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HYPOGLYCEMIC POTENTIALS OF NEWBOULDIA LAEVIS STEM BARK EXTRACT IN ALLOXAN-INDUCED DIABETIC RATS

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Abstract

This study was carried out to investigate the antidiabetic of the methanol extract of Newbouldia stem bark and to determine its safety and toxicity. Alloxan (150 mg/kg) was administered to albino rats via the oral route. The diabetic rats were then placed in 6 groups, following stabilization of hyperglycemia. The first group was non-diabetic, the second and third group were the diabetic control. The next three groups received, each day, 100, 200 and 400 mg/kg body weight of the methanol extract Newbouldia laevis and the second group received a reference standard, metformin (200mg/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. Blood glucose levels from day 1 to 14 days of treatment increased significantly ($p < 0.05$) in all the treatment groups, except group 3 (positive control) which showed no significant difference compared to the normal control group. This study supports the use of Newbouldia laevis in traditional medicine as well as highlights the need to further explore the potentials of the plant extract as an antidiabetic.

Keywords: Hypoglycemic, Newbouldia laevis, Stem Bark, Extract, Alloxan-Induced, Diabetic Rats

INTRODUCTION

Diabetes mellitus (DM) is one of the most common non-communicable diseases globally. It is either the fourth or fifth leading cause of death in developed and developing countries of the world (Kaviarasan et al., 2010). DM has been reported to be one of the most common chronic disease with 387 million reported cases in 2014 and an expected 600 million more in 2035 (Chang et al., 2014). Thus, DM has become a major global health problem in developed countries and now more evidently in developing countries (Kaur and Meena, 2012). Symptoms of DM include classical hyperglycemia, polyuria, polydypsia, weight loss, sometimes with polyphagia, and blurred vision. DM is classified into Type-1 or Type-2 Diabetes depending on the aetiology (Ezeigbo, 2010). Amongst the different forms of diabetes, type 2 diabetes (T2D) is the most common with 95% prevalence globally (Chen et al., 2020). T2D is caused by impaired insulin action and secretion due to insulin resistance. In order to maintain glucose homeostasis, over secretion of basal insulin by the beta cells occurs which ultimately leads to pancreatic beta cells dysfunction. Several studies have shown that long-term feeding on high fat diet (HFD) may contribute to the development of metabolic syndrome and eventually metabolic related diseases such as diabetes mellitus (Ezuruike and Prieto, 2014).

Although, diet restrictions, exercise and numerous anti-diabetes drugs (e.g. metformin, glibenclamide, vildagliptin, etc.) have been employed in the management of T2D, the morbidity and mortality rates of the disease still remains of public health concern (Abdel-lateif et al., 2016). In spite of the health benefits of the current antidiabetic drugs, each drug has its own range of side effects which may compromise the disease status or even worsen the condition in some cases. Some of the side effects of antidiabetic drugs which may offset their benefits include weight gain, hyperinsulinemia, hypoglycemia, edema and volume expansion (Modi, 2007). Thus, the prevalence of the disease continues to rise worldwide and there is little that could be done to prevent its complications. Therefore, there is the need to search for better antidiabetic remedies.

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 (Jayakumar, 2010). More than 400 plants are being used in different forms for their hypoglycemic effects in treating diabetes (Ezeigbo, 2010). In Nigeria, traditional medicine occupies a unique position in health care delivery, especially among the rural populace. 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 (De-Silva, 1997). 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 antibiotic's family (Lewis et al., 2001). In recent times, quite a number of some plants have been used as herbal medicines due to the presence of phytochemicals in them (Riby, 2006).

Newbouldia leaves is a boundary tree called 'Aduruku' in Hausa, 'Ogirisi' in Igbo and 'Akoko' in Yoruba languages. it 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 meters in the west of its range, but can attain a height of up to 20 meters in the east. It has many stem forming clumps of gnarled branches (Arbonnier, 2004). 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 (Bafor and Sanni, 2009). The leaves of the plant were reported to have hepatoprotective activity (Hassan et al., 2010). The plant has antidiabetic, uterine contractile, antihypertensive, analgesic and anti-inflammatory properties (Tanko et al., 2008).

