Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Biochemistry

ISSN 1815-9923
DOI: 10.3923/ajb.2016.82.89

Research Article

Antidiabetic and Antidyslipidemic Effect of Ethanolic Extract of *Alternanathera pungens* on Alloxan-Induced Diabetic Rats

[1]Owa Stephen Olugbemiga, [1]Oyewale Damilola Grace, [1]Taiwo Adeolu Adeola, [2]Edewor-Ikupoyi Theresa Ibibia, [1]Okosun Jenifer Akhere, [1]Otohiniyi David Adeiza, [1]Akujobi Yvonne Oluchi, [1]Nwonuma Charles Obiora and [1]Adeyemi Oluyomi Stephen

[1]Unit of Biochemistry, Department of Biological Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria
[2]Department of Chemical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Osun State, Nigeria

Abstract

Diabetes mellitus is a metabolic disorder affecting about 5-10% of the world's population. Presently, there is no known cure for diabetes but it could be managed by the use of agents that exhibit hypoglycemic effect. Insulin is a well known agent that has this effect; however, it comes with side effect and contraindications. This underscores the search for newer treatments. The ethanolic extract of *Alternanthera pungens* was evaluated for antidiabetic and antidyslipidemic properties in alloxan-induced diabetic rats. Thirty five male Wistar rats were assigned into 7 groups of five. All rats, except the negative control group, were induced into diabetes by single intraperitoneal injection of 150 mg kg^{-1} alloxan. Induced-diabetic rats, apart from the diabetic (positive) control group, were treated daily by oral administration of either glibenclamide at 5 mg kg^{-1} b.wt., or the ethanolic extract of *Alternanathera pungens* at 50, 100, 200 and 300 mg kg^{-1} b.wt. Treatment lasted for 20 days. Data show that the extract treatment significantly reduced the blood glucose to levels lower than in the positive control group. Further, the extract improved the diabetic-related alteration of rat serum lipid profile. The antidiabetic and antidyslipidemic activity of the extract compared competitively with a well-known antidiabetic drug glibenclamide and lends credence to the folk use of *Alternanathera pungens*.

Key words: *Alternanthera pungens,* diabetes mellitus, insulin, alloxan, glibenclamide

Received: September 24, 2015 **Accepted:** December 01, 2015 **Published:** February 15, 2016

Citation: Owa Stephen Olugbemiga, Oyewale Damilola Grace, Taiwo Adeolu Adeola, Edewor-Ikupoyi Theresa Ibibia, Okosun Jenifer Akhere, Otohiniyi David Adeiza, Akujobi Yvonne Oluchi, Nwonuma Charles Obiora and Adeyemi Oluyomi Stephen, 2016. Antidiabetic and Antidyslipidemic Effect of Ethanolic Extract of *Alternanathera pungens* on Alloxan-Induced Diabetic Rats. Asian J. Biochem., 11: 82-89.

Corresponding Author: Adeyemi Oluyomi Stephen, Biochemistry Unit, Department of Biological Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) is debilitating metabolic disorder associated with carbohydrate metabolism and is a major cause of disability and hospitalization (Berger *et al*., 1999; Whiting *et al*., 2011; Xie *et al*., 2011; Eckel *et al*., 2005) invoking a huge financial burden ($92 billion per year in the USA) (Zhang *et al*., 2010). The diabetic population is increasing with hundreds of millions at risk of the disorder (Sy *et al*., 2005).

