Effect of *Butea monosperma* Lam. leaves and bark extracts on blood glucose in streptozotocin-induced severely diabetic rats

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ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder that has significant impact on the health, quality of life and life expectancy, as well as on the health care system. *Butea monosperma* Lam. Kuntze (Fabaceae), commonly known as palash, is widely used in the treatment of various diseases and disorders including diabetes. **Materials and Methods:** The present study was planned to evaluate the antidiabetic effect of aqueous extracts of *B. monosperma* leaves and bark in streptozotocin-induced severely diabetic rats. The animals were divided into four groups, with each consisting of six rats, viz. control, diabetic control, leaf extract-treated and bark extract-treated groups. Treatment was continued for 6 weeks. The biochemical estimations included blood glucose and serum insulin levels. Histopathology of pancreas was also performed. **Results:** The results indicated that both leaf and bark extracts of *B. monosperma* produced insignificant antihyperglycemic activity. The leaf and bark extracts reduced blood glucose to an extent of 28% and 11%, respectively. It was also evidenced that both leaf and bark extracts did not increase insulin synthesis or secretion and did not improve pancreatic architecture as reflected by the histopathologic studies. **Conclusions:** The findings of the study emphasize that *B. monosperma* does not possess significant antidiabetic activity in severe experimental diabetes at the dosage tested.

Key words: *Butea monosperma*, diabetes, insulin, pancreas, streptozotocin

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that has significant impact on the health, quality of life and life expectancy, as well as on the health care system.[1] Type 2 diabetes represents a syndrome with disordered metabolism of carbohydrate; its most prominent clinical feature is hyperglycemia.[1] Medicinal plants continue to play an important role in the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have an access to modern treatment. The increased demand is also due to the side effects associated with the use of insulin and oral hypoglycemic agents.[2] To date, over 400 traditional medicinal plant treatments for diabetes have been reported, but only a small number of these have received scientific and medical evaluation to assess their efficacy.

One such well-known traditionally used medicinal plant is *Butea monosperma* Lam. Kuntze (Fabaceae), commonly known as palash,[3] a medium-sized tree, native of the mountainous regions of India and Burma.[4] Various medicinal properties are attributed for different parts of this plant in Ayurveda.[5] The root bark is used as an aphrodisiac, analgesic and anthelmintic.[4] The stem bark is used for the treatment of dyspepsia, diarrhea, dysentery, diabetes, ulcers, sore throat and snake bites.[6,7] Some scientific studies have established the antidiabetic activity of various parts of this plant. Ethanol extract of the flowers at a dose of 200 mg/kg exhibited significant reductions in blood glucose, serum cholesterol and improved glucose tolerance, high density lipoprotein (HDL)-cholesterol and albumin levels in alloxan-induced diabetic rats.[8] Bavarva *et al.* reported that oral administration of the ethanol extract of the seeds exhibited significant antidiabetic effect in non–insulin-dependent diabetes mellitus (NIDDM) rats.[3] Deore

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et al. reported that the crude aqueous extracts of the bark exhibited significant hypoglycemic and antihyperglycemic effects in normal and alloxan-induced diabetic albino rats, respectively.[9] In view of the above, the present study was undertaken to evaluate the effect of aqueous extracts of *B. monosperma* leaves and bark in streptozotocin (STZ)-induced severely diabetic rats.

MATERIALS AND METHODS

Drugs and chemicals

STZ was purchased from Sigma-Aldrich, Bangalore, India. Serum insulin was determined using Elisa kit purchased from Linco Research, Inc., Missouri, USA. Urea and creatinine were determined using standard diagnostic kits purchased from Aggappe Diagnostics, Ernakulam, India. Blood glucose was determined using one touch glucometer (Accu-chek, Roche Diagnostics, Mannheim, Germany). All other reagents and chemicals used in the study were of extra pure analytical grade.

Plant material

The leaves and the bark of *B. monosperma* were collected from Kollegal, Chamarajanagar district, Karnataka, India, and subsequently identified by Dr. Shivprasad Hudeda, JSS Ayurvedic College, Mysore, and a voucher specimen is retained in the laboratory for future reference (RL2 003/2008-09). The bark and leaves were cleaned, dried in a hot air oven (50°C), powdered, passed through 60 mesh sieve (BS) and stored in an airtight container at 4°C till further use.

