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**Research Article**

# EVALUATION OF ANTI-DIABETIC EFFECTS OF ETHANOLIC EXTRACT OF *ALBIZIA LEBBECK* IN RATS

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**ABSTRACT**

Diabetes Mellitus, one of the major diseases affecting human population all over the world has caused significant morbidity and mortality. The management of this condition has raised the demand of safe and cost-effective remedial measures due to several side effects associated with the present use of modern medicines. Thus, it is crucial to explore other options for diabetes management such as the use of medical plants. *Albizia lebbeck* is one of the known plant species used by traditional medicine practitioners for the treatment of various ailments including inflammatory conditions, pain and diabetes. Even though the plant species has been extensively studied, scientifically, no evidence exists in literature to corroborate the claim made by traditional medicine practitioners of its therapeutic success in the treatment of diabetes and pain. Therefore, the objective of this present study was to investigate the anti-diabetic activity of *Albizia lebbeck* using bark ethanol extract of the plant species on Albino rats.



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## INTRODUCTION

Diabetes mellitus (DM) is a group of complex metabolic diseases characterized by hyperglycemia resulting from a defect in insulin secretion and insulin action, or both, that ultimately affects carbohydrate, fat, and protein metabolism [1]. DM is a growing public health concern worldwide. Approximately one billion people suffer from chronic hyperglycemia globally, a major public health problem. The International Diabetes Federation estimates that 10.5% (537 million) of adults aged 20–79 years are currently living with DM, and this prevalence is expected to increase to 11.3% (643 million) by 2030 and 12.2% (783 million) by 2045. With 1.541 million adults having impaired glucose tolerance (IGT), their risk for type 2 diabetes is increased [2]. Obesity and overweight are important public health problems as their prevalence rates continue to rise. Worldwide, over 1.9 billion and 650 million adults are reported to be overweight and obese, respectively [3]. Further, >60% live in Asia, mainly in India and China [4]. Obesity increases the risk of developing diseases such as DM, cancers, cardiovascular disease, and musculoskeletal and neurological disorders [5]. These in turn affect the health of citizens and directly impact the pro-ductivity and economy of the country.

Several therapeutic agents are used to treat obesity and diabetes. However, most of these agents pose undesirable side effects such as, lactic acidosis, hyperglycemia, diarrhea, and flatulence, which impose an economic burden [6]. Therefore, extensive research is going on worldwide to find alternative therapeutic strategies to minimize the side effects and cost. The major drug therapy for type II DM comprises insulin secretagogues, biguanides, insulin sensitizers, α-glucosidase inhibitors, incretin mimetics, amylin antagonists, and sodium-glucose co-transporter-2 (SGLT2) inhibitors. Despite the appreciable therapeutic benefits, the conventional dosage forms depict differential bioavailability and short half-life, mandating frequent dosage, and causing greater side effects leading to therapy ineffectiveness and patient non-compliance.

Increasing physical activity, and managing fat and sugar-rich products, is the keys to managing obesity [7]. Indian medicinal plants and their plant products have traditionally been used since ancient times. These plant-based foods have novel protection, and are used as an alternative medicine to treat several medical conditions. Purified and crude extract contains numerous active phytochemicals which exert anti-inflammatory and antioxidant properties by regulating

several signalling pathways [8, 9]. The high degree of safety, good effectiveness, wide availability, acceptability, and affordability make plant-based therapy a preferable choice [10].

*Albizia lebbeck* (L) Benth. (Family – Fabaceae) is commonly known as Lebbeck Tree. ) is one of them which are described in Unani classical literature for its potent pharmacological action and medicinal uses [11, 12]. It is a large deciduous perennial tree resembles very much with the tamarind tree. The plant is found in tropical and sub-tropical areas of India, from the plains up to 900 meters in the Himalayan region. However, the above plant is claimed to possess antidiabetic activity, but no scientific evidence is supported [13, 14]. Therefore, study was undertaken to evaluate the preliminary anti-hyperglycemic effects of ethanol extract of *Albizia lebbeck.*

## MATERIALS AND METHODS

### a. Materials

#### I) Plant materials

The leaves of *Albizia lebbeck* was collected from local village of Berhampur in the month of February, 2023 and were authenticated. The whole plant was washed properly, dried under shade and stored in an air tight container.

