International Journal of Scientific Research in Chemistry (IJSRCH) | ISSN: 2456-8457
© 2018 IJSRCH | Volume 3 | Issue 5

Phytochemical Studies and Antidiabetic Activities of *Newbouldia laevis* (P. Beauv) Ethanolic Leaves Extracts in Alloxan-Induced Diabetic Rats

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ABSTRACT

The phytochemical properties of the ethanolic leaves extract of *Newbouldia laevis* and its antidiabetic properties on alloxan-induced diabetic albino rats were evaluated. The qualitative phytochemical screening revealed the presence of alkaloids, tannins, saponins, terpenes, flavonoids, cardiac glycosides and anthraquinones in *Newbouldia laevis* leaf extract. Quantitative phytochemical screening of *Newbouldia laevis* revealed the presence of flavonoids (12.22 ± 1.67 mg/kg), alkaloids (10.71 ± 1.75 mg/kg), saponins (7.01 ± 0.61 mg/kg), tannins (6.74 ± 0.36 mg/kg), cardiac glycosides (4.11 ± 0.46 mg/kg), anthraquinones (1.42 ± 0.24 mg/kg) and terpenes 1.16 ± 0.24 mg/kg. The toxicity test of the extracts produced various degree of toxicity ranging from writhing, decreased respiration, convulsion to mortality. The intensities of these effects were proportional to the dose administered. The LD_{50} for *Newbouldia laevis* was 447.21 mg/kg. The results of the influence of ethanolic leaves of *Newbouldia laevis* on alloxan-induced diabetic rats showed significant ($p<0.05$) reduction in the blood glucose level (BGL) as time of treatments increases. The leaves extracts of *Newbouldia laevis* significantly ($p<0.05$) decreased the blood glucose level of diabetic rats on day 14 compared to diabetic untreated (control) rats. The BGL of diabetic rats treated with oral hypoglycemic agent alone were significantly ($p<0.05$) different from control group on day 14. The presence of these biologically active compounds suggest that the plant could serve as potential sources of drugs and their secondary metabolites could exert some biological activities when taken by animals. The extracts of *Newbouldia laevis* also produced a similar reduction in blood sugar level as the standard hypoglycemic drug (Glibenclamide). This may be due to its ability to enhance insulin secretion like the standard drug. This positive result confirms why herbalists use this leaf for the management of diabetes.

Keywords : Albino Rats, Alloxan, Antidiabetic, Diabetes, Glibenclamide, *Newbouldia laevis,* Phytochemicals

I. INTRODUCTION

Diabetes mellitus (DM) is one of the most common non-communicable diseases globally [1]. It is either the fourth or fifth leading cause of death in developed and developing countries of the world [2]. Symptoms of DM include classical hyperglycaemia, polyuria, polydypsia, weight loss, sometimes with polyphagia, and blurred vision. Diabetes mellitus is classified into Type-1 or Type-2 depending on the aetiology [3].

Plant drugs are frequently considered to be less toxic and free from side effects than synthetic chemicals [4].

Before the advent of insulin and chemical drugs for the treatment of diabetes as with other ailments, plant-based medications have been used as traditional remedies for the treatment of many diseases including diabetes mellitus [5, 6, 3]. More than 400 plants are being used in different forms for their hypoglycaemic effects in treating diabetes [7, 3]. In Nigeria, traditional medicine occupies a unique position in health care delivery, especially among the rural populace. However, the activities of herbalists are surrounded with a lot of secrecy and lack of scientific procedure, hence the need to standardize the practice of traditional medicine [8, 3].

The use of medicinal plants for treatment and management of diseases is gaining prominence worldwide especially in the developing countries where 80 % of the population still depends on traditional healing methods [9, 10]. This surge in the use of herbal medicines is probably due to the perceived failure of some synthetic drugs in the treatment of some diseases, the side effects associated with most drugs and the incidence of drug resistance especially among the antibiotics family [11]. In recent times, quite a number of some plants have been used as herbal medicines due to the presence of phytochemicals in them [12]. Many of these discovered phytochemicals seem to fight diseases and lower the rates at which they occur [13].

