

Original Research Article

Effect of Methanolic Stem Bark Extract of *Acacia nilotica* (Bagaruwa) on Renal Functions in Alloxan Induced Diabetic Albino Rats

ABSTRACT

Aims: Diabetic Mellitus (DM) is a complex metabolic disorder characterize by high blood sugar which gives rise to many complications including diabetic nephropathy. While there were enormous reports on the uses and efficacy of various medicinal plants, very few information are available on their safety and side effects while been used to treat certain ailment. The aim of this study is to determine the effect of methanolic stem bark extract of *Acacia nilotica* on kidney functions in alloxan induced diabetic albino Rats..

Methodology: Thirty (30) young albino rats were grouped into six (6), comprising of five (5) rats per group. Diabetes was induced by single intraperitoneal injection of freshly prepared alloxan monohydrate and blood glucose level was determined forty eight hours (48 hrs) after injection. After induction of diabetes, Metformin and selected doses of *Acacia nilotica* extract were orally administered for 4 weeks after which serum biochemical markers were determined.

Results: . *Acacia nilotica* and metformin decreased fasting blood glucose levels compared to diabetic control Group. No significant changes were observed in electrolytes level with the exception of urea and creatinine that significantly ($P < 0.05$) decreased at 1200mg/kg and 600mg/kg respectively compared to control.

Conclusion: The present study revealed that, the plant extract possessed hypoglycemic effect and that it may have nephrotoxic protection effect.

Keywords: *Acacia nilotica*, Stem bark, Diabetic Mellitus, metformin, Fasting Blood Sugar, Electrolytes, Albino rats

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Comment [S2]: stem bark

1. INTRODUCTION

Diabetic Mellitus (DM) is one of the most common metabolic disorders of great concern all over the world. DM is caused due to lack of insulin or ineffective production of insulin in the pancreas and lead to abnormalities in carbohydrate, protein and lipid metabolism. A total of 463 million people were reported to have DM in 2019 and this number is predicted to rise up to 578 million by the year 2030 and 700 million by 2045 [1]. High blood sugar gives rise to many complications like diabetic retinopathy, diabetic nephropathy, atherosclerosis, hypercoagulability, cardiovascular diseases, coronary heart disease, abdominal obesity, hypertension, hyperlipidemia, cerebrovascular disease, coronary artery disease, foot damage, skin complications, alzheimer's disease, hearing impairment, and depression [1].

Acacia nilotica known as “Bagaruwa” in Hausa language of Northern Nigeria is a proverbial, medium sized tree and broadly scattered in tropical and subtropical countries [2]. The stem bark extract of *Acacia nilotica* have been reported to contain wide range of phytochemicals, among which are flavonoid, triterpenes, alkaloids, cardiac glycosides, carbohydrate, anthraquinone, unsaturated steroids and saponins [3]. Different parts of the plant have been shown to possessed, vasoconstriction, antibacterial, anti-hypertensive, hypoglycemic, anti-inflammatory, antitumor, antifungal, anti-trypanosomal, Antidiarrheal, Antihyperlipidemic and anthelmintic activity [3, 4, 5, 6, 7, 8, 9, 10, 11].

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Therapeutic evaluation of medicinal plants has recently witnessed a growing interest amongst researchers worldwide [12]. While there were enormous reports on the uses and efficacy of various medicinal plants, very few information are available on their safety and side effects while been used to treat certain ailment. Therefore, the aim of this research work is to investigate the effect of methanolic *A. nilotica* stem bark extract on kidney functions in alloxan induced diabetic albino rats.

2. MATERIAL AND METHODS

2.1 Plant collection and Authentication

Stems barks of *A. nilotica* were collected from Sokoto south local government area of Sokoto State, in May 2020. It was authenticated in the botany unit, Biology department of Sokoto State University. The collected sample was cut into small pieces and air dried at room temperature for four weeks. It was then ground into fine powder by a mechanical grinder, followed by sieving through a 40 mesh sieve. The grounded sample was packed in clean dry plastic air tight bag.

