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ANTI-DIABETIC ACTIVITY OF ALBIZIA AMARA BOIVIN LEAF ON STREPTOZOTOCIN INDUCED DIABETES IN RATS

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ABSTRACT

The incidence of diabetes mellitus is increasing worldwide in adults and is one of the leading cause of death. It is predicted that by 2030, the number of people with diabetes will increased in India, China and the United States. The recently used effective drugs for diabetes mellitus are insulin injection and hypoglycemic agents, but these drugs are having undesirable adverse effects. Therefore, the present study aimed to evaluate the anti-diabetic activity of *Albizia amara* Boivin leaf extracts on streptozotocin induced diabetic rats. The Diabetes was induced in male albino wistar rats by single intraperitoneal injection of streptozotocin (STZ) (50 mg/kg b.w.). The diabetic rats were administered orally with *Albizia amara* Boivin leaf methanolic extract at the dose of 250, 500mg/kg b.w. and glibenclamide (600µg/kg b.w.) as standard by oral route for 45 days. Methanolic extracts of *Albizia amara* Boivin leaf and glibenclamide treated diabetic rats showed significant reduction in blood glucose and glycosylated hemoglobin levels; in addition, plasma insulin and hemoglobin levels were elevated. Results of methanolic extract of *Albizia amara* were comparable to the standard drug glibenclamide. The present findings support the usage of the plant extracts for the treatment of diabetes and exhibited anti-diabetic activity when compared with standard hypoglycemic agent.

KEY WORDS: Diabetes, *Albizia amara*, glibenclamide, streptozotocin

1. INTRODUCTION:

The increasing worldwide incidence of diabetes mellitus in adults comprises a global public health burden. It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes **(Frode and Medeiros, 2008).** By definition, diabetes mellitus is categorized as a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.

The recently used effective drugs for diabetes mellitus are insulin injection and hypoglycemic agents, but these drugs are having undesirable adverse effects and have no effects on diabetes complications in long term **(Malviya *et al.,* 2010).**

Therefore, it is essential to find effective drugs with minimum side effects in treating diabetes. Medicinal plants are one of the good source as alternative or complementary therapy for this and other diseases. Although there are various plants have been traditionally used throughout history to reduce blood glucose and improve diabetes complications **(Bahmani *et al.,* 2014).**

2. MATERIALS AND METHODS:

2.1. Plant material collection, processing and preparation of extracts

The leaves of *Albizia amara* Boivin were collected from the nearby regions of Bhopal in the month of December. The plant was identified by Department of Botany, Safia College, Bhopal M.P. Leaves were shade dried for a month, and powdered in mechanical grinder and stored in airtight container.

The air dried powdered leaves (1000g) were defatted with petroleum ether and remaining marc was extracted with methanol (70%v/v) and concentrated in rotary evaporator under reduced pressure to get methanol extract (120.2g) **(Mukherjee, 2010).**

2.2. Preliminary phytochemical screening

The freshly prepared extract was subjected for qualitative chemical tests for the presence various phytoconstituents viz. alkaloids, glycosides, tannins, flavonoids and phenolic compounds, fats, resins, steroids, proteins and amino acids etc **(Farnsworth, 1966).**

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2.3. Chemicals

STZ and glibenclamide was obtained from Sigma-Aldrich Company (Bangalore, India). The other experimental chemicals used were of analytical grade and were purchased from Hi Media (Mumbai, India).

2.4. Acute oral toxicity studies

Healthy Male Albino mice 220-250 g (Wistar strain) were selected for acute oral toxicity. Animals were acclimatized with free access to food and water. The study group used 6 animals in each group. Animals were kept fasting for overnight providing only water, after that the extracts were administered orally at the dose level of 5 mg/kg bodyweight intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals then the dose assigned was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If the mortality was not observed, the procedure was repeated for further higher dose such as 50, 250, 500 and 2000mg/kg body weight **(Anonymous, 2001; Kokate, 2005).**

2.5. Experimental animals

Adult male albino Wistar rats with body weight (b.w.) above 180g -200g were taken for the study. They have been housed at poly propylene confines and kept up in standard environment (12 h light and 12 h dark cycle, (25 ± 3) °C). The rats were fed with standard rat pellet diet and given water *ad libitum* and maintained at Central Animal House. All studies were conducted as per the guideline of CPCSEA, and the study was endorsed by the Institutional Animal Ethical Committee of Vedica College of B. Pharmacy, RKDF University, Bhopal. Animals were adapted for 3 days in the research laboratory before start of the experiments.

