Antihyperglycemic and antioxidative potential of hydroalcoholic extract of Butea monosperma Lam flowers in alloxan-induced diabetic mice

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Daily treatment of alloxan-induced diabetic animals with 50% ethanolic extract of B. monosperma flowers (BMEE) for 45 days significantly lowered blood glucose level thereby preventing steep onset of hyperglycemia which was observed after alloxan administration and maintained body weight and blood glucose level close to the values observed in normal control and glibenclamide-treated diabetic mice. Moreover, the level of serum total cholesterol, triglyceride, low-density lipoprotein and very low-density lipoprotein cholesterol were also lowered, whereas the level of high-density lipoprotein cholesterol, which was reduced in untreated diabetic animals, was significantly elevated. Oxidative damage in the liver, pancreas and kidneys of diabetic mice as evidenced by a marked increment in the level of thiobarbituric acid reactive substances and also a distinct diminution in glutathione content was nullified by BMEE. Activities of antioxidant enzymes were also assessed in all the experimental groups. These enzymes registered a decline in their activity in diabetic animals thus revealing the damaging effects of free radicals generated due to alloxan exposure but their activities were reverted towards near normal range in BMEE-administered mice thus indicating the antioxidant efficacy of the drug in resisting oxidative damage.

Keywords: Alloxan, Antidiabetic, Antioxidative, Butea monosperma, Diabetes mellitus, Ethanol extract, Oxidative stress

Hyperglycemia in diabetes mellitus (DM) may directly or indirectly contribute to excess formation of free radicals, which ultimately leads to oxidative stress. Increased free radical generation and oxidative stress are hypothesized to play an important role in pathogenesis of diabetes and its secondary complications such as nephropathy, retinopathy, neuropathy and cardiomyopathy. These complications may be delayed, decreased or prevented by maintaining blood glucose values close to normal by treatment with antidiabetic drugs. Current drugs used for the treatment of diabetes involve different mechanisms for bringing down blood sugar level in normal or glycemic conditions but are associated with several side effects, hence there is need for effective, safe and better oral hypoglycemic agents. Several plants are known for controlling diabetes but only a few have been scientifically evaluated and the active principles have been isolated.

Butea monosperma Lam (Leguminosae), known as "Flame of the Forest" in English, "Palash" in Hindi, "Kimsuk" in Bengali, "Urasu" in Tamil, "Moduga" in Telugu and "Muriku" in Malayalam, is highly esteemed to possess various medicinal properties. In a preliminary study, the use of flowers has been reported for relieving burning sensation, in treatment of gout, leprosy and other skin diseases. In Unani system of medicine, they are used as aphrodisiac, expectorant, tonic, emmenagogue, diuretic and in biliousness. Further, this plant also possesses the potential in treating a number of ailments where the free radicals have been reported to be the major factor contributing to the disorders. But the protective effect of B. monosperma flowers, in controlling the complications of diabetes induced oxidative stress has not been worked out. The present study is an attempt in this direction. In this study, the effect of 45 days chronic oral treatment with ethanolic extract of B. monosperma flowers (BMEE) (300 mg/kg body wt) on diabetes and resultant oxidative stress has been investigated by evaluating its antihyperglycemic, hypolipidemic and antioxidative properties in alloxan-induced diabetic mice.
Material and Methods

Preparation of extract — Flowers of B. monosperma were collected from Sanjay Van, Newai (Rajasthan, India) and taxonomically identified by Dr. G. S. Shekhawat (Botanist, Department of Bioscience & Biotechnology, Banasthali University, Rajasthan, India). Shade dried flowers were powdered. The powder was soxhlet extracted with 50% ethanol and concentrated to dryness under reduced pressure at 60°C±1°C in a vacuum rotatory evaporator. The concentrated extract was further dried at 40°-45°C in hot air oven till solid to semi-solid mass was obtained, 3.8% w/w (with respect to crude material). This extract (BMEE) was stored in refrigerator below 10°C. The suspension of BMEE prepared in 20% tween 20 in normal saline was used in each day of the experiment.