N. laevis is a medium size angiosperm which belongs to the *Bignoniaceae* family. It is a fast-growing evergreen shrub or small tree. It only reaches a height of 3 - 8 metres in the west of its range, but can attain a height of up to 20 metres in the east. It has many stem forming clumps of gnarled branches [14].

The tree, and especially the bark, is widely used in traditional medicine in Africa. *Newbouldia laevis* is one of such plants that its leaves are used in Southeastern Nigeria to hasten parturition and to expel the placenta after delivery. Agents that stimulate uterine contraction are classified as oxytocics and are employed clinically for the induction and support of labour as well as in the management of the third stage of labour [15].

The bark has analgesic and stomachic properties. A decoction is used in the treatment of coughs, diarrhea and dysentery, while it is also given to children for the treatment of epilepsy and convulsions. A decoction of the bark, combined with chillies, is used in the treatment of chest pains [15].The dried bark and young twigs, pounded up with spices such as *Xylopiasp*, are given in decoction or infusion to treat such complaints as uterine colic, dysmenorrhoea, etc [15]. Thus, the aim of this study was to assess the phytochemical properties and antidiabetic effect of the *Newbouldia laevis* ethanolic leaf extract on albino rat.

II. METHODS AND MATERIAL

A. Study Area

This research was carried out in Uyo Local Government Area of Akwa Ibom State, Nigeria. Uyo is a city in South-South Nigeria found between latitude 5.02^0N and longitude 7.92^0E; it has an average temperature of 25.1-27.8^{0}C and an annual rainfall range of 33-37.8mm with the land mass of 115 km^2 and the population of 1,400 thousands persons/km^2.

B. Plants Collection and Authentication

The leaves of *Newbouldia laevis* were collected from plants growing in the University of Uyo farm, University of Uyo, Akwa Ibom State. The plant samples used for this research work were authenticated by a Plant Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

C. Collection and Maintenance of Experimental Animals

Male and female albino rats weighing 150 – 250g were obtained from the animal house of the

Department of Biochemistry, University of Uyo and randomly assigned to five groups of five rats in each group; Two groups served as a positive control and a negative control, while the other three groups served as test groups, which were used for application of *Newbouldia laevis* leaves extract.

D. Preparation of Plant Extracts

The fresh leaves of *Newbouldia laevis* were air dried for 7 days and ground into powdered form, 400g of each plant samples were extracted using 6000ml of 70% ethanol and shaken intermittently for 72 hours. It was filtered and the filtrate concentrated (dried) *in-vacuo* at 40^0C in a water bath. The extract was weighed and stored in 150ml beaker, labeled and covered with foil paper and preserved in the refrigerator at 4^0C for use in qualitative and quantitative phytochemical screening, acute toxicity test, and anti-diabetic tests.

E. Preparation of Extract and Fraction Solutions

The extract and fraction solutions were prepared by dissolving about 1.5g (using high performance profile design compact weighing balance: CS200) of the extract and fractions in 10 ml of distilled water, to give an effective concentration.

The formula:

$$\text{Dosage} = \frac{\text{Mg/kg} \times \text{Wt. of animal (g)}}{1000 \text{ conc. (mg/ml)}}$$

was used to calculate the volume of the extract/fractions solution to be administered to each animal. The solutions were prepared fresh daily before administration [16].

