CHAPTER-I
RESULTS & DISCUSSION

Protective effect of Butea monosperma on diabetic rats: antidiabetic, antioxidant, hypolipidemic and histological evidences
INTRODUCTION

Diabetes mellitus (DM) is the most serious, non-communicable metabolic disorder characterized by abnormalities in insulin secretion and insulin resistance of major target tissues [Green and Feinglos, 2007]. The International Diabetes Federation (IDF) estimates that worldwide there are 285 million people with diabetes in 2010 and this will increase to 438 million by 2025 (54% increase) (IDF Diabetes Atlas, 2009). DM is classified as Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM) on etiological basis. T1DM is a chronic metabolic disorder characterized by high blood glucose levels due to an absolute or relative deficiency of circulating insulin levels. T2DM is the most common form of diabetes affecting ≈90% of the diabetic people [Khunti and Ganguli, 2000]. It is associated with a variety of conditions and diseases characterized by hyperglycemia resulting from a defect in insulin secretion or function, or both [Mahler and Adler, 1999; Green and Feinglos, 2007]. Chronic elevation of blood glucose leads to tissue damage, with consequent often serious complications [Henquin et al., 1992; Hamden et al., 2008]. DM often associated with a variety of metabolic and physiologic complications including elevated blood pressure, cardiovascular disease, dyslipidemia (high triglyceride levels and low levels of high-density lipoproteins) and high cholesterol level [Ahmed and Goldstein, 2006]. Long-term complications of DM include retinopathy, cerebro-vascular disease, coronary heart disease, nephropathy, neuropathy and peripheral vascular disease. Diabetes imposes a large economic burden on the individual, national healthcare system and economy. Although insulin and other types of insulin analogues hypoglycemic agent are currently available for treating DM, there is a growing interest in herbal remedies due to the side-effects associated with the existing therapeutic hypoglycemic agents [Holman and Turner, 1991; Kameswara et al., 1997]. This has led to the belief that natural products are safe because they are more harmonious with biological systems [Atal, 1983; Erasto, 2003]. The pancreatic β-cell possesses the ability to respond to minor increases in the plasma glucose levels, thereby maintain the blood glucose level [Hellestrom, 1997]. Progressive destruction of pancreatic β-cell leading to decreased insulin production and subsequent hyperglycemia that is observed in DM (T1DM and T2DM). In particular, liver is an insulin-sensitive tissue and plays a major role in maintaining glucose homeostasis by regulating the interaction between the glucose utilization and gluconeogenesis [Ferre et al., 1996]. Therefore, damage to pancreas and liver is of decisive importance in the development and progression of both type of diabetes. Thus, effect of dietary supplement on tissues such as liver and pancreas that regulates glucose metabolism is an interesting area to explore.
Much clinical and experimental evidence have evaluated the strong association between hyperglycemia, oxidative stress and diabetic complications [Baynes and Thorpe, 1999; Kuyvenhoven and Meinders, 1999]. Oxidative stress is increased in diabetes because of multiple factors. Hyperglycemia itself generates reactive oxygen species (ROS), which in turn cause lipid peroxidation and membrane damage via auto-oxidation of glucose [Hunt et al., 1988]. Furthermore, alteration of the GSH redox state by activation of NADPH-dependent aldose reductase (polyol pathway) and non-enzymatic glycosylation of protein have also been shown to be a source of free radicals [Ceriello et al., 1992]. Excessive production of ROS causes oxidative damage to macromolecules such as lipid (lipid peroxidation [LPO]), protein (protein carbonyl [PC] formation), nucleic acids and inactivation of anti-oxidants enzymes [Strain, 1991; Gutteridge, 1995] and eventually cell death. Under physiologic conditions, our body uses a wide range of anti-oxidant defenses including reduced glutathione (GSH) and anti-oxidant enzymes: glutathione-s-transferase (GST), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) to protect against the adverse effects of ROS [Halliwell and Gutteridge, 1990; McLennan et al., 1991].

One promising aspect of understanding the role of oxidative stress in diabetic complications is the ability of antioxidant supplementation to attenuate the adverse effects of diabetes. Many medicinal plants have been used in diabetes treatment for a long time [Grover et al., 2002; Modak et al., 2007] and these herbal formulations are being investigated for their antioxidant as well as antidiabetic potentials [Mutalik et al., 2005; Prince et al., 1999]. *Butea monosperma* (BM) Lami. (family: Fabaceae) commonly called the Palash, is a well-known traditionally used medicinal plant and possesses a number of pharmacotherapeutic effects such as antihepatotoxic, antifungal, estrogenic, anti-inflammatory, antistress and anticonceptive [Budia and Khadeb, 2007]. The major chemical constituents of BM flower extract are butrin, isobutrin, medicarpin, flavone glycoside, butin, palasonin, coreopsin and β-sitosterol. Most studies of BM as a hypoglycemic and hypolipidemic agent have been done with the alloxan model of diabetes [Somani et al., 2006; Sharma and Garg, 2009; Panda et al., 2009]. Recently, Bavarda and Nanasiinahcharya, [2008] showed the anti-hypoglycemic and anti-hyperlipidemic effects of BM in a neonatal rat model of T2DM. In the present study, we evaluated the beneficial effects of BM on hyperglycemia, hyperlipidemia and oxidative damage in liver and pancreas using both type of diabetic model (TIDM and T2DM) in rats. The efficacy of BM for diabetes treatment was compared with the standard drug. Our results support the efficacy of BM in reducing glucose level and improving diabetic complications in non-genetic rat models of diabetes (TIDM and T2DM).
MATERIALS AND METHODS

Chemicals
As described in materials and methods, section-III.

