Antihyperglycemic, antihyperlipidemic potential and histopathological analysis of ethyl acetate fraction of *Callistemon lanceolatus* leaves extract on alloxan induced diabetic rats

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**Summary**

**Objective:** The present research work was carried out to investigate antihyperglycemic, antihyperlipidemic and histopathological analysis of ethyl acetate fraction of *Callistemon lanceolatus* methanolic leaves extract (CLEE).

**Method:** The alloxan- induced diabetic rat were treated by administering oral doses (200 and 400 mg/kg body weight) of CLEE for 21 days. Blood glucose levels and body weights of rats were measured weekly on days 0, 7, 14 and 21. Other biochemical parameters, e.g. liver profile, renal profile and total lipid levels were determined in normal and alloxan induced diabetic rats after oral administration of the extract for 21 days.

**Results:** Daily oral administration of CLEE and glibenclamide (10 mg/kg) showed beneficial effects on blood glucose level (P<0.001) as well as improving kidney, liver functions and hyperlipidaemia due to diabetes. Furthermore, the extract has a favorable effect on the histopathological changes of the pancreas, liver and kidney in alloxan induced diabetes.

**Conclusion:** *C.lanceolatus* possesses antihyperglycemic property as well improves body weight, liver profile, renal profile and total lipid levels in alloxan-diabetic rats.

**Key words:** Alloxan; Antihyperglycemic; Antihyperlipidemic; *Callistemon lanceolatus*; Diabetic rat; Serum cholesterol

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**Introduction**

Diabetes is a metabolic disorder characterized by hyperglycemia resulting deficiency of insulin secretion by pancreas, ineffectiveness of produced insulin, or both [1]. There has been increasing demand for the use of natural products with antidiabetic activity. Due to undesirable side effects of synthetic drugs as well as not suitable for use during pregnancy, hypoglycemic agents from plants origin become much important [2-4]. Medicinal plants and their products have been used in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity [5-6]. In accordance to the recommendations by the WHO (World Health Organisation) expert committee on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become more important [7].

*Callistemon lanceolatus* (Family: Myrataceae) is commonly known as bottle brush. It is shrub or small tree grow upto 7.5 m in height. Because of its ornamental value, it is cultivated in gardens [8-9]. An aqueous extract of the leaves and flowers shows antifungal and antibacterial activity. The essential oils of the leaves of this plant possess antimicrobial, fungitoxic, antinociceptive and anti-inflammatory activities [10-11].

Many people have been using *C.lanceolatus* leaves to treat diabetes-related problems in Haryana regions of India. After literature survey, we have not found any research dealing with antihyperglycemic use of the plant. So, the present research work was done to explore the antihyperglycemic and antihyperlipidemic activities of isolated ethyl acetate fraction of methanolic extract of *C.lanceolatus*. Histopathological studies of liver, kidney and pancreas were also performed of normal and diabetic rats.

**Materials and methods**

**Plant material**

*C.lanceolatus* leaves were collected from the campus of Kurukshetra University, Kurukshetra, India and were identified by Dr. H.B. Singh, Scientist F & Head, Raw Material Herbarium & Museum, NISCAIR, New Delhi, India. A voucher specimen of the plant is preserved in the herbarium (NISCAIR/RHMD/Consult/-2009-10/1381/182/2).
Preparation extract and fraction

*C. lanceolatus* leaves were dried under shade and powdered to coarse particles. The powdered material were defatted with petroleum ether (60-80°C) in Soxhlet extraction apparatus at 60°C and further the same amount material was successively extracted with dichloromethane and methanol. The extracts were dried at 45°C in rotary evaporator to produce a semisolid mass and stored in airtight containers in refrigerator below 10°C. The dried methanolic extract was suspended in distilled water and successively partitioned with hexane, ethyl acetate and n-butanol in separating funnel. The fractions were dried in rotary evaporator and collected. Ethyl acetate fraction showed good antihyperglycemic activity in oral glucose tolerance test. So, ethyl acetate fraction was taken for further studies (*Callistemon lanceolatus* methanolic leaves extract; CLEE).

Chemicals

Alloxan was purchased from Loba Chemie Pvt. Ltd. Mumbai, India. Total cholesterol, triglyceride, high-density lipoprotein, serum creatinine, serum urea, serum alkaline phosphatase (ALP), serum alanine (ALT) and aspartate transaminase (AST) standard kits were obtained from Erba Diagnostics Mannheim GmbH, Germany. All reagents used in study were of analytical grade.

