

## **SAnti-Diabetic Effect of Ethanolic Flower Extracts of *Newbouldia laevis* (Bignoniaceae) on Blood Glucose Levels of Streptozocin-Induced Diabetic Wistar Rats**

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**Abstract:** This study was undertaken to evaluate the hypoglycemic effect of ethanolic flower extract of *Newbouldia laevis* on blood glucose levels of streptozocin-induced diabetes rats. Three doses of the extract (250, 500 and 1000 mg kg<sup>-1</sup>) were administered intraperitoneally. After 2 h of extract administration there was no significant change in the blood glucose levels in all the three doses of the extract administered. Also after 4, 8 and 24 h of extract administration there was a significant ( $p < 0.05$ ) decrease in the blood glucose levels in doses of the extract administered. The preliminary phytochemical screening revealed the presence of cardiac glycosides, tannins, flavonoids and steroids. The median Lethal Dose (LD<sub>50</sub>) in rats was calculated to be 1264.9 mg kg<sup>-1</sup> body weight. The ethanolic flower extract of *Newbouldia laevis* possess anti-diabetic effect in streptozocin induced in diabetic rats.

**Key words:** *Newbouldia laevis*, hypoglycemic activity, streptozocin, diabetes mellitus, phytochemicals

### INTRODUCTION

Diabetes is a disorder of carbohydrate, fat and protein attributed to a diminished production of insulin or mounting resistance to its action. Chronic hyperglycemic during diabetes causes glycation of the body protein that in turn leads to secondary complications affecting eyes, kidney, nerves and arteries (Kameswara *et al.*, 1999). Along with hyperglycaemia and abnormalities in serum lipids (Virella and Virella, 2003; NCEP, 2002). Diabetes is associated with microvascular and macrovascular complications which are the major causes of morbidity and death in diabetic subjects (Nagappa *et al.*, 2003). It can be manage by exercise, diet and pharmaceutical drugs, which are either too expensive or have undesirable sides effects or contraindications (Serrano, 1990). The search for more effective and safer hypoglycemic agents therefore, has continued to be an area of research of interest (Krishna *et al.*, 2004; Pepato *et al.*, 2003). The World Health Organization has recommended and encouraged the use of alternative therapy especially in countries where access to the conventional treatment of diabetes is not adequate (WHO, 1980).

The plant *Newbouldia levis* (P. Beauv) seem or boundary tree is locally called as Aduruku in Hausa, Ogirisi in Igo and Akoko in Yoruba languages (Hutchinson and Dalziel, 1963) is a medium sizes angiosperm which belongs to the *Bignoniaceae* family. It grows to height of about 7-8m, more usually as a shrub of-3 m, many-stemmed forming clumps of gnarled branches (Arbonnier, 2004; Usman and Osuji, 2007). It is a native to tropical African and grows on moist and a well-drained soil extends from Guinea Savannah to the dense Forest zones.

The present study was designed to test the hypoglycemic effect of ethanolic flower extracts of *Newbouldia laevis* in streptozocin-induced diabetes.

### MATERIALS AND METHODS

**Plant material:** The flowers of *Newbouldia laevis* were collected from Kundingi Village-Samaru Zaria, in the Month of September 2006. The plant specimen was identified by Mal U.A. Gallah of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria, where a voucher specimen was deposited.

**Extract preparation:** The flowers of *Newbouldia levis* was dried under shade for several day and the pulverized into fine powder. About 200 g of the powdered form was extracted exhaustively with 95% (v/v) ethanol in water using continous soxhlet apparatus. The extract was concentrated under reduced pressure to yield a dark green mass which weight 30 g.

**Chemicals used:** All chemicals and drugs were obtained commercially and were of analytical grade.

**Acute toxicity study:** The Lethal Doses (LD<sub>50</sub>) of the plant extract was determined by method of Lorke (1983) using 13 mice. In the first phase rats were divided into 3 groups of 3 rats each and were treated with the ethanolic extract of the plant at doses of 10, 100 and 1000 mg kg<sup>-1</sup> body weight intraperitoneal. They were observed for 24 h for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also treated with the ethanolic extract at doses of 600, 1600, 2900 and 5000 mg kg<sup>-1</sup> bodyweight (*i.p.*). The median Lethal Dose (LD<sub>50</sub>) was calculated using the second phase.

