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ANTIDIABETIC ACTIVITY OF ETHENOLIC EXTRACT *ALBIZZIA LEEBECK* IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia and abnormalities in carbohydrate, fat and protein metabolism. It results from defects in insulin secretion, insulin sensitivity, or both. Chronic microvascular, macrovascular, and neuropathic complications The present study was carried out to evaluate the antidiabetic activity of *Albizzia leebeck* ethanolic leaf extracts in alloxan induced diabetic rats for 7 days. The oral administration of ethanolic leaf extract of *Albizzia leebeck* were found to have potent antidiabetic activity that reduces blood sugar level in alloxan induced diabetic rats. In this study, the antihyperglycemic effect of extract at 200mg/kg b.w.p.d were investigated. Glibenclamide was used as reference drug at 2.5mg/kg b.w.p.d. Fasting blood glucose, serum insulin and liver enzyme levels was evaluated in normal, diabetic and treated rats.

Key words: Diabetes mellitus, *Albizzia leebeck* , Alloxan, Hypoglycemic Effect, Hypolipidemic Effect, Glibenclamide .

1. INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders with micro-and macrovascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world [1, 2]. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus [3]. There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents [4,6]. There are numerous traditional medicinal plants reported to have hypoglycemic properties such as *Allium sativum* (Garlic), *Azadirachta*

indica (Neem), *Vinca rosea* (Nayantara), *Trigonella foenum* (Fenugreek), *Momordica charantia* (Bitter ground), *Ocimum santum* (Tulsi). Many of these are less effective in lowering glucose levels in severe diabetes.

Albizzia leebeck, (Mimosaceae) is an herbaceous subshrub also known as siris, Kalindi, Chichola. *Albizzia lebbeck* has a long history of use in Indian traditional medicine particularly for the treatment of asthma and allergic disorder. The leaves are good for ophthalma. The bark of *Albizzia lebbeck* has been used in Ayurveda for treatment of bronchitis, asthma, leprosy, eczema, diseases of the blood, leucoderma, skin disease, piles, pruritus, paralysis, gum inflammation and worm infestation. The root is used in hemicrania. The flowers are given for asthma and for snake bite. All parts of plant are recommended for treatment of snake bite [10].

2. MATERIALS AND METHODS

2.1. Plant Material

During summer the leaf of matured *albizzia leebeck* was freshly collected from Ujjain (M.P) india. leaves are chopped , shade dried and coarsely powdered. An authenticated voucher specimen (no.328/bot/safia/12) of the plant has been preserved in our department for future reference.

2.2. Alcoholic Extraction

The whole plants were collected and shadow dried. The powder was macerated with ethanol at room temperature for 3 days. The extract were kept in refrigerator for futher use. A dose determination process was carried out by administering 100mg/kg, 200mg/kg, 400mg/kg (60mg/ml) b.w.p.d., and 1600mg/kg, 2000mg/kg(200mg/ml) b.w.p.d were dissolved in distilled water (5ml). 200mg/kg b.w.p.d. and above were found to have similar and better hypoglycemic activity. Thus 200mg/kg b.w.p.d was chosen for the treatment.

2.3. PHYTOCHEMICAL STUDY

Table No. 1 Phytochemical study

COMPOUND	CHEMICAL TEST	PLANT EXTRACT
Alkaloid	Dragendorff test	-
Carbohydrate	Molish test	-
Protein	Biuret test	-

Glycosides	Borntrager test	-
Acetic acid	Ninhydrin test	+
Saponin	Foam test	+
Steroid	Salkowaski test	-
Tannin	FeCl3 test	+
Flavonoid	Shinoda test	+
Triterpenoid	Libermann test	-

2.4. Animals

Wistar albino rats (8–10 weeks) of both sexes were obtained from the animal house of Sapience bioanalytical laboratory, Indrapuri, Bhopal. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours ad libitum.