Animals

The Wistar strains of albino rats of either sex (15 males; 15 females), weighing about 150-250 g were used in the study. Animals were maintained under standard environmental conditions, i.e. ambient temperature of 22±2°C and at 45-55% relative humidity, 12 h each of dark and light cycle and fed with a standard pellet rats diet ad libitum, obtained from Ashirwad Industries, Chandigarh, India. Water was supplied ad libitum. All the studies were conducted in accordance with the Animal Ethical Committee of the University.

Induction of diabetes

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg, i.p.; Loba Chemie Pvt. Ltd., Bombay, India) in sterile saline. Twelve days after Alloxan injection, rats with blood glucose level of >200 mg/dl were separated and used for the study. Blood glucose level was measured using blood glucose test strips with Elegance CT-X10 glucose meter (Convergent Technologies GmbH & Co. KG, Frankenberg, Germany) at weekly intervals till the end of study (i.e. 3 weeks).

Experimental design

Overnight fasted rats were divided into five groups and for each group six animals and treated orally once a day for 21 days as follows:

- **Group I.** Normal healthy control: received only vehicle (Tween 80, 1% v/v)
- **Group II.** Diabetic control: received vehicle (Tween 80, 1% v/v)
- **Group III.** Diabetic rats treated with CLEE (200 mg/kg body weight)
- **Group IV.** Diabetic rats treated with CLEE (400 mg/kg b.w.)
- **Group V.** Diabetic rats treated with glibenclamide (10 mg/kg b.w.)

Blood glucose estimation and body weight measurement were done on days 0, 7, 14 and 21 after administration of extract orally.

**Table 1.** Effects of glibenclamide and CLEE on the blood glucose and insulin levels in diabetic rats (Means ± S.E.M.)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose level (mg/dl)</th>
<th>Serum insulin (IU/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial day 0</td>
<td>Day 7</td>
</tr>
<tr>
<td>Normal + Vehicle</td>
<td>115.72 ± 4.5</td>
<td>113.34 ± 3.8</td>
</tr>
<tr>
<td>Diabetes + Vehicle</td>
<td>253.52 ± 2.45</td>
<td>296.54 ± 4.35*</td>
</tr>
<tr>
<td>Diabetes + CLEE (200)</td>
<td>258.32 ± 3.47</td>
<td>230.27 ± 2.38**</td>
</tr>
<tr>
<td>Diabetes + CLEE (400)</td>
<td>278.73 ± 2.4</td>
<td>207.13 ± 3.7**</td>
</tr>
<tr>
<td>Diabetes + Glibenclamide</td>
<td>255.24 ± 2.28</td>
<td>201.23 ± 3.52</td>
</tr>
</tbody>
</table>

*P<0.05 compared to normal controls; **P<0.05, and ***P<0.01 compared to diabetic controls.

**Table 2.** Effects of glibenclamide and CLEE on the body weights in diabetic rats (Means ± S.E.M.)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial day 0</td>
</tr>
<tr>
<td>Normal + Vehicle</td>
<td>215.2 ± 2.35</td>
</tr>
<tr>
<td>Diabetes + Vehicle</td>
<td>224.34 ± 2.7</td>
</tr>
<tr>
<td>Diabetes + CLEE (200)</td>
<td>225.25 ± 3.47</td>
</tr>
<tr>
<td>Diabetes + CLEE (400)</td>
<td>223.48 ± 2.48</td>
</tr>
<tr>
<td>Diabetes + Glibenclamide</td>
<td>222.23 ± 2.71</td>
</tr>
</tbody>
</table>

*P<0.05 compared to normal controls; **P<0.05 compared to diabetic controls.
The protein level was elevated in alloxan induced diabetes. Glibenclamide significantly decreased the elevated serum insulin level in diabetic rats (Table 1). The insulin level was also increased in the diabetic rats after administration of CLEE. The insulin level was also increased in the diabetic rats after administration of CLEE.

Effect on body weight of rats
There was continuous reduction in body weight in diabetic rats (Table 2). Glibenclamide as well as the fraction (CLEE 200 and 400 mg/kg) treatment significantly (P<0.05) improved the body weight of diabetic rats.

