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Research Article

Effect of Ethanol Leaf Extract of *Newboulda Laevis* on Blood Glucose Levels of Diabetic Rats

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Abstract

Purpose: *To investigate anti-diabetic effect of the ethanol leaf extract of Newbouldia laevis (P. Beauv) in alloxan-induced diabetic rats.*
Methods*: Alloxan (150 mg/kg) was administered to wistar albino rats via the intraperitoneal route. The diabetic rats were then placed in 5 groups, following stabilization of hyperglycemia. The first group was untreated, the next three groups received, each day, 100, 200 and 400 mg/kg body weight of the ethanol extract Newbouldia laevis and the fifth group received a reference standard, glibenclamide (5 mg/kg). Treatment was via the oral route for 14 days and fasting blood sugar level was monitored over this period. Acute toxicity (oral and intraperitoneal) studies on the extract was carried out, as well as phytochemical screening of the extract.*
Results: *All doses of the extract (100, 200, and 400 mg/kg) significantly ($p < 0.05$, $p < 0.0001$, $p < 0.05$, respectively) lowered fasting blood glucose level, notably at the 4^{th}, 8^{th} and 14^{th} day. Glibenclamide (5 mg /kg) also significantly lowered fasting blood glucose ($p < 0.0001$). The results on acute toxicity revealed that for the oral and intraperitoneal route, mortality was at 8 and 1 g/kg, respectively while LD_{50} was 6 g/kg, indicating the high safety status of the plant. Phytochemical screening revealed the presence of saponins, tannins, alkaloids and flavonoids.*
Conclusion: *This study supports the use of Newboulda laevis in traditional medicine as well as highlights the need to further explore the potentials of the plant extract as a antihyperglycemic agent.*

Keywords: *Diabetes mellitus, Newbouldia laevis, Antidiabetic, Hypoglycemia, Blood glucose*

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INTRODUCTION

The reasons to study medicinal plants include the widespread use of plants in folk medicines, good safety profile of plants and their ready availability [1]. Plant resources are a veritable source of pharmaceuticals and therapeutics, but they have not been adequately documented [2].

Traditional medicinal practice has existed in Africa and other cultures for centuries since man came into being but recently it has been neglected due to undue pressure from orthodox medical practitioners and the unscientific background of its method of operation [3]. There is renewed worldwide interest in traditional medicine which is derived from the realization that orthodox medicine is not widespread in poor countries whereas healthcare has virtually been sustained by these cultural alternatives [3].

Diabetes is a chronic metabolic disorder which is characterized by hyperglycaemia as a result of the diminished production of insulin or mounting resistance to its action [4]. Chronic hyperglycaemia during diabetes causes glycation of body proteins thereby affecting the eyes, kidney, nerves and arteries [4].

The plant, *Newbouldia laevis* (P.Beauv), is called *Aduruku* in Hausa, *Ogirisi* in Ibo and *Akoko* in Yoruba languages of Nigeria [5]. It is a medium size angiosperm which belongs to the Bignoniaceae family. It grows to a height of about 7 - 8m, but more usually as a shrub of 3m. It has many stem forming clumps of gnarled branches [6,7]. The plant, native to tropical Africa, is used traditionally for the treatment of diabetes, diarrhoea, dysentery and in pregnancy [6].

This study was designed to test the hypoglycaemic effect of the ethanol extract of the leaves of *Newbouldia laevis* on alloxan induced diabetic rats, because of its local use amongst the binins, ibos and yourubas speaking areas in Nigeria in the treatment of diabetes mellitus [6].

EXPERIMENTAL

Plant material

The leaves of *Newbouldia laevis* were collected from Ugbowo area of Benin City, Nigeria, in the month of July 2009. The plant was identified by Mr S Nweke of the Department of Pharmacogonosy, Faculty of Pharmacy, University of Benin, Benin City. Botanical authentication of the plant was confirmed at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria where a voucher specimen (No FHI 107753) was deposited for future reference.

Extract preparation

The leaves of *Newbouldia laevis* were air-dried for several days and then pulverized into fine powder. About 500 g of the powdered leaves was extracted exhaustively via marceration for 72 h with 2.8 L of 70 % absolute ethanol. Filtration was carried out using a funnel and filter paper. The extract was concentrated in an evaporating dish over a hot water bath. The yield was 38.28 %.