2.2. Preparation of plant extract

One hundred grams (100 g) powder of the plant sample was later extracted in 90% methanol (600 ml) for 72 h. The extracts were filtered using clean cloth and Whatman No. 1 filter paper. The filtrate was concentrated in vacuum at 30°C and stored in sterile sample containers at 40°C until further use.

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2.3. Experimental Animals

Thirty (30) healthy adult albino rats were used in the study. They were obtained from the Animal Research Centre (ARC) of the Ahmadu Bello University (ABU), Zaria, Nigeria. The animals were allowed to acclimatize for a period of two weeks in the animal house at the department of Biochemistry in Sokoto State University, Sokoto, prior to the study. The rats were housed in polypropylene cages, maintained under standard laboratory conditions. The animals were maintained on 12 h light, dark cycle, and fed with standard mice pellets.

The total of thirty (30) albino rats were grouped into six (6), comprising of five (5) rats per group (n = 5). The groups were as follows:

Group 1: (Normal control) received distilled water only.

Group 2: (Diabetic control) received Alloxan monohydrate without treatment.

Group 3: (Positive control) Received conventional drug (Metformin 250mg/kg)

Group 4: Test group (*A. nilotica*, 300mg/kg)

Group 5: Test group (*A. nilotica*, 600mg/kg)

Group 6: Test group (*A. nilotica*, 1200mg/kg)

The animals were fasted for 8 hours, but allowed free access to water. Diabetes was induced experimentally by single intraperitoneal injection of freshly prepared alloxan monohydrate (150 mg/kg) in groups 2, 3, 4, 5 and 6. Forty eight hours after injection, blood glucose was determined using glucose analyzer model with glucometer strips. Rat with blood glucose level above 2000 mg/L (>11.1 mmol/L), were considered diabetic and suitable for use in the study. After induction of diabetes, Metformin and selected doses of *A. nilotica* extract were orally administered for 4 weeks.

2.4. Estimation of serum biochemical markers

At the end of the 28 day treatment period, the rats were fasted overnight, anaesthetized with chloroform and then decapitated. Blood samples were collected via cardiac puncture into non-EDTA tubes. It was allowed to coagulate, before centrifugation and the serum separated was used for biochemical analysis. Using corresponding commercially available diagnostic kits, spectrophotometric estimations of Fasting Blood glucose (FBG), Electrolytes (Na⁺, K⁺, Cl⁻, and HCO³⁻), Urea and Creatinine were performed as primary markers of diabetic nephrotoxicity.

2.5. Statistical analysis

The results were expressed as the Mean ± SD (n=5). The results were statistically analyzed using Statistical Package for Social Science (SPSS) version 20. One-way ANOVA was used followed by Duncan's test for parametric multiple comparisons between the control and the treatment groups. Differences were considered significant at p<0.05.

3. RESULTS AND DISCUSSION

The results from this study show that the FBG level in non-DM group was invariable throughout the experimental period and the FBG level before the treatment was significantly higher among the groups compared with non-DM groups (Table 1). After treatment the mean value for the final fasting blood glucose (FFBG) in DM control group was significantly higher compared to non-DM control group (Table 1). However, the level of FBG in *A. nilotica* stem bark extract treated groups decreased compared with positive controls. Similarly metformin treated group significantly decreases when compared with positive control.

