Original Research Article

Anti-diabetic Effect of the Methanolic Leaf Extract of *Axonopus Compressus* (P. Beauv) in Alloxan Induced Diabetic Rats

ABSTRACT

Aims: This study is a preliminary step in investigating the anti-diabetic effect of the methanolic extract of the leaf of *Axonopus Compressus (P. Beaux)* in alloxan-induced diabetic rats.

Study design/Methodology: Intraperitoneal (i.p.) injection of alloxan monohydrate at the dose of 180mg/kg b.w was carried out to induce diabetes in the rat models. Different doses (250, 500 and 1000mg/kg b.w.) of the extract was administered per oral through gastric gavage to the alloxan induced diabetic rats, and 2mg/kg b.w. of glibenclamide was used as the standard drug, and only tween 20 solution (10 ml/kg) given to the negative control group. AccuCheck Active® (an auto-analyzer) glucose kit was used to assay the effects of the P. *Beaux* extract and the standard drug on the fasting blood glucose level at 0, 1, 3 and 6 h using the blood collected through the snip made in the tail of the rats.

Results: the different doses of 250, 500 and 1000 mg/kg b.w. produced a significant ($p < 0.05$) reduction in the blood glucose level of the alloxan-induced diabetic animal models in the degree of 31.5%, 19.8% and 24.5% respectively, compared to the negative control group and this was time dependent (at the end of the 6th hour, although a decrease in the blood glucose was also noticed at the 1st, 3rd hours). 2 mg/kg glibenclamide decreased the blood glucose levels by 69.9% while the blood glucose level of the negative control group increased by 15.2% at the 6th hour.

Conclusion: it is obvious form the above findings that that Axonopus compressus deserves further investigations into the active ingredients and structural allucidation to validate the anti-diabetic property it possess.

Keywords*: Axonopus compresus, Alloxan, Diabetes, glibenclamide*

1. INTRODUCTION

A quick review of evaluations and projections by the World Health Organization suggests that, in 2014 the global prevalence of diabetes had an estimate of 9% among adults (18 years and above) [1]. In 2012, about 1.5 million deaths were estimated to be directly caused by diabetes, with over 80% of those deaths occurring in the low- and middle-income countries [2], and that diabetes will rank the 7th leading cause of death in 2030 [3]. Diabetes is a major degenerative disease in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders [4]. However it is the most common endocrine disorder that impairs glucose homeostasis resulting in severe diabetic complications including retinopathy, nephropathy and also causing neurological disorders due to perturbation in glucose uptake [5]. It is a chronic disease characterized by high blood glucose levels due to inability of the beta cells of the islets of Langerhans in the pancreas to produce insulin, leading to insulin deficiency or as a result of defective responsiveness of insulin receptors at the body tissues to insulin. Currently available therapy for diabetes include insulin and various oral antidiabetic agents such as sulphonylureas, metformin, α-glucosidase inhibitors, and

troglitazone [6]. These drugs are used as mono-therapy or in combination to achieve better glycemic control. The use of the above oral agents causes a number of serious adverse effects which includes digestive disorders (nausea, vomiting, cholestase), blood disorders (hemolytic anemia), and hypoglycemia. As a consequence there continues to be the high demand for new oral anti-diabetic drug with little or no side effect. Plants are well known in traditional herbal medicine for their hypoglycemic activities and available literatures indicate that there are more than 800 plant species showing hypoglycemic activity [7]. There has been increasing demand for the use of plant products with anti-diabetic activity due to its low cost, easy availability and less side effects. Therefore, plant materials are continuously scrutinized and explored for their effects as hypoglycemic agents. *Axonopus compressus (P.Beaux)* also kown as carpet grass is a plant that is widely spread in most parts of Nigeria and used as forage for animals. Also it is been found that *Axonopus compressus (P.Beaux)* is prescribed to common cold and diabetic patient in folklore medicine, necessitating the idea that *Axonopus Compressus* may have anti-diabetic property hence this property shall be investigated by carrying out this study.