Animal care and monitoring — Healthy male Swiss albino mice (Mus musculus) procured from C.C.S. Haryana Agricultural University, Hisar (Haryana, India) were housed under standard laboratory conditions of light (12:12 h L: D cycle) at 23°C± 2°C and 55 ± 5% RH. The animals were given standard rat pellet feed and tap water ad libitum. After one week of acclimatization, mice were randomly divided into following 4 experimental groups of 7 animals each: A (normal control, NC), B (alloxan-treated diabetic control, DC), C (alloxan-induced diabetic + BMEE-treated, BT) and D (glibenclamide treated, GT). Maintenance and treatment of animals was done in accordance with the principles of Institutional Animal Ethics Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Chemicals — Alloxan monohydrate was purchased from SD Fine chemicals (Mumbai, India). All other chemicals used for this study were of analytical grade and obtained from HIMEDIA (India), Sisco Research Laboratories (India), Central Drug House (India) and Qualigens (India/Germany).

Experimental induction of diabetes — After fasting for 18 h, mice of group B, C and D were made diabetic by a single ip injection of alloxan with a dose of 150 mg/kg body wt, freshly dissolved in normal saline. One week after alloxan injection, the fasting blood glucose (FBG) concentration was determined by means of one touch ultra glucometer (Johnson & Johnson Company, USA) and compatible blood glucose strips. Mice showing fasting blood level greater than 140 mg/dl were considered as diabetic and selected for further study.

Experimental design — Normal and diabetic animals were randomly divided into 4 groups of 7 mice each as follows:

Group A: Normal control (untreated)
Group B: Pathogenic diabetic control (Alloxan only without any drug treatment)
Group C: Alloxan + BMEE treated (300 mg/kg body wt)
Group D: Alloxan + glibenclamide treated (10 mg/kg body wt)

The test drug and reference standard drugs were fed orally for 45 days.

Mice of group A and B received only 20% tween 20 solution orally once a day for 45 days.

The experiment was terminated in overnight fasted mice at the end of 45 days.

Blood collection and biochemical estimations in serum — For estimating fasting blood glucose (FBG) concentration, blood sample was obtained from tail tip vein of all experimental animals at different time periods i.e. before inducing diabetes, after inducing diabetes and after 45 days of BMEE treatment.

For estimating serum lipid profile, serum was isolated from the blood which was collected on day 45 of BMEE treatment and serum total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) - cholesterol were estimated by using respective diagnostic kits (Erba Mannheim Cholesterol kit, Transasia Bio-Medicals Ltd; Daman). Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) - cholesterol were calculated as per Friedeval equation.

\[ \text{VLDL-cholesterol} = \frac{\text{serum triglyceride}}{5} \]

\[ \text{LDL-cholesterol} = \text{serum total-cholesterol} - \text{VLDL-cholesterol} - \text{HDL-cholesterol} \]

Results were expressed in mg/dl.

Biochemical estimation in tissues — Tissue homogenate supernatant of all the four experimental groups was used. For this, liver, pancreas and kidney were dissected out, washed in normal saline and weight of all the organs was taken only after drying the tissue. Tissues were then homogenized in ten times its volume of 0.2 M tris HCl. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C. The

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Results were expressed in mg/dl.
supernatant so obtained was used for estimation of total protein, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), reduced glutathione (GSH), and lipid peroxidation (LPO) products (TBARS).

Statistical analysis — Results are expressed as mean ± SD. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey’s post-hoc multiple comparison test using SPSS (version 16.0) and Student’s t test using SigmaPlot (version 8.0). The values of P<0.01 and P<0.05 were considered as statistically significant.

Results

Effect on body weight — A significant (P<0.05) loss in body weight (29.6%) was persistently observed in DC group. In group C the decrease in body weight from 34.9 ± 2.5 g to 30.3 ± 3.3 g after alloxan injection was significantly (P<0.05) restored to 37.6 ± 2.7 g (19.4% increase) after 45 days of BMEE treatment and the increased weight observed after treatment was even found to exceed the value that was observed before inducing diabetes. This increase in body weight is comparable to that observed in group D i.e. glibenclamide treated group in which 20.2% increase was observed after 45 days of treatment.

