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Effect of Flavonoid Fraction of *Newbouldia Laevis* Leaf on Blood Sugar Level and Some Biomarkers of Cellular Toxicity of Alloxan Induced Diabetes in Albino Rats.

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ABSTRACT

Newbouldia laevis leaves is used extensively for its medicinal value for the treatment of diabetes mellitus in Nigeria. This study investigated the effect of flavonoid fraction of *Newbouldia laevis* leave on blood sugar levels, liver function markers and antioxidant alloxan induced diabetes in rats. Diabetes was induced by intraperitoneal injection of Alloxan monohydrate (130 mg/kg b w) to rats. The rats were randomly divided into the following groups (n = 6). Group I (normal control) received distilled water, group II received 5 mg/kg b w glibenclamide, Group III diabetic untreated control (negative control), group IV, V and VI received 200, 400 and 600 mg/kg b w of the flavonoids fraction of Newbouldia laevis, respectively orally once daily for 14 days. The results showed significant reduction ($p<0.05$) in the fasting blood sugar levels in alloxan-induced diabetic treated groups in a dose dependent manner. The flavonoid fraction significantly (<0.05) decreased AST, ALT, ALP level of the treated groups. The untreated diabetic rats were subjected to oxidative stress as indicated by significantly abnormal activity of the scavenging enzymes (superoxide dismutase and high Malondialdehyde) compared with healthy and treated rats. The outcome of the results obtained in this study provides the scientific rationale for the use of *Newbouldia laevis* as anti-diabetic agent.

Keyword: Diabetes, Flavonoid, biomarkers, amelioration, medicinal plants

INTRODUCTION

Diabetes is a well-known metabolic disorder characterized by chronic alterations in carbohydrate, fat and protein metabolisms. This disorder mostly manifest from the secretion of insufficient or dysfunctional endogenous insulin which is secreted by the beta-pancreatic cells. Although the development of diabetes has been reported to be hereditable. A type-2 diabesity model is considered apt for investigating new leads with antidiabetic potentials. Since the human condition of the disease is characterized by insulin resistance and/or the inability of the beta-cells to sufficiently compensate, it is expected therefore that animal models should also include these features as well (Anaduaka et al., 2014).

The practice of traditional medicine is as old as origin of man. The use of plants in traditional medicine referred to as herbalism or simply botanical medicine (Edeoga *et al.,* 2005) falls outside the mainstream of the Western or Orthodox medicine. It has been estimated that two third of the word population (mainly in the developing countries) reply on traditional medicine as their primary form of health care (Cacereves *et al.,* 1990). The use of traditional medicine cannot fade out in the treatment and management of diseases in African continent and this could be attributed to socio-cultural and socio-economic life styles; lack of basic health care and qualified personnel (Elujoba *et al.,* 2005). Plants contain active components such as anthraquinones, flavonoids, glycosides, saponins, and tannins etc which possess medical properties that are harnessed for the treatment of different diseases. The active ingredients for a vast number of pharmaceutically derived medications contain components originating from phytochemicals in plants. These substances that contain the healing property are known as the active principles and are found to differ from plant to plant. Among these plants are the vegetables whose part(s) are eaten as supporting food or main dishes and which could be aromatic, bitter or tasteless.

Worldwide, more than 171 million people suffer from diabetes, making this one of the most common. The countries with the largest number of diabetics are India, China, and United states (American Diabetes Association, 2007). In the past decades, research has been focused on scientific evaluation of traditional drugs of plant origin, and screening of more effective and safe hypoglycemic agents has continued to be a quarrying domain (Ndenecho, 2009).

