Comparative Studies on Anti-hyperglycemic Effects of Ethyl Acetate and Methanol Extract of *Albizia lucida* Benth Bark in Alloxan Induced Diabetic Rats

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Received: July 16, 2013 Revised: August 5, 2013 Accepted: August 8, 2013

Abstract

The decoction of the bark of *Albizia lucida* Benth was used in ayurvedic system for management of diabetes mellitus. To confirm the traditional claim, the comparative anti-hyperglycemic efficiency of ethyl acetate extract of bark of *Albizia lucida* Benth. (EEAL) and methanol extract of bark of *Albizia lucida* Benth. (MEAL) was done in alloxan induced diabetic rats. Acute and sub-acute effect of oral administration of EEAL and MEAL (200 & 400 mg/kg, b.wt) on blood glucose was observed in different time interval for 10 days. By the end of the study, the collected blood sample and pancreas was used for estimation of serum lipid profile and histological studies. EEAL and MEAL showed that the serum blood glucose and serum lipids (total cholesterol, triglyceride, VLDL-cholesterol, LDL-cholesterol) was reduced significantly (\(p < 0.01\)) in dose dependent manner when compare to control group. At the same time MEAL was showed potent effect than EEAL. EEAL and MEAL does not affect the body weight and alter the histopathology of pancreas similar to that of normal. It was concluded that EEAL extract of bark of *Albizia lucida* Benth and MEAL extract of bark of *Albizia lucida* Benth. possess anti-hyperglycemic activity in alloxan-induced diabetic model rats.

Keywords: *Albizia lucida* Benth., Ayurveda, Diabetes, Alloxan, Serum Lipid Profile, Histopathology, Anti-hyperglycemic.

1. Introduction

Diabetes is chronic metabolic disorder characterized by hyperglycemia due to absence or insufficient of circulating insulin levels (Holmann and Turner, 1991). Though insulin and various anti-diabetic drugs are available but insulin could not be used by oral and continuous use of synthetic agents leads severe side effects (Valiathan, 1998). Herbal medicines are prescribed universally even though their biological active constituents are unknown due to their potency with minimum side effects and relatively low cost (Verspohl, 2002; Villasen‘or and Lamadrí, 2006).

In folk medicine, the decoction of bark of *Albizia lucida* Benth. (Family: Mimosaceae) is considered useful in diabetes, pregnancy and stomachache. It is also used as a medicine for water buffalo when given with salt (Bulusu Sitaram and Chunekar, 2006). Therefore, the present research was undertaken to verify the anti-hyperglycemic potential of ethyl acetate extract of bark of *Albizia lucida* Benth. (EEAL) and methanol extract of bark of *Albizia lucida* Benth. (MEAL) in alloxan induced diabetic rats.

2. Materials and Methods

2.1. Plant Material and Preparation of Extract

The bark of *Albizia lucida* Benth. was collected from Tirumala hills, Chittoor district of Andhra Pradesh, India in March 2011. The plant was authenticated Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati. The bark of *Albizia lucida* Benth. was dried in shade and pulverized in the grinder-mixer to obtain a coarse powder, then passed through the 40 mesh sieve. A weighed quantity (100gm) of powder was subjected to continuous hot extraction with ethyl acetate and methanol in soxhlet apparatus for 48 hours. Then the extracts were evaporated at reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. The percentage yield of ethyl acetate and methanol extract of *Albizia lucida* Benth. was found to be 16.7 % and 18.5 % respectively.

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The preliminary phytochemical investigation was performed by using several standard phytochemical tests for the qualitative estimation of presence of various phytochemicals in ethyl acetate (EEAL) and methanol extract (MEAL) of bark of *Albizia lucida* Benth. (Harborne, 1973).

2.3. Animals

Male albino Wistar rats (200-250gm) were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati, Andhra Pradesh. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle and water was given ad libitum. The experiments were performed after approval (Approval no: SVCP/IAEC/I-009/2011-12) of the protocol by the Institutional Animal Ethics Committee (IAEC) and were carried out in accordance with the current guidelines on the care of laboratory animals.

2.4. Acute Toxicity Study

The acute toxicity of EEAL and MEAL was determined as per the OECD guideline no. 423 (Acute toxic class method). Albino Wistar rats (n=6) either sex selected and kept fasting for overnight providing only water. EEAL and MEAL was administered orally at the dose level of 2000mg/kg by oral needle and observed for 14days. Hence, 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of the doses were selected for further study (OECD, 2002).