A section of Non-Insulin Dependent Diabetes Mellitus(NIDDM) patients can be managed by diet; others require oral hypoglycemic therapy and or insulin (Kodak-Kimble *et al*., 2005). Currently available pharmaceutical options for diabetes, like oral hypoglycemic agents and insulin, have serious limitations (Saxena and Vikram, 2004), hence the search for more effective anti-diabetic agents form traditional medicines have been recommended (Mukherjee *et al*., 2006). In recent years, the popularity of complementary medicine has increased considerably. Traditional medicines offer great potential for the discovery of new antidiabetic drugs (Jung *et al*., 2006). Report confirms that medicinal plants are being used as alternative and safe antidiabetic agents (Patel *et al*., 2012). *Alternanthera pungens* is a plant popularly called khaki weed and locally called dagunro among the Yoruba of Nigeria. The leaves are widely used as vegetable. Majorly the whole plant is used for medicinal purpose. It is a kunthcreeping, prostrate perennial pioneer plant of the *Amaranthaceae* family, spreading by seed and vegetatively, with roots often developing at the nodes of spreading stems. It belongs to the kingdom Plantae, phylum Angiospermae, order Caryophyllales, family Amaranthaceae, subfamily Gomphrenoideae, genus *Alternanthera*, species *pungens*. Majorly the whole plant is used for medicinal purpose. Literature search indicates dearth of information regarding the antidiabetic effect of *Alternanthera pungens*, although, its congener "*Alternanthera sessilis*" has been analyzed and proven to have hypoglycemic and antidiabetic effect (Rao *et al*., 2011).

This study evaluated the anti-diabetic and hypolipidemic potential of *Alternanthera pungens* in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Reagents and chemicals: Alloxan monohydrate used was a product of Oxford Laboratories Reagent, London. Finetest Blood Gluco-Strips was a product of Infopia Co., Ltd, Kyunggi-Do, Korea. Glucose reagent, HDL, Total Glycerides and Total Cholesterol kits were products of Agape Diagnostics, Kerrala, India. Glibenclamide tablets were produced by Swiss Pharmacy Nigeria Ltd, Nigeria. All other chemicals and reagent were of analytical grade and used as supplied unless otherwise stated.

Experimental animals: Male Wistar rats weighing 160-200 g were obtained from Covenant farms, Olodo, Ibadan, Oyo state. The animals were housed in the Animal House of Landmark University at standard environmental condition and maintained under controlled room temperature with about 12 h light and 12 h dark cycle. Standard pellets obtained from Grand Cereals Ltd, Jos, Plateau State, Nigeria, were use as basal diet during the experimental period. The controls and experimental animal were provided food and drinking water *ad libitum*. The rats were allowed to acclimatize to laboratory environment for two weeks before the commencement of the experiment. Handling of animals was humane and consistent with guidelines as approved by the Landmark University Ethics Committee.

Preparation of leaf extract: One kilogram of the whole plant of *Alternanthera pungens* was collected from local areas of Omu-Aran, Kwara state, Nigeria. The plants were air dried and ground to get coarse powder of 40-mesh size and extracted with ethanol using the soxhlet extractor. The extract was concentrated under reduced pressure.

Experimental procedure: Diabetes was induced by intra-peritoneal injection of alloxan at the concentration of 150 mg kg^{-1} dissolved in 0.9% saline. The alloxan injection was given to rats after the rats were fasted for 12 h. In order to avoid the alloxan-induced hypoglycemic mortality, 5% glucose solution was given for 24 h to alloxan treated animals. Seventy two hour after the induction of alloxan blood samples were withdrawn from the tail and the blood glucose level was determined using a glucometer. The rats that exhibited blood glucose levels to be above 250 mg dL^{-1} were regarded as successfully induced diabetic; and were used to further the experiment.

Experimental design: The rats were assigned into seven groups, five animals per group. The subdivision of the rats was as follow:

- **Group I (positive control):** Diabetic, non-treated rats; they were induced for experimental diabetes but received 1 mL of normal saline orally for 20 days

