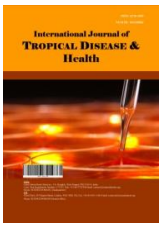

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Prophylactic Effect of Ethanol Extract of *Azadirachta indica* Leaf in Streptozotocin-induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OCE and CJO designed the study. Authors OCE and MEO performed the statistical analysis. Authors OCE, ORI and ICA wrote the protocol and wrote the first draft of the manuscript. Authors FAE, UEE and UHC managed the literature searches and the analysis of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Medicinal plants are now becoming indispensable in the treatment and management of many ailments. The unaffordability, unavailability and adverse effects of conventional therapy in the treatment and management of many diseases have geared keen interest in the use of herbal medicine. This work was carried out to investigate the prophylactic effect of the ethanol extract of *Azadirachta indica* leaf in streptozotocin-induced diabetic rats.
Methods: A total of one hundred (100) rats were randomized into four (4) groups (n=25) and used for the study. Each group of 25 rats was sub-divided into five (5) groups (n=5). The sub-groups comprise: Group A-normal control that was not treated, group B-100 mg/kg body weight of metformin and groups C to E - graded doses (100 mg/kg, 200 mg/kg and 400 mg/kg body weight) of the ethanol leaf extracts of *A. indica* leaves. The standard drug and the extracts were consecutively administered to groups B-E for 7, 14, 21 and 28 days before the induction of diabetes. Diabetes was induced intraperitoneally using 50 mg/kg bodyweight of streptozotocin.

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Results: The groups that were administered 100, 200 and 400 mg/kg bw of ethanol extract of *A. indica* showed a significant (p<0.05) increase in their weight after 21 and 28 days of pre-treatment compared with the control group that was not treated. The graded doses of the extract also have a remarkable effect in the fasting blood glucose levels which was made visible by the significant (p<0.05) reduction recorded in the fasting blood glucose levels compared with the control group that was not pre-treated and the group pre-treated with metformin.
Conclusion: The results obtained in this research suggest that ethanol extract of *A. indica* has the potential to protect against diabetes by delaying its onset. However, the longer the period of pre-treatment, the better the condition of the animals pre-treated as well as the protection as can be seen from the results of the weight and fasting blood glucose levels.

Keywords: Prophylactic; Azadirachta indica; ethanol extract; streptozotocin; diabetes.

1. INTRODUCTION

Diabetes mellitus (DM) is a diverse group of chronic metabolic disorder associated with a high disease burden in developing and developed countries, affecting the population on epidemic level. Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. In Nigeria, diabetes is a major health challenge affecting both the young and adult. Studies conducted in Nigeria indicated that the prevalence of diabetes ranged from low level of 0.8% among adults in rural highland dwellers to over 7% in urban Lagos with an average of 2.2% nationally [2]. Diabetes is a major cause of mortality both in developing and developed countries. The incidence is rising rapidly with sub-Saharan Africa experiencing the largest percentage increase between 2013 and 2035 [3]. Nigeria has the largest number of people with the disease, yet information on the diabetes mellitus is still fragmentary [3]. According to International Diabetes Federation estimates, around 415 million people had DM in 2015 and this number is expected to rise to 642 million by 2040 [4]. Around 75% of subjects with DM live in low and middle-income countries. In financial terms, the global burden of DM is enormous, with an estimated annual expenditure of 673 billion US dollars in 2015, which constituted 12% of global health spending for that year [4]. While in urban areas of low and middle-income countries, diabetes is well recognized as a public health priority; recent prevalence data suggest that diabetes is an increasing problem among rural populations as well [5].

DM can result from the body's failure to produce insulin and is refered to as Type I diabetes mellitus or Insulin-dependent diabetes mellitus (IDDM). The majority of Type I diabetes is immune-mediated in nature, where beta cells loss is a T-cell mediated autoimmune attack [6]. Type I diabetes is caused by a lack of insulin due to the destruction of insulin-producing beta cells in the pancreas [6]. Diabetes can also be caused by insulin resistance (reduced sensitivity of cells to insulin), a relative insulin deficiency, or both. When this is the cause, it is called Type II diabetes and is usually noticed in adulthood, and occurs mostly in patients that are obese [7,8,9]. DM may also be characterized by glucose intolerance during pregnancy which is associated with a variety of adverse birth outcomes, including excessive fetal weight gain and related increases in the rate of cesarean delivery and perinatal injury [10]. In this case, it is called Gestational diabetes mellitus (GDM). GDM usually results after delivery [11]. Symptoms of diabetes mellitus include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Symptoms of type II diabetes may develop gradually and can be subtle; some people with type II diabetes remain undiagnosed for years [12]. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia [13].

Diabetics must follow a daily management regimen that includes attention to food intake (carbohydrates, as well as fats and total energy intake), exercise, and blood glucose monitoring. Administration of medication (insulin or oral medications) may also be required. Patients treated with insulin or sulfonylureas are at risk for low blood glucose levels. The management of diabetics with synthetic drugs such as sulfonylure as (chlorpropamide, glibenclamide, glipizide, glyb uride, micronase, tolazamide, tolbutamide), bigua nides (metformin), meglitinides, alpha glucosida-se inhibitors, dipeptidyl peptidase inhibitors and ergot alkaloids has been a major intervention

[14]. However, these drugs are either too expensive or have undesirable side effects or contraindications. Phenformin was used from 1960s through 1980s, but was withdrawn due to lactic acidosis risk [14].

The economic burden of diabetes is enormous in terms of the direct cost of intensive monitoring and control of blood glucose and managing cardiovascular, renal, and neurological consequences [15]. This has resulted to psychological trauma in patients suffering from diabetes. In recent years, there has been renewed interest in plant medicine and plant-based therapies [16]. Many traditional plant treatments for diabetes mellitus are used throughout the world and some of these plants have been validated while a good number of them are yet to receive scientific scrutiny [17]. The use of medicinal plants to prevent and treat DM successfully over the years has attracted the attention of scientists globally.