Comment [S7]: Please mention in brief the mechanisms by which Stem Bark Extract of *Acacia nilotica* induce its protective effect

Table 1. Effect of stem bark extract of *A. nilotica* on Fasting Blood Glucose Level of experimental rats

Parameters	Normal Control	Diabetic Control	Positive Control	300mg/kg	600mg/kg	1200mg/kg
Initial FBG (mMol/L)	4.26 ± 0.53 [#]	8.16 ± 0.60*	7.76 ± 0.31	8.42 ± 1.23*	8.58 ± 1.09*	8.42 ± 0.63*
Final FBG (mMol/L)	4.31 ± 0.69 [#]	8.92 ± 0.74	6.38 ± 0.59**	7.36 ± 1.35	6.16 ± 0.78**	4.66 ± 0.67 [#]

Values are mean ± SD. Values followed by the same superscript are not statistically different (P<0.05)

The results are similar to those of Niyodusenga, Bukachi [9] who reported the glucose lowering effect of *A. nilotica* in diabetic rats. He suggested that, the hypoglycemic activity of *A. nilotica* extract possibly occurs by stimulating the activity of the β cells of the pancreas and/or due to its insulin like action on insulin sensitive cells.

Adebayo, Yakubu, Egwim4-6 [13] and Yakubu, Bilbis, Lawal4-6 [14] revealed that assessing the level of excretory metabolites such as urea, creatinine and electrolyte levels can be implored as a reliable tool to evaluate renal function.

Table 2. Effect of stem back extract of *A. nilotica* on electrolyte levels of experimental rats

Parameters	Normal Control	Diabetic Control	Positive Control	300mg/kg	600mg/kg	1200mg/kg
Na⁺ (mMol/L)	138.60±2.79	138.40±7.54	144.80±6.30	137.60±7.50	135.60±9.15	138.20±6.06
K⁺ (mMol/L)	4.24±0.49	3.48±0.43	3.80±0.93	3.76±0.86	3.04±0.68	4.34±0.57
Cl⁻ (mMol/L)	99.80±3.83	96.20±7.29	99.01±5.70	93.20±4.87	90.40±2.97	96.01±8.80
HCO³⁻ (mMol/L)	27.20±3.27	28.00±4.69	24.40±5.32	27.20±1.92	25.60±3.58	26.40±3.58
Urea (mMol/L)	4.00±0.89	6.10±0.65	4.96±0.38	4.46±0.70	4.60±0.95	4.66±1.04*
Creatinine (mg/dl)	0.44±0.20	1.02±0.13	0.70±0.26	0.68±0.16	0.62±0.15*	0.60±0.22

Values are mean \pm SD (n=5) *statistically different from diabetic control (P<0.05)

There was no significant change in the serum sodium, potassium, chloride and bicarbonate levels in the groups administered the *A. nilotica* stem back extract at tested doses compared to diabetic control (Table 2). However, there was a significant decrease (P<0.05) in the serum urea and creatinine levels in the groups administered the *A. nilotica* stem back extract at 1200mg/kg and 600mg/kg doses respectively, compared to diabetic control (Table 2). This result is not in agreement with that of Tanko, Ibrahim, Muhammad4-6 [15] who reported a significant increase in the levels of sodium and chloride with no significant change in urea,

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Comment [S9]: Please change it to Adebayo et al [13]

Comment [S10]: Please change it to Yakubu [14]

Comment [S11]: Please change it to Tanko et al [15]

creatinine and potassium. Similarly, Abdulhamid, Ismail [12] observed significant changes in the level of sodium, potassium and creatinine.

It was reported that, changes in serum potassium level usually alternate with those of sodium [16]. Conversely, our result shows that, the serum level of both potassium and sodium did not significantly change, compared to control.

4. CONCLUSION

The present study showed that methanolic stem bark extract of *A. nolitica* produced hypoglycemic effect in alloxan-induced diabetic rats, at the dose tested. Moreover, the plant extract cause slight decrease in the level of electrolytes after treatment at selected doses with significant decrease in urea and creatinine.