- **Group II (negative control):** Non-diabetic, non-treated rats; they were given 1mL of normal saline for 20 days
- **Group III:** Diabetic, treated with standard reference drug glibenclamide at 5 mg kg^{-1} b.wt., dissolved in 1 mL normal saline, administered orally for 20 days
- **Group IV:** Diabetic, treated with *A. pungens* extract at a dose of 50 mg kg^{-1} b.wt., of extract, given orally for 20 days
- **Group V:** Diabetic, treated with *A.* pungens extract at a dose of 100 mg kg^{-1} b.wt., of extract, given orally for 20 days
- **Group VI:** Diabetic, treated with *A.* pungens extract at a dose of 200 mg kg^{-1} b.wt., of extract, given orally for 20 days
- **Group VII:** Diabetic, treated with *A.* pungens extract at a dose of 300 mg kg^{-1} b.wt., of extract, given orally for 20 days

Administrations were done orally, using sterile cannulas. The rats were weighed before and after acclimatization and weekly after the beginning of the experiment and 24 h before the rats were sacrificed. The study lasted for 21 days. Blood was collected from the tail of the rats to determine the blood glucose level on the 7th and 14th day, On the 21st day after the rat were sacrificed, the blood was collected into a fluoride oxalate anti-coagulated bottle to determine the final blood glucose concentration.

Blood sample collection: At the end of the experiment, the rats were sacrificed under anesthesia using diethyl ether 24 h after the final treatment. The blood was collected from the neck region after the jugular vein was cut with surgical blade. Two milliliter of blood was collected into the fluoride oxalate-bottle for blood glucose analysis. Another 4 mL was collected into plain sample bottle for biochemical assay of lipid profile. The blood was spun at 4000 g for 5 min (Anke TDL-5000B, Shanghai, China) and the serum aspirated by Pasteur pipette.

Biochemical assays: The plasma concentration of glucose, total cholesterol, triglyceride, High Density Lipoprotein Cholesterol (HDL-C) and Low Density Lipoprotein Cholesterol (LDL-C) were measured using commercial reagent assay kits (Randox Diagnostics, Crumlin, UK).

Determination of plasma glucose using enzymatic method: Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacted, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinone imine dye as indicator. The absorbance was then read at 520 nm using spectrophotometer (Jenway UV/Vis Spectrophotometer, Staffordshire, UK).

Serum lipid estimations: Serum cholesterol, serum triglycerides and HDL-cholesterol were assayed using Randox assay kit (Crumlin, UK). LDL–cholesterol was estimated using the expression; LDL = Total cholesterol+(HDL- (triglyceride/5) (Friedewald *et al*., 1972). Serum VLDL-cholesterol was determined using the Friedwalds expression; VLDL = Triglyceride/5 (Friedewald *et al*., 1972).

Statistical analysis: The measurements were statistically tested on Statistical Package for Social Sciences (SPSS, V 19, IBM 2010) to determine if the extracts were comparable to, or significantly different in effect from the standard drug glibenclamide. Analysis of variance (ANOVA) was used and p was set at 0.05.

RESULTS

Effect of *Alternanthera pungens* extract on rat serum glucose: In diabetic untreated rats, the serum glucose level remained significantly high relative to the glibenclamide and/or extract treated groups throughout the experimental period (Fig. 1). In diabetic rats treated with 50, 100, 200 and 300 mg kg^{-1} *A.* pungens extract, the sugar level reduced significantly compared to rats treated with glibenclamide. By day 21 the *A. pungens* extract has restored the serum glucose level to normal comparable to the negative control. Treatment with the extract produced faster glucose level control and lower than the antidiabetic drug glibenclamide.

Effect of *Alternanthera pungens* extract on rat weight: The untreated diabetic rats lost weight (Fig. 2). Glibenclamide-treated diabetic rats significantly lost weight but not as much as untreated diabetic rats. All *A. pungens* extract (50-300 mg kg^{-1}) treatment improved the rat weight.

Effect of *Alternanthera pungens* extract on rat serum total cholesterol: Both glibenclamide and the *A. pungens* extracts lowered the rat serum total cholesterol (Fig. 3).