Drugs and chemicals

Alloxan and glibenclamide (Sigma-Aldrich, UK), and 95 % ethanol (Sosol Inc, South Africa) were used. All other chemicals and drugs used were of analytical grade.

Experimental animals

Adult albino rats and mice of both sexes and weighing 135 - 255 g and 20 - 30 g, respectively, were used for the study. The animals were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria. They were kept in standard cages in a well-ventilated room, fed with standard growers mash (Bendel Feeds and Flour Mills

Ltd, Ewu, Edo State, Nigeria) and allowed water *ad libitum*.

Ethical approval for the study was obtained from the Ethical Committee on the Use of Animals for Experiments, Faculty of Pharmacy, University of Benin. The animals were handled according to the standard protocols for the use of laboratory animals [8].

Phytochemical screening

Freshly prepared extracts of *Newbouldia laevis* were subjected to preliminary phytochemical screening for various constituents. The methods of analysis employed was as previously described [9,10].

Acute toxicity studies

The acute toxicity of the plant extract was evaluated using the method described by Lorke [11]. The mice were divided into 5 groups of 5 mice each. The first group was treated with 2 ml/kg of normal saline. This served as the control. The second, third, fourth and fifth groups were treated with the ethanol extract of the plant at doses of 1,000, 2,000, 4,000 and 8,000 mg/kg, respectively. All administrations were carried out orally via an oro-gastric syringe. The mice were then observed for 24 h for signs of toxicity and mortality. Intraperitoneal acute toxicity test was similarly carried out using the same series of doses but on separate groups of mice. [11].

Induction of diabetes mellitus

Twenty five albino rats of both sexes weighing 135 - 255g were used. The rats were fasted overnight prior to injection with alloxan dissolved in normal saline at a dose of 150 mg/kg intraperitoneally for 5 days. Rats with blood glucose levels greater than 200 mg/dl were considered diabetic and used for this investigation [12].

Antidiabetic treatment

The alloxan-diabetic albino rats which were fasted were placed in 5 groups (groups 2 - 6) of 5 rats each and treated as follows: group 1 (normal rats) received normal saline orally for 14 days; group 2 were untreated diabetic rats; groups 3, 4 and 5, which were all diabetic, received orally 100, 200, 400 mg/kg of the extract, respectively, for 14 days. The 6^{th} group (diabetic) received 5 mg/kg of glibenclamide orally for 14 days. These doses were selected after an initial preliminary work done using various doses on diabetic rats.

Measurement of blood glucose levels

All blood samples were collected by cutting the tail-tip of the overnight fasted rats. Blood samples for fasting blood glucose determination were collected at intervals of 2, 4, 8, 24 h and on the 14^{th} day following treatment. Measurement of the blood glucose level (mg/dl) was carried out using Acco-Chek Active blood pressure monitor and results recorded [13].

Statistical analysis

All data were expressed as mean ± SEM and where applicable, the data were analysed statistically by Student's t-test using Graph Pad Instat software, version 2.05a. $P < 0.05$ was taken as indicative of significant difference.

RESULTS

Phytochemical analysis

The results on the phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids, tannins and cardiac glycosides.

Acute toxicity

The sign of toxicity were noticed from 30 min to 4 h following the administration of the extract for both oral and intraperitoneal

routes. There was prostration and increased sensitivity to sound after about 1 h. There was also increased feed intake after about 30 min of extract administration, followed by itching and decreased movement after about 3 h of extract administration. There was weight loss after 14 days, and death for the 1 and 8 g/kg groups after 24 h of extract administration in the oral and intraperitoneal routes, respectively. The median lethal dose (LD_{50}) was 6g/kg for the intraperitoneal route, and indeterminate for the oral route. These results are shown in Table 1 and Fig 1.

Anti-diabetic effect

The anti-diabetic effect of the extract is shown in Fig 2. The 100 mg/kg dose of the extract at the 2nd, 4th and 8th h and 14th day of extract administration significantly ($p < 0.05$) reduced fasting blood glucose level from 226 to 174.3, 181.5, 156.3 and 150 mg/dl, respectively.