Medicinal plants are of great importance to the health of individuals and every member of a known community. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of important phytochemical constituents [18]. Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes [19]. In the rural communities, many people depend solely on medicinal plants for the treatment of diabetes due to its easy accessibility, affordability and availability even when the efficacy of the herbal remedy has not been established. *Azadirachta indica* Juss. Meliaceae (Neem) is one of the most versatile medicinal plants with a wide spectrum of biological activity. Traditional healers make use of the leaves of *A. indica* to treat diabetes. Scientific report supports the hypoglycemic activity of Neem leaves [20]. Maragathavalli *et al*. [21] even reported that chewing of *Azadirachta indica* leaves in the morning for twenty-four days protected the body from diabetes. However, there are limited researches on the prophylactic property of the ethanol extract of *A. indica* leaves. This research focuses on the prophylactic effect of ethanol extract of *Azadirachta indica* leaf in Streptozotocin-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

The leaves of *A. indica* were collected at Nnamdi Azikiwe University Awka, Anambra State. The sample was identified and validated by a taxonomist in the Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka. The voucher number assigned to *A. indica* leaf as deposited in the herbarium of the Department of Botany, Nnamdi Azikiwe University, Awka is 14.

2.2 Preparation of Ethanol Extract of *A. indica* Leaf

The leaves were hand-picked, thoroughly washed and air dried at room temperature for two (2) weeks. The dried leaves were pulverized using Corona manual grinding machine. Exactly 1 kg of the ground powdered leaves of *A. indica* was soaked in 5 litres of 80% ethanol for 24 hours for ethanol extraction. The ethanol extraction was sieved with muslin cloth and filtered using Whatman filter paper. The filtrate was concentrated using rotary evaporator. The ethanol extract was stoppered in universal bottles and preserved in the refrigerator until use. The extracts were thereafter prepared by solubilizing it with distilled water before administration.

2.3 Investigation of Prophylactic (Protective) Effect of Ethanol Extract of *A. Indica* Leaves

A total of one hundred (100) rats were randomized into four (4) groups (n=25) and used for the study. Each group of 25 rats was sub-divided into five (5) groups (n=5). The twenty-five (25) rats were grouped as follows: Group A was the normal control (not pre-treated), group B was pre-treated with standard drug (100 mg/kg bw. metformin), groups C to E were pre-treated orally with 100 mg/kg, 200 mg/kg and 400 mg/kg body weight of the ethanol leaf extract of *A. indica* respectively. Groups B to E were consecutively pre-treated for 7 days, 14 days, 21 days and 28 days before the induction of diabetes. After each period of pre-treatment, diabetes was induced intraperitoneally using 50 mg/kg bodyweight of streptozotocin to check the ability of the extract to prevent or delay the onset of diabetes. Initial bodyweights and fasting blood glucose levels of the rats were determined before initiating the

daily administration of the extract and were also monitored at two days interval to know the effect of the extract on the body weight and fasting blood glucose levels before the induction of diabetes. The onset of diabetes was established by increase in the normal fasting blood sugar level (between 60 mg/dl to 120 mg/dl) to above 200 mg/dl [22]. Forty-eight (48) hours after the induction of diabetes with streptozotocin the weights of rats, the fasting blood glucose levels as well as other symptoms of diabetes were determined and monitored.

2.4 Statistical Analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) software for windows version 21 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD. Statistical analysis of the results obtained were performed by using ANOVA to determine if significant difference exists between the mean of the test and control groups. The limit of significance was set at $p<0.05$.

3. RESULTS

3.1 Result of Weight after Pre-Treatment with Ethanol Extract of *A. indica* Leaf Extract

The groups that were pre-treated for seven days with 100, 200 and 400 mg/kg bw of ethanol extract of *A. indica* before the induction of diabetes showed 5.94%, 5.72% and 5.47% reduction respectively in their weight 48 hours after the induction of diabetes compared with the group pre-treated with metformin (7.25%) and the untreated group (9.89%) (Fig. 1b). The result shows that a better protection against weight loss was observed in the group treated with 400 mg/kg bw (Fig. 1a). The groups that were pre-treated for fourteen days with 100, 200 and 400 mg/kg bw of ethanol extract of *A. indica* before the induction of diabetes showed 6.45%, 6.94% and 4.86% reduction respectively in their weight 48 hours after the induction of diabetes compared to the group pre-treated with metformin (8.85%) and the untreated group (9.67%) (Fig. 2b). The result shows that at two weeks all the doses tested showed better protection against weight loss. However, the group that was pre-treated with 400 mg/kg bodyweight showed a far much better protection after two weeks (Fig. 2a).

The groups that were pre-treated for twenty-one days with 100, 200 and 400 mg/kg bw of ethanol extract of *A. indica* before the induction of diabetes showed 6.94%, 5.24% and 5.45% reduction respectively in their weight 48 hours after the induction of diabetes compared with the group pre-treated with metformin (8.04%) and the untreated group (9.90%) (Fig. 3b). The result shows that a better protection against weight loss was observed in the group pre-treated with 400 mg/kg bw (Fig. 3a). The groups that were pre-treated for twenty-eight days with 100, 200 and 400 mg/kg bw of ethanol extract of *A. indica* before the induction of diabetes showed 4.14%, 4.99% and 4.80% reduction respectively in their weight 48 hours after the induction of diabetes compared with the untreated group (10.8) and the group pre-treated with metformin (6.02%) (Fig. 4b). The result shows that a better protection against weight loss was observed in the group pre-treated with 100 mg/kg bw followed by the group pre-treated with 400 mg/kg bw of ethanol extract of *A. indica* for a period of twenty-eight days (Fig. 4a).