Effect of *Alternanthera pungens* extract on rat serum triglyceride level: Glibenclamide caused lowered the rat serum triglyceride level (Fig. 4). The *A. pungens* extract at 50,

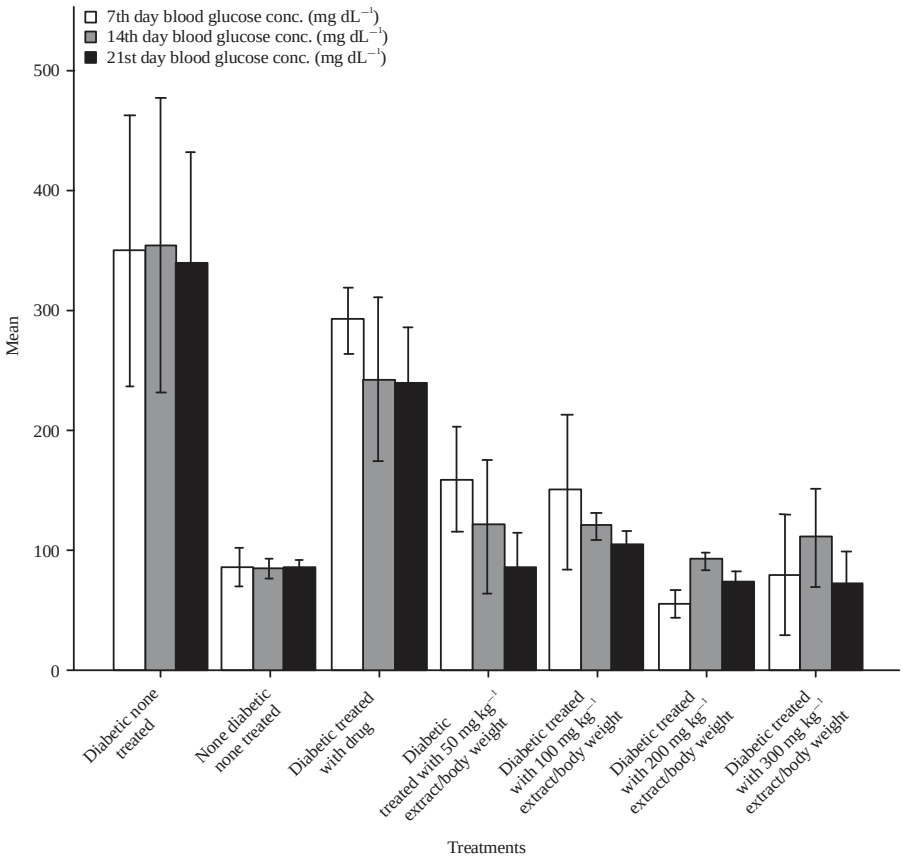

Fig. 1: Effect of the *Alternanathera pungens* extract on rat serum glucose levels, error bars represent 95% CI