Preparation of aqueous extracts

Aqueous extracts were prepared by extracting the powders of *B. monosperma* leaves (BML) and *B. monosperma* bark (BMB) with hot water (70°C) in a mechanical shaker (24 h), followed by filtration and freeze drying.

Experimental design and induction of diabetes

Healthy adult male Wistar rats (24) between 8 and 9 weeks of age and weighing 130 ± 15 g were housed in polyacrylic cages and maintained under standard experimental conditions (12 h photo period; 27 ± 2°C; 45–60% RH). Animals were fed a standard laboratory diet and provided with food and water *ad libitum*.

The rats were divided into the following four groups, with each group consisting of six animals.
Group I: Normal healthy rats served as control
Group II (BML): Diabetic rats treated with *B. monosperma* leaf extract (500 mg/kg BW, p.o.)
Group III (BMB): Diabetic rats treated with *B. monosperma* bark extract (500 mg/kg BW, p.o.)
Group IV (DC): Untreated diabetic rats served as diabetic control

Diabetes was induced in the experimental groups by a single intramuscular injection of STZ (55 mg/kg BW) dissolved in citrate buffer (pH 4.5) after 24 h of fasting. Hyperglycemia was confirmed by elevated blood glucose levels after 72 h, and rats with fasting blood glucose levels >450 mg/dL were selected for the study. Body weights were determined weekly, while blood glucose was determined fortnightly.

After 6 weeks, the rats were euthanized and the blood was collected by direct cardiac puncture and centrifuged (2500 ×*g*, 20 min). The serum separated was used for the assay of insulin, urea and creatinine. Pancreas were excised and fixed in a 10% solution of formaldehyde, and then dehydrated in graduated ethanol (50–100%), cleared in xylene and embedded in paraffin. The sections (4–5 μm) were examined with a photomicroscope (400×) after staining with hematoxylin and eosin (H and E) dye.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) followed by Tukey's multiple comparisons test for significant differences using SPSS 14.0 software. The values were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The present study reports the effect of aqueous extracts of *B. monosperma* leaves and bark on glucose metabolism in STZ-induced severely diabetic rats. STZ-induced diabetic rats have been widely used as a model for evaluation of antidiabetic activity. The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues.[10]

STZ is known to induce characteristic loss of body weight by an increased muscle wasting.[11] In the present study, a progressive loss of body weight was observed in BML-treated, BMB-treated and untreated diabetic rats compared to control rats [Figure 1]. It is generally observed that uncontrolled diabetes results in loss of body weight. Further, STZ administration resulted in significant ($P \leq 0.05$) hyperglycemia in all the experimental groups. Although oral administration of *B. monosperma* leaf and bark extracts (500 mg/kg) significantly decreased ($P \leq 0.05$) blood glucose compared to initial levels, the levels did not reach nearer to control levels. The total reduction in fasting blood glucose concentration brought about by the leaf and bark extracts was 28 and 11%, respectively [Figure 2]. These findings are consistent with an earlier study, wherein aqueous extract of the bark caused 28% reduction in blood glucose in alloxan-induced diabetic mice after 5 h of administration.[9]

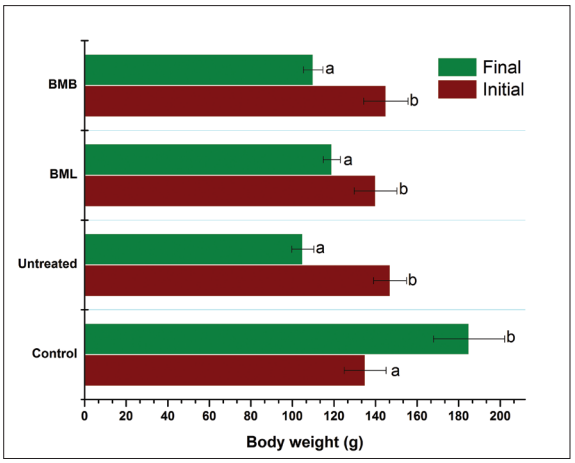

Figure 1: Body weight changes of various groups. (BML: Butea monosperma leaf extract, BMB: Butea monosperma bark extract)