Effect on fasting blood glucose level — Treatment of normal control group (group A) with saline alone does not affect the normal blood glucose concentration throughout the study whereas alloxan injection to B, C and D group resulted in a significant (P<0.05) increase in blood glucose level from 72.7 ± 7.4 mg/dl to 244.3 ± 11.6 mg/dl, 89.4 ± 9.2 mg/dl to 230.0 ± 37.6 mg/dl and 96.1 ± 9.4 mg/dl to 360.6 ± 37.6 mg/dl in DC, BMEE-treated and GT animals respectively. These increased values are considerably higher than that of group A in which fasting blood glucose concentration ranges between 79.6 ± 7.7 to 80.7 ± 8.3 mg/dl throughout the study period. However, a significant (P<0.05) fall in blood glucose level of diabetic mice i.e. 50.7% (from 230.0 mg/dl to 113.3 mg/dl) was observed in group C after 45 days of BMEE treatment and this antidiabetic attribute was even greater than that of glibenclamide which produced 49.3% reduction (i.e. from 360.6 ± 37.6 mg/dl to 182.7 ± 14.5 mg/dl) in blood glucose levels of group D after 45 days of treatment (Table 1).

Effect on serum lipid profile — Compared to the normal control group A, serum TC, TG, LDL and VLDL - cholesterol levels increased significantly (P<0.05) in untreated diabetic control group B, whereas HDL - cholesterol has been significantly (P<0.05) reduced in this group. Treating group C with BMEE for 45 days, decreased serum TC, TG, LDL and VLDL - cholesterol and increased HDL-cholesterol significantly (P<0.05) as compared to diabetic control mice. Similar pattern was also observed in glibenclamide-treated group D (Table 2).

Other biochemical parameters — In diabetic animals, there was a significant (P<0.05) decrease in tissue GSH, SOD (hepatic), CAT and GSH-Px whereas the level of TBARS was increased significantly (P<0.05). However, treatment with BMEE significantly (P<0.05) reverted all the parameters (except SOD, where the elevation observed was insignificant, P>0.05) towards normalization (Table 3).

Table 1 — Effect of 45 days treatment of ethanolic extract of B. monosperma flowers on body weight and fasting blood glucose (FBG) level in alloxan-induced diabetic mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Day</th>
<th>NC</th>
<th>DC</th>
<th>BT</th>
<th>GT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g)</td>
<td>BD*</td>
<td>27.4 ± 3.2</td>
<td>29.7 ± 3.3</td>
<td>34.9 ± 2.5</td>
<td>22.4 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>27.4 ± 3.2</td>
<td>18.4 ± 3.5</td>
<td>30.3 ± 3.3</td>
<td>19.4 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>27.9 ± 3.1</td>
<td>20.9 ± 4.5</td>
<td>37.6 ± 2.7</td>
<td>24.3 ± 3.0c</td>
</tr>
<tr>
<td>FBG Level (mg/dl)</td>
<td>BD*</td>
<td>79.6 ± 7.7</td>
<td>72.7 ± 7.4</td>
<td>89.4 ± 9.2</td>
<td>96.1 ± 9.4</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>79.6 ± 7.7</td>
<td>244.3 ± 11.6</td>
<td>230.0 ± 37.6</td>
<td>360.6 ± 37.6</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>80.7 ± 8.3</td>
<td>232.9 ± 34.9</td>
<td>113.3 ± 8.6</td>
<td>182.7 ± 14.5</td>
</tr>
</tbody>
</table>

*Before diabetes
Student’s t test is significant at P<0.05. *significant (P<0.05) difference compared to basal values; †insignificant (P>0.05) difference compared to basal values and ‡significant (P<0.05) difference compared to values obtained after alloxan injection.

NC = Normal control; DC = Diabetic control; BT = BMEE treated; GT = Glibenclamide treated
The undamaged or residual pancreatic islets to release may be due to increased peripheral glucose utilization (50.7%) in FBG levels. This hypoglycemic action animals with BMEE caused a significant reduction in hyperglycemia. Further, the hypoglycemic potential of BMEE was comparable with that of glibenclamide and can be correlated with the previous research findings.

However, continuous treatment of diabetic animals with BMEE caused a significant reduction (50.7%) in FBG levels. This hypoglycemic action may be due to increased peripheral glucose utilization by or by potentiating the insulin effect via stimulation of the undamaged or residual pancreatic islets to release insulin. Moreover, significant reduction in blood glucose level by glibenclamide treatment strengthens the above explanation since it also exerts its hypoglycemic effect by increasing insulin secretion. Further, the hypoglycemic potential of BMEE was comparable with that of glibenclamide and can be correlated with the previous research findings.