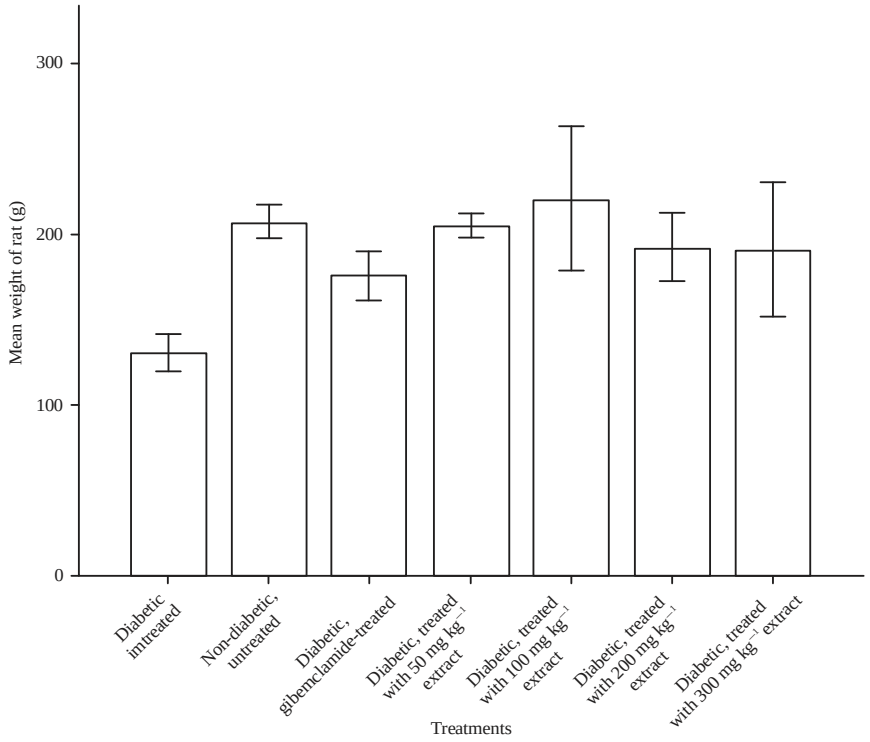

Fig. 2: Effect of the *Alternanathera pungens* extract on rat weight, error bars represent 95% CI

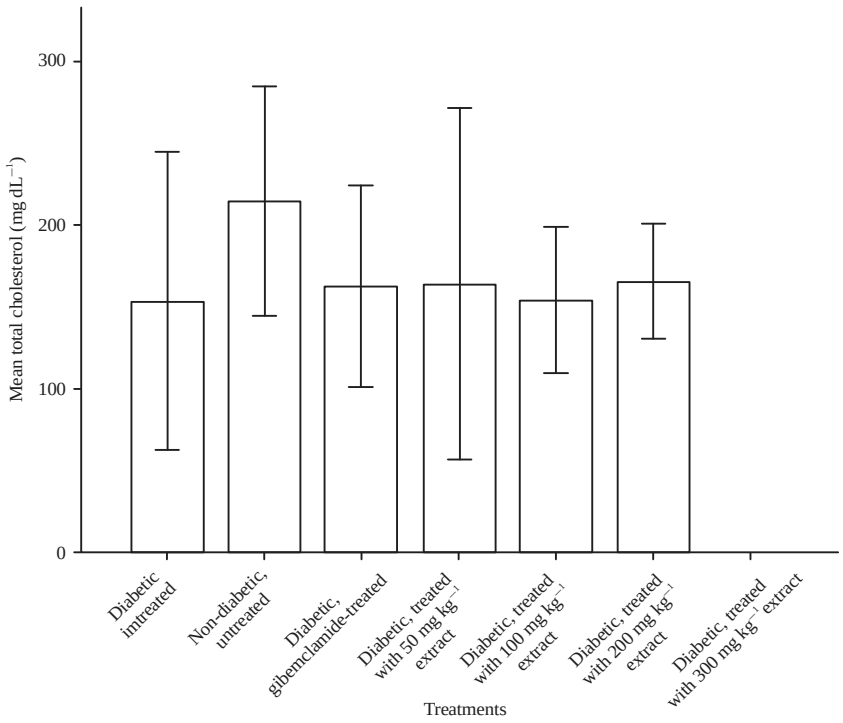

Fig. 3: Effect of *Alternanathera pungens* extract on serum total cholesterol, error bars represent 95% CI