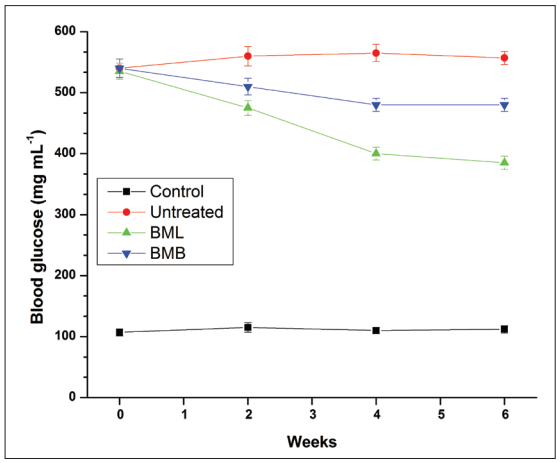

Figure 2: Changes in blood glucose concentration of various groups. (BML: Butea monosperma leaf extract, BMB: Butea monosperma bark extract)

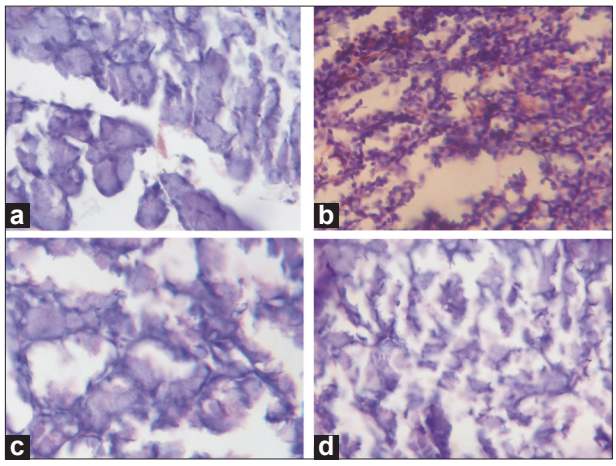

Figure 3: (a) Control rats showing normal pancreatic tissue. (b) Untreated diabetic rats showing degenerative changes and mononuclear infiltrate in the pancreatic tissue. (c) Butea monosperma leaf extract-treated rats showing vacuolation and mononuclear infiltrate in the pancreatic tissue. (d) Butea monosperma bark extract-treated rats showing vacuolation and mononuclear infiltrate in the pancreatic tissue.

Table 1: Serum insulin levels of various groups

Groups	Serum insulin (mIU/L)
Control	$42^{b} \pm 1.37$
Untreated	$21^{a} \pm 0.91$
BML	$23^{a} \pm 0.79$
BMB	$21^{a} \pm 0.73$

Values are Mean ± SD ($n = 6$). Values carrying different superscript letters, a and b, differ significantly ($P \leq 0.05$), BML: *Butea monosperma* leaf extract, BMB: *Butea monosperma* bark extract

STZ is reported to cause rapid and irreversible necrosis of pancreatic β-cells. Serum insulin levels were determined followed by histopathology of pancreas to study the insulin synthesis and secretion by residual β-cells after STZ administration and their differentiation and proliferation. The results indicated no significant increase in serum insulin levels in both BML- and BMB-treated rats compared to untreated diabetic rats, which were significantly lower ($P \leq 0.05$) than that of control rats [Table 1]. These observations were further substantiated by the histopathology of the pancreas, wherein the sections from all the experimental groups showed significant loss of pancreatic architecture. In control animals, the pancreatic sections showed normal cells with well-preserved cytoplasm [Figure 3a], while in untreated diabetic rats the sections showed intense degenerative necrosis and inflammation with neutrophil infiltration of the pancreatic cytoplasm [Figure 3b]. Although the leaf and bark extracts controlled hyperglycemia to some extent, they did not cause any significant improvement in pancreatic architecture compared to untreated diabetic rats, except for slight a reduction of mononuclear infiltrate and inflammatory changes [Figure 3c and d].

CONCLUSION

The results of the present study indicate that *B. monosperma* leaves and bark possess insignificant antihyperglycemic activity in severely diabetic rats. It was also evidenced that both leaf and bark extracts do not increase insulin synthesis or secretion and do not contribute to improve pancreatic architecture in STZ-induced severely diabetic rats at the dosage tested.

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