Preparation of extract and phytochemical screening
The BM flower extract was subjected to qualitative phytochemical analysis as described in materials and methods, section-III.

Determination of total phenolic and flavonoid content
In the present study, total phenolic and flavonoid content present in extract were estimated as described earlier in materials and methods, section-III.

In vitro antioxidant activity
Reducing power activity, hydrogen peroxide scavenging activity and DPPH scavenging capacity of the extract was determined as described earlier in materials and methods, section III.

EFFICACY OF BM FOR THE TREATMENT OF TYPE 1 DIABETES

Experiment 1: Evaluation of therapeutic dose of BM against type I diabetes mellitus (TIDM) and check whether the required therapeutic dose is safe or produces some type of toxicity

Experimental procedure and induction of TIDM
Experiment was carried out to evaluate the therapeutic dose of BM for the treatment of diabetes. Experimental setup and induction of TIDM was done according to procedure as described in materials and methods, section-III.

Estimation of fasting blood glucose
On the morning of the end of experiment, after overnight fasting, the rats were anesthetized by ether inhalation and blood was collected from the dorsal aorta. Serum was separated and blood glucose level was measured as described in materials and methods, section-III.

Experiment- II: Effect of BM on biochemical and morphological alterations in STZ-induced type 1 diabetic rats.

Experimental design and development of the STZ model of type1 diabetes
As described in materials and methods, section-III. Thirty two rats were divided into four groups of eight animals each as described earlier.
Biochemical analyses
Metabolic markers (OGTT, FBG, HbA1c, insulin, amylase, hepatic glycogen, FFAs, lipid profile (TC, TG, HDL-C, LDL-C, VLDL-C)), inflammatory markers (NO, IL-1β, TNF-α), and renal function markers (BUN, Scr, ALP) were performed as described in materials and methods, section-III. Oxidative damage parameters (TBARS, MDA, PC, GSH, GST and CAT) and protein content are described in materials and methods, section-III.

Histological examinations
Histopathological study is described in materials and methods, section-III.

Efficacy of BM for the treatment of type 2 diabetes

Experiment I: Evaluation of therapeutic dose of BM against type 2 diabetes mellitus (T2DM) and check whether the required therapeutic dose is safe or produces some type of toxicity

Experimental procedure and induction of T2DM
As described in materials and methods, section-III.

Estimation of fasting blood glucose
On the morning of the end of experiment, after overnight fasting, the rats were anesthetized by ether inhalation and blood was collected from the dorsal aorta. Serum was separated and blood glucose level was measured as described in materials and methods, section-III.

Experiment II: Effect of Butea monosperma (BM) on biochemical and morphological alterations in HFD/STZ-induced type 2 diabetic rats.

Experimental design and development of HFD/STZ model of type 2 diabetes
Experiment was carried out as described in material and methods, section-III. Rats were divided described in materials and methods, section-III.

Biochemical analyses and histological examinations
Metabolic markers (OGTT, FBG, HbA1c, insulin, FFAs, hepatic glycogen, lipid profile (TC, TG, HDL-C, LDL-C, VLDL-C)), inflammatory markers (NO, IL-1β, TNF-α), and renal function markers (BUN, Scr, ALP) are described in materials and methods, section-III. Oxidative damage parameters (TBARS, MDA, PC, GSH, GST and CAT) and protein content were determined as described in materials and methods, section-III. Histological studies were done described in materials and methods, section-III.

Statistical analysis
As described in material and methods, section-III.
RESULTS

Phytochemical Screening
The compounds that are responsible for therapeutic effect are usually the secondary metabolites. The extract of the plant material was subjected to preliminary phytochemical screening for the detection of various plant constituents [Kokate et al., 1996]. A phytochemical screening of the powder revealed the presence of flavonoids, phytosterols, glycosides, saponins, phenolic compounds and fat. Tests for tannins, alkaloids and fats were negative (Table 1).

Total phenolic and flavonoid content of BM methanolic extract
Phenolics and flavonoids are the most widespread secondary metabolite in plant kingdom. For phenolic content, values were expressed as gallic acid equivalents 16.75±1.51 mg/100 mg of dried bark extract. For flavonoid content, values were expressed as rutin equivalents 58.54±1.77 mg/100 mg of dried bark extract.