2.5. Anti-hyperglycemic Efficiency of *Albizia lucida* Benth. Extracts

2.5.1. Induction of diabetes

Male wistar albino normoglycemic rats were injected intraperitoneally with alloxan monohydrate dissolved in normal saline at the dose of 120 mg/kg b.wt (Lachin and Reza, 2012; Chaudhary et al., 2012). After 3 days, the fasting blood glucose was checked and above 250 mg/dl of blood glucose reached rats was considered as diabetic rats. These diabetic rats were segregated into seven groups of six rats each group. Group I (Normal control): Vehicle 1% w/v CMC; 5ml/kg, b.w. p.o; Group II (Alloxan induced Diabetic control) received only vehicle (1%CMC; 5ml/kg, b.w. p.o); Group III & IV - Alloxan induced diabetic rats received the EEAL 200 & 400 mg/kg/day p.o suspended in 1% w/v CMC; Group V & VI – Alloxan induced diabetic rats received the MEAL 200 & 400 mg/kg/day p.o suspended in 1% w/v CMC; Group VII (Standard) Alloxan induced diabetic rats received Glibenclamide (2.5 mg/kg p.o) suspended in 1% w/v CMC, respectively.

2.5.2. Acute experimental study

Blood glucose level of all group rats were checked after 0, 1, 3, 6 & 9h of oral administration of single dose of test drugs.

2.5.3. Sub-acute experimental study

The same treatments continued with same dose once daily for 10 days. The blood samples collected from tail vein and measured the glucose level using commercially available glucose strips (Accu-Chek) using one-touch glucometer (Johnson-Johnson, India) on initial day, 3, 7, 10th day respectively. After terminate the study, the animals were sacrificed by cervical dislocation. The blood was collected without anticoagulant and serum was separated by centrifugation at 6000 rpm for 5min. the pancreas organ of rats also separated carefully for histological study.

2.5.4 Estimation of serum lipid profile

Serum total cholesterol (TC), triglycerides (TG), LDL-C, VLDL, HDL-C were measured for all animals (n=6) from each group by commercially available diagnostic kits (Span Diagnostics, India). The serum low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels were calculated by Friedewald formula (Friedewald et al., 1972): VLDL = TG / 5; LDL = TC - HDL - VLDL.

2.6 Histological Assessment of Pancreas

The separated pancreas was kept in 10% formaldehyde solution for histopathological studies and standard procedure followed for fixation. This study was done at pathology department of Sri Venkateswara University, Tirupati, Andhra Pradesh.

2.7 Statistical Analysis

The present research observations were signified as Mean ± Standard Error Mean. Statistical significance of dissimilarities amid the groups was evaluated by one way and multiple way analysis of variance (ANOVA) followed by Turkey test. *P* values less than 0.05 were deliberated as significance.

3. Results

3.1. Preliminary Phytochemical Investigation of EEAL & MEAL

Ethyl acetate and methanol extracts of bark of *Albizia lucida* Benth. were showed proteins, steroids, alkaloids, flavonoids, terpenoids, tannins and phenolic compounds and saponins.

3.2. After single dose of EEAL & MEAL on serum blood glucose level in diabetic rats

EEAL and MEAL extracts were significantly (*P*<0.01) decreased the serum blood glucose level. In acute experimental study, single dose response of EEAL and MEAL in alloxan induced diabetic rats at 0, 1, 3, 6 & 9h. Table 1 and Figure 1 showed that the methanolic extract of bark of *Albizia lucida* Benth. (MEAL 200 and 400mg/kg, p.o) effectively (*P*<0.01) reduced blood glucose level of diabetic rats (47.59% and 53.34%, respectively) than ethyl acetate extract of bark of *Albizia lucida* Benth. (EEAL 200 and 400mg/kg, p.o) (26.34% and 37.49%, respectively). These reduced values were very close to that of glibenclamide (2.5mg/kg p.o) (61.74%). After oral administration of MEAL, acute reduction (*P*<0.01) of glucose level in time dependent manner when compared to EEAL.
3.3. After Multidose of EEAL & MEAL on serum blood glucose level in diabetic rats

In sub-acute experimental study, the blood glucose was determined on initial day, 3, 7, 10th day after oral administration of EEAL and MEAL (200 & 400mg/kg, p.o) (Table 2 and Figure 2). Both doses of MEAL (200 and 400mg/kg, p.o) showed significant (P<0.01) chronic reduction of serum blood glucose level (54.59% and 57.73%, respectively) when compared to diabetic rats. MEAL effect was near to that of (P<0.01) of glibenclamide (2.5mg/kg p.o) (67.06%). Anti-hyperglycemic effect of MEAL was greater effect (P<0.01) than EEAL 200 and 400mg/kg, p.o (40.89% & 48.96%, respectively).

