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Anti-Diabetic Effect of Methanolic Extract of *Albizia lebbeck* (L.) Benth Leaf on Alloxan-Induced Diabetic Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DYB, OHA, AIE and JAA designed the study, wrote the protocol and the first draft of the manuscript. Authors DYB, OHA, AJA, AAA, ONI, EOO, EK and JAA identified and harvested the plant materials and processed it. All authors took part in managing the laboratory animals during the experiment, analyses of samples and the literature searches. Authors DYB, OHA, AJA, AAA and EK carried out the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: Diabetes Mellitus is a debilitating metabolic disease characterized by hyperglycemia due to insufficient insulin or inability of insulin receptors to respond positively to circulating insulin. Prevalence of the disease is on the global increase annually with tendency to rise from 380 to over 592 million by 2035 in developing countries. Toxicity of some Orthodox drugs in use has led to searches for alternative sources for treatment and management of the disease.
Aim: This work was aimed at investigating the effect of methanolic extract of *Albizia lebbeck* leaf on Alloxan monohydrate induced diabetic albino rats.
Materials and Methods: Qualitative Phytochemical analysis of the plant extract was carried out in Biochemistry Division of National Veterinary Research Institute Vom, Plateau State, Nigeria. Forty female Wistar albino rats were used for the study, divided into five groups (A-E) in two replicates. Group A- negative control (non-induced, not treated) and group B- positive control (induced with Alloxan - 120mg/kg body weight (b.w), administered intra-peritoneally but not treated). Groups C- and D- induced and treated with 100 and 200mg/kg b.w extract respectively. Group E- non-induced but treated with 100mg/kg b.w of extract. Diabetes was established on day 3 after induction. Blood samples were collected through retro-orbital plexus into Fluoride oxalate bottles on days 0, 3, 5 and 9. Glucose was estimated by Glucose Oxidase method.
Results/Conclusion: Phytochemical analysis of the extract showed the presence of saponins, tannins, steroids, cardiac glycosides, flavonoids, alkaloids and terpenes. Glucose analysis results showed dose dependence and statistically significant reduction ($p<0.05$) in blood glucose from 3rd, 5th and 9th days post induction following treatment with *Albizia lebbeck* in group C- 14.5±3.5, 10.4±4.6 and 8.4±3.7; in group D- 14.0±4.7, 10.2±3.3 and 6.5±2.5; and in group E- 5.0±0.6, 3.7±0.5 and 3.5±0.5 respectively. We conclude that methanolic leaf extract of the plant *Albezia lebbeck* (L.) Benth, possesses anti-diabetic properties effective on both normal and Alloxan-induced diabetic rats.

Keywords: Albino rats; methanolic; alloxan; anti-diabetic; Albizia lebbeck; phytochemical analysis.

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia which is attributed to an insufficient supply of insulin [1]. It also occurs when the insulin receptors are resistant to the functions of circulating insulin [2]. Guariguata *et al* [3] postulated that by 2035 the number of adults with diabetes will rise from 382 million to 592 million particularly in developing countries. Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in the increasing prevalence of diabetes cases in the past two decades [4]. Diabetes mellitus is the sixth leading cause of death globally [5].

Although various conventional oral hyperglycemic agents along with insulin are in use for the treatment and management of diabetes mellitus, many of these drugs have various toxic side effects [6, 7], such as hepatotoxicity (troglitazone) and cardiac failure (rosiglitazone) [8]. For these reasons, there is urgent need to develop hypoglycemic agents from natural sources that are cost effective, sustainable and without adverse effects to reduce the risk associated with morbidity and mortality associated with diabetes mellitus. Several studies in this direction have been conducted [9-12], and the search continues.

Albizia lebbeck is a plant species of the genus Albizia of the family Mimosaceae, of the order Rosales, of the series Calciflorae, of the subclass Polypetalae, of the class Dicotyledonae, division Magnoliophyta, of the super division Spermatophyta, of the sub-kingdom Tracheobionta and of the kingdom Plantae [13]. It is also known as the 'Woman's tongue tree' or 'Shirish plant' in English [14], 'Abun mamaki' by the Hausas in Nigeria; is a perennial and medium sized tree 12-21m in height. It is distributed throughout India and in tropical and subtropical regions of Asia and Africa. The tree *Albizia lebbeck* can easily be cultivated and grown in arid environment with good water requirement. The leaf can be described as alternate, stipulate, evenly bipinnate, with grooves on upper side, tapering and about 9'' long [15]. Various parts of the plant have been used as remedies for many ailments. For example, the juice from the fresh leaves is used in the treatment of eye problems [16], cold, cough and respiratory disorders