2.6. Experimental induction of diabetes (Frode, 2008; Gupta, 2009)

Diabetes was prompted through single intraperitoneal injection of freshly prepared streptozotocin (STZ) (50 mg/ kg b.w.) in 0.1 M citrate buffer (pH = 4.5) to overnight starved rats. Diabetic rats were permitted to drink 20% glucose solution overnight to overcome the initial drug induced hypoglycemic death. The blood glucose level was measured after three days, and rats with glucose levels >200 mg/dL were considered as diabetic. At the time of induction, control rats were injected with 0.2 mL of vehicle (0.1Mcitrate buffer, pH 4.5) alone.

2.7. Experimental design

2.7.1. OGTT model of normal rats

Overnight fasted normal rats were divided into five groups of six in each. The single dose (1000 mg/kg bw) of each prepared combination was administered orally in the rats with the help of gastric canula. The test drugs were administered in the animals one hour prior to the glucose load (2g/kg b. w.). The blood samples were withdrawn from the animals by puncturing the retro-orbital plexus under mild anaesthesia five minutes before the administration of test drugs. Later on blood samples were withdrawn at 0, 30, 60, 90 and 120 min after glucose administration, the serum glucose was estimated by Glucose oxidase-peroxidase method **(Trinder, 1969).**

2.7.2. STZ-induced diabetes model

In this experiment 30 rats were used. They were separated into five groups of 6 rats each. The methanolic extracts of *Albizia amara* Boivin leaf and glibenclamide 0.5 mL of 0.9% saline and administered orally (45 days).

Group I. Control rats (were given 0.5 mL of 0.9% saline orally for 45 days).

Group II. Diabetic group (STZ 50 mg/kg b.w.).

Group III. Diabetic rats were given methanolic extracts of *Albizia amara* Boivin Linn leaf (250 mg/kg b.w. dissolved in 0.5 mL of 0.9% saline) orally for 45 days.

Group IV. Diabetic rats were given methanolic extracts of *Albizia amara* Boivin leaf (500 mg/kg b.w. dissolved in 0.5 mL of 0.9% saline) orally for 45 days.

Group V. Diabetic rats were given Glibenclamide (600 µg/ kg b.w. dissolved in 0.5 mL of 0.9% of saline) for 45 days

The blood samples were collected one hour before dosing on day 0, 15th, 30th and 45th of the study through retro-orbital plexus under mild anesthesia. The blood serum was separated by centrifugation at 3000 rpm for 15 min. The resultant supernatant was analysed for SGL and other biochemical estimation. The body weight of all the animals was recorded proceeding to the treatment **(Frode, 2008; Gupta, 2009).**

2.8. Biochemical analysis

2.8.1. Estimation of blood glucose

Glucose level in plasma was determined by glucose oxidase/ peroxidase method using areagent kit. In brief, to 0.01 mL of plasma, standard and distilled water (blank) into 3 test tubes, 1.0 mL of the enzyme was added, mixed and kept at 37 °C for 15 minutes. The color developed was read at 505 nm against reagent blank **(Sundaram *et al.,* 2014).**

2.8.2. Qualitative determination of plasma insulin

The plasma insulin was assayed by Enzyme Linked Immunosorbent Assay (ELISA) method using Boehringer- Mannheim kit. In brief, 0.1 mL of plasma was injected into the plastic tubes coated with monoclonal anti-insulin antibodies. Phosphate buffer and anti-insulin POD conjugate was added to form anti-insulin antibody–POD conjugate. Substrate chromogen solution was then added to form indicators reaction. A set of standards were also treated in a similar manner. After the development of color the absorbance was read at 420 nm **(Trinder, 1969).**

2.8.3. Determination of hemoglobin

Hemoglobin content in blood was determined by the cyanmethemoglobin method. In brief, the reaction mixture in a volume of 5.02 mL contained 5 mL of Drabkin's reagent and 0.02 mL of blood. The reaction mixture was kept at room temperature for 5 min to ensure the completion of the reaction. The solution was read at 540 nm together with the standard solution of cyanmethemoglobin **(Andersen *et al.,* 1993).**