3.2 Result of Fasting Blood Glucose Levels after Pre-Treatment with *A. indica* Leaf

The result of the daily fasting blood glucose levels of the different group of rats administered 100 mg/kg, 200 mg/kg and 400 mg/kg b.w of ethanol extract of *A. indica* for 7, 14, 21 and 28 days before the induction of diabetes are shown in Figs. 5a, 5b, 6a, 6b, 7a, 7b, 8a and 8b. Also the fasting blood glucose levels of the rats were recorded 48 hrs after the induction of diabetes. The difference in percentage increase in blood glucose levels between the normal untreated rats and those administered extract at all dose levels remain about the same after 7 days and showed marked difference from 14 days and increases significantly ($p<0.05$) as the duration of pre-treatment increased from 21 days to 28 days.

There was no significant ($p<0.05$) difference in the fasting blood glucose levels for the group of animals pretreated for seven days with the ethanol leaf extracts of *A. indica* compared with the untreated group after the induction of diabetes (Fig. 5a and 5b). The result of the daily fasting blood glucose levels of the different group of rats administered graded doses of *A. indica* extract for fourteen (14) days before the induction of diabetes is shown in Fig. 6. The groups that were pre-treated with *A. indica* leaf extract for fourteen days showed a significant ($p<0.05$) reduction in the fasting blood glucose level after the induction of diabetes compared with the normal rats that were not treated (Fig. 6a

and 6b). There was a significant (p<0.05) reduction in the percentage fasting blood glucose level of the groups treated with graded doses of *A. indica* leaf for a period of 21 days compared with the normal untreated group and the group pre-treated with a standard antidiabetic drug (Fig. 7a and 7b). The result of the fasting blood glucose levels of the different group of rats administered graded doses of *A. indica* leaf extract for twenty-eight (28) days before the induction of diabetes is shown in Fig. 8. The groups that were pretreated for twenty-eight days with ethanol extract of *A. indica* leaf before the induction of diabetes showed a significant (p<0.05) decrease in their fasting blood glucose levels after the induction of diabetes compared with the normal untreated rats (Fig. 8a and 8b).

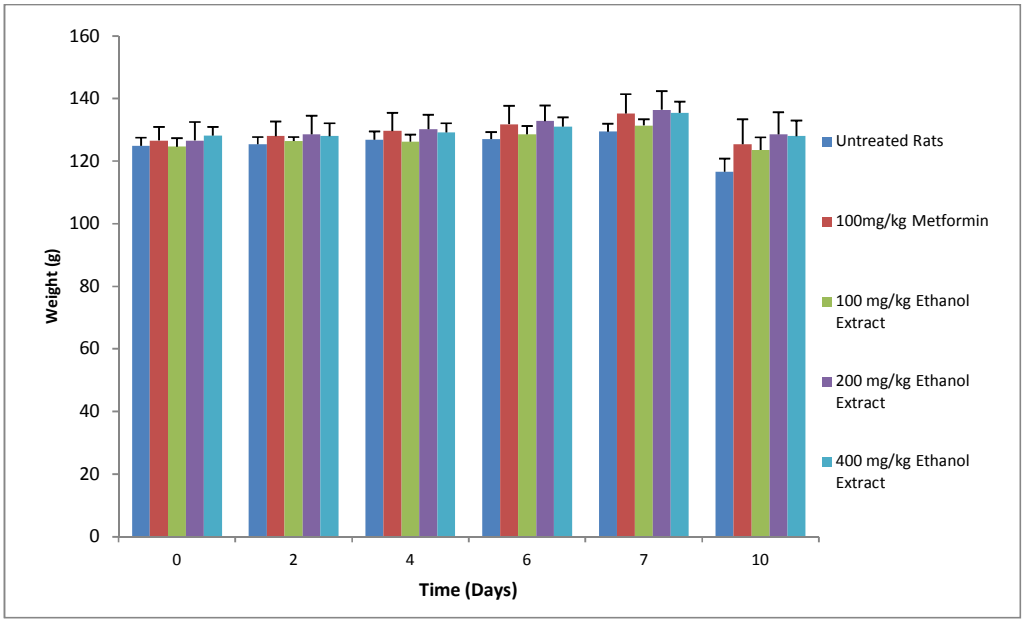

Fig. 1a. Weight (g) profile of rats during one-week pre-treatment with 100 mg/kg, 200 mg/kg and 400 mg/kg bw. of ethanol extract of *A. indica* leaf expressed as mean ± SD

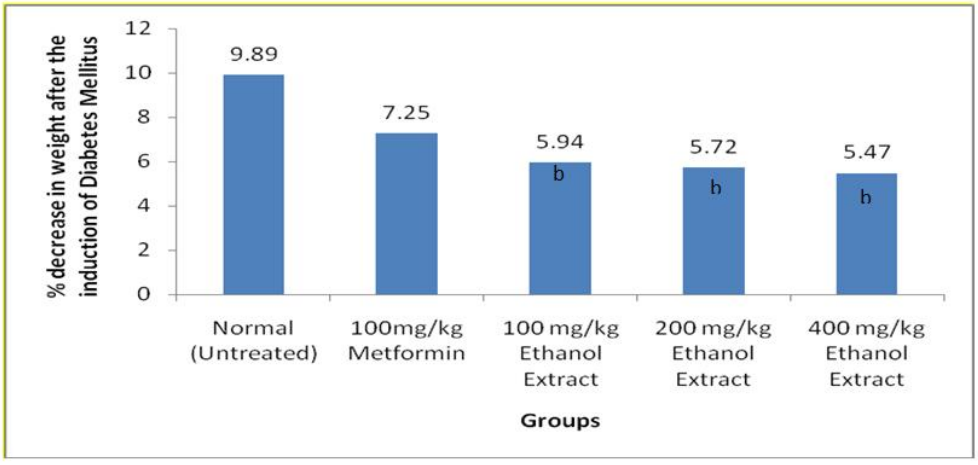

Fig. 1b. Percentage decrease in Weight after the induction of Diabetes Mellitus at the completion of seven days pretreatment