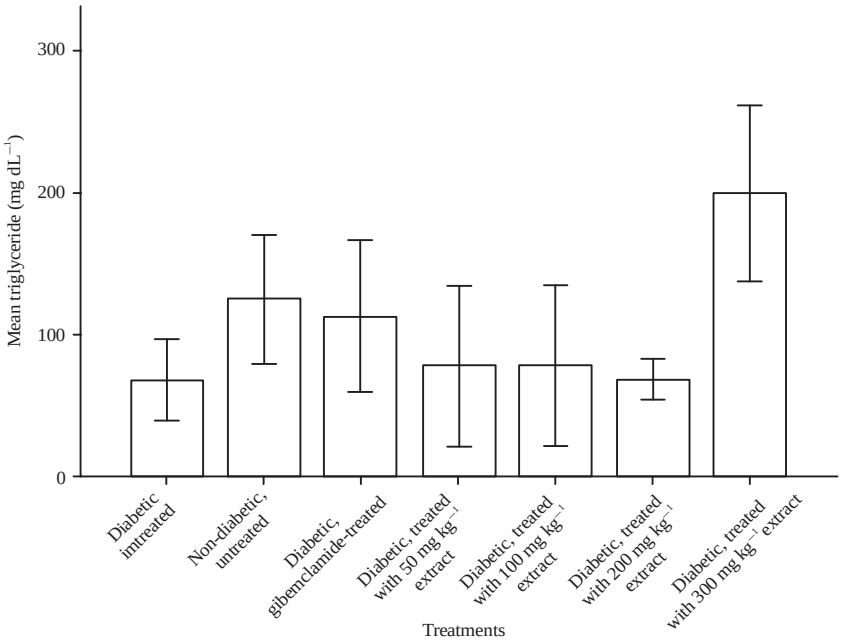

Fig. 4: Effect of *Alternanathera pungens* extract on rat serum triglyceride level, error bars represent 95% CI

100 and 200 mg kg^{-1} also lowered the rat serum triglyceride. In contrast, the extract at 300 mg kg^{-1}, raised the rat serum triglyceride significantly relative to the untreated diabetic group.

Effect of *Alternanthera pungens* extract on rat serum HDL level: Induction of diabetes reduced the rat serum HDL level relative to the negative control (Fig. 5). Treatment with 50, 200 and

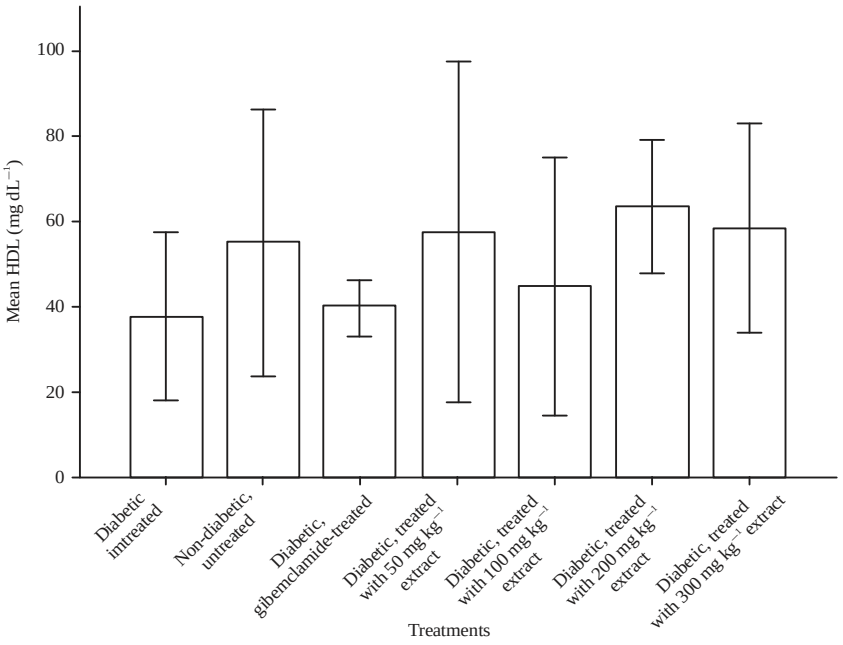

Fig. 5: Effect of *Alternanathera pungens* extract on rat serum HDL level, error bars represent 95% CI