**Phytochemical screening:** The preliminary phytochemical screening of the crude ethanolic extract of *Newbouldia laevis* flower was carried out in order to ascertain the presence of its constituents utilizing standard conventional protocols (Markham, 1982; Harborne, 1993; Sofowora, 1993; Trease and Evans, 2000).

**Animals and induction of diabetes mellitus:** Twenty five Wistar rats of both sexes weighing 120-180 g were used for the study of the effects of ethanolic flower extract of *Newbouldia levis* on the blood glucose levels of the animals. They were kept in standard cages at 25°C and 12 h light/dark condition in the animal room of the Department of Human Physiology, ABU, Zaria. The animals were fed on commercial feeds and were given water *ad libitum*. The animals were fasted from feeds for 12 h before the commencement of each experiment, but were allowed water *ad libitum*. The rats were injected with streptozocin dissolved in citrate buffer pH 4.5 in a dose of 60 mg kg<sup>-1</sup> body weight intraperitoneal. Since Streptozocin is capable of producing fatal hypoglycemia as a result of massive pancreatic release of insulin, the rats were treated with 20% glucose solution intraperitoneally after 6 h (Stanley *et al.*, 2001). They were kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia. After a period of 3 days of streptozocin administration rats with blood glucose levels greater than 200 mg dL<sup>-1</sup> were considered diabetic and used for this research work.

**Experimental design:** The Streptozocin-induced diabetic Wistar rats were randomly assigned into 5 groups (1-5) of five rats (n=5) each as follows, namely

- Group 1-Received normal saline *i.p.*
- Group 2-Received Biphasic Isophane Insulin 6 i.u kg<sup>-1</sup> *i.p.* (Stanley *et al.*, 2001).
- Group 3-Received 250 mg kg<sup>-1</sup> body weight of the ethanolic flower extract *i.p.*
- Group 4-Received 500 mg kg<sup>-1</sup> body weight of the ethanolic flower extract *i.p.*
- Group 5-Received 1000 mg kg<sup>-1</sup> body weight of the ethanolic flower extract *i.p.*

**Determination of blood glucose levels:** All blood samples were collected by cutting the tail-tip of the rats. Blood samples for blood glucose determination were collected from the tail at intervals of 0, 2, 4, 8 and 24 h. Determination of the blood glucose level was done by the glucose-oxidase principle (Beach and Turner, 1958) using the ONE TOUCH Basic (Lifescan, Milpitas, CA) instrument and results were reported as mg dL<sup>-1</sup> (Rheney and Kirk, 2000).

**Statistical analysis:** Blood glucose levels were expressed in mg dL<sup>-1</sup> as mean ± SEM. The data were statistically analyzed using ANOVA with multiply comparisons versus control group. The values of p<0.05 were considered as significant (Duncan *et al.*, 1997).

## RESULTS

**Phytochemical analysis:** Freshly prepared extracts were subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of cardiac and steroidal glycosides, tannins, flavonoids, while phytochemicals such as alkaloids, saponins were absent.

**Acute toxicity study (LD<sub>50</sub>):** The sign of toxicity were first noticed after 2-4 h of extract administration. There was decreased locomotor activity and decreased in sensitivity to touch. Also there was decreased feed intake and prostration after 4 h of extract administration. The median Lethal Dose (LD<sub>50</sub>) in rats was calculated to be 1264.9 mg kg<sup>-1</sup> body weight. Table 1 showed the results of the effects of three doses (250, 500 and 1000 mg kg<sup>-1</sup>) of the ethanolic flower extract of *Newbouldia levis*,

Table 1: Effect of ethanolic flower extract of *Newbouldia laevis* on streptozocin-induced diabetic Wistar rats