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control

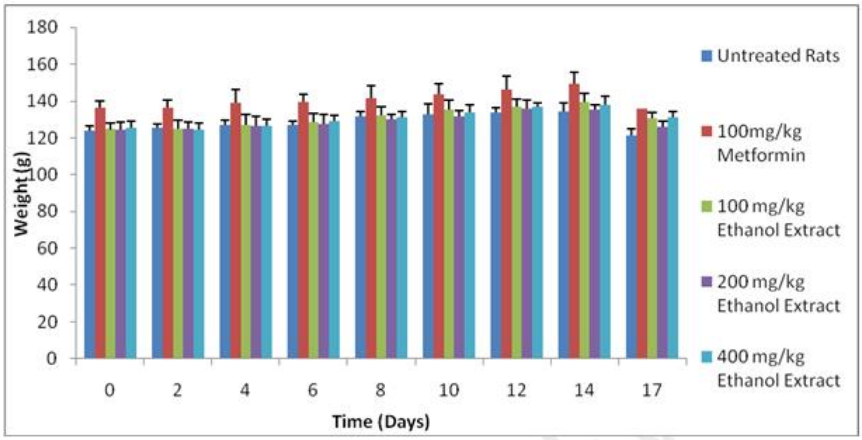

Fig. 2a. Weight (g) profile of rats during two weeks pre-treatment with 100 mg/kg, 200 mg/kg and 400 mg/kg bw. of ethanol extract of *A. indica* leaf expressed as mean ± SD

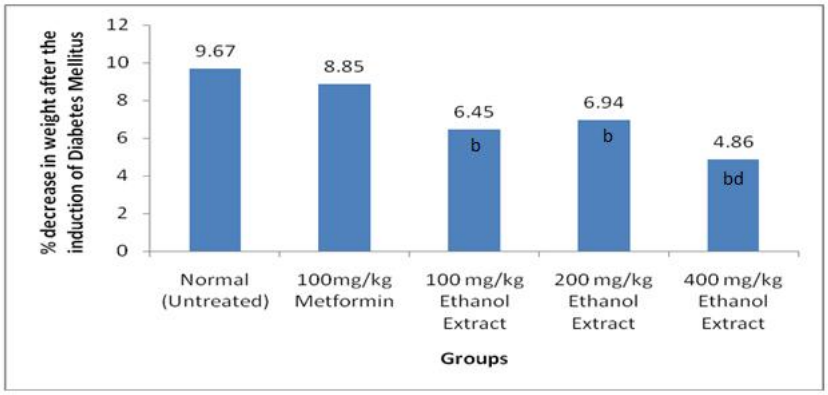

Fig. 2b. Percentage decrease in Weight after the induction of Diabetes Mellitus at the completion of fourteen days pretreatment

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control

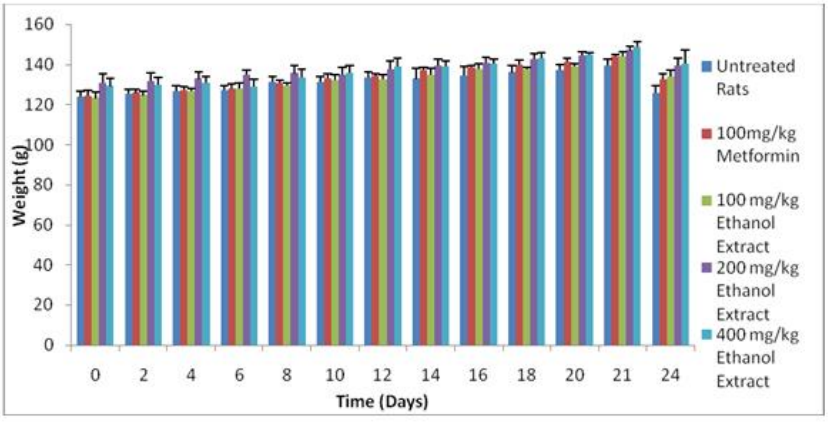

Fig. 3a. Weight (g) profile of rats during three weeks pre-treatment with 100 mg/kg, 200 mg/kg and 400 mg/kg bw. of ethanol extract of *A. indica* leaf expressed as mean ± SD

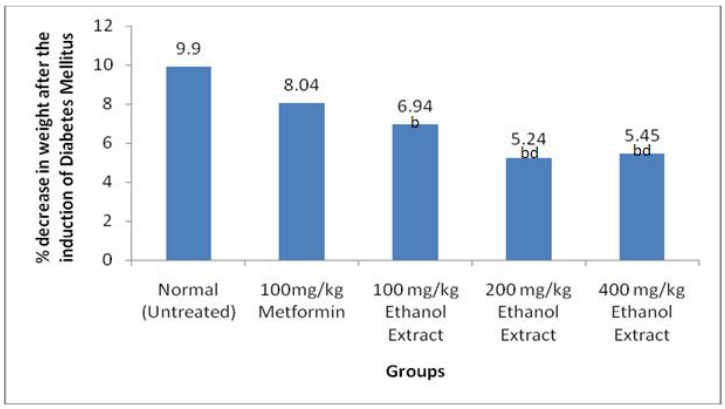

Fig. 3b. Percentage decrease in Weight after the induction of Diabetes Mellitus at the completion of twenty-one days pretreatment

