ANTIDIABETIC ACTIVITY OF BARK PART ETHANOLIC EXTRACT OF ALBIZIA LEBBECK LINN IN ALLOXAN INDUCED DIABETIC RAT.

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ABSTRACT

The current study was designed to investigate the hypoglycemic effect of ethanolic extract of Albizia lebbeck bark (EAL) in alloxan induced diabetic rats. The effect of extract was observed by checking the biochemical, physiological and histopathological parameters in diabetic rats. Diabetes was induced by administering alloxan monohydrate (150 mg/ Kg body weight; intraperitoneal). After the oral administration of ethanolic extract at doses of 100mg/kg, 200mg/kg & 400mg/kg body weight, blood glucose levels and body weights were monitored at specific interval. In our study, both Glibenclamide (10mg/kg) and EAL significantly decrease fasting blood glucose and increases the body weight in alloxan induced diabetic rats as comparable to the animals in diabetic control group. The present study shows significant antidiabetic activity of EAL as compared to standard drug Glibenclamide.

KEYWORDS: Albizia lebbeck, alloxan, antidiabetic, Glibenclamide, EAL, intraperitoneal.

INTRODUCTION

Nature has been a source of medicinal treatment for thousands of years. Now days, the search for new chemotherapeutic agents has been expended to the whole biodiversity. An increasing number of chemotherapeutic agents are discovered as a result of chemical studies directed towards the isolation of the active substances from plants used in traditional
medicine. Despite the great success achieved in natural products chemistry and drug development, we have barely begun to tap the potential of our molecular diversity.

Diabetes mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Defective insulin secretion is the major cause for chronic hyperglycemia resulting in impaired function or serious damage to many of the body’s systems, like eyes, kidneys, nerves, heart and blood vessels.\(^1\),\(^2\)

The common signs and symptoms are excessive thirst and urination, weight loss or gain, fatigue, and influenza–like symptoms. Early diabetes symptoms can be very mild and often even unnoticeable. Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world.\(^3\),\(^4\)

Diabetes mellitus is a progressive metabolic disease and it has affected considerable percentage of population throughout the world. Epidemiologic data indicated that 2.8% of the world's population was diabetic in the year 2000 and it may progress to 4.4% of the world's population by 2030. It affects all age groups of people and ethnic groups.\(^5\) In India, statistical analysis revealed that the number of diabetics will rise to 57 million in the year of 2025 compared to 15 million diabetics in 1995.\(^6\) Moreover, diabetic complications lead to morbidity and mortality due to multiple defects in its pathophysiology.\(^7\) Presently, research is focused on traditional medicinal plants and herbs, which are used as potential alternative source to treat diabetes with its multiple pharmacologic actions.\(^8\) Several phytoconstituents possessing antidiabetic activity were isolated and studied from many medicinal plants, but still scientists continue their research on medicinal plants to bring good antidiabetic lead or drugs to the healthcare community.

**MATERIAL AND METHODS**

**Collection of Crude Drugs**

The whole plant of *Albizia lebbeck* was collected during the month of Oct-Nov from the Gwalior, District of M.P. The Plant was Identified and authenticated by Dr. N.K. Pandey (Research officer Ayurveda) National Research institute for Ayurveda HRD, Gwalior (M.P) (Ref.no.5-4/12-13/NRIASHRD /TECH/SURVEY2067).
Preparation of Extract
The collected plant bark was segregated from the extraneous material and dried in shade to prevent the loss of active ingredients and then powdered roots was passed through the sieve (coarse 10/40). Powdered bark extracted with ethanol in soxhlet extractor at room temperature. The extraction was continued for 12 cycles or until the solvent in the thimble was clears. The extracts freed of the solvent under reduced pressure yielding brown semi-solid mass. This extracts dissolved or suspended in distilled water, its pH brought to 7.0 and used for the hypoglycemic activity studies. Extract obtained was stored under refrigerating condition.

Chromatographic Studies
Chromatographic studies were carried out following Harborne (1998), Stahl (2005) and Wagner et al (1996). Thin layer chromatography fingerprinting was performed to detected presence of various phytoconstituents. The TLC plates were prepared by using silica gel G and activated at 105°C for 1 hr prior to experiment. Extract was applied on the silica gel plates as a single spot using a capillary tube. Ethanol fraction was developed in ethyl acetate: methanol: toluene: water (5:4:7:0.5) as solvent.

Plates were developed for a migration distance of about 80% of the total height of the plate and then after drying, plates were observed under 254 and 365 nm. And finally after exposing to iodine vapours. The Rf value was calculated for each observed spot.

Preparation of Extract
Locally collected plant bark (3.0 kg) were shade-dried and then powdered roots and passed through the sieve (coarse 10/40). Powdered bark extracted with ethanol in soxhlet extractor at room temperature. The extraction was continued for 12 cycles or until the solvent in the thimble was clears. The extracts freed of the solvent under reduced pressure yielding brown semi-solid mass. This extracts dissolved or suspended in distilled water, its pH brought to 7.0 and used for the hypoglycemic activity studies. Extract obtained was stored under refrigerating condition.