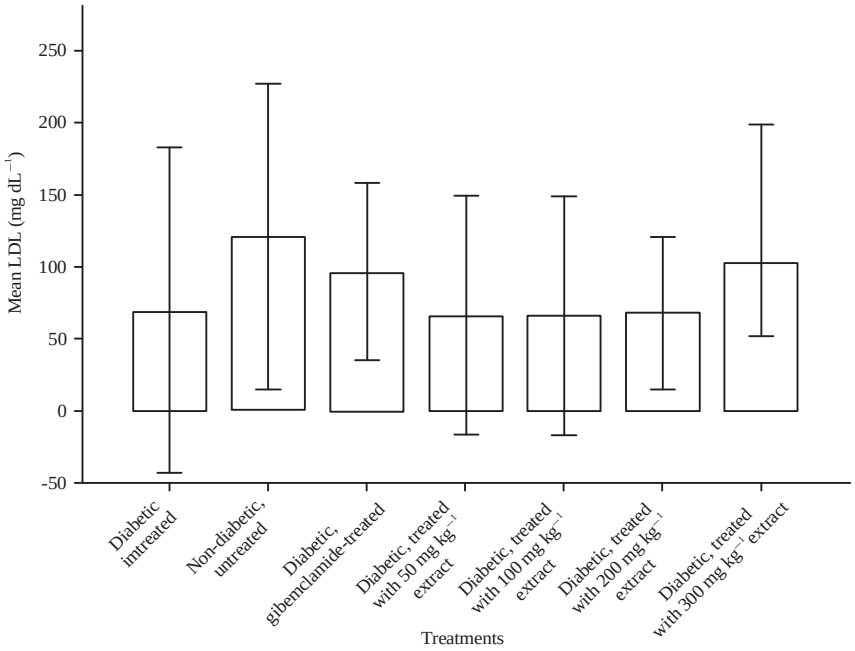

Fig. 6: Effect of *Alternanathera pungens* extract on rat serum LDL level, error bars represent 95% CI

300 mg kg^{-1} *A. pungens* extract improved and restored the rat serum HDL level.

Effect of *Alternanthera pungens* extract on rat serum LDL: Treatment with glibenclamide, as well as treatment at all doses of the *A. pungens* extract altered the rat serum LDL (Fig. 6). But the alteration did not follow a definite pattern.

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. Most of these plants have phytoconstituents such as glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect (Malviya *et al*., 2010). In this study, the ethanolic extract of *A. pungens* was evaluated for its antidiabetic and antidyslipidemic properties in alloxan induced diabetic rats.

The treatment of the experimental diabetic rats with *A. pungens* significantly lowered the rat blood glucose level relative to the untreated diabetic control. This underscores the hypoglycemic effect of *A. pungens.* The *A. pungens* extract treatment compared competitively and favorably with the reference drug glibenclamide. Previously, Tan and Kim (2013) demonstrated the hypoglycemic potential of *Alternanthera sessilis.* Further, treatment of diabetic rats with the *A. pungens* extract improved weight loss. Diabetes mellitus comes with polyuria, polyphagia, polydipsia, hyperglycemia, weakness in the muscle and loss of weight (Rohilla and Ali, 2012). The diabetic-induced weight loss is usually explained as partially due to increased muscle wasting (Lau *et al*., 2003). Diabetic rats treated with the *A. pungens* extract conserved body weight, much better than those treated with the reference drug, glibenclamide.

Also, the treatment with *A. pungens* extract improved and restored the rat serum lipid status. The capacity of the *A. pungens* extract to reduce the serum total cholesterol and triglyceride levels while elevating the HDL level indicates potential to support cardiac functions. Diabetes is associated with an increased risk for premature arteriosclerosis due to increase in triglycerides and low density lipoprotein levels. It is an independent predictor of high risk for Coronary Heart Disease (CHD). The CHD morbidity is 2-4 times higher in patients with diabetes than non-diabetics and the mortality from CHD is up to 100% higher in diabetic patients than in the non-diabetics over a 6-year period (Sharma *et al*., 2008).

We demonstrated that *A. pungens* possess antidiabetic activity comparable with the reference drug, glibenclamide. Data show that the diabetic control was more effective with the 50 mg kg^{-1} of *A. pungens* extract treatment. Though its mechanism of action is unknown yet, however, the hypoglycemic activity of medicinal plants have been linked to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes (Patel *et al*., 2012).