A similar effect was seen with the 200 mg/kg in which there was also significant reduction of fasting blood glucose levels from 294.5 to 244 ($p < 0.05$), 144.8 ($p < 0.0001$), 110.0 ($p < 0.0001$) and 145.3 mg/dl ($p < 0.0001$) on 4th, 8th and 24th h, and14th day, respectively. The 400 mg/kg dose showed the highest hypoglycaemic effect with significant ($p < 0.05$, $p < 0.0001$) reductions in blood glucose level from 266.3 to 161.0, 158.5, 131.5 and 101.5 mg/dl on the 2nd, 4th and 8th h, and 14th day. A significant blood sugar level reduction ($p < 0.0001$) was seen with glibenclamide over the same treatment period.

Table 1: Oral acute toxicity data in mice for ethanol leaf extract of *Newboulda laevis* (n = 6)

Treatment	Log-dose	Mortality (%)
Control normal saline)	0	0
Extract (1 g/kg)	3.000	0
Extract (2 g/kg)	3.301	0
Extract (4 g/kg)	3.602	0
Extract (8 g/kg)	3.903	40

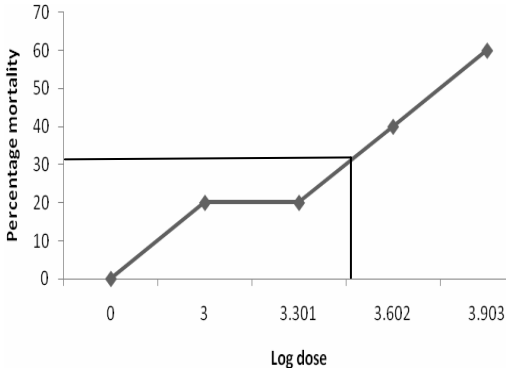

Fig 1: Acute toxicity studies (intraperitoneal) of ethanol leaf extract of *Newboulda laevis* in mice (n = 6, LD_{50} = 6 g/kg)

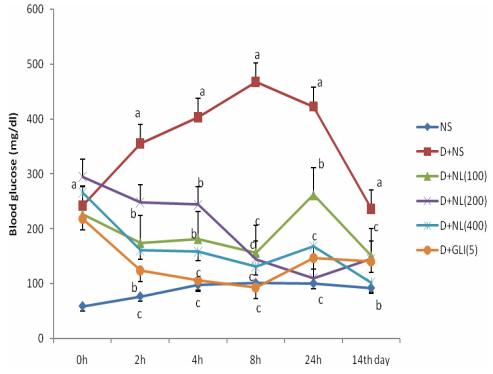

Fig 2: Anti-diabetic effect of ethanol leaf extract of *Newboulda laevis* in rats (n = 6, error bars = SEM). **Key:** NS = normal saline; D+NS = diabetic given normal saline; D+NL(100) = diabetic group given 100 mg/kg extract; D+NL(200) = diabetic given 200 mg/kg extract; D+NL(400) = diabetic given 400 mg/kg extract; D+GLI(5) = diabetic given 5 mg/kg glibenclamide; $^{a}p < 0.0001$ significantly different from control; $^{b}p < 0.05$ and $^{c}p < 0.0001$ significantly different from untreated diabetic rats given normal saline

DISCUSSION

Oral acute toxicity studies showed that the extract produced no mortality except at the dose of 8g/kg. This indicates that the extract has a wide margin of safety and LD_{50} could not be ascertained since no mortality was recorded at lower doses. Intrapentoneal (*ip*) administration of the extract caused mortality at 2 g/kg dose, with an LD_{50} of 6 g/kg which

confirms the safety of the plant. Induction of diabetes using alloxan has been described as a useful experimental model for studying the effect of hypoglycemic agents [14]. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, resulting in the destruction of pancreatic β-cells and diabetes [14].