Experimental Animals
Wistar albino rats of both sexes weighing between 150-200 gm were obtained from the animal house of shri ram college of Pharmacy, gwalior (M.P.), India .The animals were housed in polypropylene cages at 24±2°C and fed with commercial pellet diet and water ad
libitum. All the animal experiments were carried out in accordance with the guidelines of Committee for the Purpose of Care and Supervision on Experimental Animals (CPCSEA) and the study was approved by the Institutional Animal Ethics Committee (IAEC) (Reg.No.891/AC/05/CPCSEA).

**Acute Toxicity Studies**[^9]

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the extract was administered orally at the dose level of 100 mg/kg body weight by intragastric tube and observed for 14 days. Mortality was not observed, and the procedure was repeated for further higher dose such as 200, 400 mg/kg body weight. Then intermittently and at the end, number of deaths was noted to calculate LD50.

**Induction of Diabetes**

A cohort of male Wistar rats was fasted overnight for at least 8 hours. Hyperglycemia was induced in each fasted rat by administering alloxan monohydrate (150 mg/Kg body weight; intraperitoneal) in normal saline. The control cohort was administered normal saline intraperitoneally. After 48 hrs, alloxanization in blood samples collected by tail tipping method using glucometer, rats with marked hyperglycemic fasting blood glucose more than 200mg/dl were selected and used for the study. All the animals were allowed free access to water, pellet diet and maintained at room temperature in poly-ethylene cages.

**Experimental design**[^10]

a) **Group-A** Normal control administered with 0.9% sodium chloride (NaCl)
b) **Group-B** Alloxan induced diabetic control administered with 0.9% NaCl
c) **Group-C** Alloxan induced diabetic control administered with Glibenclamide (GLB) at 10 mg/kg bw
d) **Group-D** Ethanolic Extract of bark(100 mg/kg)
e) **Group-E** Ethanolic Extract of bark (200 mg/kg)
f) **Group-F** Ethanolic Extract of bark (400 mg/kg)

**Biochemical Parameters Estimation**

**Blood Glucose**

The treatment was started from the same day except normal control and diabetic control Groups for a period of 10 days orally. During this period, animals in all groups had free
Access to standard diet and water. Blood glucose levels were estimated on 0st, 4th, and 7th and 10th day of the treatment. Blood was withdrawn from the tail vein and glucose levels were estimated using glucometer strip and a glucometer (sugar check, Mumbai).

**Body Weight**

Decrease in body weight seen in diabetes. The body weight of the rats was recorded on 0st, 4th, 7th and 10th day of the treatment with the help of electronic balance.

**Statistical Analysis**

All the data are expressed as mean ± SEM were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett’s test using spss 16.0 windows version and values of \( P < 0.05 \) were considered as statistically significant.

**Results and Discussion**

In the present study the hypoglycemic activity of ethanolic extract of *Albizia lebbeck* bark was evaluated in alloxan induced diabetic rats. The continuous treatment of bark extract for a period of 10 days produced a significant decrease in blood glucose level and changes body weight (in table no. 5 & 6) in diabetic rats which is comparable to that of standard drug Glibenclamide which is used in treatment of type II diabetes mellitus. The standard drug Glibenclamide stimulates insulin secretion from beta cells of islets of langerhans. From the study, it is suggested that the possible mechanism by which the plant extract decreases the blood glucose level may be by potentiation of insulin effect either by increase in pancreatic secretion of insulin from beta cells of islets of langerhans or by increase in peripheral glucose uptake.\[63\]

During the course of 10 days alloxan induced diabetes mellitus average body weight was recorded on day 1, day 4, day 7 and day 10. Day 1 was compared with day 10. Diabetic control rats showed significant decrease in body weight from day 1 to day 10. Glibenclamide (standard antidiabetic drug) produced significant decrease in body weight on day 10. *Albizia lebbeck* at the dose of 100, 200 and 400 mg/kg b.w showed significant increase in body weight.

In Diabetes mellitus, besides hyperglycemia, cardiovascular disease (CVD) is a major cause of death in the world and is mainly due to atherosclerosis (hardening of the arteries). Abnormal blood lipids are risk factors for \[64\] so the prevention of cardiovascular disease in diabetic patients is necessary. It has also been seen that the liver function tests which include serum aminotransferases i.e ALT, AST, Alkaline phosphatases (AP) and Bilirubin are raised.
in diabetes \(^{11}\) Prevention of liver injury in type-II diabetes due to insulin resistance is necessary. \(^{12}\)

*Diabetes mellitus* is a metabolic disorder characterized by hyperglycemia and alterations in carbohydrate, fat and protein metabolism, associated with absolute or relative deficiencies in insulin secretion or insulin action.