2. MATERIAL AND METHODS / METHODOLOGY

Fresh leaves of Axonopus compresus were collected from natural habitat in Micheal Okpara University of Agriculture Umudike in the month of June 2010. Their botanical identity was confirmed by Dr Dike of forestry department, college of Natural Resources and Enviromental management, Micheal okpara University of Agriculture Umudike Nigeria. The leaves were washed with distilled water without squeezing to remove debris and dust particles. The leaves were dried at room temperature and they were pulverized with manual blender. 92.35 grams of the powdered leaves was cold macerated in 80% methanol for 48 hours with intermittent shaking every two hours. The extract material was filtered with Whatmann filter papers No 1 into a weighed beaker. The obtained crude extract was stored in a beaker in a refrigerator below 10^{o}c until time of use. Adult male wistar albino rats (120 ±20g) and mature albino mice of both sexes (22±6g) were used throughout the experiments. The animals were procured from the laboratory animal unit of faculty of veterinary Medicine, University of Nigeria, Nsukka. The animals were allowed to acclimatize to the environment for a period of 7 days before the start of the experiment. Standard environmental conditions such as temperature (28±1c) and relative humidity (46 ± 6%) with 12 hour light-dark circle and adequate ventilation were maintained in the animal house. The animals were kept in plastic cages and commercial pelleted feed (Vital Feed ® Nigeria). Ethical conditions governing the conducts of the experiment with life animals as stipulated by Ward and Elsea [8] were strictly observed.

2.1 Grouping of the Animals

Twenty-five adult male wistar albino rats were weighed using citizen electronic weighing balance and randomly grouped into five groups (A –E) of five rats per group.

2.2 Anti-Diabetic Study

The study was carried out using the method described by Venogopal *et al*, [9] with slight modifications. The fasting blood glucose level of the adult albino rats were measured with an autoanalyzer (AccuCheck Active (®)) glucose kit after an overnight fast with water made available to them. Diabetes was then induced in the rats by single intraperitoneal injection of alloxan monohydrate dissolved in distilled water at the dose of 180 mg/kg b.w. The fasting blood glucose level of each of the rats were checked from the 5^{th} day. After 8 days, animals with fasting blood glucose levels of 250 mg/dl and above were considered to be diabetic and were used for the study.

2.3 Study Design

The rats were randomly regrouped into five groups of five rats each.

Group A – served as the negative control and received tween 20 solution (solvent used to dissolve the extract) 10 ml/kg..

Group B – received the extract at the dose of 250 mg/kg b.w.

Group C – received the extract at the dose of 500 mg/kg b.w.

Group D – received the extract at the dose of 1000 mg/kg b.w.

Group E – served as the positive control and received glibenclamide the standard reference drug at the dose of 2 mg/kg b.w.

2.4 Fasting Blood Sugar Test

Blood samples from the tail snip was used to measure the blood glucose levels using AccuCheck Active (®)autoanalyzer and glucose test strips (GCO1465997, Roche Diagnostic Mannheim, Germany). The

fasting blood glucose level of the rats were determined at zero hour before oral administration of drug and extracts and then determined at 1, 3 and 6 hours in each case after drug and extract administration.

2.5 Statistical Analysis

Results were presented as mean ± standard error of mean (SEM) and the statistical analysis was done using one way analysis of variance (ANOVA). The differences between the mean were tested using Post Hoc LSD. A p-value of $p < 0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSION

Table 1: Mean blood glucose levels during a preliminary anti-diabetic study of the leaf extracts of *axonopus compresus* in alloxan induced diabetic rats.

	Fasting blood glucose level mg/dl			
Group Treatment	0 hour	1 hour	3 hour	6 hour
A) Alloxan + Tween 20	393.0±5.7	457.6±6.2	452.2±5.7	463.6±5.5
B) Alloxan + ACE (250mg/kg)	331.6±5.9	271.0±4.3	265.2±4.9*	227.2±3.4*
C) Alloxan + ACE (500mg/kg)	430.2±5.7	379.6±4.2	356.2±4.1	255.4±6.5*
D) Alloxan + ACE (1000mg/kg)	342.2±5.0	268.4±4.4	298.0±5.0*	258.4±4.4*
E) Alloxan + Glibenclamide	308.2±1.8	168.8±1.4*	116.0±0.7*	92.6±1.3*

Values are postprandial blood glucose levels in each group expressed as Mean ± SEM; n = 5 animals in each group; $P < 0.05$ is considered significant when compared with the untreated alloxan induced diabetic rats in control group (using one way analysis of variance).