#### II) Animals

As per the OECD draft guidelines 423 received from CPCSEA, young female albino mice were used for acute toxicity study. Whereas other *in vivo* methods were carried out by using Sprague-Dawley (SD) rats of both sexes. All the animals for the *in vivo* studies, with no prior drug treatment, were procured from the animal house of Royal College of Pharmacy and Health Sciences (R.C.P.H.S.), Berhampur and housed in polypropylene cages with clean sterilized husk bedding (six mice or three rats/ cage). Bedding was changed every alternate day to maintain proper hygienic condition. Animals were maintained under controlled room temperature (22 ± 2°C) and humidity (55 ± 5°C) with a 12:12 hour light: dark cycle.

The animals were fed with standard laboratory food pellets and pure drinking water *ad libitum*. The animals were acclimatized to laboratory hygienic conditions in the departmental laboratory for 7 days before commencing the experiment. The ethical clearance was granted for the study by Institutional animal ethics committee (IAEC) of Royal College of Pharmacy and Health Sciences (R.C.P.H.S.), Berhampur.

#### III) Chemicals

Glibenclamide was obtained from Dr. Reddy's Laboratories, Hyderabad. Blood glucose test-strips of Contour plus

of Bayer Health Care and Diagnostic kits of Crest Biosystems, a division of Coral clinical systems, India were purchased. All other chemicals used for study were of analytical grade.

**b. Methods**

**1) Preparation of extracts**

Dried and powdered plant materials (100 gm) were extracted by successive extraction process using soxhlet apparatus. Solvents were chosen depending upon their increase in polarity like Petroleum Ether (60-80° C), Chloroform, and Ethanol. The extraction was carried out for 72 hours for each solvent. All the extracts were dried using rotary vacuum evaporator and freeze dryer. Their percentage yields were determined and stored in desiccator until further use.

**2) Phytochemical screening**

Extracts obtained from the above extraction process were analyzed for presence of various phytoconstituents such as alkaloids, glycosides, flavones, tannins, terpenes, sterols, saponins, fats and sugars by the method of preliminary phytochemical study (colour reactions) [15, 16, 17] .

**3) Acute toxicity studies**

The acute oral toxicity studies of extracts were carried out as per the OECD guidelines. Administration of stepwise doses of dried extracts of *Albizia lebbeck*, from the dose of 100 mg/kg up to2000 mg/kg, to young female albino mice and observed the signs of toxicity in the tested animals [18].

The albino mice were divided into different groups of six animals each. The control group received 5 ml/kg of distilled water orally. The other groups received the ethanolic extracts of *Albizia lebbeck* at dose levels of 100, 500, 1000, 1500, 2000mg/kg through oral route.

After administration of dose the animals were observed continuously for the first 4 hr and occasionally up to 24 hr and at the end of 72 hr for recording mortality, if any [19]. Additional observations like behavioral changes, somato motor activity, tremors, convulsions, tonic extension, stub tail, muscle spasm, loss of righting reflex, ataxia, sedation, hypnosis, lacrimation, diarrhoea, salivation, writhing, changes in skin, fur, eyes, mucous membranes etc were recorded [19]. One tenth of upper limit dose; and its half and double dose were selected as the levels for examination of therapeutic activity.

**4) Oral Glucose Tolerance Test**

After acclimatization for 7 days in the departmental laboratory, the OGTT was performed in overnight fasted normal rats as per the method described by Jarald*et al*., 2008 [20].The rats were randomly divided into five groups of six

rats each and administered different drugs as per the schedule given in Table 1.