Treatment	Blood glucose levels (mg dL <sup>-1</sup> )				
	0 h (mg mg)	2 h (mg mg)	4 h (mg mg)	8 h (mg mg)	24 h (mg mg)
Group 1 Control <sup>(NS)</sup>	273±36.2	315.1±34.7	317.4±27.2	314.4±24.2	312.6±18.9
Group 2 (Insulin 6.i.u kg <sup>-1</sup> )	277.4±21.5 <sup>ns</sup>	238.6±18.1 <sup>ns</sup> (24%)	187.0±5.04 <sup>*</sup> (41%)	163.6±12.3 <sup>*</sup> (48%)	120.6±18.6 <sup>*</sup> (61%)
Group 3 (250mg kg <sup>-1</sup> )	271.4±25.9 <sup>ns</sup>	240.4±22.0 <sup>ns</sup> (24%)	201.6±11.2 <sup>*</sup> (36%)	174.4±5.92 <sup>*</sup> (45%)	133.6±9.64 <sup>*</sup> (57%)
Group 4 (500mg kg <sup>-1</sup> )	277.2±33.7 <sup>ns</sup>	252.2±23.4 <sup>ns</sup> (20%)	223.2±21.1 <sup>*</sup> (30%)	186.0±13.5 <sup>*</sup> (41%)	169.2±10.6 <sup>*</sup> (46%)
Group 5 (1000mg kg <sup>-1</sup> )	273.6±46.0 <sup>ns</sup>	264.2±37.9 <sup>ns</sup> (16%)	245.6±37.6 <sup>ns</sup> (23%)	220.4±33.9 <sup>*</sup> (30%)	207.2±33.0 <sup>*</sup> (34%)

Values are given as mean ± SD for 5 rats in each group, experimental groups are compared with diabetic control. Values are statistically significant at \*p<0.05

<sup>ns</sup>=not significant. Figures in parentheses represent percentage glycemic change

% Glycaemic change =  $\frac{\text{Glucose concentration (2, 4, 8 or 24)} - \text{fasting blood glucose}}{\text{Fasting blood glucose}} \times 100$

Insulin and control groups in streptozocin-induced diabetic Wistar rats. The dose of Insulin and the three doses of the extract did not show any significant change in the blood glucose levels when compared to untreated control after 2 h of extract administration while after 4 h of extract administration there was a significant decrease in the blood glucose level in 250 and 500 mg kg<sup>-1</sup> when compared to control untreated. However, after 8 and 24 h of treatments there was a significant decrease (p<0.05) in the blood glucose levels when compared to untreated control in all the three doses given.

## DISCUSSION

Streptozocin-induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycaemic agents (Szkudelski, 2001). Streptozocin selectively destroyed the pancreatic insulin secreting β-cells, leaving less active cell resulting in a diabetic state (Kamtchouing *et al.*, 1998; Szkudelski, 2001).

Many secondary metabolites participate in a variety of anti-diabetic functions *in vivo* (Kako *et al.*, 1997). The glycemic change in blood glucose levels of diabetic rat at different time intervals after intraperitoneal administration of ethanolic flower extract of *Newbouldia laevis* at the doses of 250, 500 and 1000 mg kg<sup>-1</sup> as showed in (Table 1).

In relation to the diabetes rats that received 250, 500 and 1000 mg kg<sup>-1</sup> bodyweight of ethanolic flower extract of *Newbouldia laevis* there was no significant change in the blood glucose levels when compared to the control untreated group after 2 h of extract administration. In regard to the dose of 250 and 500 mg kg<sup>-1</sup> of the *Newbouldia laevis* it significantly (p<0.05) lowered the blood glucose level when compared to control after 4 h of extract administration with percentage glycemic change of 36 and 30%, respectively. Also in regard to the dose of 1000 mg kg<sup>-1</sup> there was no significant change in the blood

glucose levels. After 8 h of extract administration there was a significant change in the blood glucose level of all the doses given when compared to control with percentage glycemic change of 45, 41 and 30%, respectively. Also, 24 h of extract administration there was a significant change in the blood glucose level of all the doses given when compared to control with percentage glycemic change of 57, 46 and 24%, respectively. In relation to the reference drug biphasic insulin 6.i.u/kg<sup>-1</sup> there was a significant decrease in the blood glucose level when compared to control group. The dose of 250 mg kg<sup>-1</sup> which is the lowest dose was found to be more effective in the glycaemic change than the other two doses of the extract 500 and 1000 mg kg<sup>-1</sup> body weight. The extract might possess Insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic Gluconeogenesis. The phytochemical studies of ethanolic flower extract of *Newbouldia laevis* revealed the presence of flavonoids isolated from the other plant has been found to stimulate secretion or possess an insulin like-effect (Marles and Farnsworth, 1995).

## CONCLUSION

In conclusion, the experiment evidence obtained in the present laboratory animal study indicate that ethanolic flower extract of *Newbouldia laevis* possess anti-diabetic properties which suggest the presence of biologically active components which may be worth further investigation and elucidation.

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