentage Max. Reduction
		0 h	1 h	3 h	6 h	9 h	
Extract	648mg/kg	404.3 ± 6.3	174.4 ± 3.4*	121.2 ± 7.0*	99.0 ± 1.0**	191.7 ± 3.7*	75.5
Tolbutamide	500mg/kg	337.5 ± 4.7	212.3 ± 7.0	104.7 ± 6.2**	160.2 ± 5.1	168.1 ± 2.9	69.0
Distilled water	3 ml/kg	356.3 ± 6.5	365.7 ± 5.8	355.9 ± 1.0	356.7 ± 3.0	356.7 ± 6.0	—

Values are expressed as mean ± SEM; * $P < 0.05$ *vs* respective control; ** $P < 0.01$ *vs* respective control; n=5.

within 1 h post administration which climaxed in the sixth hour post administration. The hypoglycaemic effect of the extract did not persist beyond the first eight hours.

When compared with tolbutamide treated controls, the extract produced noticeable percentage maximal reduction in the mean fasting blood sugar levels in normoglycaemic animals. The extract achieved its maximum blood sugar level lowering effect on the normoglycaemic animals faster than tolbutamide treated controls but the latter experienced more persistent control of the mean blood sugar level than the extract and a more significant percentage maximal reduction of the blood sugar level which invariably showed that the extract has lesser hypoglycaemic effect in normoglycaemic rabbits than tolbutamide.

But in the hyperglycaemic animals, the extract of *P. nitida* seed has significant blood sugar level lowering activity even within 1 h post administration and the maximal reduction of blood sugar level highest in 6 h post administration while the maximal reduction of blood sugar level by tolbutamide occurred in 3 h.

Although the maximal reduction of blood sugar level was achieved earlier with tolbutamide but the extract exhibited a more persistent hypoglycaemic activity in alloxan-induced diabetic animals. This points to a mechanism of action different from that of tolbutamide, and not related to insulin secretion from pancreatic β-cell.

In diabetes, causes and sites of intervention in the biochemical processes are many and include the action of hormones and chemical mediators as well as vascular modifications of the pancreas and insufficient insulin production [5]. Alloxan is known to permanently destroy the pancreatic β-cells [6] but the extract lowered the blood sugar levels in alloxanized rabbits which is an indication that the extract has extra pancreatic effects.

These may have been caused by the presence of several biologically active secondary metabolites although the plants seed has been known to be very rich in indole alkaloids highly implicated in the inhibition of phosphoenol pyruvate carboxykinase, an important enzyme in glyconeogenesis, thus producing hypoglycaemia [18].

Inspite of these tentative mechanisms for the hypoglycaemic activity of *P. nitida*, further investigation, isolation and characterization of the plants active constituents would be necessary, especially as the folkloric use in the management of diabetes is not so popular.

References

1. Undie AS, Akubue PI. (1986) *J. Ethnopharmacol.* 15: 133 - 144.

2. Iwu MM,Okunji CO, Akah PA, Tempesta MS, Corley DG. (1990) *Planta Med.* 56: 119 - 120.

3. Iwu MM. (1980) *Planta Med.* 39: 247.

4. Reiter HD (1995) *Phytomed.* 2 (10): 78 - 91.

5. Akah PA, Okafor CL. (1992) *Phytother. Res.* 6: 171 - 173.

6. Ezugwu CO, Okonta JM, Esimone CO. (2000) *J. Nat. Remed.* 1(1): 60 - 63

7. Keay RWJ, Onochie CFA, Stemfield DD. (1964) *Nigerian Trees, Ibadan Federal* Dept. of Forest Res: Ibadan; 2, 378 - 396.

8. Irvine FR. (1961) *Woody plant of Ghana,* Oxf. University Press: London; 629 - 630.

9. Francoise G, Ake-Assi-1, Holenz J, Bringmann G. (1996) *J. Ethnopharmacol.* 54: 2 - 3, 113 - 117.

10. Dalziel JM. (1958) *Useful plants of West Tropical Africa,* Crown Overseas Agents for Colonies: London; 65.

11. Iwu MM. (1982) *Ethnomed.* 7: 387.

12. Hamet C. (1940) *In: Medicinal plants in Nigeria,* Nigeria College of Arts, Science and Technology: Ibadan; 77 - 78.

13. Arens H, Borde HO, Ulbrich B, Stockigt J. (1982) *Planta Med.* 46: 210 - 214.

14. Levy J, Lemen J, Jannot MM. (1963) *Tetrahedr.* 19: 1265.

15. Trease GE, Evans WC. (1991) *Pharmacognosy,* 13th edn, Bailliere Tindall: London; 167 - 197, 386.

16. Tainter MC, Miller LC. (1944) *Proc. Soc. Expt. Biol. Med.* 57: 261.

17. Stroev EA, Makarowa VG. (1989) *Lab. Manual in Biochem,* Mir Publishers: Moscow; 143.

18. Webb Leyden J. (1966) *Enzyme and Metabolic Inhibitors,* Academic Press: N.U; (3) 321 - 326.