[17,18]. The bark is known to cure diseases of blood, leucoderma, itching skin disease, pile, excessive perspiration, inflammation, bronchitis, toothache and strengthening of the gums and teeth [19]. The aqueous extract of the bark (2 teaspoonful daily for one week before menses) has been used by females to prevent conception [20]. The flowers are applied externally to boils, eruptions and swellings for quick healing [21]. Other pharmaceutical activities of the plant include anti-asthmatic activity, effect on anaphylactic shock, anti-tussive activity, allergic conjunctivitis, anti-fertility activity, anti-diarrheal activity, antimicrobial activity, anti-inflammatory activity, analgesic, immunomodulatory activity, hypoglycemic activity with ethanol extract, inhibition of cognitive behavior activity and anxiety [15,21]. This study investigated the anti-diabetic effect of methanolic leaf extract of *Albizia lebbeck* in Alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Fresh leaves of *Albizia lebbeck* were obtained from the Federal College of Forestry Jos, Plateau State, Nigeria; authenticated and deposited at the herbarium of the same institution with specimen voucher number 154.

2.2 Methanolic *Albizia lebbeck* Extract Preparation

The plant materials were air-dried under shade at room temperature (20-25^{o}C) for two weeks and grinded into fine powder using a blender. One hundred gram (100g) of the powder was soaked in 80% methanol for 72 hours with intermittent shaking. After 72 hours the mixture was filtered using a clean, dry and white muslin cloth. The filtrate was again filtered with Whatman No 1 filter paper. The filtrate was concentrated in an oven at 41^{o}C. The methanol extract was stored in an air-tight container and kept in the refrigerator at 2-8^{o}C until required for use. Percentage yield of the extract was 3.16%.

2.3 Experimental Animals

Forty female albino rats weighing between 68-145g were used in the study. The animals were obtained from the Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Nigeria. They were housed in the University of Jos animal unit and cared for as recommended in the 'Guide for the care and use of laboratory animals' prepared by the National Institute of Health (NIH) [22]. The animals were maintained on pelletized growers' feed produced at the Dagwom farm Division of the National Veterinary Research Institute, Vom, Plateau State, Nigeria. They were given water *ad libitum*. The animals were acclimatized for two weeks on a regular feed and water prior to the experiment.

2.4 Induction of Diabetes

Diabetes was induced by a single dose of intraperitonial injection with Alloxan monohydrate (120mg/kg body weight) dissolved in sterile normal saline; and administered after an overnight fast. Blood glucose levels of rats were determined on day 3 after induction using the Glucose Oxidase method. Experimental animals with blood glucose levels of 10mmol/L and above from day 3 were considered diabetic and administered the extract once daily until termination of experiment.

2.5 Administration/Treatment with *Albizia lebbeck* Methanolic Leaf Extract and Blood Sample Collection

Animals that had plasma glucose levels of 10mmol/L and above were treated with *Albizia lebbeck* leaf extract orally using a ryheal tube. Blood samples were collected through the reto-orbital plexus of the animals using sterile plain capillary tubes into Fluoride oxalate bottles; and mixed very well to avoid clotting. Within 3 hours, samples were centrifuged at 3000rpm for 5 minutes and the plasma separated, and used for glucose assay immediately.

3. EXPERIMENTAL DESIGN

The rats were divided into five groups of eight rats each, with each group further subdivided into two replicates having four rats in a cage. The groups were treated as follows:

Group A: Negative control group – non-induced and untreated rats
Group B: Positive control group – diabetes induced and untreated rats
Group C: Test group 1 – diabetes induced and treated rats with extract (100mg/kg body weight)
Group D: Test group 2 – diabetes induced and treated rats with extract (200mg/kg body weight)

Group E: Test group 3 – non-induced but treated rats with extract (100mg/kg body weight)

The powdered extract was reconstituted in sterile distilled water and administered orally by intubation to the groups that received the extract as above. Blood samples were collected on days 3, 5 and 9 of experiment and assayed for glucose using Randox kit according to Glucose Oxidase method.