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control

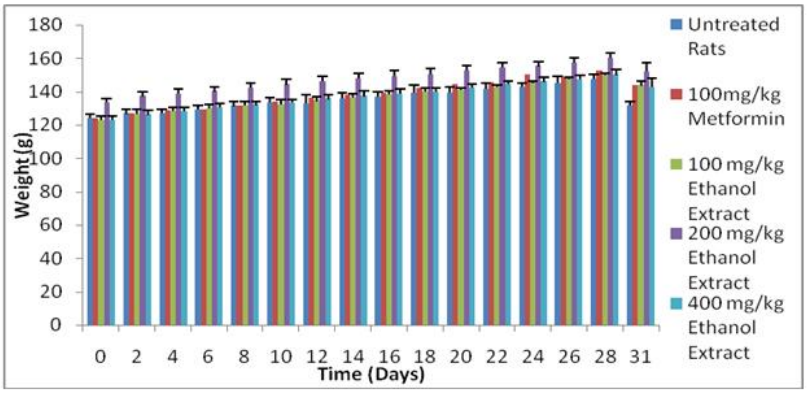

Fig. 4a. Weight (g) profile of rats during four weeks pre-treatment with 100 mg/kg, 200 mg/kg and 400 mg/kg bw. of ethanol extract of *A. indica* leaf expressed as mean ± SD

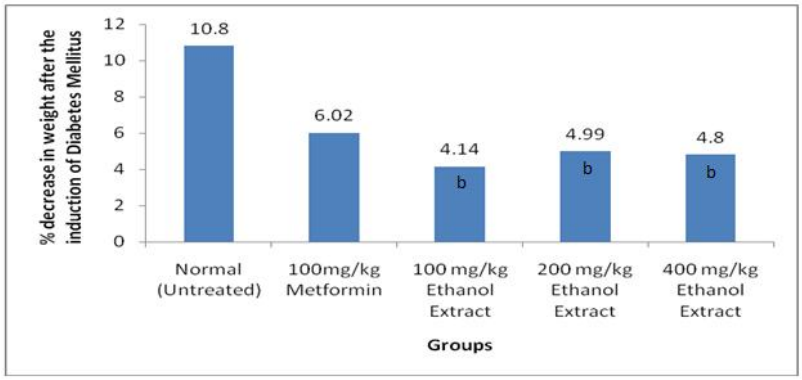

Fig. 4b. Percentage decrease in Weight after the induction of Diabetes Mellitus at the completion of twenty-eight days pretreatment

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control

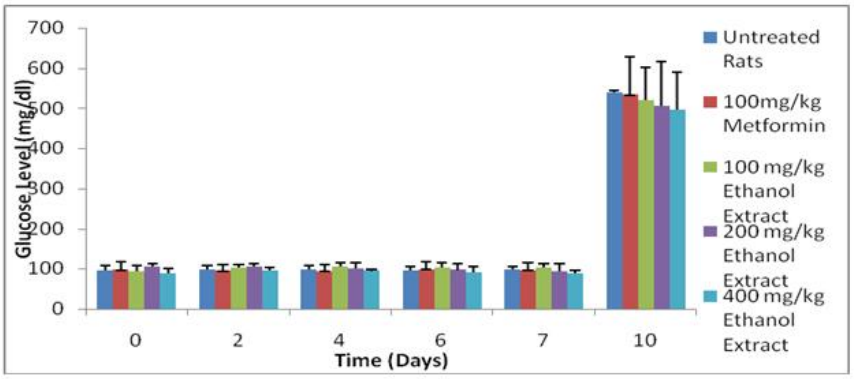

Fig. 5a. Fasting blood glucose (mg/dl) levels during one-week pre-treatment with 100 mg/kg, 200 mg/kg and 400 mg/kg bw. of ethanol extract of *A. indica* leaf expressed as mean ± SD

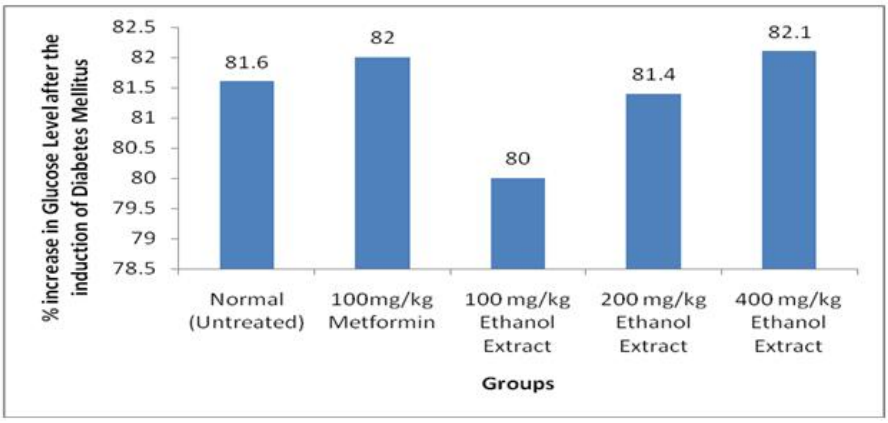

Fig. 5b. Percentage increase in Fasting Blood Glucose level after the induction of Diabetes Mellitus at the completion of seven days pretreatment

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control

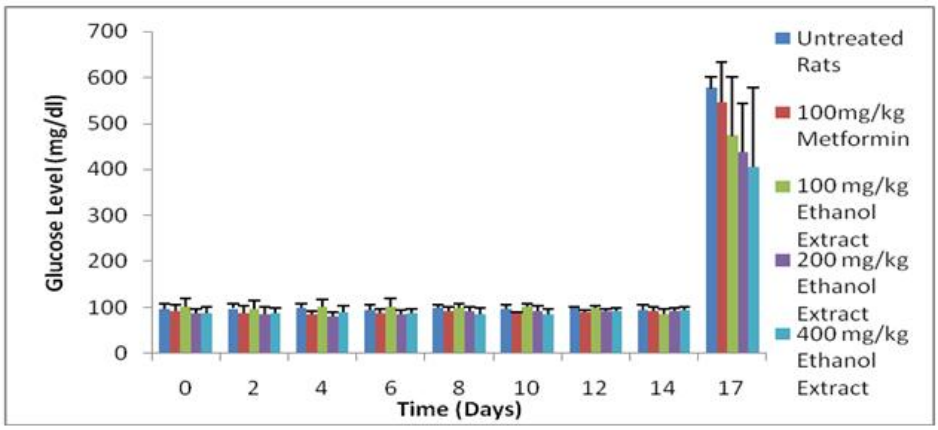

Fig. 6a. Fasting blood glucose (mg/dl) levels during two weeks pre-treatment with 100 mg/kg, 200 mg/kg and 400 mg/kg bw. of ethanol extract of *A. indica* leaf expressed as mean ± SD