2.5. Oral Glucose Tolerance Test

Rats were divided into four groups containing six animals in each group. All animals fasted before treatment. Group I was kept as vehicle control which received only distilled water daily, group II Alloxan induced diabetic rats and serve as diabetic control, group III received ethanolic extract 200 mg/kg., group IV received Glibenclamide (GBC) at the dose of 2.5mg/kg body weight once in a day. Blood samples were collected from tail vain just prior to drug administration. Serum glucose level was measured immediately by using glucose estimation kit (Span Diagnostic Pvt. Ltd. Surat, India).

2.6. Acute Oral Toxicity Studies

Albizzia leebeck at the dose range of 100 mg–2000 mg/kg were administered orally to different group of rats comprised of six rats in each group. Mortality was observed after 72 hours. Acute toxicity was determined according to the toxicity guidline no. 423[14].

2.7. Experimental Design

Three groups of rats, four in each received the following treatment schedule. Group I: Normal control (saline).Group II: Alloxan treated control (150 mg/kg. ip), Group III: Alloxan (150 mg/kg.ip) + *albizzia leebeck* leaf extract (200 mg/kg, p.o),Group IV: Alloxan (150 mg/kg. ip) + Standard drug, Glibenclamide (2.5 mg/kg, p.o).Leaf extract and standard

drug glibenclamide (2.5 mg/kg) and saline were administered with the help of feeding cannula. Group I serve as normal control, which received saline for 7 days. Group II to Group IV are diabetic control rats. Group III (which previously received alloxan) are given a fixed dose whole plants extract (200 mg/kg, p.o), and Group IV received standard drug glibenclamide (2.5 mg/kg) for 7 consecutive days.

2.8. Induction of Diabetes in Experimental Animals

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg) [15]. First weighed individually for each animal then Alloxan was admistered according to the body weight and then solubilized with 5 ml sterile water for injection just prior to administered. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection.

2.9. Collection of Blood Sample and Blood Glucose Determination

Blood samples were drawn from tail tip of rat at weekly intervals till the end of study (i.e., 1 weeks). Fasting blood glucose estimation and body weight measurement were done on 1st and 7th day of the study. Blood glucose estimation can be done by one touch electronic glucometer using glucose test strips. The serum samples were subjected to biochemical parameters examination like SGOT, SGPT, HDL, LDL, VLDL, Triglyceride and Cholesterol levels were estimated by using standard kits (Span diagnostics Ltd).

On day 7, blood was collected from tail vain from overnight fasted rats and fasting blood sugar was estimated [16]. The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formaline solution, and immediately processed by the paraffin technique. Sections of 5μ thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination.

2.10. Statistical Analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean±standard error of mean (S.E.M.) and The results were analyzed statistically by software (Graph pad instat 3) using ANOVA followed by *Bonferronni's multiple comparison test*. The minimum level of significance was fixed at $p<0.05$.

3. Results

3.1 Glucose Tolerence

The effects of extracts of *Albizzia leebeck* (200 mg/kg) on glucose tolerance test are shown in table 2. The supplementation of *Albizzia leebeck* improved the glucose tolerance in the fasted normal rats. After that serum glucose level was lowered significantly ($P < .05$) at 90 minutes and varied significantly ($P < .01$) lowered at 150 minutes. Extract also showed significant hypoglycemic effect after 90 minutes of treatment.

3.2. Experimental Results

The acute oral toxicity study of *Albizzia leebeck* showed no mortality upto 2000 mg/kg.

Table 2 -Observation of acute toxicity study of ethanolic Extract of *Albizzia leebeek*.

S. No.	Dose (mg/kg) p.o.	No. of animals	Observation
1	100	3	All animals survived
2	200	3	All animals survived
3	500	3	All animals survived
4	1000	3	All animals survived
5	2000	3	All animals survived

The anti-hyperglycemic effect of the extracts on the fasting blood sugar levels of diabetic rats is shown. Administration of alloxan (150 mg/kg, i.p.) lead to 1.5-fold elevation of fasting blood glucose levels, which was maintained over a period of 1 weeks. Two weeks of daily treatment of various extract of *Albizzia leebeck* lead to a dose-dependent fall in blood sugar levels by 25%–50%. Effect was maximum till 7 days of treatment. Vehicle control animals were found to be slightly increased in their body weight but diabetic rats showed significant reduction in body weight during 7 days (table 2).