2.8.4. Estimation of glycosylated hemoglobin (HbA1c)

The saline washed erythrocytes (0.5 mL) were lysed with 5.5 mL of water, mixed and incubated at 37°C for 15 minutes. The contents were centrifuged and the supernatant was discarded, then 0.5 mL of saline was added, mixed and processed for estimation. To 0.02 mL of aliquot, 4 mL of oxalate hydrochloric solution was added and mixed. The contents were heated at 100 °C for 4 hours, cooled and precipitated with 2 mL of 40% TCA. The mixture was centrifuged and to 0.5 mL of supernatant, 0.05 mL of 80% phenol and 3.0 mL of concentrated H_2SO_4 were added. The color developed was read at 480 nm after 30 minutes **(Drabkin and Austin, 1932).**

2.9. Statistical analysis

All the results are expressed as mean ± SEM, analysed by one-way ANOVA followed by Tukey's multiple comparison analysis as post-hoc test. The $P<0.05$ was considered to be statistically significant.

3. RESULTS:

3.1. Phytochemical Screening

The phytochemical screening of methanolic extract of *Albizia amara* revealed the presence of alkaloids, terpenoids, sterols, glycosides, phenolic compounds, tannins, flavonoids, carbohydrates, proteins and saponins.

3.2. Acute Toxicity studies

Acute oral toxicity studies showed no mortality up to the dose of 2000 mg/kg body weight. So, the selected extract is safe for long term use.

3.3. OGTT study

Figure 1 shows the changes in the blood glucose levels in normal control and different experimental groups after oral administration of glucose (2g/kg b.w.). In all experimental groups oral feeding of glucose induced a significant elevation in blood glucose after 30 minutes as compared with corresponding values on 0 min. After 120 min the blood glucose level in normal control rats (group I) tend to return near normal level. The untreated diabetic rats (group II) showed maximum increase in blood glucose after 60 min and mild decline after 90 min. In diabetic rats treated with 500 mg/kg b.w. of methanolic extract of *Albizia* amara leaf (group IV) or Glibenclimide (group V), the blood glucose level showed continues decline after 60 min and after 120 min the level reached to near initial value at 0 min.

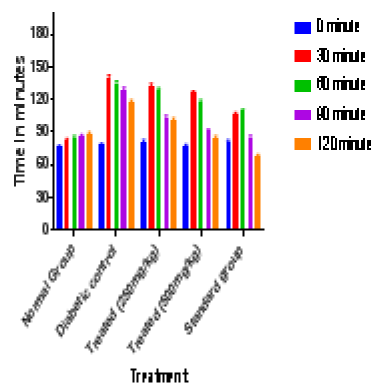

Figure 1 Hypoglycemic effect of *Albizia amara* leaves methanolic extract (250 and 500 mg/kg) in oral glucose tolerance test (OGTT)

3.4. Effect on body weight

Changes in body weight of the normal control, diabetic control and experimental rats are depicted in figure 2. Diabetic rats treated with methanolic extract of *Albizia amara* leaf extract at different doses (250 and 500 mg/kg b.w./day) showed stastically significant dose dependent increase in mean body weight (P≤0.001).

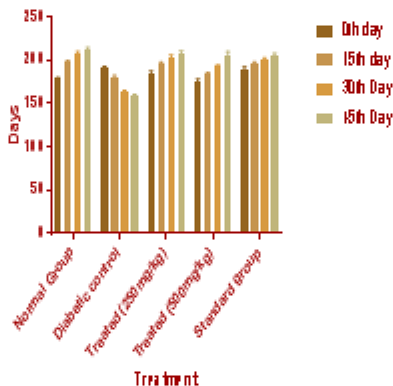

Figure 2: Effect of methanolic extract of *Albizia amara* Linn leaves on body weight (g) in STZ-induced diabetic rats

3.5. Effect on blood glucose level

The fasting blood glucose levels in diabetic rats treated with 250 and 500 mg/kg b.w./day doses methanolic extract of *Albizia amara* leaf showed significant dose dependent decline after 15, 30, and 45 days of treatment as represented in figure 3. In diabetic rats receiving glibenclamide treatment, the fasting blood glucose level also reduced significantly (P ≤ 0.01) after 15, 30, and 45 days of treatment as compared with corresponding values on 0 days.