IN VITRO ANTIOXIDANT ACTIVITY
Reducing power activity of BM methanolic extract
For the measurements of the reducing ability, the Fe"⁻⁻Fe²⁺ transformation was investigated in the presence of BM. Fig. 1(A). depicts the reductive capabilities of BM extract compared to ascorbic acid. The reducing power of extract of BM was very potent and the power of the extract was increased with increasing dosage. The result shows that BM could reduce the most Fe⁺⁺ ions, which had a lesser reductive activity than the reference standard of ascorbic acid.

Hydrogen peroxide scavenging activity of BM methanolic extract
As shown in Fig. 1(B). BM also demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner with an IC₅₀ of 12.61 µg/ml. The IC₅₀ value of the extract was found to be comparable to reference standard ascorbic acid (IC₅₀ 2.24 µg/ml).

DPPH antioxidant of BM methanolic extract
Comparison of the antioxidant activity of the extract and ascorbic acid is shown in Fig. 1(C). The methanol extract of BM exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC₅₀) at a concentration of 23.38 µg/ml. The IC₅₀ value of the extract was found to be comparable to reference standard ascorbic acid (IC₅₀ 3.7 µg/ml).
Table 1: Phytochemical screening of methanol extract of BM flower

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Constituent</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Sterols</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>Phenolic</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Proteins and amino acid</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Fat</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Sugars and carbohydrate</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Resins</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: (−) shows the absent of constituent in the extract, (+) shows the present of constituent and (+++) shows the present of constituent in higher amount.

Table 2: Total phenolic and flavonoid content of BM

<table>
<thead>
<tr>
<th>Part used</th>
<th>Total phenolic content (mg/100mg extract) Mean ± S.E.M</th>
<th>Total flavonoid content (mg/100mg extract) Mean ± S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower</td>
<td>16.75±1.51</td>
<td>58.54±1.77</td>
</tr>
</tbody>
</table>

Values are the average of triplicate experiments and represented as Mean ± S.E.M. The total phenolic content was expressed as gallic acid equivalent in mg/100mg of the extract. The total flavonoid content was expressed as rutin equivalents in mg/100mg of the extract.
Fig 1 (A). Reducing power of ascorbic acid and methanol extract of BM flowers. (B) \( \text{H}_2\text{O}_2 \) scavenging activity of ascorbic acid and methanol extract of BM flowers. (C) DPPH radical scavenging activity of ascorbic acid and methanol extract of BM flowers. Values are the average of triplicate experiments and represented as Mean ± S.E.M.
EFFICACY ON TYPE 1 DIABETES

Effect of BM on hyperglycemia in the STZ group (Dose evaluation)
The STZ group showed significant ($P < 0.05$) increase in FBG compared to the control group. In the BM (300mg/kg) treated group, the levels of FBG was significantly ($P < 0.05$) attenuated as compared to other doses of BM (200mg/kg; 400mg/kg) in STZ-induced diabetic rats. Only BM (300mg/kg) treatment did not show any significant change in the FBG compared to the control group. GL also produced significant ($P < 0.05$) reduction in FBG level.

STUDY ON TYPE 1 DIABETES WITH SELECTED DOSE

Effect of BM supplementation on OGTT in the STZ-induced rat model of diabetes
Blood glucose level of the control, the STZ group and the STZ + BM groups at different time points (0, 30, 60 and 120 min) after oral administration of glucose (2 gm/kg) shown in Fig.3. In the STZ group, the peak increase in blood glucose level was observed after 60 min and remained high over next 60 min. BM and GL treatment significant ($P < 0.05$) decreased blood glucose level at 60 and 120 min in the STZ + BM group compared to the STZ group.

Effect of BM treatment on body weight in the STZ-induced rat model of diabetes
STZ rats had significantly increased body weight ($P < 0.05$) compared to control. Administration of BM and GL in the STZ + BM or STZ + GL group restored body weight significantly ($P < 0.05$) when compared to the STZ group. There was no significant difference in body weight between control animals and only BM-treated animals (Table 3).

Effect of BM treatment on FBG in the STZ-induced rat model of diabetes
Table 4 shows the effect of BM on the FBG level. A significant ($P < 0.05$) increase in FBG was observed in STZ group compared to the control rats. BM treatment significantly ($P < 0.05$) reduced FBG in the STZ + BM group compared to the STZ group. Only BM treatment did not show any significant change in the FBG compared to the control rats. The four-week treatment with BM resulted in significant ($P < 0.05$) antihyperglycemic effect and was comparable to standard drug, GL.
Effect of BM treatment on HbA1c in the STZ-induced rat model of diabetes

Effect of BM on HbA1c level in the STZ is shown in Table 4. A significant \( (P < 0.05) \) increase in HbA1c level was observed in the STZ group compared to the control rats. BM and GL treatment in the STZ + BM or STZ + GL group significantly \( (P < 0.05) \) decreased the HbA1c level. There was no significant change in HbA1c level in BM treatment in the control + BM treated rats when compared to control rats.