Antihyperlipidemic effect
Diabetes is also associated with altered lipid profile. There was a significant increase of serum total cholesterol, triglycerides, and significant decrease in HDL cholesterol in diabetic rats as compared to that of normal control. Glibenclamide as well as both CLEE regimens significantly decreased (P<0.05) the levels of cholesterol and triglycerides. HDL cholesterol level was enhanced (Table 3) after 21 days of CLEE treatment.

Effect on other biochemical parameters
ALT, AST, ALP and bilirubin levels were significantly elevated in alloxan induced diabetes. The rats treated with CLEE showed significant (P<0.05) reduction in the elevated levels of liver transaminases in a dose dependent manner. Bilirubin level was also decreased in diabetic rats after CLEE treatment. Total protein level was decreased significantly in diabetic rats and after 21 days of CLEE treatment increased again significantly as shown in Table 4. CLEE also reduced the elevated levels of creatinine and urea in the alloxan induced diabetic rats (Table 5).

Histopathology of organs
The liver (Fig.1) showed normal hepatic cells with well preserved cytoplasm, nucleus, nucleolus and central vein in control group. In the group II diabetic rats, the normal lobular structure was preserved; the central vein was prominently preserved; t

**Biomedical parameters**

After blood glucose estimation on day 21, whole blood was collected by cardiac puncture from rats under mild ether anesthesia. Serum cholesterol, triglycerides, creatinine, bilirubin, urea, alkaline phosphatase, HDL and total proteins were also evaluated in normal and alloxan-induced diabetic rats. Serum alanine transaminase (ALT) and serum aspartate transaminase (AST) were measured by autoanlyser (Erba Chem 7, Mannheim, Germany). Serum insulin levels were determined using insulin ELISA kit.

**Statistical analysis**

All results are presented as mean ± standard error of the mean (S.E.M.) The statistical analysis involving two groups was evaluated by means of Student’s t-test whereas one way analysis of variance (ANOVA) followed by post-hoc Dunnett’s multiple comparison were used for statistical comparison between control and various treated groups. Statistical significance was accepted at the P<0.05 values.

**Results**

**Antihyperglycemic activity**

Single dose alloxan monohydrate significantly increased the blood glucose. After the daily oral administration with CLEE (200 and 400 mg/kg, p.o.) for 21 days, significant decreases (P<0.001) in the blood glucose levels were observed in the diabetic rats (Table 1). The insulin level was also slightly increased in the diabetic rats after administration of CLEE.

**Table 3. Effects of glibenclamide and CLEE on the lipid profile (mg/dl) in diabetic rats (Means ± S.E.M.)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL cholesterol (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal + Vehicle</td>
<td>7.26 ± 2.18</td>
<td>0.45 ± 1.24</td>
<td>43.22 ± 2.34</td>
</tr>
<tr>
<td>Diabetes + Vehicle</td>
<td>5.26 ± 1.29</td>
<td>0.94 ± 1.29*</td>
<td>102.26 ± 4.87</td>
</tr>
<tr>
<td>Diabetes + CLEE (200)</td>
<td>6.83 ± 2.25**</td>
<td>0.52 ± 1.43**</td>
<td>65.87 ± 4.46</td>
</tr>
<tr>
<td>Diabetes + CLEE (400)</td>
<td>7.23 ± 3.37***</td>
<td>0.43 ± 1.58***</td>
<td>44.28 ± 3.15***</td>
</tr>
<tr>
<td>Diabetes + Glib.</td>
<td>7.21 ± 1.25**</td>
<td>0.38 ± 1.83***</td>
<td>45.56 ± 3.54***</td>
</tr>
</tbody>
</table>

*P<0.05 compared to normal controls; **P<0.05, and ***P<0.01 compared to diabetic controls.