From this study, we can conclude that the ethanolic extract of *Albizia lebbeck* bark have beneficial effects on blood glucose levels and body weight. However, further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research. \(^{13}\)

### RESULTS AND OBSERVATIONS

Table 1: Effect oral treatment of bark extracts of *Albizia lebbeck* on glucose level changes alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood glucose level mg/dl</th>
<th>Initial</th>
<th>0th day</th>
<th>4th day</th>
<th>7th day</th>
<th>10th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Saline</td>
<td></td>
<td>71.4 ± 2.5</td>
<td>68.6 ± 3.5</td>
<td>69.8±4.4</td>
<td>70.6 ± 3.2</td>
<td>75.5 ± 2.4</td>
</tr>
<tr>
<td>Group II</td>
<td>Saline + Alloxan (150mg/kg)</td>
<td></td>
<td>255.42±2.4</td>
<td>260.6±3.4</td>
<td>273.4±3.2</td>
<td>281.4±2.5*</td>
<td>298.0±3.5*</td>
</tr>
<tr>
<td>Group III</td>
<td>Glibenclamide (10mg/kg) + Alloxan (150mg/kg)</td>
<td></td>
<td>250.0 ± 3.9</td>
<td>189.4±3.6</td>
<td>140.3±3.4*</td>
<td>88.5 ± 3.6*</td>
<td>69.2 ± 4**</td>
</tr>
<tr>
<td>Group IV</td>
<td>EAL (100mg/kg) + Alloxan (150mg/kg)</td>
<td></td>
<td>252.6 ± 3.2</td>
<td>195.6±3.5</td>
<td>186.7±4.4*</td>
<td>170.7±3.5*</td>
<td>123.2±2.4**</td>
</tr>
<tr>
<td>Group V</td>
<td>EAL (200mg/kg) + Alloxan (150mg/kg)</td>
<td></td>
<td>258.1 ± 2.7</td>
<td>196.8±3.6</td>
<td>167.8±4.8*</td>
<td>102.8±2.5**</td>
<td>95.0 ± 3.4**</td>
</tr>
<tr>
<td>Group VI</td>
<td>EAL (400mg/kg) + Alloxan (150mg/kg)</td>
<td></td>
<td>256.2 ± 2.0</td>
<td>190.6±3.4</td>
<td>160.8±4.6*</td>
<td>98.1 ± 3.2**</td>
<td>73.5 ± 2.2**</td>
</tr>
</tbody>
</table>

Alloxan monohydrate (150mg/kg) was administrated i.p. sterile saline, single dose, 5 days before the administration of the extract of bark. Standard drug glibenclamide and ethanolic extract of bark administrate orally for 10 days in single dose daily, 5 days after confirmation of hyperglycemia n= 6 (no. of animal in each group).

Statistic significance test was done by one way ANOVA followed by Dunnett’s test using spss 16.0 windows version.
Table 2 Effect of oral treatment of Albizia lebbeck bark extract on body weight changes in alloxan-induced diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Body Weight (gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0th day</td>
</tr>
<tr>
<td>Group I</td>
<td>Saline</td>
<td>200.6 ± 3.0</td>
</tr>
<tr>
<td>Group II</td>
<td>Saline + Alloxan (150mg/kg)</td>
<td>190.7 ± 2.9</td>
</tr>
<tr>
<td>Group III</td>
<td>Glibenclamide (10mg/kg) + Alloxan (150mg/kg)</td>
<td>198.7 ± 5.3</td>
</tr>
<tr>
<td>Group IV</td>
<td>EAL (100mg/kg) + Alloxan (150mg/kg)</td>
<td>192.8 ± 3.0</td>
</tr>
<tr>
<td>Group V</td>
<td>EAL (200mg/kg) + Alloxan (150mg/kg)</td>
<td>193.2 ± 3.8</td>
</tr>
<tr>
<td>Group VI</td>
<td>EAL (400mg/kg) + Alloxan (150mg/kg)</td>
<td>196.5 ± 3.3</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM for six animals.*p<0.05, **p<0.01 compared to diabetic control group.

Alloxan monohydrate (150mg/kg) was administrated i.p. sterile saline, single dose, 5 days before the administration of the extract of bark. Standard drug glibenclamide and ethanolic extract of bark administrate orally for 10 days in single dose daily, 5 days after confirmation of hyperglycemia n= 6 (no. of animal in each group).

Statistic significance test was done by one way ANOVA followed by Dunnett’s test using spss 16.0 windows version.

Fig.1. Effect of oral treatment of Abizia lebbeck bark extract on blood glucose level changes in alloxan-induced diabetic rats.
Statically significance test was done by any way ANOVA followed by Dunnett’s test
*p<0.05 compared to disease control group Control value for blood glucose level- 75.5 ± 2.4
All values are mean ± SEM of 6 animals per group.

**Fig.2. Effect of oral treatment of *Abizia lebbeck* bark extract on body weight changes in
alloxan-induced diabetic rats.**

Statically significance test was done by any way ANOVA followed by Dunnett’s test
*p<0.05 compared to disease control group Control value for blood glucose level- 209.2 ±
1.5. All values are mean ± SEM of 6 animals per group.

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