Effect of BM supplementation on amylase activity in the STZ-induced rat model of diabetes

Effect of BM on amylase activity in diabetic and control groups is shown in Table 4. A significant \( (P < 0.05) \) decrease in amylase activity was observed in the STZ group compared to the control rats. Administration of BM in the STZ + BM group increased amylase activity significantly \( (P < 0.05) \) when compared to the STZ group. Supplementation of GL showed significant \( (P < 0.05) \) improvement in the amylase activity when compared to diabetic animals. Only BM treatment did not show any significant change in amylase activity when compared to the control rats.

Effect of BM treatment on insulin in the STZ-induced rat model of diabetes

Significant \( (P < 0.05) \) decrease in insulin level was observed in the STZ group compared to the control rats (Table 4). Administration of BM in the STZ + BM group increased insulin level significantly \( (P < 0.05) \) when compared to the STZ group. Diabetic rats given GL showed significant \( (P < 0.05) \) improvement in the insulin level when compared to diabetes group. Only BM treatment did not show any significant change in insulin level when compared to the control rats.

Effect of BM on hepatic glycogen content in the STZ-induced rat model of diabetes

Effect of BM treatment on the hepatic glycogen content in the STZ group is shown in Table 4. The STZ group showed the decreased levels of glycogen content compared to the control group. The glycogen levels were significantly increased by BM and GL treatment in the STZ + BM or STZ + GL group compared to the STZ group. The results obtained for the glycogen content indicates that there was no significant difference in glycogen content between the control and control + BM group.
Fig 2. Different BM doses were used for evaluating the dose showing maximum antihyperglycemic activity and was compared to GL. Values are expressed as mean ± S.E.M.

Fig 3. The STZ group showed the increase in blood glucose level at 60 min and remained high over next 60 min compared to the control group (\( P < 0.05 \) STZ vs. control group). BM and GL treatment decreased the blood glucose at both time points in the STZ + BM or GL group compared to the STZ group (\( P < 0.05 \) STZ + BM or STZ + GL vs. STZ group). Values are expressed as mean ± S.E.M. (\( n = 8 \)).
Table 3. Effect of BM supplementation on body weight in the STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Parameters/Group</th>
<th>Initial Body weight (g)</th>
<th>Final Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (Control)</td>
<td>190±5.0</td>
<td>218±4.5</td>
</tr>
<tr>
<td>C + BM</td>
<td>179±8.5</td>
<td>171±8.0</td>
</tr>
<tr>
<td>STZ</td>
<td>185±6.5</td>
<td>166±5.5</td>
</tr>
<tr>
<td>STZ + BM</td>
<td>194±8.0</td>
<td>189±6.5</td>
</tr>
<tr>
<td>STZ + GL</td>
<td>196±6.0</td>
<td>184±7.5</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=8). The body weight of all animals was measured initially and after the end of experiment. STZ group showed a significant decrease in body weight compared with the control group (P < 0.05 STZ vs. control group). BM and GL treatment significantly augmented body weight compared with the STZ group (P < 0.05 STZ vs. STZ + BM or STZ + GL group).

Table 4. Effect of BM treatment on FBG, HbA1c, amylase, insulin and hepatic glycogen in the STZ-induced rat model of diabetes

<table>
<thead>
<tr>
<th>Parameters / groups</th>
<th>Control (C)</th>
<th>C + BM</th>
<th>STZ</th>
<th>STZ + BM</th>
<th>STZ + GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dl)</td>
<td>112.98±3.2</td>
<td>114.32±3.9</td>
<td>258.95±5.3</td>
<td>164.53±4.6</td>
<td>136.33±4.1</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.8±0.24</td>
<td>6.7±0.28</td>
<td>12.23±0.34</td>
<td>9.69±0.31</td>
<td>8.53±0.37</td>
</tr>
<tr>
<td>Amylase (U/dl)</td>
<td>34.65±8.5</td>
<td>33.32±8.9</td>
<td>11.32±1.8</td>
<td>23.51±1.3</td>
<td>25.41±1.1</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>2.5±0.10</td>
<td>2.4±0.11</td>
<td>0.22±0.05</td>
<td>1.2±0.05</td>
<td>1.6±0.04</td>
</tr>
<tr>
<td>Hepatic glycogen (mg)</td>
<td>218.37±4.6</td>
<td>220.11±4.8</td>
<td>96.87±2.8</td>
<td>148.62±3.6</td>
<td>151.62±3.5</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=8). The STZ group showed a significant decrease in amylase, insulin and hepatic glycogen with significant increase in FBG and HbA1c compared to the control group (P < 0.05 STZ vs. control group). BM and GL treatment significantly restored these parameters in the STZ + BM or STZ + GL group compared to the STZ group (P < 0.05 STZ vs. STZ + BM or STZ + GL group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared to the control or STZ group.
**Effect of BM on serum lipid profile and FFAs in the STZ-induced rat model of diabetes**

The effects of BM on lipid profile in the STZ rats shown in Table 5. STZ group showed a significant ($P < 0.05$) increment in serum TC, TG, FFAs, LDL-C and a significant ($P < 0.05$) decrement in serum HDL-C compared to the control group. Treatment of BM in the STZ + BM group significantly ($P < 0.05$) restored all the changes in the lipid profile. Diabetic rats given GL showed significant ($P < 0.05$) improvement in these parameters when compared to diabetes group. BM treatment did not show any significant changes in the lipid profile when compared to the control group.