Alloxan is one of the usual substances used for the induction of type 1 diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas [10]. It causes a massive reduction in insulin release by the destruction of beta cells of the islets of Langerhans, thereby inducing hyperglycemia which is characteristically similar to type 1 diabetes in human [11]. In the present study, hypoglycemic activity of the methanolic extract of *A. compresus* leaves was evaluated in alloxan-induced diabetic rats by testing its effect on fasting blood glucose level using Accu-check active ® auto analyzer glucometer kit. The extract was given at the doses of 250, 500, and 1000 mg/kg b.w. to the alloxan induced diabetic rats while glibenclamide (2mg/kg) was used as standard reference drug and the negative group was given only the tween 20 (solvent) solution at the dose of 10 ml/kg after induction of diabetes. From the results of this work, methanolic leaf extract of *Axonopus compressus* at all the doses (250, 500 and 1000 mg/kg) used caused a respective time dependent and significant ($p < 0.0001$) reduction (by 31.5%, 19.8% and 24.5%) of the blood glucose levels in the diabetic rats when compared to the negative control group at the 6th hour. Glibenclamide, the reference drug used in carrying out this study produced a better significant reduction in the fasting blood glucose level of the alloxan induced diabetic rats when compared to the extract. Glibenclamide belongs to the class of oral hypoglycemic agents called sulphonylureas. Sulphonylureas functions by inhibiting ATP-sensitive K+ channels leading to a reduced efflux of potassium concentration. This will create a sufficient cellular depolarization which will cause the opening of the voltage-dependent calcium channels. It is the increase in intracellular Ca^{2+} that causes insulin secretion [12]. Sulphonylureas also functions to inhibit glucagon secretion and sensitize target tissues to the action of insulin [12]. Plant extract that reduces blood sugar level has been suggested to have an antidiabetic activity and could be acting like a drug [13, 14]. The result of this study shows that the extract reduced the fasting blood glucose level of the alloxan induced diabetic rats. This suggest that *A. compressus* extract could stimulate insulin secretion from the remnant beta-cells or from

regeneration beta-cells like the reference drug. According to J. C. harborne 1998 and Arika et al, 2015 [15,16], plants are known to possess various phytochemicals which are potent in ameliorating various diseases and infections such as diabetes so one could suggest that the hypoglycermic activity of the extract could be as a result of a synergistic effect of the phytochemicals inherent in the plant. Also mineral elements such as Magnesium, potassium, Calcium, Manganese, Iron, Zinc, Chromium, Cupper, and Vanadium have been implicated to contribute to the antidiabetic effect of most antidiabetic medicinal plants due to their function in most metabolic pathways of the body [17], so I could say that the antidiabetic properties of *Axonopus compressus* could be as a result of the mineral elements contained in them. Moreso, the reduction in the fasting blood glucose level in alloxan- induced diabetic rats in this study could suggest that the extract might have worked via several mechanisms such as slowing down absorption of sugar from the gut, decreasing the release of glucose by the liver, or increasing glucose uptake by the fat and muscle cells [18] [19].

CONCLUSION

This proposed mechanism of action may await further studies for its authentication. Also, an estimation of insulin level, insulin receptor and other biochemical parameters can give an insight into the mechanism of the anti-diabetic activity exhibited by the extract. However, the findings suggest that *Axonopus compressus* may possess antidiabetic property.

REFERENCES

1. Global status report on noncommunicable diseases 2014. Geneva, World Health Organization, 2012.

2. World Health Organization. Global Health Estimates: Deaths by Cause, Age, Sex and Country, 2000-2012. Geneva, WHO, 2014.

3 Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*, 2006, 3(11):e442.

4. Edem, D. O., (2009). Hyperglycemic Effect of Ethanolic Extract of Alligator pear seed (persea Americana Mill) in Rats. European Journal of scientific Research, 33(4), 669-678

5. Vivek, K. S., Suresh, K., Hitesh, J. P., and Schivakumar, H., (2010). Hypoglycemic activity of ficus glomerate in Aloxan-induced diabetic rats. International Journal of Pharmaceutic Sciences Review and Research, 1(2):18-22.