UNDER PEER REVIEW

REFERENCES

1. Shubham K, Anu M, Dinesh B, Amit M. Herbal Medicines for Diabetes Management and its Secondary Complications. *Current Diabetes Reviews*. 2021;17,437-56.
2. Tripathi P, Singh A. *Acacia nilotica*, *Albizia saman*, *Azadirachta indica*: Ethnobotany and Medicinal Uses. *Modern Research in Botany*. 2020;1,96–103.
3. Goronyo JI, Ibrahim YKE, Tytler BA, Hussaini M. In vivo antitrypanosomal activities of *Acacia nilotica* stem bark methanol extract in Wistar rats infected with *Trypanosoma brucei* brucei. *AROC in Natural Products Research*. 2022;2,21-7.
4. Aremu O, Olayemi O, Ajala T, Isimi Y, Oladosu P, Ekere K, et al. Antibacterial evaluation of *Acacia nilotica* Lam (Mimosaceae) seed extract in dermatological preparations. *J Res Pharm*. 2020;24,170-81.
5. Bachaya HA, Iqbal Z, Khan MN, Sindhu ZU, Jabbar A. Anthelmintic activity of *Ziziphus nummularia* (bark) and *Acacia nilotica* (fruit) against Trichostrongylid nematodes of sheep. *Journal of Ethnopharmacology*. 2009;123,325–9.
6. Dongmo AB, Nguenefack T, Lacaille-Dubois MA. Antinociceptive and anti-inflammatory activities of *Acacia pennata* wild (Mimosaceae). *Journal of Ethnopharmacology*. 2005;98,201–6.
7. Jodi SM, Omoniyi S, Ungo-kore HY, Ngwai YB. Antifungal activities of honey and acacia *nilotica* extracts. *FUDMA Journal of Sciences*. 2020;4,779 - 83.
8. Lam SK, Ng TB. A dimeric high-molecular-weight chymotrypsin inhibitor with antitumor and HIV-1 reverse transcriptase inhibitory activities from seeds of *Acacia confusa*. *Phytomedicine*. 2010;7,621–5.
9. Niyodusenga A, Bukachi FO, Kiama TN. Cholesterol lowering Effects of *Acacia nilotica* subalata in Normal and Type 2 Diabetic Male Rats. *Rwanda Medical Journal*. 2019;76,1-5.
10. Shoge M, Amusan T. Phytochemical, Antidiarrhoeal activity, Isolation and Characterisation of 11-Octadecenoic Acid, Methyl ester Isolated from the seeds of *Acacia nilotica* Linn. *Journal of Biotechnology and Immunology*. 2020;2,
11. Salissou MTM, Tanko Y, Yaro AH, Magaji RA. Analgesic and Anti-inflammatory activities of N-buthanol portion of *Acacia nilotica* pods in mice and wistar rats. *int J Sci Res Man*. 2022;10,2321-3418.
12. Abdulhamid A, Ismail AM, Sani I, Sulaiman A, Kabir A. Acute and Sub-chronic Toxicity Evaluation of Crude Methanolic Leaves Extract of *Acacia nilotica* (Linn.). *Res J Med Plants*. 2019;13,109-18.
13. Adebayo JO, Yakubu MT, Egwim CE, Owoyele BV, Enaibe BU. Effect of ethanolic extract of *Khaya senegalensis* on some biochemical parameters of rat kidney. *Journal of Ethnopharmacology*. 2003;88,69-72.
14. Yakubu MT, Bilbis LS, Lawal M, Akanji MA. Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine *Biokemistri*. 2003;15,50-6.

15. Tanko Y, Ibrahim RA, Muhammad A, Eze ED, Jimoh A, Mohammed KA, et al. Evaluation of the Effect of Crude Extract and Aqueous Fraction of *Acacia nilotica* Leaves on Haematological Indices and Serum Electrolytes Levels on Diabetic Wistar Rats. *Journal of Advances in Biology & Biotechnology*. 2015;4,1-7.

16. Ikpi DE, Obembe AO, Nku CO. Aqueous leaf extract of *rothmannia longiflora* Improves basal metabolic rate and electrolyte parameters in alloxan induced diabetic rats. *Nigerian Journal of Physiological Sciences*. 2009;24,67-71.

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