In the present study, dyslipidemia observed in alloxan-induced diabetic mice is in accordance with the previous research findings. The diabetes-induced hyperlipidemia may be due to excess mobilization of fat from adipose tissue because of underutilization of glucose. The hypolipidemic

Discussion

The dose of alloxan, 150 mg/kg destroys pancreatic β-cells which ultimately results in hyperglycaemia. The increased blood glucose level in diabetic animals as compared to normal ones may be due to glycogenolysis or gluconeogenesis in the form. However, continuous treatment of diabetic animals with BMEE caused a significant reduction (50.7%) in FBG levels. This hypoglycemic action may be due to increased peripheral glucose utilization by or by potentiating the insulin effect via stimulation of the undamaged or residual pancreatic islets to release insulin. Moreover, significant reduction in blood glucose level by glibenclamide treatment strengthens the above explanation since it also exerts its hypoglycemic effect by increasing insulin secretion. Further, the hypoglycemic potential of BMEE was comparable with that of glibenclamide and can be correlated with the previous research findings.

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Table 2 — Effect of 45 days treatment of ethanolic extract of B. monosperma flowers on serum lipid profile in alloxan-induced diabetic mice

<table>
<thead>
<tr>
<th>Parameters (mg/dl)</th>
<th>NC</th>
<th>DC</th>
<th>BT</th>
<th>GT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>84.9 ± 2.9</td>
<td>214.7 ± 3.7</td>
<td>169.9 ± 11.4</td>
<td>75.0 ± 3.9</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>72.8 ± 3.1</td>
<td>158.3 ± 4.4</td>
<td>99.6 ± 4.5</td>
<td>58.1 ± 5.2</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>26.9 ± 3.5</td>
<td>25.9 ± 0.9</td>
<td>127.3 ± 11.6</td>
<td>46.4 ± 3.8</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>43.5 ± 3.6</td>
<td>167.1 ± 3.3</td>
<td>22.7 ± 4.2</td>
<td>16.9 ± 1.9</td>
</tr>
<tr>
<td>Very-low density lipoprotein</td>
<td>14.6 ± 0.5</td>
<td>31.7 ± 0.9</td>
<td>19.9 ± 2.2</td>
<td>11.6 ± 1.2</td>
</tr>
</tbody>
</table>