It was also observed that, in Mexico, N. leavis is well-known in folkloric for its use in controlling hyperglycemia associated with diabetes (Andrede-Cetto and Heinrich, 2005). This inspired Bosha et al., (2019) to examine the effect of methanol extract of the leaves on experimental diabetic rats induced by intraperitoneal injection of 150 mg/kg alloxan monohydrate. It was reported that after 24 hrs of administration, the extract (250 mg/kg) suppressed fasting blood glucose level by 60.2% compared to 51.5% by glibenclamide (2 mg/kg). To investigate the possible mechanism of hypoglycemic properties of the plant´s

leaves, Kolawole et al., (2013) observed that methanol leaves extract significantly reduced postprandial glucose levels and inhibited pancreatic α-amylase activity in diabetic rats at 500 mg/kg (IC50 = 58.7 µg/mL) relative to 92.3 µg/mL of acarbose (50 mg/kg). The authors further assessed the inhibitory effects of the extract on baker's yeast and rat intestinal α-glucosidases and rat pancreatic α-amylase, in vitro. The extract was reported to significantly inhibit both baker's yeast and rat intestinal α-glucosidases with IC50 values of 2.2 µg/mL and 43.5 µg/mL, respectively compared to 3.8 µg/mL and 62.7 µg/mL, respectively by acarbose. These results showed that the extract acts by inhibiting the two enzymes that play the key roles in increasing blood glucose level. Based on the reported antidiabetic activities of the plant leaves, Mbagwu et al., (2021) evaluated the inhibitory effects of ethanol extract of the leaves on α-amylase activity and reported that the extract potently inhibited the enzyme with IC50 value of 102.91 mg/mL. Apigenin isolated from the methanol fraction of the leaves dichloromethanol-methanol extract exhibited antidiabetic property (Osigwe et al., 2017b).

Diabetes we know is a lifelong multifactorial disease with micro- and macro-vascular complications. This has prompted different pharmacological and non-pharmacological therapeutic agents and measures to be implemented to benefit diabetic patients with the aim of enhancing their quality of life (American Diabetes Association Standards of medical care in diabetes, 2011). The currently available treatment for diabetes mainly manages to reduce and regulate glucose metabolism (McCrimmon et al., 2010). The first line of intervention for diabetic patients is to change their lifestyle to a healthier diet and to physical activities (Paulweber et al., 2010).

The administration of insulin is routinely used for type 1 diabetic patients as their pancreatic β- cells are incapable of secreting insulin, and type 2 diabetic patient's due to their inability to respond to circulating insulin (Bodmer et al., 2008). Managing diabetes is also achieved by using some antidiabetic compounds that reduce blood glucose levels. In addition, surgical operations, like bariatric surgery, can help obese patients with their diabetes management if other interventions become difficult to contain the disease and its complications (Catalan et al., 2001).

Managing diabetes is not simple as it requires continuous support, medical attention, and education to patients to prevent serious complications. Sustainable management of diabetes is a global necessity due to the increase in the morbidity rate of the disease.

MATERIALS AND METHODS

Plant Material

Stem bark of Newbouldia laevis were collected from the uncultivated farm land of Wukari Taraba State, Nigeria. The stem bark was taxonomically identified and authenticated in the Department of Plant Science of Modibbo Adama University of Technology, Yola, Nigeria. The stem bark was washed with distilled water before being air-dried for thirty days and then ground into powder by grinding with a mortar and pestle. One hundred and fifty grams (150g) of the powdered stem bark was cold macerated in 500ml of methanol in an Erlenmeyer flask and shaken at one-hour intervals. Then was left to stand at room temperature for 48 hours and filtered using Whatman's filter paper. The extracts were then concentrated to dry using a rotary evaporator and stored frozen until needed.

Breeding of Animals (Albino Rats)

40 male albino rats (weighing between 150-190g.) are used for this experiment. The rats are purchased from the National Veterinary Research Institute, Vom, Plateau State. The rats are maintained under standard laboratory conditions and are allowed free access to standard diet and water ad libitum. They are allowed to acclimatize for 24 hours.