CONCLUSION

In conclusion, the *A. pungens* extract possess strong hypoglycemic and anti-dyslipidemic effect which could be explored for the control of diabetes-related conditions. Further this study provides scientific basis for the folk use of the *A. pungens* plant for the treatment of diabetes.

ACKNOWLEDGMENT

Authors appreciate the laboratory staff at the Department of Biological Sciences, Landmark University, Omu-Aran, Nigeria.

REFERENCES

Berger, B., G. Stenstrom and G. Sundkvist, 1999. Incidence, prevalence and mortality of diabetes in a large population. A report from the Skaraborg Diabetes Registry. Diabetes Care, 22: 773-777.

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

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

Eckel, R.H., S.M. Grundy and P.Z. Zimmet, 2005. The metabolic syndrome. Lancet, 365: 1415-1428.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasm a, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Jung, M., M. Park, H.C. Lee, Y.H. Kang, E.S. Kang and S.K. Kim, 2006. Antidiabetic agents from medicinal plants. Curr. Med. Chem., 13: 1203-1218.

Kodak-Kimble, M.A., L.Y. Young, W.A. Kradjan, B.J. Guglielmo and R.L. Corelli, 2005. Applied Therapeutics: The Clinical Use of Drugs. 8th Edn., Lippincot Williams and Wilkins, Philadelphia, pp: 487-509.

Lau, A.J., M.J. Holmes, S.O. Woo and H.L. Koh, 2003. Analysis of adulterants in a traditional herbal medicinal product using liquid chromatography-mass spectrometry-mass spectrometry. J. Pharm. Biomed. Anal., 31: 401-406.

Malviya, N., S. Jain and S. Malviya, 2010. Antidiabetic potential of medicinal plants. Acta Pol. Pharm. Drug Res., 67: 113-118.

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

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

Rao, R.K.V., K.R.S.S. Rao, R. Nelson, K. Nagaiah and V.J.S. Reddy, 2011. Hypoglycemic and anti diabetic effect of *Alternanthera sessilis* in normal and streptozotocin (STZ)-induced rat. J. Global Trends Pharma. Sci., 2: 325-335.

Rohilla, A. and S. Ali, 2012. Alloxan induced diabetes: Mechanisms and effects. Int. J. Res. Pharmaceut. Biomed. Sci., 3: 819-823.

Saxena, A. and N.K. Vikram, 2004. Role of selected Indian plants in management of type 2 diabetes: A review. J. Alter. Complement Med., 10: 369-378.

Sharma, M., J. Fernandes, D. Ahirwar and R. Jain, 2008. Hypoglycemic and hypolipidimic activity of alcoholic extract of citrus aurantium in normal and alloxan-induced diabetic rats. Pharmacologyonline, 3: 161-171.

Sy, G.Y., A. Cisse, R.B. Nongonierma, M. Sarr, N.A. Mbodj and B. Faye, 2005. Hypoglycaemic and antidiabetic activity of acetonic extract of *Vernonia colorata* leaves in normoglycaemic and alloxan-induced diabetic rats. J. Ethnopharmacol., 98: 171-175.

Tan, K.K. and K.H. Kim, 2013. *Alternanthera sessilis* red ethyl acetate fraction exhibits antidiabetic potential on obese type 2 diabetic rats. Evidence-Based Complement. Alternat. Med. 10.1155/2013/845172

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

Whiting, D.R., L. Guariguata, C. Weil and J. Shaw, 2011. IDF diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res. Clin. Pract., 94: 311-321.

Xie, X., X. Meng, X. Zhou, X. Shu and H. Kong, 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.

Zhang, P., X. Zhang, J. Brown, D. Vistisen, R. Sicree, J. Shaw and G. Nichols, 2010. Global healthcare expenditure on diabetes for 2010 and 2030. Diabetes Res. Clin. Pract., 87: 293-301.