**Effect of BM on renal function markers in the STZ-induced rat model of diabetes**

Effects of BM on renal markers (BUN, Scr and ALP) were measured to demonstrate renal function in serum of the STZ group. There were no significant changes in BUN, Scr, and ALP in the control + BM group while these factors were significantly ($P < 0.05$) increased in the STZ group compared to the control group. Following administration of GL diabetic rats also showed a significant reduction in the levels of BUN, Scr and ALP. BM treatment significantly ($P < 0.05$) decreased these markers in the STZ + BM group compared to the STZ group (Table 6).

**Effects on serum TNF-α, IL-β and NO levels**

Significant ($P < 0.05$) increased in the levels of serum TNF-α, IL-β and NO was observed in STZ group compared to control group. BM and GL administration in STZ + BM or STZ + GL group however decreased significantly ($P < 0.05$) the serum TNF-α, IL-β and NO levels when compared to STZ group. Only BM treatment did not show any significant difference in the levels of TNF-α, IL-β and NO when compared to the control rats.
Table 5. Effect of BM treatment on serum lipid profile and FFAs.

<table>
<thead>
<tr>
<th>Parameters / groups</th>
<th>Control (C)</th>
<th>C + BM</th>
<th>STZ (mg/dl)</th>
<th>STZ + BM</th>
<th>STZ + GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>132.84±2.3</td>
<td>134.91±2.4</td>
<td>268.51±4.4^a</td>
<td>201.21±3.4^b</td>
<td>195.52±3.0^b</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>121.35±2.2</td>
<td>126.93±2.1</td>
<td>223.81±3.9^b</td>
<td>168.58±3.1^b</td>
<td>160.75±3.2^b</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>39.26±1.8</td>
<td>36.21±2.2</td>
<td>19.83±1.2</td>
<td>28.32±1.7^b</td>
<td>33.12±1.9^b</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>68.71±1.4</td>
<td>73.31±1.6</td>
<td>203.91±3.9^a</td>
<td>144.42±2.8^b</td>
<td>133.54±2.5^b</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>24.87±0.93</td>
<td>25.38±0.96</td>
<td>44.76±1.6</td>
<td>33.31±0.9^b</td>
<td>30.42±0.9^b</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.39±0.03</td>
<td>0.41±0.04</td>
<td>0.78±0.07</td>
<td>0.65±0.05^b</td>
<td>0.56±0.04^b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=8). The STZ group showed a significant decrease in HDL-C with the significant increase in TC, TG, LDL-C, VLDL-C and FFAs in STZ group compared to the control group (P < 0.05 vs, control group). BM and GL treatment reversed these effects compared to the STZ group (P < 0.05 STZ vs, STZ + BM or STZ + GL group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or STZ group.

Table 6. Effect of BM treatment on renal function markers (BUN, Scr and ALP) in serum of the STZ induced rat model of diabetes

<table>
<thead>
<tr>
<th>Parameters / groups</th>
<th>Control (C)</th>
<th>C + BM</th>
<th>STZ (mg/dl)</th>
<th>STZ + BM</th>
<th>STZ + GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mg/dl)</td>
<td>16.39±1.03</td>
<td>17.98±1.08</td>
<td>45.62±1.25^a</td>
<td>34.31±1.19^b</td>
<td>28.22±1.22^b</td>
</tr>
<tr>
<td>Scr (mg/dl)</td>
<td>1.21±0.06</td>
<td>1.23±0.05</td>
<td>4.01±0.08</td>
<td>2.78±0.07^b</td>
<td>2.56±0.06^b</td>
</tr>
<tr>
<td>ALP (units/dl)</td>
<td>23.41±0.94</td>
<td>24.61±0.98</td>
<td>54.71±1.1^a</td>
<td>34.20±1.06^b</td>
<td>22.22±1.1^b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=8). The STZ group showed a significant increase in renal function markers (BUN, Scr and ALP) in serum of the STZ group compared to the control group (P < 0.05 HF/DSTZ vs, control group). BM and GL treatment significantly modulated these parameters in the treatment groups compared to the STZ group (P < 0.05 STZ vs, STZ + BM or STZ + GL group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or STZ group.
Fig 4. STZ group showed significant increase in TNF-α, IL-1β and NO levels compared with the control group (*P < 0.05 STZ vs. control group). BM and GL supplementation significantly decreased these parameters in the STZ + BM or STZ + GL group compared with the STZ induced diabetic group (*P < 0.05 STZ + BM or STZ + GL vs. STZ group). Values are expressed as mean ± S.E.M. (n = 8).
Effect of BM on TBARS and MDA contents in the STZ-induced rat model of diabetes

There were no significant changes in TBARS and MDA contents in control + BM treated group compared to the control group. These parameters were significantly (P < 0.05) increased in the STZ group compared to the control group. Levels of TBARS and MDA in the STZ group decreased significantly (P < 0.05) with BM and GL treatment (Fig. 5 A. and B.).