**Table 4. Effects of glibenclamide and CLEE on liver parameters in diabetic rats (Means ± S.E.M.)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Protein (g/dL)</th>
<th>Bilirubin (mg/dL)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal + Vehicle</td>
<td>7.26 ± 2.18</td>
<td>0.45 ± 1.24</td>
<td>43.22 ± 2.34</td>
<td>59.35 ± 3.49</td>
<td>123.35 ± 3.43</td>
</tr>
<tr>
<td>Diabetes + Vehicle</td>
<td>5.26 ± 1.29</td>
<td>0.94 ± 1.29*</td>
<td>102.26 ± 4.87</td>
<td>113.23 ± 3.45</td>
<td>198.26 ± 4.37*</td>
</tr>
<tr>
<td>Diabetes + CLEE (200)</td>
<td>6.83 ± 2.25**</td>
<td>0.52 ± 1.43**</td>
<td>65.87 ± 4.46</td>
<td>62.34 ± 3.76**</td>
<td>143.57 ± 4.38**</td>
</tr>
<tr>
<td>Diabetes + CLEE (400)</td>
<td>7.23 ± 3.37***</td>
<td>0.43 ± 1.58***</td>
<td>44.28 ± 3.15***</td>
<td>57.50 ± 3.13***</td>
<td>124.35 ± 1.76***</td>
</tr>
<tr>
<td>Diabetes + Glib.</td>
<td>7.21 ± 1.25**</td>
<td>0.38 ± 1.83***</td>
<td>45.56 ± 3.54***</td>
<td>58.86 ± 3.58**</td>
<td>125.25 ± 3.25***</td>
</tr>
</tbody>
</table>

*P<0.05 compared to normal controls; **P<0.05, and ***P<0.01 compared to diabetic controls.
Table 5. Effects of glibenclamide and CLEE on kidney parameters (mg/dl) in diabetic rats (Means ± S.E.M.)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum urea</th>
<th>Serum creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.25 ± 1.58</td>
<td>0.63 ± 1.34</td>
</tr>
<tr>
<td>Diabetes</td>
<td>59.24 ± 1.57</td>
<td>0.97 ± 0.54*</td>
</tr>
<tr>
<td>CLEE (200)</td>
<td>35.47 ± 2.51</td>
<td>0.74 ± 1.35**</td>
</tr>
<tr>
<td>CLEE (400)</td>
<td>32.37 ± 1.73**</td>
<td>0.64 ± 2.47***</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>35.35 ± 0.87**</td>
<td>0.65 ± 0.62***</td>
</tr>
</tbody>
</table>

*P<0.05 compared to normal controls; **P<0.05, and ***P<0.01 compared to diabetic controls.

Discussion

Alloxan has a destructive effect on the beta cells of the pancreas [3]. The pancreas is the primary organ involved in sensing the organism’s dietary and energetic states via glucose concentration in the blood. In response to elevated blood glucose insulin is secreted. Histopathological study of diabetic rats showed degeneration of pancreatic islet cells, which was due to alloxan used in this study. This probably gave rise to insulin deficiency. Insulin deficiency (or diabetes mellitus) causes excessive elevation of blood glucose and underutilization leading to hyperglycemia [12]. Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose, increased cholesterol, increased levels of alkaline phosphatase and transaminases etc [13-14]. CLEE and glibenclamide were found to reduce the hyperglycemia significantly in alloxan-induced diabetic animals during 21 days treatment. The active fraction CLEE has also increased the insulin level in diabetic rats. So, one possible antidiabetic mechanism of C.lanceolatus extract may be stimulation of pancreas for insulin secretion. The weight loss in the diabetic group was improved by CLEE treatment. This may be due to control the urinary glucose and protein release during treatment.

Repeated administration of the CLEE for 21 days significantly decreased cholesterol and triglyceride levels. This hypolipidemic effect may be due to decreased cholesterologenesis and fatty acid synthesis [15]. HDL cholesterol level was also significantly improved by the fraction.
Serum transaminases, i.e. AST, ALT and ALP levels, are markers of hepatic diseases. In diabetic rats, serum level of these enzymes was elevated [16] and it was significantly reduced by CLEE and glibenclamide treatment. Serum protein and bilirubin levels were also normalized by the treatment.

Serum urea and creatinine are markers of renal diseases. CLEE improved renal functions in levels. Alloxan has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free radical damage [17]. Histopathological studies of liver, pancreas and kidney tissues revealed that CLEE has protective effect on the organs.
In conclusion, the result of the present study showed that CLEE reduced hyperglycemia and hyperlipidemia diabetes-induced rats. The fraction also improved kidney and liver functions. It has inhibited the histopathological changes of the pancreas, liver and kidney in alloxan induced diabetes. However, the exact chemical compounds responsible for the antihyperglycemic and antihyperlipidemic effects of C. lanceolatus need to explore in further studies.

References