Table 3 — Effect of 45 days treatment of ethanolic extract of B. monosperma flowers on other biochemical parameters in alloxan-induced diabetic mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NC</th>
<th>DC</th>
<th>BT</th>
<th>GT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic GSH</td>
<td>16.0 ± 1.7</td>
<td>10.5 ± 0.6</td>
<td>27.1 ± 1.6</td>
<td>32.5 ± 2.7</td>
</tr>
<tr>
<td>Hepatic TBARS</td>
<td>43.9 ± 6.3</td>
<td>1425 ± 122</td>
<td>244.7 ± 28.7</td>
<td>1001 ± 85.6</td>
</tr>
<tr>
<td>Hepatic SOD</td>
<td>308.6 ± 9.2</td>
<td>2381 ± 22.3</td>
<td>2588 ± 37.8</td>
<td>1922 ± 6.2</td>
</tr>
<tr>
<td>Hepatic CAT</td>
<td>233.0 ± 12.7</td>
<td>1767 ± 19.9</td>
<td>3865 ± 42.5</td>
<td>1574 ± 12.0</td>
</tr>
<tr>
<td>Hepatic GSH-Px</td>
<td>689.5 ± 27</td>
<td>453.1 ± 20.7</td>
<td>555.1 ± 20.3</td>
<td>287.5 ± 27.9</td>
</tr>
<tr>
<td>Pancreatic GSH</td>
<td>14.8 ± 1.8</td>
<td>10.4 ± 0.3</td>
<td>27.5 ± 2.3</td>
<td>32.2 ± 0.9</td>
</tr>
<tr>
<td>Pancreatic TBARS</td>
<td>9.6 ± 1.5</td>
<td>419.6 ± 25.8</td>
<td>87.9 ± 12.8</td>
<td>345.6 ± 30.5</td>
</tr>
<tr>
<td>Pancreatic SOD</td>
<td>321.7 ± 20.4</td>
<td>317.6 ± 19.6</td>
<td>340.0 ± 28.0</td>
<td>358.0 ± 46.8</td>
</tr>
<tr>
<td>Pancreatic CAT</td>
<td>266.6 ± 18.4</td>
<td>167.5 ± 6.7</td>
<td>350.9 ± 24.2</td>
<td>277.1 ± 15.6</td>
</tr>
<tr>
<td>Pancreatic GSH-Px</td>
<td>721.0 ± 71.7</td>
<td>478.0 ± 20.9</td>
<td>697.8 ± 45.2</td>
<td>534.1 ± 19.1</td>
</tr>
<tr>
<td>Renal GSH</td>
<td>15.8 ± 1.8</td>
<td>10.8 ± 0.4</td>
<td>27.5 ± 2.4</td>
<td>32.6 ± 0.8</td>
</tr>
<tr>
<td>Renal TBARS</td>
<td>110.1 ± 4.9</td>
<td>964 ± 119</td>
<td>525.8 ± 17.8</td>
<td>638.8 ± 61.7</td>
</tr>
<tr>
<td>Renal SOD</td>
<td>333.1 ± 29.2</td>
<td>299.6 ± 28.8</td>
<td>364.3 ± 24.4</td>
<td>294.6 ± 26.8</td>
</tr>
<tr>
<td>Renal CAT</td>
<td>272.1 ± 21.5</td>
<td>161.9 ± 10.6</td>
<td>426.7 ± 40.8</td>
<td>245.8 ± 16.4</td>
</tr>
<tr>
<td>Renal GSH-Px</td>
<td>723.2 ± 75.2</td>
<td>377.7 ± 42.9</td>
<td>788.3 ± 57.1</td>
<td>420.2 ± 16.6</td>
</tr>
</tbody>
</table>

Student’s t test is significant at P<0.05. a significant (P<0.05) difference compared to NC; b significant (P<0.05) difference compared to DC; c significant (P<0.05) difference and d<0.05 difference compared to GT.

Other abbreviations are same as in Table 1.
action of BMEE may be due to its potential for inhibiting lipid peroxidation\textsuperscript{30,32}.

The reactive oxygen species (ROS) are a common contributory factor in the development of diabetes complications and there are many reports indicating alterations in the antioxidant parameters during diabetes induced oxidative stress\textsuperscript{33}. Reduced glutathione (GSH) is essential to maintain structural and functional integrity of cells. Apart from its direct free radical scavenging properties and ability to conjugate with several electrophilic intermediates that are capable of initiating lipid peroxidation, GSH also acts as the physiological co-substrate of the conjugating enzyme system\textsuperscript{32}. The distinct diminution of GSH content in tissues of diabetic animals and the subsequent improvement towards their near normal values after BMEE treatment reveals the protective properties of BMEE in combating oxidative damage due to diabetes\textsuperscript{34,30,1}.

Enhanced level of TBARS in the tissues of diabetic animals indicates excessive formation of free radicals and activation of lipid peroxidative system. But treatment of group C animals with BMEE reduced the level of TBARS in this group thus unveiling its anti-lipid peroxidative potential. This finding is in correlation with findings of other investigators\textsuperscript{32,34}.

Besides, a decline in the content of antioxidant marker enzymes such as CAT, GSH-Px and SOD in tissues of diabetic mice definitely indicates the extent of free radical induced damage due to hyperglycemia. It is now well known that when there is an imbalance between free radical production and antioxidant defence, oxidative stress occurs resulting in deregulation of cellular functions\textsuperscript{35,32}. On the contrary, an antioxidant drug is expected to bring an alleviation of this type of cellular functions. The profound increment in the activities of the antioxidant enzymes in BMEE treated group C unravels the efficacy of the drug in resisting oxidative damage due to diabetes.

Based on findings of the present study, it can be concluded that hypoglycemic effect of \textit{B. monosperma} may be mediated through modulation of cellular antioxidant defence system thus proving itself to be a promising plant in respect to its antioxidant potential to alleviate diabetes and necessitates further study in this direction.

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References