A very large area of Nigeria ecological zones is populated with many plant species which have found their usefulness either directly or indirectly for humans. The medicinal values of many of these plants cannot be over emphasized in the light of oral traditions and folklores from the distant past that have continued to extol the healing virtues of these plants and their extracts. Generally, the active principles found in *Newbouldia leaves* can be extracted and used in different forms which include infusions, syrups, concoctions, decoctions, infused oils, essential oils, ointments and cream (Kutete *et al.,* 2007)

in the treatment/ management and prevention of some diseases. Plants have been used medicinally in all civilization. Despite the effectiveness of chemically synthesized medicine, screening for plant drugs will continue for the development of new pharmaceuticals to resolve both old and new health problems. The plant kingdom offers a wide range of natural antioxidants and medicinal values (Lewis and Manony, 1977). Dietary plants with proven antioxidant properties may function as a direct anti-radical chain breaker of free radical propagation, interaction with transition metals, and inhibition of reactive oxygen species (ROS) generating enzymes.

Newbouldia leaves is one of such medicinal plants whose medicinal values have stood the test of time. *Newbouldia leaves* (*Bignonicaceae*) is commonly known as African Border tree or boundary treet (Gbile and Adesina, 1986). It is called "Aduruku" in Hausa; "Ogirisi" in Igbo; "Ikhimi" in Edo and "Akoko" in Yoruba languages. It grows to a height of about 7.8 (up to 15 metres), more usually a shrub of 2-3 metres, many-stemmed forming clumps of gnarled branches. It is easily recognized by its short branches, coarsely toothed leaflets and purple and white flowers. *Newbouldia leaves* are native to tropical Africa and grow from Guinea Savannahs to dense forests, or moist and well-drained soils (Burkill, 1984). One remarkable thing about this plant is that it hardly dies hence it is used to indicate boundary marks among the Igbo people of South Eastern Nigeria (Gill, 1992).

This study was designed to test the effect of flavonoid fraction of *Newbouldia laevis* leaves on the blood sugar level, glycated hemoglobin and biomarkers of cellular toxicity in alloxan induced diabetic rats.

MATERIALS AND METHODS

Collection of Plant Leave

The leaves of *Newbouldia laevis* were collected from Umuerim Area of Owerri City, Nigeria, in the month of July 2019. The plant was identified by Dr C.N Duru from the Department of Microbiology, Federal university of technology Owerri, Imo state. Botanical authentication of the plant was confirmed at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria where a voucher specimen (No FHI107753) was deposited for future reference.

Chemicals and Reagents

The chemicals and reagents used were of analytical grade and include: Absolute methanol, Petroleum ether, Diethyl ether, Ethyl acetate, Alloxan monohydrate

Preparation and Extraction Flavonoid Fraction

The dried sample was separated by soxhlet extraction by Subamanian and Nagarajan (1969) method. The leaves were washed, shade dried and grinded into coaxed form and soak with methanol for 48 hours. Mixture was then filtered; the filtrate was evaporated to dryness (paste) at 65C in a rotary evaporator to extract flavonoid from the sample. The dried extract was kept in a clean, cool and dried container. The extract was collected and was concentrated and re-extracted with petroleum ether (40-65c, fra 1), diethyl ether (fra ii) and diethyl acetate (fra iii) in succession. the mixture was filtered and the filtrate was extracted with diethyl acetate in a separating funnel. The ethyl acetate layer (upper layer) was dried in water bath for flavonoid bound.

Experimental Animals

Thirty-six (36) albino rats of the wilstar strain aged 10-12 weeks weighing (80-120g) of both sexes was purchased from relief market Owerri. They were housed in a standard transparent cage with wheat husk bedding, renewed every 24 hours. They were kept under controlled room temperature and humidity in a 12 hour light-dark cycle. Animals were acclimatized for 2 weeks to laboratory conditions before starting the experiment. The rats were given standard laboratory diet and water *ad libitum.* Care of experimental animals was taken as per the guidelines given by committee for the purpose control and supervision on experiments on animals (CPCSEA), Ministry of Environment and Forests (Animal welfare division), Umuahia Abia State, Nigeria.

Experimental Design

Total 36 albino rats were allocated randomly into 6 groups (6 animals per group). After induction of diabetes, treatment began as follows;

Group 1 normal rats (administered with 0.2ml distilled water).