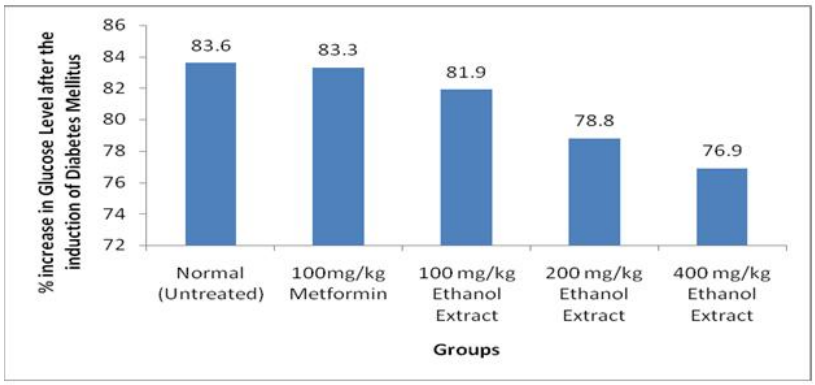

Fig. 6b. Percentage increase in Fasting Blood Glucose level after the induction of Diabetes Mellitus at the completion of fourteen days pretreatment

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control

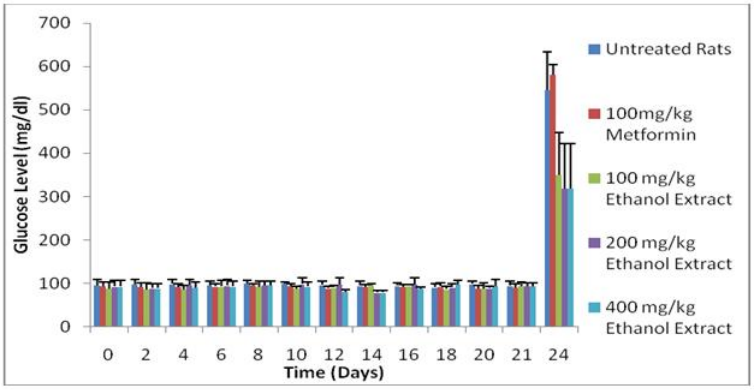

Fig. 7a. Fasting blood glucose (mg/dl) levels during three weeks pre-treatment with 100 mg/kg, 200 mg/kg and 400 mg/kg bw. of ethanol extract of *A. indica* leaf expressed as mean ± SD

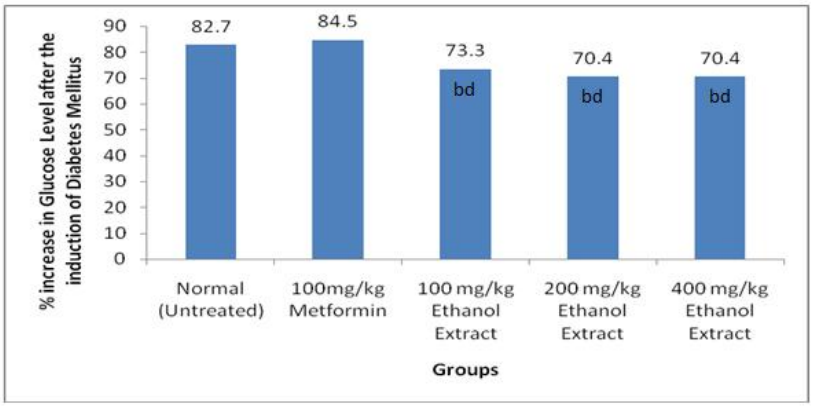

Fig. 7b. Percentage increase in Fasting Blood Glucose level after the induction of Diabetes Mellitus at the completion of twenty-one days pretreatment

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control

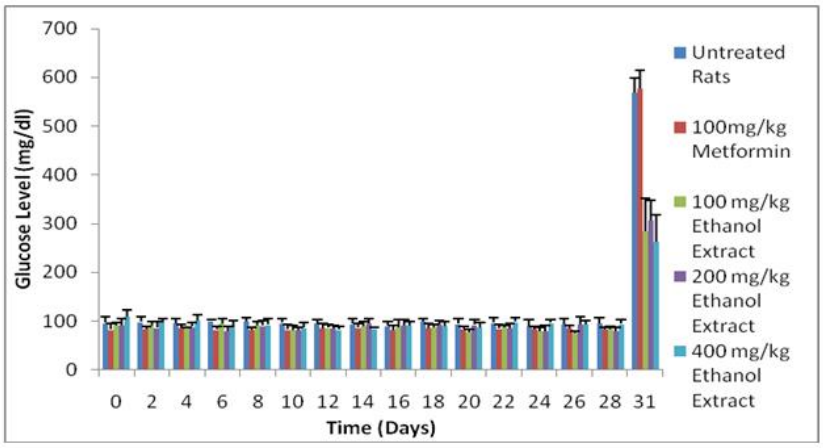

Fig. 8a. Fasting blood glucose (mg/dl) levels during four weeks pre-treatment with 100 mg/kg, 200 mg/kg and 400 mg/kg bw. of ethanol extract of *A. indica* leaf expressed as mean ± SD