F. Treatment Application and Experimental Design

Three groups out of five groups of rats were administered with ethanolic extract of *Newbouldia laevis*. Group one and two were used as control (positive and negative control). The positive control was orally given soluble fraction of standard drug, fed with normal feed and distilled water. Group two were fed with normal feed and distilled water. Group three were administered orally with 40% (LD) soluble fraction of ethanolic extract of *Newbouldia laevis*, normal feed and distilled water. Group four were fed orally with 60% (MD) soluble fraction of ethanolic extract of *Newbouldia laevis*, allowed free access to normal feed and distilled water. Group four were administered orally with 100% (HD) soluble fraction of ethanolic extract of *Newbouldia laevis*, allowed free access to normal feed and distilled water daily for 14 days (two weeks). The animals were anaesthetized and sacrificed with chloroform. Blood samples were collected with 5 ml syringe by cardiac puncture into ethylene diaminetetraacetic acid (EDTA) sequestrene bottles to prevent coagulation and used for determination of antidiabetic activities within 24 hours of sample collection.

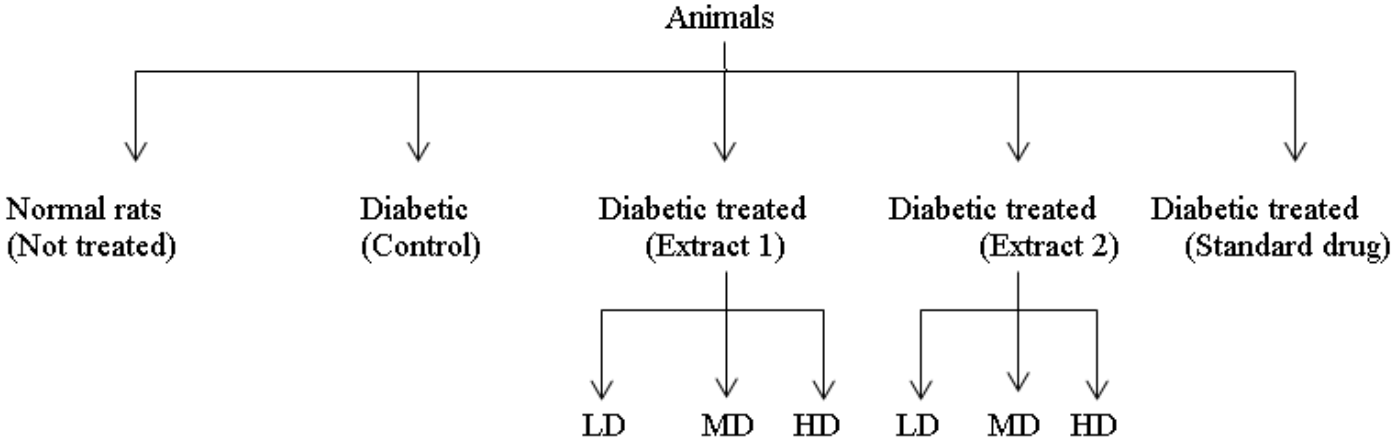

Figure 1. Treatment Application and Experimental Design

G. Determination of Median Lethal Dose (LD_{50})

Albino rats weighing 25 – 32g were kept in five cages (5 per cage) and handled according to standard guidelines for the use and care of laboratory animals. Food was withdrawn for 18 hours before the onset of the experiment [17]. The five groups of mice were administered with 4000mg/kg, 3000mg/kg, 2000mg/kg, 1000mg/kg and 500mg/kg of *Newbouldia laevis* extract. The groups were observed for mortality rate within 24 hours and the median lethal dose (LD_{50}) was calculated according to the methods of Lorke [18] with this formula:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where: D_0 = Maximum dose producing 0% mortality

D_{100} = Minimum dose producing 100% mortality

H. Determination of Weight, Feed and Water intake of the Rats

Feed and water intakes were measured every day at the same hour during the experimental periods while the body weight of the animals was measured at zero day and every seven days for the period of 21 days using high performance profile design compact weighing balance (DW 1100) [19].

I. Determination of Blood Glucose

Blood samples were collected at 0, 30, 60 and 120 minutes post-treatment by a tail tip-snip cut. The plasma glucose concentration of the rats was determined with the aid of an electronic glucose meter (Accu-Chek Advantage) and glucose strips (Accu-Chek Advantage II). The mean antidiabetic response observed was recorded as mmol/L and used to determine the dose response effect of *N. laevis* [3].