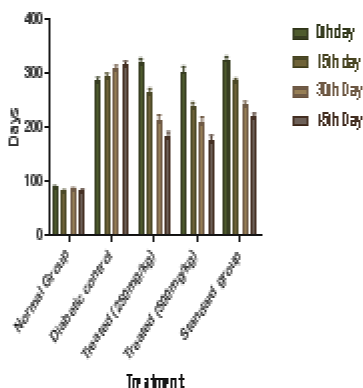

Figure 3: Effect of methanolic extract of *Albizia amara* leaves on fasting blood glucose (mg/dL) in STZ-induced diabetic rats

3.6. Effect on serum insulin and glycosylated hemoglobin

The methanolic extract (250 and 500mg/kg) showed significant dose dependent decrease in total glycosylated Hb levels compared to diabetic control group (Figure 4). The methanolic extract (250 and 500 mg/kg) showed significant dose dependent increase in plasma insulin levels compared to control group.

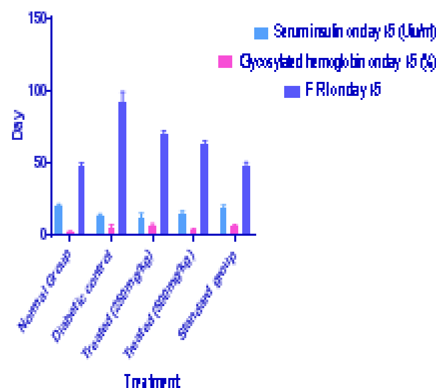

Figure 4: Effect of methanolic extract of *Albizia amara* leaves on serum insulin and glycosylated hemoglobin in rats

3.7. Effect on serum triglyceride levels

Methanolic extract (250 and 500 mg/kg) showed significant dose dependent decrease in TG levels compared to control and other groups, depicted in figure 5.

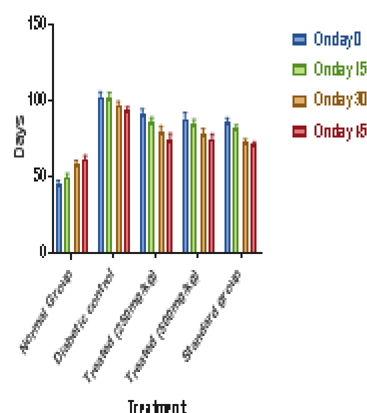

Figure 5: Effect of methanolic extract of *Albizia amara* leaves on serum triglyceride levels in rats

3.8. Effect on lipid parameters

Methanolic extract of *Albizia amara* leaves (250 and 500mg/kg) showed significant dose dependent decrease in LDL and VLDL levels compared to

control group. Methanolic extract (250 and 500 mg/kg) showed significant dose dependent increase in HDL levels compared to control group as represented in figure 6.

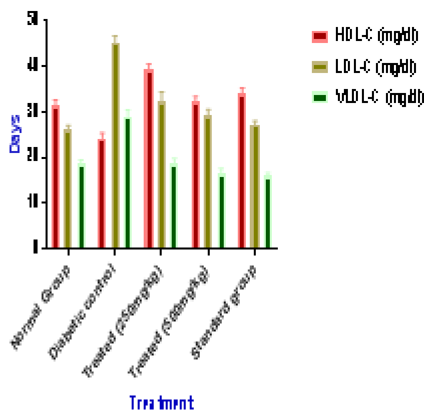

Figure 6 Effect of methanolic extract of *Albizia amara* leaves on lipid parameters in rats

3.9. Effect on serum total cholesterol levels

Methanolic extract of *Albizia amara* leaves (250 and 500 mg/kg) showed significant dose dependent decrease in total cholesterol levels compared to control group (figure 7).

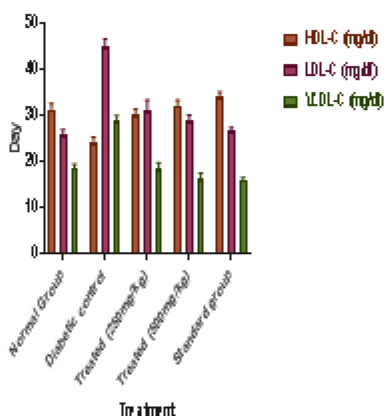

Figure 7: Effect of methanolic extract of *Albizia amara* leaves on serum total cholesterol levels in rats

3.10. Effect on renal function biomarkers

Methanolic extract of *Albizia amara* leaves (250 and 500 mg/kg) showed significant dose dependent decrease in SGOT, SGPT, urea and creatinin levels compared to control group, represented in figure 8.