3.4. After Multidose of EEAL & MEAL on serum lipid profile and body weight in diabetic rats

The serum blood glucose was raised (P<0.01) accompanied by an increase in serum lipid profile such as total cholesterol (TC), triglycerides (TG), LDL-C, VLDL & HDL-C in alloxan-induced diabetic rats (Table 3 and Figure 3). MEAL (200 and 400mg) afforded greater reduction of TC, TG, LDL-C & VLDL, whereas HDL-C level was significantly (P<0.01) increased. Anti-hyperlipidemic effect of MEAL was great efficient than EEAL. Standard group rats showed significant (P<0.01) anti-hyperlipidemic effect with glibenclamide (2.5mg/kg p.o).

3.5. Histopathological studies on islets of pancreas

Pancreatic beta cells of normal rats showed adorned islets with well defined border and normal allocation of islets of langerhans. The histopathological studies of alloxan induced diabetic rat pancreas illustrates that a atrophy, suppress the number of beta cells of islets of langerhans was noted when compared to control group. Necrosis and damage of islets was caused by alloxan. The oral administration of EEAL and MEAL to groups III, IV, V & VI of experimental rats showed in the recovery of beta cells necrosis and the restoration of the islets similar to that of control and standard groups (glibenclamide 2.5mg/kg p.o) treated animals (Figure 5).

Table 1. After single dose of EEAL & MEAL on serum blood glucose in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Blood Glucose (mg/dl) (% of glucose reduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>I - Normal control</td>
<td>86.67±1.856**a</td>
</tr>
<tr>
<td>(1% w/v CMC)</td>
<td></td>
</tr>
<tr>
<td>II - Alloxan induced Diabetic control</td>
<td>258.00±2.113</td>
</tr>
<tr>
<td>(200 mg/kg, p.o)</td>
<td>264.50±2.513b</td>
</tr>
<tr>
<td>IV - Alloxan + EEAL (400 mg/kg, p.o)</td>
<td>265.83±2.600b</td>
</tr>
<tr>
<td>V - Alloxan + MEAL (200 mg/kg, p.o)</td>
<td>267.17±4.102b</td>
</tr>
<tr>
<td>VI - Alloxan + MEAL (400 mg/kg, p.o)</td>
<td>261.83±2.857b</td>
</tr>
<tr>
<td>VII - Alloxan + Glibenclamide (2.5mg/kg, p.o)</td>
<td>266.17±3.380b</td>
</tr>
</tbody>
</table>

All values expressed in mean ± SEM for six animals in each group (n=6).
All values compared with diabetic control groups. * p < 0.05 ; ** p < 0.01;

a – Normal control group I V/S diabetic control group II; b – Groups III, IV, V, VI & VII V/S diabetic control group II.
**Figure 1.** After single dose of EEAL & MEAL on serum blood glucose in alloxan induced diabetic rats

All values expressed in mean ± SEM for six animals in each group (n=6);
All values compared with diabetic control groups. * p < 0.05 ; ** p < 0.01;

- a – Normal control group I Vs diabetic control group II;
- b – Groups III, IV, V, VI & VII Vs diabetic control group II.

**Table 2.** After Multidose of EEAL & MEAL on serum blood glucose in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Blood Glucose (mg/dl)</th>
<th>(% of glucose reduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I - Normal control (1% w/v CMC)</td>
<td>86.67±1.856**a</td>
<td>87.50±2.802**a</td>
</tr>
<tr>
<td>II - Alloxan induced Diabetic control</td>
<td>258.00±2.113</td>
<td>297.83±1.990</td>
</tr>
<tr>
<td>III - Alloxan + EEAL (200 mg/kg, p.o)</td>
<td>264.50±2.513b (21.74)</td>
<td>207.00±0.966**b (30.56)</td>
</tr>
<tr>
<td>IV - Alloxan + EEAL (400 mg/kg, p.o)</td>
<td>265.83±2.600b (32.92)</td>
<td>178.33±1.333**b (43.94)</td>
</tr>
<tr>
<td>V - Alloxan + MEAL (200 mg/kg, p.o)</td>
<td>267.17±4.102b (34.00)</td>
<td>176.33±1.726**b (45.66)</td>
</tr>
<tr>
<td>VI - Alloxan + MEAL (400 mg/kg, p.o)</td>
<td>261.83±2.857b (41.18)</td>
<td>154.00±1.238**b (54.17)</td>
</tr>
<tr>
<td>VII - Alloxan + Glibenclamide (2.5mg/kg, p.o)</td>
<td>266.17±3.380b (63.68)</td>
<td>96.67±0.919**b (64.81)</td>
</tr>
</tbody>
</table>
Figure 2. After Multidose of EEAL & MEAL on serum blood glucose in alloxan induced diabetic rats
All values expressed in mean ± SEM for six animals in each group (n=6);
All values compared with diabetic control groups. * p < 0.05 ; ** p < 0.01;
a – Normal control group I Vs diabetic control group II; b – Groups III, IV, V, VI & VII Vs diabetic control group II.