Table 3 - Effect of ethanolic extracts of *Albizzia leebeek* on body weight in alloxan induced diabetes.

S.no	Treatment	Body weight (g)		% Change in Body weight
		Initial	7^{th} day	
1	Normal	138.24±2.52	135.66±2.41	1.86

2	Diabetic control	124.21±2.05	112.35±2.36	9.54
3	Standard (Glibenclamide)	120.19±3.45	123.32±2.45	2.53
4	EEAL	135.16±3.14	127.46±2.72	5.69

Alloxan caused body weight reduction, which is reversed by whole plant extract at dose (200mg/kg) is more effective after 7 days of treatment. Alloxan treatment will increase the serum enzymes levels such as cholesterol, LDL, alkaline phosphatase and decrease the HDL level, but glibenclamide (5mg/kg) and whole plant extracts of *Albizzia leebeck* reversed the above alloxan induce changes (Table 3).

Table 4- Effect of ethanolic extracts of *Albizzia leebeek* on mean fast glucose concentration (mg/dl) profile and serum biochemical parameters in Alloxan (150mg/kg,ip) induced diabetic albino rats after 7 days of treatment.

Groups	Glu_Initial Day (mg/dl) (Mean ± SD)	Glu_7th day (mg/dl) (Mean ± SD)	HDL (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	VLDL (mg/dl)
Group I	117 ± 1.2	87.35 ± 3.36	33.36 ± 2.15	90.43 ± 2.34	88.03±2.63	6239 ± 2.36.	6239 ± 2.36.
Group II	371 ± 5.4	166.26 ± 4.63 a***	40.28 ± 2.02	96.06 ± 3.23	94.20±4.08	66.51 ± 2.23	66.51 ± 2.23
Group III	158 ± 3.6	111.24 ± 3.31 a**, b***	31.04 ± 2.32	89.03 ± 3.62	90.37±3.81	65.86 ± 3.47	65.86 ± 3.47
Group IV	135 ±4.6	137.39 ± 4.52 a***,b***,c**	35.21 ± 1.45	95.31 ± 4.27	92.43±4.41	68.37 ± 3.15	68.37 ± 3.15

The data in the table is the **mean ± SEM** (n = 6 wistar rats per groups), *p<0.05, ** p<0.01, ***p<0.001 compared with multiple groups using *Bonferronni's* multiple comparison test followed by one way **ANOVA**.

a - Significance difference as compare to Control (Group-I)

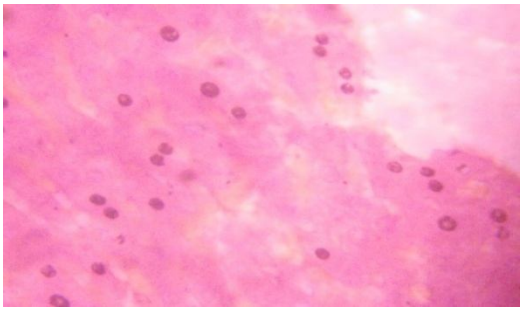
b- Significant inhibition as compare to Negative control (Group-II)

c- Significance difference as compare to Standard (Group-III)

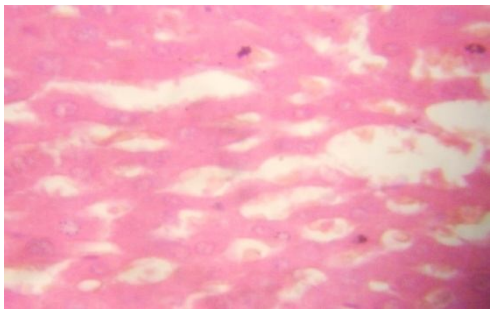
Table 5- Effect of ethanolic extracts of *Albizzia leebeek* on serum liver enzyme in Alloxan induced diabetes in rats.