2 Positive controls (diabetic rats treated with 5mg/kg gilbenclamide)

3 Negative control: diabetes rats only

4 Diabetic rats treated with 200mg/kg of extract

5 Diabetic rats treated with 400mg/kg of extract

6 Diabetic rats treated with 600mg/kg of extract

Note: Diabetes was induced using 140mg/kg of alloxan.

Determination of Blood Glucose Level

The study lasted for 14 days. Blood was collected from the tail of the rats to determine the blood glucose level on the 4th and 9th day, On the 14th day after the rat were sacrificed, the blood was collected into a fluoride oxalate anti-coagulated bottle to determine the glucose level.

Biochemical Estimations

Serum ALT, ALP, AST activity were estimated by the method of Reitman and Frankel (1957). Superoxide dismutase activity was assayed by the method of Arthur and Boyne (1985) as adopted in Randox kit. Malondialdehyde activity was assayed by the method of Sinha (1972).

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 instant software, version 2.05a. $p<0.05$ was taken as indicative of significant difference.

RESULTS AND DISCUSSION

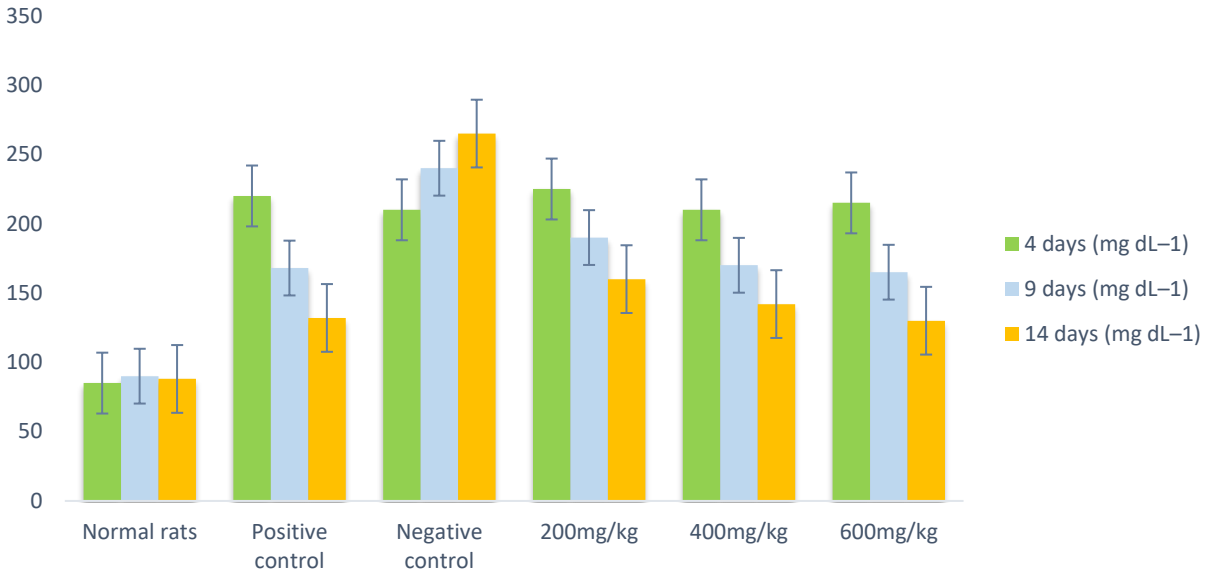

Fig 1: Shows the Effect of the flavonoid fraction of *Newbouldia laevis* leave on the blood sugar level of alloxan induced diabetic rat.

The fraction produced a significant anti-diabetic activity with significant reduction in the blood glucose compared to negative control. Treatment of the diabetic rats with gilbenclamide (5 mg/kg b.w) also showed significant reduction in their blood glucose.