Table 3. After Multidose of EEAL & MEAL on serum lipid profile in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Lipid Profile (mg/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>LDL- C (mg/dl)</th>
<th>VLDL- C (mg/dl)</th>
<th>HDL- C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I - Normal control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1% w/v CMC)</td>
<td></td>
<td>119.67±1.282**a</td>
<td>115.83±1.493**a</td>
<td>40.33±1.833**a</td>
<td>23.17±0.299**a</td>
<td>56.17±0.833**a</td>
</tr>
<tr>
<td>II - Alloxan induced</td>
<td></td>
<td>233.33±2.951</td>
<td>163.33±1.667</td>
<td>168.00±2.266</td>
<td>32.67±0.333</td>
<td>29.67±0.494</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III - Alloxan + EEAL</td>
<td></td>
<td>173.83±1.014**b</td>
<td>150.00±1.238**b</td>
<td>106.33±1.077**b</td>
<td>30.00±0.248**b</td>
<td>37.50±0.563**</td>
</tr>
<tr>
<td>(200 mg/kg, p.o)</td>
<td></td>
<td>147.83±1.579**b</td>
<td>146.80±1.703**b</td>
<td>80.80±1.838**b</td>
<td>28.37±0.141**b</td>
<td>37.67±0.803**</td>
</tr>
<tr>
<td>IV - Alloxan + MEAL</td>
<td></td>
<td>139.83±0.543**b</td>
<td>141.33±1.116**b</td>
<td>69.90±0.834**b</td>
<td>28.27±0.223**b</td>
<td>41.67±0.422**</td>
</tr>
<tr>
<td>(200 mg/kg, p.o)</td>
<td></td>
<td>123.83±1.014**b</td>
<td>126.50±1.118**b</td>
<td>52.20±1.108**b</td>
<td>25.30±0.224**b</td>
<td>46.17±0.401**</td>
</tr>
<tr>
<td>V - Alloxan + Glibenclamide (2.5mg/kg, p.o)</td>
<td>116.67±1.282**b</td>
<td>115.00±0.730**b</td>
<td>42.83±1.237**b</td>
<td>23.00±0.146**b</td>
<td>50.83±0.909**</td>
<td></td>
</tr>
<tr>
<td>VI - Alloxan + MEAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(400 mg/kg, p.o)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII - Alloxan + Glibenclamide (2.5mg/kg, p.o)</td>
<td>116.67±1.282**b</td>
<td>115.00±0.730**b</td>
<td>42.83±1.237**b</td>
<td>23.00±0.146**b</td>
<td>50.83±0.909**</td>
<td></td>
</tr>
</tbody>
</table>

All values expressed in mean ± SEM for six animals in each group (n=6);
All values compared with diabetic control groups. * p < 0.05 ; ** p < 0.01;
a – Normal control group I Vs diabetic control group II; b – Groups III, IV, V, VI & VII Vs diabetic control group II.
Figure 3. After Multidose of EEAL & MEAL on serum lipid profile in alloxan induced diabetic rats

All values expressed in mean ± SEM for six animals in each group (n=6).

All values compared with diabetic control groups. * p < 0.05 ; ** p < 0.01;

a – Normal control group I Vs diabetic control group II; b – Groups III, IV, V, VI & VII Vs diabetic control group II.