4. BIOCHEMICAL ANALYSIS

4.1 Determination of Plasma Glucose by Glucose Oxidase method of [23]

4.1.1 Procedure

A total of 42 tubes were arranged on a test tube rack comprising 40 tests, 1 standard and 1 blank. One (1) ml of reagent was dispensed into each of the tubes. Ten microliter (10 µl) of plasma was added into the 40 tests according to their numbers, while 10 µl of standard was added to the standard tube and 10 µl of D/W was added into the blank tube. Five milliliter (5ml) of Randox Glucose reagent was added into all the tubes and incubated at room temperature for 10mins; and read spectrophotometrically at a wavelength of 540nm. The Optical Density (OD) obtained was used to calculate the concentration of glucose in each sample.

4.2 Phytochemical Analysis of *Albizia lebbeck* Benth by Method of Soforowa [24]

Phytochemical analysis was carried out on the plant in Biochemistry Division of the National Veterinary Research Institute, Vom, Plateau State, Nigeria. The tests were qualitative analysis, so results were obtained by inference and there was no calculation involved.

4.2.1 Tannins

Procedure: To 0.5g of the plant extract, 1ml of distilled water was added. It was filtered, and 5% Ferric chloride reagent was added to the filtrate. Blue-black, green, or blue green precipitate indicates the presence of tannins.

4.2.2 Saponins

Procedure: To 0.5g of the plant extract in a test tube was water added and the mixture shaken. If there is frothing, the tube is warmed. Persistent frothing indicates a preliminary evidence for the presence of saponins.

4.2.3 Cardiac glycosides

Procedure: To 0.1g of the plant extract dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution, 1.0ml of concentrated Sulphuric acid (H_2SO_4) was added to the mixture. A brown ring indicates the presence of deoxysugar characteristic cordenolides.

4.2.4 Steroids and Terpenes

Procedure: To 0.1g of the plant extract was added 1ml of chloroform to dissolve. Then 1ml of acetic anhydride and 2 drops of concentrated H_2SO_4 were added. A pink colour which changes to bluish green on standing is indicative of the presence of steroids and terpenes.

4.2.5 Alkaloids

Procedure: To 0.5g of the plant extract was added 3ml of 1% aqueous HCl on a steam bath. Two test tubes were arranged and 1ml of the solution added to each of the tubes. A few drops of Mayers reagent was added in one of the tubes and of picric solution to the other. Precipitation with either of these reagents is preliminary evidence of the presence of alkaloids.

4.2.6 Flavonoids

Procedure: To 5ml of detanned solution, 5ml of 20% NaOH was added. A yellow solution indicates the presence of flavonoids.

4.2.7 Antraqquinones

Procedure: To 5g of the extract was added 10 ml benzene. The mixture was filtered and ammonia solution added. The Ammonical layer becomes pink to red indicating the presence of anthraquinones derivatives.

4.3 Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0 and all data were expressed as mean ± Standard Deviation (SD). The results were analyzed by one-way ANOVA to determine significant results. Differences between groups were considered significant at $p<0.05$.

5. RESULTS

The results of the experiment are presented in tables below:

Table 1 shows the effect of methanolic leaf extract of *Albizia lebbeck* on blood glucose levels of Alloxan induced diabetic rats. After induction of diabetes with Alloxan, there was a statistical significant increase ($p<0.05$) in the blood glucose levels of rats in group B, C and D when compared with group A (negative control).

Blood glucose levels during the treatment period on the 5th and 9th day of administration of the extract, at the dosage of 100 and 200mg/kg body weight to groups C and D (diabetic treated groups), showed that there was statistically significant decrease ($p<0.05$) in the blood glucose levels when compared with group B (diabetic control). There was also statistically significant decrease ($p<0.05$) in blood glucose levels of rats in group E (non-induced treated groups) administered 100mg/kg body weight when compared with group A (negative control). The negative control rats maintained their glucose levels within normal range from start of experiment to the end; while the diabetic control animals in group B, had their fasting blood glucose levels on the rise from the baseline to the end of the experiment. It was also observed that animals in the treatment groups (C, D and E) showed a progressive reduction in their fasting blood glucose levels during the treatment period of the experiment as seen in group C- 14.5±3.5, 10.4±4.6 and 8.4±3.7; in group D- 14.0±4.7, 10.2±3.3 and 6.5±2.5; and in group E- 5.0±0.6, 3.7±0.5 and 3.5±0.5. In this research, a classical and significant feature was observed in relation to the non-diabetic treated animals (group E), where the results showed a consistent reduction in glucose. This corroborates with the results of diabetic rats treated with the extract, and which showed statistically significant reduction in their glucose levels, confirming the hypoglycemic effect of *Albizia lebbeck* in experimental diabetes mellitus and in normal or non-diabetic rats.