Table 1: Effect of the flavonoid fraction of *Newbouldia laevis* leave on liver function parameters of alloxan induced experimental rats

GROUP	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal rats	83.54±2.73c	91.79± 1.50b	111.38±2.40
Positive control	68.22 ± 2.73a	71.99± 1.50a	93.29± 2.40
Negative control	105.43±2.73d	111.18± .50d	136.46±2.40
200mg/kg	81.23±.73 c	84.17± 1.50c	103.12±2.40b
400mg/kg	73.67±2.73b	89.55±1.50b	95.50±2.40
600mg/kg	66.55±2.73a	71.85±1.50a	92.73± 2.40a

Values are mean±SD (n=6). Alphabet with different superscript differs significantly p(<0.05).

The result in Table 1 showed that the negative control (untreated diabetic rats) had significant ($p<0.05$) increase in the activity of ALP, AST, ALT but the administration of the different doses of the administration of the different doses of the extracts resulted in significant decrease in ALP, AST and ALT activity.

Table 2: Effect of the flavonoid fraction of *Newbouldia laevis* leave on endogenous antioxidants of alloxan induced experimental rats

GROUP	SOD (U/L)	MDA (ng/ml)
Normal rats	$45.14±0.73^{a}$	$61.79± 6.50^{b}$
Positive control	$68.22 ± 2.73^{c}$	$51.99± 5.20^{a}$
Negative control	$51.23±1.73^{b}$	$90.18± 3.50^{d}$
200mg/kg	$61.23±0.73^{d}$	$64.17± 4.70^{c}$
400mg/kg	$63.67±2.7^{cd}$	$59.55±2.50^{b}$
600mg/kg	$66.55±2.73^{c}$	$51.85±1.10^{a}$

Values are mean±SD (n=6). Alphabet with different superscript differs significantly (p <0.05).

The result in Table 2 showed significant (p<0.05) increase in the MDA level of the negative control (untreated diabetic rats) compared to the fraction treated groups and positive control. The administration of the different doses of the fraction resulted in significant (p<0.05) reduced the MDA level.

DISCUSSION

Diabetes mellitus is a metabolic disorder affecting about 5-10% of the world's population (Patel *et al*., 2012; Xie *et al*., 2011). There in presently no known cure for diabetes (Mukherjee *et al*., 2006). Meanwhile, more than 400 plant species have demonstrated hypoglycemic activity (Colca, 2006; De Sousa *et al*., 2004; Patel *et al.*, 2012; Verspohl, 2002). This motivates further research efforts to discover new antidiabetic agents from natural plants. The effects of flavonoid fraction of *Newbouldia laevis* leave extract on blood glucose levels, liver function indices, endogenous antioxidants and lipid profile in diabetic rats were evaluated in this study. Glucose lowering effect flavonoid fraction of *Newbouldia laevis* extract might be due to stimulation of surviving β-cells of islets of Langerhans leading to more insulin release (Jaganjac *et al.*, 2013). Alloxan toxicity could lead to destructive changes in the hepatocytes related liver injury. An alterations in the serum levels of liver enzymes is associated with hepatic injury which could the reason for the elevated liver marker enzymes in negative control rats. An increase in ALP activity can be a sign that the membrane integrity of the cell is being lost due to peroxidation. Scientific reports have shown that ALT participates in gluconeogenesis and its transcription is suppressed by insulin and that increased activity is therefore an indication of impairment of insulin signaling and not hepatocyte injury (Miikue-Yobe et al., 2015).

An obvious rise in the levels of serum liver function marker enzymes was evident in the diabetes untreated (negative) control. The outcome of the study is in line with several studies that have reported similar elevation in the activities of serum liver marker enzymes of alloxan induced diabetes in rats (Rawi et al., 2011; Gometi et al., 2014). The treatment of alloxan induced diabetes with flavonoid fraction of Newbouldia laevis or glibenclamide significantly reduced the elevated transaminases and ALP activities. The reduction may be accredited to hepatoprotective and antioxidant activity of flavonoid fraction of Newbouldia laevis. Antioxidants are known to reduce the development of chemically induced liver damage (Chen and Yen, 2007).