J. Qualitative Phytochemical Screening

The methods of [20, 21, 22] were used for qualitative phytochemical screening of the leaf extracts. These included tests for saponins, tannins, flavonoids, anthraquinones, terpenes, phlobatannins alkaloids and cardiac glycosides. The experiment was carried out in the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo.

K. Antidiabetic Analysis

The alloxan-induced diabetic albino rats were fasted and placed in 5 groups (groups 2 - 6) of 5 rats each and treated as follows: group 1(control rats) received normal saline orally for14 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 6th group (diabetic) received 5 mg/kg of glibenclamide orally for 21 days. These doses were selected after an initial preliminary work done using various doses on diabetic rats.

L. Statistical Analysis

The statistical analysis was carried out to find the effect of *Newbouldia laevis* diet on antidiabetic parameters in rats. One way analysis of variance was adopted for comparison and the results are expressed as standard error of mean (S.E.M.). The significant difference between means was determined using one way ANOVA at significance level of $p < 0.05$ according to the methods of [23].

III. RESULTS AND DISCUSSION

Qualitative phytochemical screening of ethanolic leaf extracts of *Newbouldia laevis* revealed the presence of some bioactive constituents, as summarized in Table 1. Ethanolic leaf extracts of *Newbouldia laevis* showed the presence of alkaloids, tannins, saponins, terpenes, flavonoids, anthraquinones and cardiac glycosides.

Quantitative phytochemical screening of *N. laevis* recorded that flavonoids had the highest value (12.22 ± 1.67 mg/kg), followed by alkaloids with (10.71 ± 1.75 mg/kg), saponins had (7.01 ± 0.61 mg/kg), tannins (6.74 ± 0.36 mg/kg), cardiac glycosides (4.11 ± 0.46 mg/kg), anthraquinones (1.42 ± 0.24 mg/kg) while terpenes recorded the lowest value with 1.16 ± 0.24 mg/kg (Table 2).

Table 1. Qualitative phytochemical screening of *Newbouldia laevis*

Constituents	Test	Observations	*N. laevis*
Alkaloids	Dragendoff's test	Formation of red precipitate	+++
Tannins	Ferric Chloride Test	A blue-green precipitate	+++
Saponins	Frothing Test	Appearance of froth.	+++
	Forming Test	Formation of 1 layer of form	+++
Terpenes	Libermanns Burchard's test	Formation of brown ring at the junction	+
Flavonoids	Shinoda's Test	a red colour indicated the presence of flavonoids	++
Phlobatannins		No visible colour	ND
Anthraquinone	Borntrager's test	the presence of a pink in the ammoniacal (lower) phase	ND
	Sulphuric acid test	a violet coloration in the ammonia layer (Lower phase)	ND
Cardiac glycocides	Salkowski test	a reddish-brown colour at the interphase	+
	Keller-kellani's test	brown ring obtained at the interface	+

+ = Trace, ++ = Moderate, +++ = Abundant, ND = Not detected

Table 2. Quantitative phytochemical screening of *N. laevis*

Phytochemicals	*N. laevis* (mg/kg)
Alkaloids	10.71 ± 1.75
Flavonoids	12.22 ± 1.67
Tannins	6.74 ± 0.36
Saponins	7.01 ± 0.61
Cardiac glycosides	4.11 ± 0.46
Terpenes	1.16 ± 0.04
Anthraquinones	1.42 ± 0.24