**Table 1: Schedule of drug administration in different groups of OGTT**

| Groups | Treatment groups | Treatments and Dose |
|---|---|---|
| Group-I | Normal control | Distilled Water (5 ml/kg) |
| Group-II | Glucose loaded Control | Distilled Water (5 ml/kg) + Glucose (4 mg) |
| Group-III | Standard | Glibenclamide (5 mg/kg) + Glucose (4 mg) |
| Group-IV | EEAL-200 | EEPN (200 mg/kg) + Glucose (4 mg) |
| Group-V | EEAL-400 | EEPN (400 mg/kg) + Glucose (4 mg) |

Antihyperglycemic activity was studied in glucose overloaded hyperglycemic rats. The rats were fasted for 12hr (free access to water) and administered the different drugs to respective groups as per the schedule. Zero minute blood sugar level was determined from overnight fasted animals. After 30 min of the drug treatment (p.o.), the rats of all groups were orally fed with glucose 4 gm/kg. Blood glucose concentration was determined after 30, 60, 90 and 120 min of glucose loading. The blood samples were collected from the tail tips of rat and glucose concentration was measured by using Glucometer and Glucometer strips.

**5) Hypoglycemic activity**

The hypoglycemic activity was performed in overnight fasted normal rats as per the method described by Jarald*et al*., 2008 [20].After acclimatize for 7 days in the departmental laboratory, the rats were randomly divided into four groups of six rats each and administered the drugs as per the schedule given in the Table 2.

**Table 2: Schedule of drug administration in different groups of hypoglycemic activity study**

| Groups | Treatment groups | Treatments and Dose |
|---|---|---|
| Group-I | Normal Control | Distilled Water (5 ml/kg) |
| Group-II | Standard | Glibenclamide (5 mg/kg) |
| Group-III | EEAL-200 | EEAL (200 mg/kg) |
| Group-IV | EEAL-400 | EEAL (400 mg/kg) |

The hypoglycemic activity was studied in normal rats. The rats were fasted for 12hr (free access to water) and administered the different drugs to

respective groups as per the schedule. Zero min blood sugar level was determined from overnight fasted animals i.e. before oral administration of drug. The blood glucose concentration was also measured after 30, 60, 90 and 120 min of oral administration of drug. The blood samples were collected from the tail tip of the rats and measured the glucose concentration by using glucometer.

**6) Statistical analysis**

The values are expressed as mean ± SEM. The results were analyzed for statistical significance using one-way ANOVA (and nonparametric), followed by Bonferroni's Multiple Comparison Test (Graph pad prism 5.04 version). *P* <0.05 was considered statistically significant.

**RESULTS AND DISCUSSION**

**1. Percentage of yield (w/w), colour and consistency of different extracts:**

The extract of EEAL was filtered and then it was concentrated by distilling off the solvent to obtain the crude extract. The extractive values, colours tabulated below.

**Table-3: Percentage of yield (w/w) and colour of different extracts of EEAL**

| SL. NO. | SOLVENT | % YIELD (W/W) | COLOUR | CONSISTENCY |
|---|---|---|---|---|
| 01 | Petroleum Ether (60-80) | 2.34% | Pale yellow | Greasy mass |
| 02 | Ethanol | 11.75% | Reddish brown | Dry power |

**2. Phytochemical studies:**

The ethanol extract of EEAL obtained from the above extraction process were analysed for different phytoconstituents present in it by the method of qualitative phytochemical analysis. The results are as follows.

**Table- 4: Phytochemical Study of EEAL**

| Sl. No. | Phytoconstituents | Presence/Absence |
|---|---|---|
| 1 | Alkaloid | + |
| 2 | Carbohydrate | + |
| 3 | Glycoside | + |
| 4 | Tannins | + |
| 5 | Protein and Amino acid | + |
| 6 | Gum and Mucilage | - |

| | | |
|---|---|---|
| 7 | Flavones and Flavonoids | + |
| 8 | Saponins | + |
| 9 | Steroids and Sterols | - |
| 10 | Triterpenoids | + |

Preliminary qualitative phytochemical screening of EEAL showed the presence of alkaloid, phenolics, tannins, saponins, triterpenoids, flavones and flavonoids. As flavones and flavonoids are responsible for most of pharmacological activity by their antioxidant's activity, further in-vivo study was carried out.