Acute Toxicity Studies

The LD50 was to be carried out using the method of Medinat et al., (2018) with slight modification for oral routes in rats. The method consists of two phases using 24 rats. In the first phase 3 groups of 3 rats each were administered the extracts in doses of 100, 200, and 400 mg/kg body weight orally and were observed for signs of toxicity and death for 24hours. In the second phase, 3 groups each containing 1 rat were administered new doses of the extract: 100, 200 and 400 mg/kg body weight orally, they were also being observed for any sign of toxicity and death for 24 hours.

Induction of Diabetes mellitus

Experimental diabetes was induced in rats, which had fasted for 12 hrs by a single intravenous injection through the tail vein of a freshly prepared solution of alloxan. The rats were allowed to drink 5% glucose solution overnight to overcome drug-induced hypoglycemia. Estimation of fasting blood glucose (FBG) was done 72 hours after injection of alloxan to confirm induction of diabetes and then on the 7th day to investigate the stability of diabetic condition. FBG was estimated by One Touch® glucometer

(Lifescan, Inc. 1995 Milpas, California, USA). Blood sample for the FBG determination was obtained from the tail vein of the rats and those with blood glucose value ≥ 200 mg/dl were selected for the study.

Treatment of Experimental Animals

Rats were divided into a group of non-diabetic and three groups of alloxan- diabetic rats. Each of the four groups consisted of six rats. The grouping was as follows:

Group 1 = non-diabetic control;

Group 2 = diabetic control;

Group 3 = diabetic rats treated with metformin (200mg/kg);

Group 4 = treatment 1 (100mg/kg of extract)

Group 5 = treatment 2 (200mg/kg of extract)

Group 6 = treatment 3 (400mg/kg of extract)

The drugs were administered orally every day for 28 days using a syringe fitted with a sterile cannula. Rats in group 1 and 2 were treated orally with distilled water for the four weeks. On day 29, the rats were euthanized under chloroform vapor. The jugular vein was exposed and cut with a sterile scalpel blade, and rats were bled into specimen bottles. Blood samples were transferred to sterilized centrifuge tubes and allowed to clot at room temperature. The blood samples were centrifuged for 10 min at 1500 rpm. The serum obtained was used for serum insulin assay.

Liver Function Test

The blood samples were taken from the heart of the rats and centrifuged for five minutes at 3000 rpm to prepare the serum for biochemical analysis (Miranda, 2001). The ALT, AST, ALP, Bilirubin, Protein and albumin levels were determined by colorimetric methods.

Statistical Analysis

All data were expressed as mean ± SEM and where applicable, the data were analyzed 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

Table 1: Fasting blood glucose levels.

FBG	**Group 1**	Group 2	Group 3	**Extracts**		
				Group 4	Group 5	Group 6
	Normal Control	**Negative control**	**Positive Control**	**100mg/kg**	**200mg/kg**	**400mg/kg**
Day 1	5.65 ± 0.19[a]	23.78 ± 0.30[c]	6.23 ± 0.47[a]	24.45 ± 0.56[b]	30.24 ± 1.34[e]	28.57 ± 0.81[d]
Day 3	5.76 ± 0.21[a]	24.73 ± 0.58[c]	5.49 ± 0.37[a]	16.83 ± 0.66[b]	25.68 ± 0.31[c]	28.49 ± 1.49[d]
Day 7	5.74 ± 0.16[a]	25.37 ± 1.93[d]	5.41 ± 0.33[a]	18.66 ± 0.67[c]	13.22 ± 0.64[b]	17.19 ± 0.55[c]
Day 14	5.25 ± 0.22[a]	27.07 ± 0.51[d]	4.78 ± 0.18[a]	14.40 ± 0.48[b,c]	11.97 ± 0.21[b]	14.51 ± 0.39[b,c]

Results are expressed as mean ± standard deviation of group results obtained (n=5). FBG =fasting Blood Glucose

Means in the same row having different superscripts are statistically significant (p <0.05).