Data is presented as mean ± standard error (±SEM) of triplicate values

The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic ailments have been reported [24]. The medicinal value of plants lies in their inherent chemical substances which have a definite

physiological action on the human body [25]. Different phytochemicals have been found to possess a wide range of activities which may help in protection and prevention against chronic diseases. For example alkaloids are known to protect against diabetes, liver infection and malaria. Saponins protect the body against hypercholesterolemia and possess antibiotic properties while terpenes show analgesic properties [26]. Esenowo *et al.* [27] reported that tannins, flovanoids and cardiac glycosides in *Peristrophe bicalculata* have the potency to promote haemopoictic indices and restore loss of blood during excessive bleeding. Similar reports were obtained for *Morinde citrotolia* and *Digitalis pupurea* by Farine *et al.* [28]; Trease and Evans [20]. They reported the presence of cardiac glycosides in these plants and stated that they can be used in the treatment of diseases associated with the heart. These plants are currently used by herbalists to treat tumour [29]. Flavonoids containing plants have been used as diuretic, laxative, emollient and poultice [30]. Therefore the use of these plants rich in flavonoids and other chemical substances in traditional medicine lends credence to the medicinal potentials of these plants. Terpenes in modern clinical studies have supported the role of plants as anti-inflammatory and analgesic agent [31].

To access the toxicity of *N. laevis,* the rats were treated intraperitoneally with a single dose of 0.09 – 0.64 mg/kg of *N. laevis* soluble fraction after being starved for 18 hours. Intraperitoneal route was chosen because of its sensitivity and rapid results. These ethanolic leaf extracts produced various degree of toxicity ranging from writhing, decreased respiration, excitation, decreased motor activity, convulsion to mortality (Table 3). The intensities of these effects were proportional to the dose administered. The intraperitoneal LD_{50} for *N. laevis* it was 447.21 mg/kg.

Table 3. Toxicity of *N. laevis* ethanolic leaf extract on albino mice

Groups	Dose (mg/kg)	Average weight of mice (g)	Number of mice per group	Percentage mortality
I	100	21	5	0/3
II	200	22	5	0/3
III	300	22	5	0/3
IV	400	23	5	0/3
V	500	20	5	3/3

$LD_{50} = \sqrt{AB}$

Where A = 400 (Maximum dose producing 0% mortality)

B = 500 (Minimum dose producing 100% mortality)

$LD_{50} = \sqrt{400}\ x\ 500$

LD_{50} = 447.21 mg/kg

Table 4 summarizes the effect of ethanolic leaves extract of *N. laevis* on alloxan-induced diabetic rats. The results showed a significant (p=0.05) reduction in the blood glucose level (BGL) as the time of treatments increases. At 0 hour, the negative control (normal saline) recorded 332.0 ± 19.0 mg/dl, positive control (Glibenclamide) was 340.0 ± 19.9 mg/dl, low dose (308.0 ± 19.2 mg/dl), middle dose (316.0 ± 25.2) while high dose recorded 314.0 ± 7.42 mg/dl. While high dose recorded 314.0 ± 7.42 mg/dl. At 24 hours, the following values were recorded; negative

control (352.0 ± 18.1 mg/dl), positive control (192.0 ± 12.7 mg/dl), low dose (184.0 ± 4.26 mg/dl), middle dose (122.0 ± 3.06 mg/dl) and high dose (120.0 ± 7.94 mg/dl). At the end of the treatments i.e. 14 days, the increase was persistent in the negative control (321.0 ± 9.54 mg/dl), progressive reduction was recorded in the positive control (97.3 ± 7.31 mg/dl), low dose was 139.0 ± 4.91 mg/dl, middle dose (95.3 ± 4.63 mg/dl) while high dose recorded 89.7 ± 2.33 mg/dl. Comparatively, the values recorded at 14 days of constant treatment in positive control, middle dose and high dose were within the same range (89.7 – 97.3 mg/dl) (Table 4).