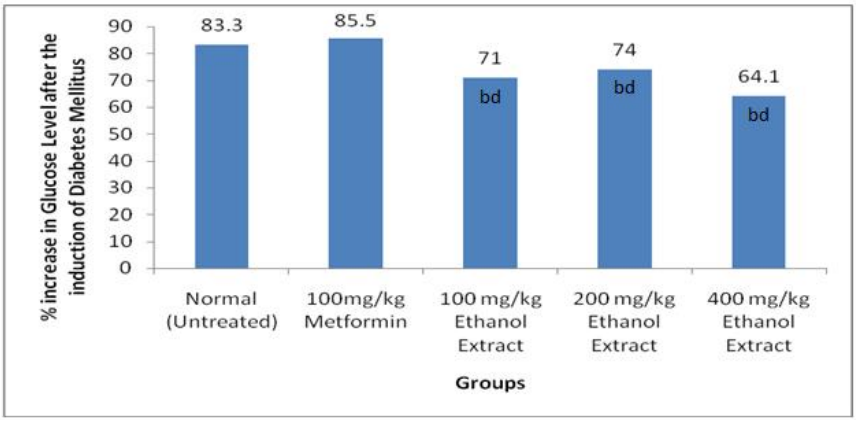

Fig. 8b. Percentage increase in Fasting Blood Glucose level after the induction of Diabetes Mellitus at the completion of twenty-eight days pretreatment

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control

4. DISCUSSION

Azadirachta indica is widely used in ethnomedicine for the treatment and management of different ailments. In the present study, ethanol extract of *A. indica* leaf improved diabetic condition in rats as a result of pre-treatment. Most diseases are difficult to manage when the immune system is already hampered. It will be preferable to prevent the onset of the disease or delay the progression of the disease thereby minimizing the effect of the disease when it is fully blown and enhancing the various methods adapted for its management. This study borders on the prevention of the onset of diabetes by pretreatment with the ethanol extract of *A. indica* leaves thereby boosting the immunity of the experimental animals.

The results of the animal study suggest that the ethanol extract has antidiabetogenic effects by reducing the severity of the symptoms of diabetic condition after the induction of diabetes. The percentage reduction in weight after the induction of diabetes for the rats pretreated for a period of twenty-eight days at a dose of 400 mg/kg bodyweight of ethanol extract of *A. indica* is 4.80% which is lower compared with the percentage reduction in weight of the untreated rats which is 10.8% (Fig. 4). This difference is statistically (p<0.05) significant which showed that the pretreated groups have a better chance

of withstanding weight loss which is one of the symptoms of diabetes.

Figs. 5, 6, 7 and 8 reveal that blood glucose profile of animals pretreated with the various doses of extract (100 mg/kgbw, 200 mg/kgbw and 400 mg/kgbw) for 7, 14, 21 and 28 days fared better compared with the group that was not pre-treated and the group pre-treated with a standard antidiabetic drug. The result revealed that there was a dose-dependent percentage reduction in the fasting blood glucose levels for 14, 21 and 28 days of pre-treatment. Looking at the percentage increase in blood glucose levels forty-eight hours after the induction of diabetes, it was obvious that the percentage increase differed markedly (p<0.05) from that of both the untreated rats and those administered the standard drug metformin after 14, 21 and 28 days compared with the groups that were pre-treated with the ethanol extract. This suggests that consistent pre-administration of *A. indica* leaf extract with increased duration reduces the severity of diabetes. Medicinal plants contain important phytochemicals which can be responsible for its use in the prevention, treatment and management of diseases. Medicinal plants have been reported to have antioxidant activity [23].

Reactive oxygen species (ROS) are one of the main culprits in the pathogenesis of various diseases. Therefore, neutralization of ROS is one of the important steps in the diseases prevention [24]. Antioxidants deactivate ROS often before they attack targets in biological cells [25] and equally play role in the activation of antioxidative enzyme that plays role in the control of damage caused by ROS. Some of the phytochemicals contained in medicinal plants confer its antioxidant activity on the experimental animals. Leaf and bark extracts of *A. indica* have been studied for their antioxidant activity and results of the study obviously indicated that all the tested leaf and bark extracts of neem have significant antioxidant properties [26].

The inference in this work is that *A. indica* leaf has a dose-dependent antidiabetogenic property which its effect becomes more pronounced with duration of administration. The ethanol extract of *A. indica* leaf extract ameliorated some of the symptoms observable in the diabetic untreated rats. The rats pre-treated with the ethanol extract of *A. indica* leaf showed evidence of recovery from the abnormalities created by the induction of diabetes. This agrees with the results of the study reported by Baligar et al. [27] which showed that pretreatment with neem at the higher dose levels moderately restores the rat liver to normal. Also, these results agree with the earlier work by Ezeigwe et al. [28] which reported that pre-treatment with aqueous extract of *A. indica* delayed the onset of diabetes in alloxan-induced diabetic rats.

5. CONCLUSION

The results obtained suggest that the longer the period of pre-treatment with the ethanol extract of *A. indica*, the better the protection against the onset of diabetes. Also, the ethanol extract of *A. indica* leaves exerted a dose-dependent protection and delayed the onset of diabetes. It was most effective at the dose level of 200 and 400 mg/kg bodyweights of the extract. This plant can be recommended for use since it possesses a high protective effect against diabetes and can be easily accessible and affordable by the populace.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that “Principles of Laboratory Animal Care” were followed. All experiments have been examined and approved by the ethics committee of Nnamdi Azikiwe University, Awka, Nigeria in accordance with the Institutional Animal Care and Use policy in Research, Education and Testing.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. Diabetes