**3. Acute Toxicity Study**

A summary of acute toxicity of ethanolic extracts of *Albizia lebbeck* is furnished in the Table-5.

**Table-5: Acute toxicity studies of extracts of *Albizia lebbeck***

| **Treatment** | **Dose (mg/kg)** | **No. of Mice** | **No. of Death** | **signs of toxicity** | **$LD_{50}$** |
|---|---|---|---|---|---|
| Control (Distilled water) | 10 ml/ kg | 6 | 0 | - | - |
| Ethanolic extracts of *Albizia lebbeck* (EEAL) | 100 | 6 | 0 | - | > 2000 mg/kg |
| | 500 | 6 | 0 | - | |
| | 1000 | 6 | 0 | - | |
| | 1500 | 6 | 0 | - | |
| | 2000 | 6 | 0 | - | |
| | 2000 | 6 | 0 | - | |

In all the cases, no death was observed. Additional observations like changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern were also found to be normal. Attention was also given to observation of tremors and convulsions, were also absent in all groups. The results were recorded in the Table-5.

The acute toxicity study revealed that the ethanol extracts of *Albizia lebbeck* did not show any signs of toxicity or mortality even at the dose level of 2000 mg/kg body weight. As per the ranking system European Economic Community (EEC) for acute oral toxicity, the $LD_{50}$ dose of 2000 mg/kg and above is categorized as unclassified and low toxicity (EC Directive 83/467/EEC, 1983) reported by Lorke(1983), Shanmugasundaram*etal*.(2006) and Mishra*etal*.(2010).

Overall results, suggested the $LD_{50}$ value is greater than 2000 mg/kg. Hence therapeutic dose was calculated as $1/10^{th}$ of highest tolerable dose i.e. 200 mg/kg body weight. Therefore, $1/5^{th}$ (400 mg/kg), $1/10^{th}$ (200 mg/kg) and $1/20^{th}$ (100 mg/kg) of 2000 mg/kg were used for further pharmacological investigations (Gupta *et al*., 2011).

The ethanol extracts of *Albizia lebbeck* was individually screened for their hypoglycemic activities by using different animal experimental models.

4. **Pharmacological Study:**

**4.1. Hypoglycaemic Activity**

In the hypoglycaemic activity study glucose concentration was estimated at 0, 30, 60, 90 and 120 min after the drug administration and results were recorded as follows in Table-6.

**Table-6: Effect of EEAL on blood glucose conc. in normal fasted Rats**

| Groups | Mean blood glucose (mg/dl) at different time | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Normal control | 83.52±2.88 | 85.37±3.24 | 83.57± 2.72 | 84.69 ±2.14 | 84.82 ±3.04 |
| standard | 85.14±3.27 | 57.86±3.75*** | 46.66 ±2.74*** | 38.85 ±2.27*** | 35.16±2.21*** |
| EEAL - 200 | 84.15±2.51 | 83.88±2.64 | 79.63± 2.26 | 75.19 ±2.89 | 72.88± 2.71 |
| EEAL - 400 | 85.13±4.45* | 76.16±2.47* | #66.13± 2.75* | #60.86± 2.82* | #56.35± 2.72* |

*The results were expressed as mean ± SEM, n=6.*

**P< 0.05; compared Standard and Test groups vs Normal control group.*

*'#'- Indicates there is no significant difference between standard and test drug at P< 0.05 significant level.*

The Percentage change in blood glucose was calculated by using the formula:

**% Change = [(Tc – Tt) / Tc] x 100**

Where Tc = Values of normal control group and

Tt = Values of treated (Standard or extract) group.

**Table-7: Percentage change in blood glucose concentration of different groups**

| Groups | Percentage change in blood glucose conc. at different time | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Standard | 0 | 32.94 | 44.57 | 54.76 | 58.33 |
| EEAL - 200 | 0 | 2.35 | 4.81 | 10.71 | 14.28 |
| EEAL - 400 | 0 | 10.58 | 20.48 | 28.57 | 33.33 |

There was significant difference found in the blood glucose concentration of glibenclamide treated animals, compared to normal control group animals. Glibenclamide treated animals showed significant reduction of blood glucose concentration. The EEAL (400 mg/kg) showed significant hypoglycaemic effect.