Concentration of Selected Liver Function Parameters

The results of selected liver function indices showed that AST decreased significantly (p <0.05) in all the treatment groups; ALT increased significantly (p <0.05) in group 4, 5 and 6, and decreased significantly in group 3, while group 2 showed no level of significance compared to normal control; ALP increased significantly (p <0.05) in group 2 and 6, decreased significantly (p <0.05) in group 4, while group 3 and 5 showed no significance compared to normal control. TP increased significantly (p <0.05) in all groups except group 3 which no level of significance; ALB decreased significantly (p <0.05) in group 4, 5 and 6, increased significantly (p <0.05) in group 3, while group 2 showed no level of significance; GLB decreased significantly (p <0.05) in group 3 and 4, while the rest of the groups showed no significant difference. TB increased significantly (p <0.05) in all the treatment groups except group 2 which showed no level of significance; DB increased

significantly in group 3, 4 and 6, decreased in group 2, while group 5 showed no significant difference compared to normal control (group 1); INDB increased significantly ($p < 0.05$) in all the treatment groups except group which showed no significant difference.

Table 2: Liver function parameters

Parameters	Group 1 (Normal control)	Group 2 (negative control)	Group 3 (positive control)	Group 4 (100 mg/kg extract)	Group 5 (200mg/kg xtract)	Group 6 (400mg/kg xtract)
AST (IU/L)	45.09 ± 4.1^{d}	11.90 ± 1.16^{a}	12.00 ± 1.11^{a}	31.53 ± 2.65^{c}	13.92 ± 0.59^{a}	22.62 ± 0.66^{b}
ALT (IU/L)	20.00 ± 0.82^{b}	11 18± 0.85^{b}	15.15 ± 0.24^{a}	25.38 ± 0.82^{c}	28.06 ± 0.34^{c,d}	32.17 ± 2.46^{d}
ALP (IU/L)	80.21 ± 0.88^{b}	225. 80 ± 6.24^{d}	80.69 ± 2.98^{b}	56.01 ± 1.21^{a}	81.56 ± 2.13^{b}	94.86 ± 1.90^{c}
TP (gm/dL)	14.13 ± 1.09^{d}	12.95 ± 0.75^{c}	14.42 ± 0.52^{d}	10.32 ± 0.82^{a,b}	9.76 ± 0.82^{a,b}	8.70 ± 1.16^{a}
ALB (gm/dL)	9.30 ± 0.09^{c}	8.82 ± 0.65^{c}	11. 60 ± 0.26^{d}	7.17 ± 0.29^{b}	5.51 ± 0.20^{a}	4.65 ± 0.27^{a}
GLB (gm/dL)	4.83 ± 0.19^{b,c}	4.13 ± 0.10^{b}	2.82 ± 0.36^{a}	3.15 ± 0.63^{a}	4.25 ± 0.62^{b}	4.15 ± 0.07^{b}
TB (mg/dL)	4.58 ± 0.54^{a}	4.36 ± 0.28^{a}	6.10 ± 0.35^{d}	6.06 ± 0.26^{b,c}	5.53 ± 0.46^{b}	5.88 ± 0.36^{b}
DB (mg/dL)	3.29 ± 0.24^{b}	1.46 ± 0.19^{a}	4.42 ± 0.24^{c}	4.10 ± 0.12^{c}	3.64 ± 0.45^{b,c}	4.57 ± 0.25^{c}
INDB (mg/dL)	1.29 ± 0.30^{a}	2.90 ± 0.08^{c}	1.68 ± 0.11^{b}	1.96 ± 0.14^{b}	1.89 ±0.01^{b}	1.31 ± 0.11^{a}

Results are expressed as mean ± standard deviation of group results obtained (n=5).