The untreated diabetic rats had a significantly higher ($p < 0.0001$) fasting blood glucose level than normal rats that received normal saline. This confirms induction of diabetes by alloxan. The results show a dose-dependent lowering of fasting blood glucose level in diabetic rats treated with different doses of the extract. This dose-dependent effect compares well with glibenclamide, the reference drug used, especially at the 400 mg/kg body weight dose, at which dose the extract produced a greater and more significant reduction of blood glucose level than 5 mg/kg glibenclamide on the 14th day. Glibenclamide is a standard drug that is routinely used in the treatment of diabetes [15].

The extract's ability to significantly reduce hyperglycemia induced by alloxan may indicate a similar mechanism with glibenclamide which, being a sulphonylurea stimulates the release of insulin [16]. Alloxan destroys pancreatic β-cells and thus reduces or completely inhibit insulin secretion resulting in hyperglycemia [14]. Hence the release of insulin by glibenclamide produces a lowering of hyperglycemia. The extract also produced a similar reduction in blood sugar level and it is probable that it also enhanced insulin secretion but this needs to be investigated in future studies. However, the presence of flavonoids in the extract may account for the observed hypoglyceamic effect since they have been found to stimulate the secretion of insulin [17]. Glibenclamide (an oral hpoglycemic agent) is known to act by enhancing exogenous insulin contribution which thus corrects deficiency in the endogenous insulin created by alloxan.

CONCLUSION

The ethanol leaf extract of *Newbouldia laevis* demostrated antidiabetic activity in rats and acute toxicity studies also show it to be relatively safe. The findings from this work supports its use in traditional medicine. However, further studies are required to elucidate its mechanism of action.

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COMPETING INTERESTS

The authors report no conflicts of interest and they alone are responsible for the content.

REFERENCES

1 *World Health Organization Resolution. Promotion and research development of training in traditional medicine. Technical Report, 1978, no. 622, Geneva.*

2 *Gbile ZO, Adesina SK. Nigerian flora and its pharmaceutical potentials. J. Ethnopharmacol 1986; 19:1-16.*

3 *Watt JM, Brayer-Brandwyk MG. The medicinal and poisonous plants of Southern and Eastern Africa. Livingstone : Edinburgh. 1962; p 3.*

4 *Kameswara RB, Kesavulu MM, Giri CH. Anti-diabetic and hypolipidemic effects of Momordica cymbalania Hook fruit powder in alloxan-induced diabetic rats. J. Ethnopharmacol, 1999; 67: 103-109*

5 *Hutchinson J, Dalziel JM. Floral of West Tropical Africa, 1963: Vol 2, 5p*

6 *Arbonnier M. Trees, shrubs and lianas of West African, Dry zone. CIRAD. Margra GMBH MNHN. Cote D'ivoire. 2004; p 194*

7 *Usman H, Osuji JC. Phytochemical and in vitro antibacterial assay of the leaf extract of Newboulda laevis. Afr J. Trad CAM 2007; 4: 476-480*

8 National Institute of Health, USA. Public health service policy on humane care and use of laboratory animals; 2002.
9 Evans WC. Trease and Evans Pharmacogonosy. 15th edn, Churchill Livingstone London, 2002; p 26.
10 Harbone JB. In Comparative biochemistry of flavonoids. Proc Natl Acad Sci 1993; 90: 4689-4692
11 Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1998; 54: 275-287.
12 Rees DA, Alcolado JC. Animal models of diabetes mellitus. Diab Med 2005; 22: 359-370.
13 Rheney CC, Kirk JK. Performance of three blood glucose meters. Ann Pharmacother 2000; 34: 317-321.
14 Szudelski T. The mechanism of alloxan and streptozotocin action in β cells of the rat pancreas. Physiol Res 2001; 50: 536-546.
15 Katzung BG, Trevor A, Masters SB. Basic and Clinical Pharmacology. Mc-Graw Hill; 2009; p 346.
16 Frode TS, Medeiros A. Animal models to test drugs with potential antidiabetic activity. J. Ethnopharmacol 2008; 115: 173-183.
17 Schimizu M, Ito T, Rshima S, Mayashi T, Arisawa M, Morita-Kurokowa S, Ito Hasimato Y. Inhibition of lens aldose reductase by flavonoids. Phytochemistry 1984; 23: 1885-1888.