Effect of BM on PC in the STZ-induced rat model of diabetes

PC content did not change by BM treatment in the control + BM group compared to the control group. PC content was significantly (P < 0.05) increased in the STZ group compared to the control alone. BM and GL treatment significantly (P < 0.05) decreased PC content in the STZ + BM or STZ + GL group compared to the STZ group alone (Fig. 6).

Effect of BM on GSH in the STZ-induced rat model of diabetes

Level of GSH did not affect by BM treatment in the control + BM treated group compared to the control group. However, a significant (P < 0.05) depletion in GSH was observed in the STZ group compared to the control group. BM and GL treatment significantly (P < 0.05) augmented GSH level in the treated groups compared to the STZ group (Fig. 7).

Effect of BM on GST and CAT activity in the STZ-induced rat model of diabetes

Effects of BM on the activity of GST and CAT in the STZ group and control groups (Fig. 8 A. and B.). The activity of GST and CAT in the control + BM group was attenuated but the elevation was not significant compared to the control group. On the other hand, the activity of GST and CAT was depleted significantly (P < 0.05) in the STZ group compared to the control group. BM and GL treatment significantly (P < 0.05) restored the activity of these enzymes in the treated groups compared to the STZ group.
Fig 5. (A). STZ group showed significant increase in TBARS levels compared with the control group (\( P < 0.05 \) STZ vs. control group). BM and GL significantly decreased this marker in the treatment group compared with the STZ-induced diabetic group (\( P < 0.05 \) STZ + BM or STZ + GL vs. STZ group). (B). The STZ group showed a significant increase in MDA levels compared to the control group (\( P < 0.05 \) STZ vs. control group). BM and GL treatment significantly decreased MDA in the supplemented group compared to the STZ group (\( P < 0.05 \) STZ + BM or STZ + GL vs. STZ group). Values are expressed as mean ± S.E.M. (n = 8).
Fig 6. STZ group showed significant increase in PC content compared with the control group (\( P < 0.05 \) STZ vs. control group). BM and GL supplementation significantly decreased PC content in the STZ + BM or STZ + GL group compared with the STZ induced diabetic group (\( P < 0.05 \) STZ + BM or STZ + GL vs. STZ group). Values are expressed as mean ± S.E.M. (n = 8).

Fig 7. STZ group showed significant decrease in GSH content compared with the control group (\( P < 0.05 \) STZ vs. control group). BM and GL supplementation significantly increased GSH content compared with the STZ induced diabetic group (\( P < 0.05 \) STZ + BM or STZ + GL vs. STZ group). Values are expressed as mean ± S.E.M. (n = 8).
Fig 8. (A). STZ group showed significant decrease in GST activity compared with the control group (\( P < 0.05 \) STZ vs. control group). BM and GL supplementation significantly increased enzyme activity in the supplemented group compared with the HFD/STZ induced diabetic group (\( P < 0.05 \) STZ + BM or STZ + GL vs. STZ group). (B). The STZ group showed a significant decrease in CAT activity compared to the control group (\( P < 0.05 \) HFD/STZ vs. control group). BM and GL treatment significantly increased enzyme activity when compared to the STZ group (\( P < 0.05 \) STZ + BM or STZ + GL vs. STZ group). Values are expressed as mean ± S.E.M. (n = 8).
Effect of BM on histopathological changes in the STZ-induced rat model of type 1 diabetes