Diabetes is considered by low plasma levels of both enzymatic and non- enzymatic antioxidant defenses which make the cells of diabetic patients vulnerable to oxidative stress. There is a link between depletion of antioxidant level and Diabetes and administration of antioxidants to compensate the depletion has been shown to improve diabetes and prevent its complications (Antu et al., 2014). It is hypothesized that under severe oxidative stress, there is high reactive species production which may result in the depletion of protective physiological moieties. Increase in lipid peroxidation during diabetes is as a result of inefficient or overwhelmed antioxidant system due to free radical generation. Many researchers have reported decreased activities of antioxidant enzymes and non-enzymatic antioxidants during experimental diabetes which correlates with hyperglycemia (Anaduaka et al., 2014; Abdulazeez et al., 2013). The high level of the MDA of negative control rats is taken as direct evidence for oxidative stress which may have overwhelmed the antioxidant system due to free radical generation. Administration of flavonoid fraction of Newbouldia laevis significantly reduced the MDA level in diabetic treated rats. The outcome suggests improved functionality of the antioxidant system of the diabetic treated rats which may be due to the effect of the flavonoids antioxidants present in the N. laevis.

Conclusion

There alloxan monohydrate induced elevation in blood glucose level and some biomarkers of cellular toxicity (AST, ALT, ALP, SOD and MDA) but the flavonoid fraction of *Newbouldia laevis* ameliorated the alteration therefore the flavonoid fraction of *Newbouldia laevis* leave could be used as anti-diabetic agent.

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REFERENCES

Abdulazeez, M.A., Ibrahim, K., Bulus, K., Babvoshia, H.B. and Abdullahi, Y. (2013) Effect of Combined Use of Ocimum gratissimum and Vernonia amygdalina Extract on the Activity of Angiotensin Converting Enzyme, Hypolipidemic and Antioxidant Parameters in Streptozocin-Induced Diabetic Rats. African Journal of Biochemistry Research, 7, 165-173.

American Diabetes Association (2007).Diagnosis and classification of diabetes

Anaduaka, E.G., Ogugua, V.N., Chidozie, V. and Okonkwo, C.C. (2014) Ethanol Extracts of Newbouldia laevis Stem and Leaves Modulate Serum Marker Enzymes and Antioxidant Enzymes Activities in Diabetic Rats. African Journal of Biotechnology, 13, 2265-2272. https://doi.org/10.5897/AJB2014.13753

Antu, K.A., Riya, M.P., Mishra, A., Anilkumar, K.S., Chandrakanth, C.K., Tamrakar, A.K., Srivastava, A.K. and Raghu, K.G. (2014) Antidiabetic Property of Symplocos cochinchinensis Is Mediated by Inhibition of Alpha Glucosidase and Enhanced Insulin Sensitivity. PLoS ONE, 9, e105829. https://doi.org/10.1371/journal.pone.0105829

Bhandari, U.N., Jain, M.N. & Ansari, K.K.P. (2008). Beneficial effect of Embelia ribes ethanolic extract on blood pressure and glycosylated hemoglobin in streptozotocin-induced diabetes in rats, *Fitoterapia*, 79(5), 35135.

Burkil, H.M. (1984). The useful plants of west tropical Africa. *Royal Botanical Garden Kew*, 2:458-463.

Cacereves, A, Cano, O., Agular, L. & Samayoa B. (1990). Plants used in Guatemala for the treatment of of gastrointestinal disorder 1. Screening of 84 plants against enterobacteria. *Journal of Ethnopharmacology*, 30(1): 55-7.

Carlsson, J., Gullstrand, C., Ludvigsson, J., Lundström, I. & Winquist, F. (2008). Detection of global glycosylation changes of serum proteins in type 1 diabetes using a lectin panel and multivariate data analysis. *Talanta*, 76 (2): 333-7.

Chen, H.-Y. and Yen, G.-C. (2007) Antioxidant Activity and Free Radical Scavenging Capacity of Extracts from Guava (P. guajava L.) Leaves. Food Chemistry, 101, 686-694.

Colca, J.R. (2006). Insulin sensitizers may prevent metabolic inflammation. *Biochem. Pharmacol.*, 72: 125-131.