GROUP	SGOT/AST (IU/L)	SGPT/ALT (IU/L)
Group I (Normal Control)	86.45± 3.59	82.36 ± 2.69
Group II (-ve Control)	58.47 ± 3.49	65.14 ± 3.40
Group III (Std. Glibeclamide)	77.43 ± 4.37	76.95 ± 4.18
Group IV (EEAL, 200mg/kg	66.46 ± 3.69	68.45 ± 3.47

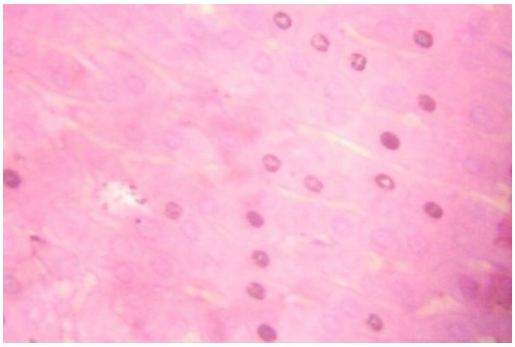
4. Histopathology study:



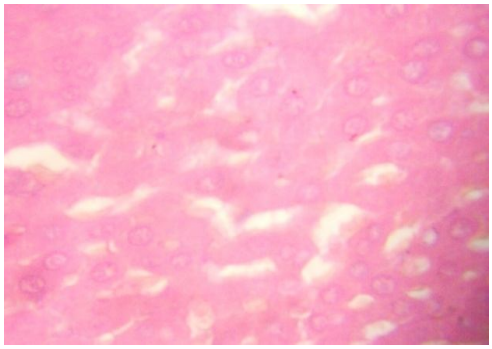
Normal control



Diabetic control



Standard (glibanclamide 2.5mg/kg)



EEAL, 200mg/kg

5. DISCUSSION

In light of the results, our study indicates that methanolic extracts of *Albizzia leebeck* have good antidiabetic activity. Alcoholic extracts of *Albizzia leebeck* exhibited significant anti-hyperglycemic activities in alloxan-induced hyperglycemic rats without significant change in

body weight; they can also improve the condition of Diabetic mellitus as indicated by parameters like body weight & lipid profile along with serum creatinine, serum urea and serum alkaline phosphatase. The renewal of beta cells in diabetes have been studied in several animal models. The total beta cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet beta cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug [24]. *Albizzia leebeck* whole plant alcoholic extracts has been shown to act by beta cell regeneration. In our studies, the damage of pancreas in alloxan-treated diabetic control rats and regeneration of beta cells by glibenclamide was observed. It is found that methanolic leaf extract at dose (200mg/kg) is effective after 7 days of treatment. Hence the above discussion revels that methanolic leaf extract at dose (200mg/kg) is more effective and shows similar curative effect as standard that is, glibenclamide (2.5mg/kg). This could be due to the possibility that some beta cells are still surviving to act upon by *Albizzia leebeck* extract to exert its insulin releasing effect.

6. CONCLUSIONS

The leaf extracts did not show a consistent effect on normal blood sugar levels but it effectively reversed the alloxan-induced changes in the blood sugar level and the beta-cell population in the pancreas. It also showed a protective effect when it was given prior to alloxan administration. The action of whole plant extracts on the pancreatic beta-cells and absence of acute toxicity may offer a new hope to the diabetics in future.

From the above discussion it conclude that alcoholic whole plant extracts of *Albizzia leebeck* at dose (200mg/kg) exhibited significant antihyperglycemic activity in alloxan-induced diabetic rats. These extracts also showed improvement in parameters like body weight and lipid profile as well as regeneration of β cells of pancreas and so might be of value in diabetes treatment. Further investigation is in necessary to determine the exact phytoconstituent(s) saponin, flavonoids, and presence of tannin may responsible for antidiabetic effect.

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