H and E staining is used to visualize and differentiate between tissue components in normal and pathological conditions (Fig 9). The histological examination of the H and E-stained control liver tissues showed normal architecture of hepatocytes. Liver section of STZ-induced diabetic group showed vacuolization of hepatocytes in the centrilobular area with remarkable dilated sinusoidal spaces. The STZ + BM group that received BM treatment showed normal hepatic parenchyma. The severity of degenerative changes was lessened by BM supplementation in the STZ + BM group compared with the STZ group. Photomicrograph from STZ + GL group animal that supplemented with standard GL, showing regularly arranged liver lobules along with portal triad with mild hyalinization of hepatic arterial wall. BM supplementation did not show any remarkable effects in the group treated with BM alone compared with the control group (data not shown). The histological examination of the H and E-stained control pancreatic section showed normal architecture of islet cells. Pancreatic section of STZ-induced diabetic group showed beta cells with reduction of cytoplasmic mass, reduction of Islet size and atrophy of beta cells with vacuolization. The beta cell cytoplasm was very scant and a few lymphocytes were also seen. These degenerative changes were lessened by BM supplementation in the STZ + BM group compared with the STZ group, indicating a partial protective effect of BM in diabetic animals. Pancreas of STZ + GL group animal showing a small islet with normal exocrine glands surrounding it. Numerous beta cells with abundant basophilic cytoplasm were seen in GL treated group. BM supplementation did not show any remarkable effects in the group treated with BM alone compared with the vehicle control group (data not shown).
Fig 9. Photograph from the control group at low power -100 x. showing normal liver architecture. PT = portal triad, CV = central vein, at high power-400 x. showing details with normal portal triad. PV = portal vein, BD = bile duct, HA = hepatic artery. The STZ group at low power-100 x. showing sinusoidal dilatation in the centrizonal area, at high power-400 x. showing vacuolated hepatocytes and remarkable sinusoidal dilatation in the centrizonal area. BM supplementation in the STZ + BM group at low power-100 x. showing histologically normal liver tissue with normal PT and CV, at high power-400 x. showing a portal triad with some inflammatory cells (arrow). PV = Portal Vein. GL supplementation in STZ + GL group at low power -100x showing regularly arranged liver lobules. PT=Portal Triad, CV=Central Vein, at high power-400x. showing a portal triad with mild hyalinization of Hepatic arterial wall. BD=Bile Duct, HA=Hepatic Artery.
Fig 10. Photograph from the control group at low power - 100 x. showing normal appearance of pancreatic tissue with a single islet of Langerhans seen in the centre. The exocrine tissue is within normal limits, IL=Islet of Langerhans, Ex=Exocrine tissue, at high power-400 x showing details of cells in the islet and Islet of Langerhans showing several beta cells with abundant basophilic cytoplasm. STZ group at low power- 100 x. showing changes in islet of Langerhans. The exocrine tissue is within normal limits, at high power-400 x. showing beta cells with reduction of cytoplasmic mass and also vacuolar change and atrophy of the beta cells. The beta cell cytoplasm is very scant and a few lymphocytes are also seen. BM supplementation in the STZ + BM group at low power-100 x. showing pancreatic tissue with a normal sized islet of Langerhans seen, at high power-400 x. showing numerous normal looking beta cells. There are however dilated vascular spaces within the islet (arrow). In the STZ + GL group at low power-100 x. showing normal sized islet of Langerhans. There are dilated spaces seen in the islet, at high power-400 x. showing a fair number of residual beta cells with prominent vacuolation and fibrosis within the islet tissue.
Chapter-I  
Butea monosperma

Efficacy on Type 2 Diabetes

Effect of BM on hyperglycemia in the HFD/STZ group (dose evaluation)

The HFD/STZ group showed significant (P < 0.05) increase in FBG compared to the control group. In the BM (300mg/kg) treated group, the levels of FBG was significantly (P < 0.05) attenuated as compared to other doses of BM (200mg/kg; 400mg/kg) in HFD/STZ-induced diabetic rats. Only BM (300mg/kg) treatment did not show any significant change in the FBG compared to the control group. Hypoglycemic activity of BM was found to be comparable with the standard drug, GL.

Study on Type 2 Diabetes with Selected Dose

Effect of BM on OGTT in the HFD/STZ-induced rat model of diabetes

Blood glucose levels of the controls, the HFD/STZ group and the HFD/STZ + BM groups at different time points (0, 30, 60 and 120 min) after oral administration of glucose (2 gm/kg) shown in Fig. 12. In the HFD/STZ group, the peak increase in blood glucose level was observed after 60 min and remained high over next 60 min. BM and GL treatment in the HFD/STZ + BM or HFD/STZ + GL group showed significant (P < 0.05) decrease in blood glucose level at 60 and 120 min when compared to the HFD/STZ group.

Effect of BM on body weight in the HFD/STZ-induced rat model of diabetes

HFD/STZ rats had significantly increased body weight (P < 0.05) compared to control. Administration of BM in the HFD/STZ + BM group restored body weight significantly (P < 0.05) when compared to the HFD/STZ group. GL treatment also showed the same effect as BM to restore body weight in diabetic animals. There was no significant difference in body weight between control animals and only BM-treated animals (Table 7).

Effect of BM on FBG level in the HFD/STZ-induced rat model of diabetes

A significant (P < 0.05) increase in blood glucose level was observed in the HFD/STZ group compared to the control (Table 8). Administration of BM or GL significantly (P < 0.05) reduced blood glucose level in the HFD/STZ + BM or HFD/STZ + GL group compared to the HFD/STZ. Only BM treatment did not show any significant change in the blood glucose level compared to the control rats.