### 4.2.Oral Glucose Tolerance Test

The glucose concentration was estimated at 0, 30, 60, 90 and 120 minutes after the glucose loading and results are recorded as follows:

**Table-8: Effect of EEAL on blood glucose conc. in glucose loaded Rats**

| Groups | Mean blood glucose concentration (mg/dl) at different time | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Normal control | 84.64±2.75 | 83.43±2.11 | 85.76±2.18 | 83.85±3.84 | 86.32±2.38 |
| Glucose loaded control | 85.34±3.43 | 145.15±2.42[a] | 158.73±3.33[a] | 143.77±3.76[a] | 123.172±3.73[a] |
| standard | 83.54±3.77 | 112.66±4.42* | 92.27±2.98* | 78.84±3.57* | 69.94±2.80* |

| | | | | | |
|---|---|---|---|---|---|
| EEAL - 200 | 86.33±4.55 | 133.73±3.58 | 142.37±2.75 | 124.14±3.52* | 109.88±2.79* |
| EEAL - 400 | 85.86±5.57 | #124.65±4.16* | #109.87±3.94* | #94.74±4.17* | #81.67±3.23* |

*The results were expressed as Mean ± SEM, n=6.*

*aP< 0.05; compared Normal control vs Glucose loaded control.*

**P< 0.05; compared Standard and Test groups vs Glucose loaded control.*

*'#'- Indicates there is no significant difference between standard and test drug at P< 0.05 significant level.*

The Percentage change in blood glucose concentration was calculated as follow:

**% Change = [(Tc – Tt) / Tc] x 100**

Where Tc = Values of Glucose loaded control group and

Tt = Values of treated (Standard or extract) group.

**Table-9: Percentage change in blood glucose concentration of different groups**

| Groups | Percentage change in blood glucose (mg/dl) at different time | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Standard | 3.17 | 22.75 | 41.77 | 45.46 | 43.92 |
| EEAL - 200 | 0 | 8.27 | 10.12 | 13.28 | 11.38 |
| EEAL - 400 | 2.80 | 14.48 | 31.01 | 34.26 | 34.14 |

After 30 min of the glucose load, there were significant rise ($p$<0.001) in the blood glucose levels of the glucose loaded control animals as compared to normal control and at the end of 2 hr, the glucose level declined. It was also revealed that, the glibenclamide (5 mg/kg) treated animals showed more glucose lowering activities than other group of animals at different time intervals. It lowers 43.92% of glucose concentration than glucose loaded control group at the end of 2 hr. EEAL of 200 and 400 mg/kg exhibited significant antihyperglycemic activity at 30, 60, 90 and 120 min after glucose load, compared to control. The EEAL at 200 and 400 mg/kg dose levels showed glucose lowering activities on dose dependent

manner. There was no significant difference found between the dose of 400 mg/kg of EEAL and glibenclamide treated animals.

## CONCLUSION

Over the past two decades, the interest in medicinal plants has grown enormously leading to routine scientific investigation of numerous plant extracts for their biological effects and potential therapeutic properties in human. A detailed investigation of plants used in local heath tradition and pharmacological evaluation of these plants and their taxonomical relatives can lead to development of invaluable plant drug for many dreaded diseases including diabetes mellitus. Thus, based on the background of diversified therapeutic values and uses in diabetes mellitus complications in folklore, the locally available plant *Albizia lebbeck* were taken up for present investigation.

The present study showed that the ethanolic heart wood extract of *Albizia lebbeck* is able to produce a consistent reduction in serum glucose dose dependent manner. There were no significant differences found in the action between glibenclamide and 400 mg/kg of EEAL treated animals. The extract has also shown the presence of active constituents responsible for significant anti hyperglycaemic supplement.

Further investigation is expected to isolate and characterize the active principle of the extracts. Clinical evaluation will throw more light on clinical usefulness, safety and efficacy of this plant extract.

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