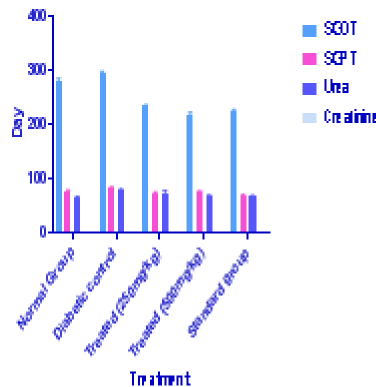

Figure 8: Effect of methanolic extract of *Albizia amara* leaves on renal function biomarkers in rats

4. DISCUSSION

In the present study, the continuous post-treatment with the methanolic extract showed significant (P<0.01) hypoglycemic activity in OGTT model. The phytochemical screening of methanolic extract of *Albizia amara* revealed the presence of alkaloids, terpenoids, sterols, glycosides, phenolic compounds, tannins and flavonoids, carbohydrates and proteins and saponins.

Induction of diabetes by STZ causes loss of body weight due to increase wasting of muscle and tissue proteins loss. In diabetic rats, reduction in body weight observed might be due to degradation in structural proteins due to carbohydrate deficiency. A significant (P<0.001) increase in body weight of diabetic rats treated with methanolic extracts of *Albizia amara* showed the blood glucose stabilization effect which in turn showed gain in body weight.

Treatment of methanolic extracts of *Albizia amara* lowers blood glucose in STZ induced diabetic rats significantly (P<0.001) and its effect was almost equal to that of glibenclamide.

The increase in level of glycosylated hemoglobinis directly proportional to the decreased level of total hemoglobin in diabetic control rats. Glycosylated hemoglobin is used as one of the most reliable marker and standard diagnosis practices for estimating the degree of protein glycation in diabetes mellitus. On oral administration of methanolic extracts of *Albizia amara* significantly (P<0.001) decreased the glycosylated hemoglobin level. A marked increase in serum concentration of TC, TG, LDL and decreased HDL was observed with

diabetic rats than normal control group which is often linked with hyperlipidaemia.

Our result showed significantly ($P<0.001$) fall in TC, TG, and LDL levels as well at the same time raised HDL level near to control on oral administration of methanolic extract (500 mg/kg) after 45 days repeatedly.

Increased serum concentration of qualitative diagnostic enzymes such as SGPT and SGOT were observed in diabetic rats indicating an altered liver function.

On treatment with methanolic extracts of *Albizia amara* significantly ($P<0.01$) reversed the elevated marker enzymes i.e. SGOT and SGPT and restored to normal values.

Reports of earlier studies suggested that various plants was proved to possessing wide variety of natural antioxidant constituents such as tannins, saponins, alkaloids, flavonoids, phenolic acids and poly phenols etc. which enhances free radical scavenging activities and responsible for treatment of diabetic related complications **(Marles, 1995).**

The Methanolic extracts have been chosen because of its expected saponins, alkaloids, flavonoids, phenolic acids and poly phenols contents that were reported to have anti-diabetic activity.

The phytochemical analysis of *Albizia amara* showed the presence of tannins, flavonoids, saponins, and sterols. Their anti-diabetic ability to regenerate the pancreatic *β*-cell has already been proved. Sterols can decrease blood sugar in experimental animal models. The antioxidant activity of the phenolic, tannins, and flavonoid compounds are attributed to its redox properties which can act as reducing agents, hydrogen donators, and singlet oxygen quenchers. Polyphenolics having hydroxyl groups are very important plant constituents which can protect body from oxidative stress **(Atangwho, 2009).** Flavones glycosides have also been reported as strong antioxidants and potent hypoglycemic agents **(Mahmoud, 2014).**

The present study clearly concluded that methanol extract *of Albizia amara* possesses the ability to control blood glucose in diabetes. Its anti-hyperglycemic potential to prevent diabetic related complications. The methanolic extracts of *Albizia amara* found to be potential as anti-diabetic extract in STZ-induced diabetic model through reducing oxidative damage and modulating antioxidant enzymes by dose dependent manner. The Results of the study scientifically validates the traditional use of *Albizia amara* leaves. It may be concluded that methanolic extract of the plant at 500mg/ kg b.w. possess hypoglycemic activities and the plant extract may be used as hypoglycemic agent.

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