6. Kameswararao, B., Kesavulu, M.M. and Apparao, C. (2003). Evaluation of antidiabetic effect of *Momordica cymbalaria* fruit in alloxan-diabetic rats. Fitoterapia. **74**:7-13.

7. Rajagopal, K. and Sasikala, K., (2008). Anti-hyperglycemic and Anti-hyperlipidemic effects of Nymphae steuata in alloxan-induced diabetic rats. Singapore Medical Journal, 49:137-141.

8. Ward, J. W., and Elsea, J. R. (1997). Animal case and use in drug rate and metabolism, methods and techniques, volume II, editors Edward R. G., Jean, L. H., Marcel, D., New York 1997. Pp 372-390.

9. Venegopal, P. M., Prince, P. S. M. and Pari, L. (1998). Hypoglycemic activity of syzigium cumini seeds: Effects on lipid peroxidation in aloxan-induced diabetic rats. Journal of Ethnopharmacology, 61:1-7

10. Jelodar, G., Mohsen, M., Shahram, S., (2003). Effect of Walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats. African Journal of Traditional, Complementary and Alternative Medicines, (3) 229-305.

11. Szkudelski, T. (2001). The mmechanism of alloxan and streptozotocin actions in beta cells of rat pancreas. Physiological Research. **50**: 536-546

12. Wadkar, K. A., Magdun, C. S., Patil, S. S. and Naikwade, N. S. (2008). Antidiabetic potential and Indian medicinal plants. Journal of Herbal Medicine and Toxicology. **2**(1): 45-50.

13. Venkatesh S., Reddy D. G., Reddy B. M., Ramesh M., and Appa Rao A. V. N. (2003), Antihyperglycemic activity of *Caralluma attenuata*. Fitoterapia **74**, 274D279.

14. Adikwu, M. U., Uzuegbu, D. B., Okoye, T. C. Uzor, P. F., Adibe, M. O., Amadi, B., (2010). Anti-diabetic effect of combined aqueous leaf extract of *Venonia Amygdalina* and Metfomin in rats. Journal of basic and clinical pharmacy, 1(3).

15. Harborne, J. B. (1998). Phytochemical methods: A guide to modern techniques of plant analysis. 3rd Edn. Chapman and Hall, London, pp. 1-3.

16. Arika, W. M., Abdirahman, Y. A., Mawia, M. M., Wambua, K. F., Nyamai, D. M., Ogola, P. E., Kiboi, N. G., Nyandoro, H. O., Njagi, S. M., Agyirifo, D. S., Ngugi, M. P., and Njagi, E. N. M. (2015). Hypoglycemic Effect of *Lippia javanica* in Alloxan Induced Diabetic Mice. Journal of Diabetes and Metabolism, **6** (11): 624-630.

17. Arika, W. M., Ogola, P. E., Nyamai, D. W., Mawia, A. M., Wambua, F. K., Kiboi, N. G., Wambani, J. R., Njagi, S. M., Rachuonyo, H. O., Emmah, K. O., Lagat, R. C., Muruthi, C. W., Abdirahman, Y. A., Agyirifo, D. S., Ouko, R. O., Ngugi, M. P. and Njagi, E. N. M. **(**2016), Mineral Elements Content of Selected Kenyan Antidiabetic Medicinal Plants. Advanced Techniques in Biology & Medicine, 4(**1**):1-6.

18. Grover, J. K., Vat, V., Rathi S.S., (2000). Antihyperglycemic effect of *Eugenia Jambolana* and *Tinopora cordifolia* in experimental diabetes and their effect key metabolic enzymes involved in carbohydrate metabolism. Journal of Ethnopharmacology. (73) 616-470.

19. Ibeh BO, Ezeaja MI. Preliminary study of antidiabetic activity of the methanolic leaf extract of Axonopus compressus (P. Beauv) in alloxan-induced diabetic rats. J thnopharmacol. 2011 Dec 8; 138(3):713-6