Table 4. Efficiency of EEAL & MEAL on body weight in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td></td>
<td>Final</td>
</tr>
<tr>
<td>I - Normal control (1% w/v CMC)</td>
<td>164.83±1.621&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>II - Alloxan induced Diabetic control</td>
<td>163.50±1.586</td>
</tr>
<tr>
<td>III - Alloxan + EEAL (200 mg/kg, p.o)</td>
<td>164.33±1.229&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV - Alloxan + EEAL (400 mg/kg, p.o)</td>
<td>163.83±0.601&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V - Alloxan + MEAL (200 mg/kg, p.o)</td>
<td>164.00±1.528&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VI - Alloxan + MEAL (400 mg/kg, p.o)</td>
<td>164.33±1.174&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VII - Alloxan + Glibenclamide (2.5mg/kg, p.o)</td>
<td>167.50±1.607&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values expressed in mean ± SEM for six animals in each group (n=6).

All values compared with diabetic control groups. * p < 0.05 ; ** p < 0.01.

a – Normal control group I Vs diabetic control group II.

b – Groups III, IV, V, VI & VII Vs diabetic control group II.

Figure 4. Efficiency of EEAL & MEAL on body weight in alloxan induced diabetic rats

All values expressed in mean ± SEM for six animals in each group (n=6).

All values compared with diabetic control groups. * p < 0.05 ; ** p < 0.01.

a – Normal control group I Vs diabetic control group II.

b – Groups III, IV, V, VI & VII Vs diabetic control group II.
II - Normal control (1% w/v CMC)                                    II - Diabetic control

III - Alloxan + EEAL (200 mg/kg, p.o)           IV- Alloxan + EEAL (400 mg/kg, p.o)

V- Alloxan + MEAL (200 mg/kg, p.o)                            VI - Alloxan + MEAL (400 mg/kg, p.o)

VII - Alloxan + Glibenclamide (2.5mg/kg, p.o)

Figure 5. Histopathological studies on pancreas

4. Discussion

Ethyl acetate extract of bark of *Albizia lucida* Benth. (EEAL) and Methanol Extract of bark of *Albizia lucida* Benth. (MEAL) was screened for discover the scientific evidence of decoction of this bark of *Albizia lucida* Benth. used for treatment of diabetes in Ayurveda (Sitaram and Chunekar, 2006). Nevertheless, presence of triterpenoid saponins which possess anti-hyperglycemic, anti-hyperlipidemic and anti-angiogenic properties has been reported in previous literature (Tan et al., 2008; Joseph and Jin, 2013).

The intraperitoneal injection of alloxan monohydrate (β-Cytotoxin) induces chemical diabetes in albino rats causes hyperglycemia, hyperlipidemia due to impaired insulin release and insulin resistance. The acute and sub-acute administration, the effect of EEAL and MEAL was reduced blood glucose significantly. Glibenclamide (sulphonylureas) produce hypoglycemia by enhancing the release of insulin and these drugs are very potent in alloxanized diabetic rats. Hence our results displayed that the acute and sub-acute anti-hyperglycemic effects of EEAL and MEAL might be potentiating the insulin secretion, it was similar to that of Glibenclamide (Meliani et al., 2011).
In alloxanized diabetes also correlated with hyperlipidemia and hypertriglyceridemia. Observation of hyperlipidemia, the total cholesterol (TC), LDL-C, VLDL was increased & fall of HDL-C due to underutilization of glucose leads to excess mobilization of fat from adipose tissue. At the same time, oral administration of EEAL and MEAL was suppresses the mobilization effect by increased the glucose utilization. Moreover, EEAL and MEAL showed significantly decreases the triglycerides (TG) by activating or releasing the lipoprotein lipase resulting in regulates the metabolism of lipids (Nammi et al., 2003).

Additionally, EEAL and MEAL treated animals body weight was not affected and it is comparable to that of normal control animals. In histological studies on pancreas, there is no necrosis and damage of islets of langerhans in oral administration of EEAL and MEAL treated groups. It was accepted the potency and safety of EEAL and MEAL.

In conclusion, our observations of the present research clearly demonstrated that Ethyl acetate extract of bark of Albizzia lucida Benth. (EEAL) and Methanol Extract of bark of Albizzia lucida Benth. (MEAL) exerts markable anti-hyperglycemic and anti -hyperlipidemic efficiency due to the presence of biologically active constituents with possible multiple mechanism involving both pancreatic and extra pancreatic effects. But still more research is needed for find out its specific mechanism of action and long term effects in diabetes mellitus.

5. Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors are grateful to Padmashree Dr. M. Mohan Babu, Chairman, Sree Vidyanikethan Educational Trust, Tirupati, India for providing the necessary facilities to carry out this work.

References