The results of the qualitative phytochemical analysis showed that the plant *Albizia lebbeck* contains saponins, tannins, steroids, cardiac glycocides, flavonoids, alkaloids and tarpenes, but negative for anthraquinones as seen in Table 2 above.

6. DISCUSSION

The study reported the anti-diabetic effects of mathanolic leaf extract of *Albizia lebbeck* on Alloxan induced diabetic rats. There was a significant increase in the level of blood glucose of rats in group B (diabetic control group); this

Table 1. Effect of Methanolic Leaf Extract of *Albizia lebbeck* on Fasting Blood Glucose Levels in Alloxan induced Diabetic rats

Group	Extract dosage mg/kg	1st day Baseline	3rd day Post-introduction	5th day	9th day
A	Negative Control	4.7±0.6	4.7±0.7	4.8±0.7	4.8±0.7
B	Diabetic control	5.2±0.7	14.7±5.1	14.8±4.1	14.8±5.2
C	Alloxan+100mg/kg.bw	4.6±0.5	14.5±3.5	10.4±4.6*	8.4±3.7*
D	Alloxan+200mg/kg.bw	4.7±0.4	14.0±4.7	10.2±3.3*	6.5±2.5*
E	Uninduced+100mg/kg.bw	5.1±0.6	5.0±0.6	3.7±0.5	3.5±0.5

Values are expressed as mean ±SD, n = 8 in each group.
Key: n= number of rats.
bw = body weight.
** = Significant decrease as compared to diabetic control group (p<0.05).*

Table 2. Results of Phytochemical analysis of Methanolic Leaf Extract of *Albizia lebbeck*

Parameter	Result
Saponins	+ve
Tannins	+ve
Steroids	+ve
Cardiac glycocides	+ve
Anthraquinones	+ve
Flavonoids	+ve
Alkaloids	+ve
Terpenes	+ve

Key: -ve = absent, +ve = present

result agrees with previous studies in rats [25-27] which showed statistically significant increase in levels of glucose in diabetes induced rats that were not treated. Oral administration of mathanolic extract of *Albizia lebbeck* at doses of 100 and 200mg/kg in groups C and D, significantly reduced the blood glucose levels when compared with group B (diabetic control). Also the administration of the extract at 100mg/kg to group E significantly reduced the blood glucose levels as compared with group A. The results from this group clearly shows that methanolic leaf extract of*Albizia lebbeck* has hypoglycemic activity. These findings are in agreement with similar work on anti-hyperglycemic and anti-diabetic activities of the aqueous leaf extracts of *Albizia lebbeck* Linn (benth) and *Psidium guajava* Linn on Alloxan and Streptozotocin induced diabetic mice [28]. It is however not known how methanolic extract of the leaf of *Albizia lebbeck* exert its hypoglycemic effect. Since Alloxan is known to destroy the β cells of the pancreas and there was significant decrease in groups C, D and E. This may be an indication that the possible mechanism of action of the extract may not be due to the ability of the extract to regenerate dead cells or stimulate insulin secretion by surviving beta cell as concluded by another group of researchers [26]. Perhaps, the extract acts directly by increasing the uptake of glucose across the membrane. The results of the phytochemical analysis of *Albizia lebbeck* which was carried out during this research is in agreement with [13]. However, the chemical constituent(s) responsible for the hypoglycemic effect observed are not known, but it has been reported that the main phytochemical constituents of *Albizia lebbeck* which include alkaloids, flavonoids, tannins and saponins may have a direct or indirect bearing to this observation [13,15,29]. This calls for further studies to identify the specific active components of the plant with a view to isolating them for development of therapeutic remedies for the treatment and management of diabetes.

7. CONCLUSION

The present research work revealed that the administration of methanolic leaf extract of *Albizia lebbeck* showed anti-diabetic activity in Alloxan induced diabetic rats and non-diabetic rats. These findings are very significant as they suggest that the plant Albizia lebbeck has the potentials to lower blood glucose in diabetes and in normal animals, which could be harnessed for use in clinical practice. Based on results obtained from this research, the researchers concluded that methanolic leaf extract of *Albizia lebbeck* is effective in lowering blood glucose levels in normal and in diabetic rats. The researchers recommend that further studies be done to understand the mechanism involved in the hypoglycemic effect of this extract in addition to its trial for the management of diabetes in human patients.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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