Means in the same row having different superscripts are statistically significant ($p < 0.05$). Legend: ALT= Alanine aminotransferase, AST= Aspartate transaminase, ALP= Alkaline phosphatase, TP= Total protein, ALB= Albumin, GLB= Globulin, TB= Total bilirubin, DB= Direct bilirubin, INDB= Indirect bilirubin

DISCUSSION

Results of blood glucose showed that administration of standard drug from day 1 to 14 was able to counteract the effect of alloxan induced diabetes to a level almost the same as normal control. Administration of 100 mg/kg body weight of Newbouldia laevis stem bark

methanolic extract showed hypoglycemic activity in day 3, day 7 and day 14 of treatment, while day one treatment did not show hypoglycemic activity; treatments with 200 mg/kg and 400mg/kg body weight of Newbouldia laevis stem bark methanolic extract showed hypoglycemic activity only in day 7 and day 14 of treatment, while day 1 and day 3 treatments showed hyperglycemic activity. This showed that the hypoglycemic activity of Newbouldia laevis stem bark methanolic extract can only be achieved after treatment for 3 to 14 days. The results of this study is in tandem with the report of Anaduaka et al., (2014) which reported the hypoglycemic activities of stem, roots and leaves extracts of Newbouldia laevis. Result of this present study supports the use of Newbouldia laevis stem bark methanolic extract as an antidiabetic agent.

Results of liver function indices showed that treatments with only 100 mg/kg and 400mg/kg body weight of Newbouldia laevis stem bark methanolic extract were able to restore serum AST activity close to normal control, while the rest of the treatments did not show any AST restoration activity. Induction of diabetes with alloxan did not affect serum ALT activity, treatment with standard drug lowered serum ALT activity, while treatments with 100 mg/kg, 200 mg/kg and 400 mg/kg body weight of Newbouldia laevis stem bark methanolic extract were able to improve serum ALT activities. Induction of diabetes significantly elevated serum ALP activity, while treatments with standard drug, 100 mg/kg, 200 mg/kg and 400 mg/kg body weight of Newbouldia laevis stem bark methanolic extract were able to restore serum ALP levels close to normal. Increased serum enzyme activities indicate cellular leakage and a breakdown of the functional integrity of the liver cell membrane (Moore et al., 1985; Imo et al., 2015). Elevated blood ALP level could be due to increased hepatic synthesis of the enzyme (Gómez, et al., 2020), or due to coronary artery disease according to Johnson et al. (2006); Schoppet and Shanahan (2008), since they encourage vascular calcification via the pyrophosphate pathway. Additionally, a high blood ALP level worsens the prognosis for those with coronary artery disease and raises the chance of death (Xiao et al., 2013; Wannamethee et al., 2013).

Induction of diabetes causes lowering of serum total protein and albumin. Only treatment with standard drug was able to restore serum total protein and albumin almost same to normal, while treatment with all the doses of the extracts did not restore serum total protein and albumin levels. Serum globulin was not affected by treatment with treatments with 200 mg/kg and 400 mg/kg body weight of Newbouldia laevis stem bark methanolic extract, while treatments with standard drug and 100 mg/kg of Newbouldia laevis stem

bark methanolic extract significantly lowered serum globulin. Total bilirubin was significantly elevated in all the treatment groups, treatments with all the doses of the extract and standard drug were able to restore serum levels of direct bilirubin and indirect bilirubin ≥ normal. The low levels of serum indirect bilirubin in all the treatment groups, suggests that bile is been expelled properly by the liver.

CONCLUSION

This study evaluated the antidiabetic and antibacterial activity of methanol extract Newbouldia laevis stem bark in alloxan-induced diabetic rats. The results obtained from this study showed that methanolic extract of Newbouldia laevis stem bark is effective against alloxan induced diabetes. The antidiabetic activity was recorded in day 3 to day 14 of treatment. Administrations of Newbouldia laevis stem bark extract showed hepatoprotective and nephroprotective activities after treatment for 14 days.

The potentials displayed in lowering blood glucose of alloxan-induced diabetic rats and restoration of levels of biochemical indices (such as AST, ALT, ALP, Urea, Sodium etc.), support the utilization Newbouldia laevis stem bark in management of diabetes, and liver disorders.

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Conflict of Interest: None Declared

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