Table 4. Anti-diabetic effects of ethanol leaves extract of *N. laevis* on alloxan induced diabetic

Dosage (mg/kg)	Fasting Blood glucose level at time interval (mg/dl)									
	Initial	0hour	1hour	2hours	3hours	5hours	7hours	24hours	7days	14days
Control (-ve control)	75±5.57^{a}	332±19.0^{a}	336±19.50^{a}	338±19.30^{a}	340±19.2^{a}	342±18.1^{a}	342±18.5^{a}	352±18.1^{a}	331±14.6^{a}	321±9.54^{a}
Glib. 5 (+ve control)	75.7±3.67^{a}	340±19.9^{a}	321±21.10^{b}	296±21.10^{b}	275±22.8^{b}	224±36.2^{b}	187±39.5^{b}	192±12.7^{b}	158±30.9^{b}	97.3±7.31^{b}
NL 44.72 (LD)	76±5.13^{a}	308±19.2^{b}	294±19.70^{c}	277±20.30^{c}	238±10.1^{c}	220±17.3^{b}	197±17.1^{c}	184±4.26^{c}	153±7.31^{b}	139±4.91^{c}
NL 89.44 (MD)	81.7±6.36^{a}	316±25.2^{c}	300±25.20^{d}	245±8.11^{d}	206±5.69^{d}	165±4.18^{c}	122±2.03^{d}	122±3.06^{d}	105±2.6^{c}	95.3±4.63^{b}
NL 134 (HD)	82.7±7.42^{a}	314±6.64^{c}	293±7.88a^{c}	266±8.65^{e}	234±9.49^{c}	189±9.94^{d}	144±9.39^{e}	120±7.94^{d}	99±2.08^{c}	89.7±2.33^{b}

Values are expressed as Mean ± SEM; Glib = Glibenclamide, HD = High dose, MD = Middle dose, LD = Low dose, *NL* = *N. laevis*, -ve = Negative, +ve = Positive. a-b Means with different superscripts along the same column are significantly different ($p<0.05$).

Induction of diabetes using alloxan has been described as a useful experimental model for studying the effect of hypoglycemic agents [32]. 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 [32]. The untreated diabetic rats had a significantly higher fasting blood glucose level than normal rats that received treatment. This confirms induction of diabetes by alloxan. The results showed a dose-dependent lowering of fasting blood glucose level in diabetic rats treated with different doses of the ethanol extracts of *N. laevis*. This dose-dependent effect compares well with glibenclamide, the reference drug used. Glibenclamide is a standard drug that is routinely used in the treatment of diabetes [33].

It is possible that the ethanolic leaf extracts of the test plants could have induced insulin secretion just like those treated with oral hypoglyceamic drug (glibenclamide). This positive result confirms why traditional medicine practitioners use the leaves of the plants in folk medicine in the treatment of

diabetes. Hence the release of insulin by glibenclamide produces a lowering of hyperglycemia. The extract of *N. laevis* 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. The preliminary identification of alkaloids, glycosides and their co-existence with phenolic acids may explain at least in part some anti-diabetic properties of the extracts. The observed anti-hyperglycemic activity was in agreement with the findings of a study, which showed that ethanol leave extract of *N. laevis* decreased blood glucose level in diabetic rats [34]. However, the presence of flavonoids in the ethanol extracts may account for the observed hypoglycaemic effect since they have been found to stimulate the secretion of insulin [35]. Glibenclamide (an oral hypoglycemic agent) is known to act by enhancing exogenous insulin contribution which thus corrects deficiency in the endogenous insulin created by alloxan.

IV. CONCLUSION

The results from this study showed that ethanolic leaf extract of *N. laevis* contain a number of bioactive constituents such as; alkaloids, tannins, saponins, terpenes, flavonoids and cardiac glycosides. The presence of these biologically active compounds suggest that the plant could serve as potential sources of drugs and their secondary metabolites could exert some biological activities when taken by animals. The results also showed that of *N. laevis* ethanol leaves extract significantly reduced blood glucose of diabetic rats on day 14 compared to diabetic untreated (control) rats. The extracts of *N. laevis* therefore produced a similar reduction in blood sugar level as glibenclamide standard drug. This positive result may explain why some people use the plant for diabetes treatment.

V. ACKNOWLEDGEMENT

The authors wish to thank the Tertiary Education Trust Fund (TETFUND) Abuja-Nigeria for the grant awarded to Eneh, Grace D. O. of the Department of Science Technology, Akwa Ibom State Polytechnic, Nigeria for this research work.

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