De-Sousa, E., Zanatta, L., Seifriz, I., Creczynski-Pasa, T.B., Pizzolatti, M.G., Szpoganicz, B. & Silva, F.R.M.B. (2004). Hypoglycemic effect and antioxidant potential of kaempferol-3,7-O-(α)-dirhamnoside from Bauhinia forcata leaves. *J. Nat. Prod.*, 67: 829-832.

Edeoga, H.O., Okwu, D.E. & Mbaebie, B.O. (2005). Phytochemical constituents of some nigeria medicinal plants. *African Journal of Biotechnology*, 4(7) 685-688.

Elujoba, A.A., Odeleye O.M. & Ogunyemi, C.M. (2005). Traditional medicine development for medical and dental primary health care delivery system in Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, 2(1): 46-6.

Gbile, Z.O. & Adesina, M.O. (1986). Ethnobotany. Taxonomy and conservation of medicinal plants: In: the state of medicinal plant research in Nigeria. Sofowara A.O. (ed). p. 19.

Gill, L.S (1992). Ethnomedical uses of plants in Nigeria. University of Benin Press, Benin City. Pp. 174-175.

Gometi, S.A., Ogugua, V.N., Odo, C.E. and Joshua, P.E. (2014) Effects of Some Antidiabetic Plants on the Hepatic Marker Enzymes of Diabetic Rats. African Journal of Biotechnology, 13, 905-909. https://doi.org/10.5897/AJB2013.13329

Jaganjac, M., Tirosh, O., Cohen, G., Sasson, S. & Zarkovic, N. (2013). 'Reactive aldehydes – Second messengers of free radicals in diabetes mellitus', *Free Radical Research*, 47(1), 39–48.

Koga, M., Murai, J., Morita, S., Saito, H. & Kasayama, S. (2013). Comparison of annual variability in HbA1c and glycated albumin in patients with type 1 vs. type 2 diabetes mellitus. *J. Diab. Complicat*, 2: 1225.

Kutete V., Eyong K.O., Folefoc G.N., Beng V.P., Hussaian, H., Krohn, K. and Nkengfack A.E. (2007). Antimicrobial activity of the methanolic extract and of the chemical constituents isolated from newbouldia laevis. Pharmazie, 62(7): 552-556.

Lewis, W.H. & Manony, P.F.E. (1977). Medical botany: plants affecting man's health. John Wiley and sons. New York, USA. P. 240

Miikue-Yobe, T.F.B., Uwakwe, A.A. and Akaninwuor, J.O. (2015) Effect of Aqueous Leaf Extract of Heinisia crinata on Hematological and Some Biochemical Indices of Toxicity in Streptozocin Induced Diabetic Rats. International Journal of Innovation Science and Research, 2, 116-126.

Mukherjee, P.K., Maiti, K., Mukherjee, K. & Houghton, P.J. (2006). Leads from Indian medicinal plants with hypoglycemic potentials. *J. Ethnopharmacol*., 106: 1-28.

Ndencho, E.N. (2009). Herbalism and resources for the development of ethnopharmacology in mount Cameroon region. *African Journal of Pharmacy and Pharmacology*, 3(3): 78-86.

Patel, D.K., Prasad, S.K., Kumar, R. & Hemalatha, S. (2012). An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian. Pac. J. Trop. Biomed.*, 2: 320-330.

Rawi, S.M., Mourad, I.M. and Sayed, D.A. (2011) Biochemical Changes in Experimental Diabetes before and after Treatment with Mangifera indica and Psidium guava Extracts. International Journal of Pharmacy and Biomedical Sciences, 2, 29-41.

Verspohl, E.J. (2002). Recommended testing in diabetes research. *Planta Medica*, 68: 581-590.

Xie, X., Meng, X., Zhou, X., Shu, X. & Kong, H. (2011). Research on therapeutic effect and hemorrheology change of berberine in new diagnosed patients with type 2 diabetes combining nonalcoholic fatty liver disease. *China J. Chin. Mater. Med.*, 36: 3032-3035.