Research and Clinical Practice. 2011; 93:299-309.
2. Olatunbosun ST, Ojo PO, Fineberg NS, Bella AF. Prevalence of diabetes mellitus and impaired glucose tolerance in a group of urban adults in Nigeria. J Natl Med Assoc. 1998;90:293-301.
3. Tukur D, Alhaji AA, Shehu AU. A review of population-based studies on diabetes mellitus in Nigeria. Sub-saharan. African Journal of Medicine. 2016;3(2):59-64.
4. International Diabetes Federation. IDF Diabetic Atlas 7th Edition. Available:http://www.idf.org/idf-diabetes-atlas-seventh-edition
[Accessed 30 Aug 2016]
5. Hwang CK, Han PV, Zabetian A, Ali MK, Narayan KMV. Rural diabetes prevalence quintuples over twenty-five years in low- and middle-income countries: A systematic review and meta-analysis. Diabetes Res Clin Pract. 2012;96(3):271–85.
6. Rother KI. Diabetes treatment bridging the divide. New England Journal of Medicine. 2007;356(15):1499-1501.
7. Quinn CH, Royak-Schaler R, Dan-Lender D, Steinle N, Gadalla SH, Zhan M. Patient understanding of diabetes self-management: Participatory decision-making in diabetes care. Journal of Diabetes Science Technology. 2011;5(3): 723-730.
8. Steinsbekk A, Rygg L, Lisulo M, Rise M, Fretheim, A. Group based diabetes self-management education compared with routine treatment for people with type 2 diabetes mellitus. A systematic review with meta-analysis. BMC Health Services Research. 2012;12:213.
9. Seung-Hyun KO, Sin AE, Jae-Hyoung PC, Sun-Hye KO, Kyung-Mi S, Seung-Hwan L. Influence of the duration of diabetes on the outcome of a diabetes self-management education program. Diabetes Metabolism Journal. 2012;36(3):222–229.
10. Horvath K, Koch K, Jeitler K. Effects of treatment in women with gestational diabetes mellitus: Systematic review and meta-analysis. British Medical Journal. 2010;340:c1395.
11. Lawrence JM, Contreras R, Chem W, Sacks DA. Trends in the prevalence of preexisting diabetes mellitus and gestational diabetes mellitus. Diabetes Care. 2008;31(5):899-904.
12. Selvin E, Steffes MW, Zhu H. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. New England Journal of Medicine. 2010;362:800–811.
13. Tobias DK, Hu FB, Chavarro J, Rosner B, Mozaffarian D, Zhang C. Healthful dietary patterns and type 2 diabetes mellitus risk among women with a history of gestational diabetes mellitus. Arch Internal Medicine. 2012;172:1566–1572.
14. Fimognari, Pastorelli R, Incalzi RA. Phenformin-induced Lactic Acidosis in an Older Diabetic Patient: A Recurrent Drama (Phenformin and Lactic Acidosis). Diabetes Care. 2006;29(4):950–951.
15. Chinenye S, Uloko AE, Ogbera AO, Ofoegbu EN, Fasanmade OA, Fasanmade AA, Ogbu OO. Profile of Nigerians with diabetes mellitus – Diabcare Nigeria study group: Results of a multicenter study. Indian Journal of Endocrinology and Metabolism. 2012;16(4):558–564.
16. Ladeji O, Omekarah I, Solomon M. Hypoglycemic properties of aqueous bark extract of oeibapentandra in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology. 2003;84: 139-142.
17. Agunu A, Sadiq Y, Gabriel OA, Zezi AU, Abdurrahman EM. Evaluation of five Nigeria medicinal plants used in treatment of diarrhea in Nigeria. Journal of Ethnopharmacology. 2005;61:209- 213.
18. Nostro A, Germanò MP, D'angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol. 2000;30:379-384.
19. Okwu DE. Evaluation of the chemical composition of indigenous spices and flavouring Agents. Global J. Pure Appl. Sci. 2001;7(3):455-459.
20. Khosla PS, Bhanwra J, Singh SS, Srivasta RK. A Study of Hypoglycaemic Effects of *Azadirachta indica* (Neem) in Normaland Alloxan Diabetic Rabbits. Indian Journal of Physiology and Pharmacology. 2000;44: 69-74.
21. Maragathavalli S, Brindha S, Kaviyarasi NS. Antimicrobial activity in leaf extract of Neem (*Azadirachta indica* linn.). International Journal of Science and Nature. 2012;3(1):110–3.
22. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216–226.
23. Rahmani AH, Aly SM. Nigella sativa and its active constituents thymoquinone shows pivotal role in the diseases prevention and

treatment. Asian Journal of Pharmaceutical and Clinical Research. 2015;8(1):48-53.

24. Mohammad AA. Therapeutic role of *Azadirachta indica* (Neem) and their active constituents in diseases prevention and treatment. Evidence-Based Complement Alternative Medicine; 2016.
[PMCID: PMC4791507]
DOI: 10.1155/2016/7382506
25. Nunes PX, Silva SF, Guedes RJ, Almeida S. Phytochemicals as Nutraceuticals-Global Approaches to their role in Nutrition and Health. InTech. Biological Oxidations and Antioxidant Activity of Natural Products; 2012.
26. Ghimeray AK, Jin CW, Ghimire BK, Cho DH. Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta indica* A. Juss grown in foothills of Nepal. African Journal of Biotechnology. 2009;8(13):3084-3091.
27. Baligar NS, Aladakatti RH, Ahmed M, Hiremath MB. Evaluation of acute toxicity of neem active constituent, nimbolide and its hepatoprotective activity against acute dose of carbon tetrachloride treated albino rats. International Journal of Pharmaceutical Sciences and Research. 2014;5(8):3455-3466.
28. Ezeigwe OC, Ononamadu CJ, Enemchukwu BN, Umeoguaju UF, Okoro JC. Antidiabetic and antidiabetogenic properties of the aqueous extracts of *Azadirachta indica* leaves on alloxan-induced diabetic wistar rats. International Journal of Biosciences. 2015;7(2):116-126.

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