Effect of BM on HbA1c in the HFD/STZ-induced rat model of diabetes

A significant (P < 0.05) increase in HbA1c level was observed in the HFD/STZ group when compared to the control rats (table 8). BM and GL treatment significantly (P < 0.05) decreased the HbA1c level. There was no significant change in HbA1c level in BM treatment in the control + BM-treated rats when compared to control rats.
Effect of BM treatment on insulin in the HFD/STZ-induced rat model of diabetes

Significant (P < 0.05) decrease in insulin level was observed in the HFD/STZ group compared to the control rats (Table 8). Administration of BM in the HFD/STZ + BM group increased insulin level significantly (P < 0.05) when compared to the HFD/STZ group. Only BM treatment did not show any significant change in insulin level when compared to the control rats.

Effect of BM on hepatic glycogen content in the HFD/STZ-induced rat model of diabetes

The HFD/STZ group showed the lowest levels of glycogen content when compared to the control and the HFD/STZ + BM group (Table 8). The glycogen levels were significantly increased in diabetic rats treated with BM when compared to the HFD/STZ group. GL also produced significant (P < 0.05) increased in glycogen content. The results obtained for the glycogen content indicates that there was no significant difference in glycogen content between the control and control + BM group.

Effect of BM on lipid profile and FFAs in the HFD/STZ-induced rat model of diabetes

The HFD/STZ group showed a significant (P < 0.05) increment in serum TC, TG, FFAs, LDL-C, VLDL-C levels while a significant (P < 0.05) decrement in serum HDL-C level compared to the control group (Table 9). Treatment of BM in the HFD/STZ + BM group significantly (P < 0.05) restored all the changes in lipid profile and FFAs compared to the HFD/STZ group. Similar effects were found with GL treatment as of BM to restore lipid abnormalities. BM treatment did not show any significant changes in lipid profile in the control + BM group compared to the control.

Effect of BM on renal markers in the HFD/STZ-induced rat model of diabetes

Effects of BM on renal markers (BUN, Scr and ALP) were measured to demonstrate renal function in serum of the HFD/STZ group. There were no significant changes in BUN, Scr, and ALP in the control + BM group, while these factors were significantly (P < 0.05) increased in the HFD/STZ group compared with the control group. BM and GL supplementation in the HFD/STZ + BM or HFD/STZ + GL group resulted in a significantly (P < 0.05) decrease in these markers compared with the HFD/STZ group (Table 10).
Figure 11. Different BM doses were used for evaluating the dose showing maximum antihyperglycemic activity. Values are expressed as mean $\pm$ S.E.M.

Fig. 12. The HFD/STZ group showed the increase in blood glucose level at 60 min and remained high over next 60 min after oral glucose administration compared to the control group ($P < 0.05$ HFD/STZ vs. control group). BM and GL supplementation decreased the blood glucose level at 60 and 120 min in supplemented groups compared to the HFD/STZ group ($P < 0.05$ HFD/STZ + BM or HFD/STZ + GL vs. HFD/STZ group). Values are expressed as mean $\pm$ S.E.M. ($n = 8$).
Table 7. Effect of BM treatment on body weight of control and experimental groups.

<table>
<thead>
<tr>
<th>Parameters/Group</th>
<th>Body weight (g)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (C)</td>
<td>187±7.5</td>
<td>285±8.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C + BM</td>
<td>180±8.5</td>
<td>269±9.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HFD/STZ</td>
<td>196±10.0</td>
<td>180±8.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HFD/STZ + BM</td>
<td>179±10.5</td>
<td>245±7.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HFD/STZ + GL</td>
<td>190±5.5</td>
<td>252±7.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=8). The body weight of all animals was taken initially and after the end of experiment. HFD/STZ group showed a significant decrease in body weight compared with the control group (P < 0.05 HFD/STZ vs. control group). BM and GL treatment significantly augmented body weight when compared with the HFD/STZ group (P < 0.05 HFD/STZ vs. HFD/STZ + BM or HFD/STZ + GL group).

Table 8. Effect of BM supplementation on FBG, HbA1c, insulin and hepatic glycogen in the HFD/STZ induced rat model of diabetes

<table>
<thead>
<tr>
<th>Parameters /groups</th>
<th>Control (C)</th>
<th>C + BM</th>
<th>HFD/STZ</th>
<th>HFD/STZ + BM</th>
<th>HFD/STZ + GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dl)</td>
<td>106.37±4.5</td>
<td>108.47±4.3</td>
<td>332.80±5.4</td>
<td>217.85±5.1</td>
<td>139.55±4.9</td>
</tr>
<tr>
<td></td>
<td>(+ 1.97%)</td>
<td>(+ 2.74%)</td>
<td>(+ 212.87%)</td>
<td>(- 52.76%)</td>
<td>(- 58.06%)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.47±0.07</td>
<td>5.62±0.07</td>
<td>10.94±0.08</td>
<td>8.97±0.03</td>
<td>8.22±0.13</td>
</tr>
<tr>
<td></td>
<td>(+ 2.74%)</td>
<td>(+ 100%)</td>
<td>(+ 100%)</td>
<td>(- 18.00%)</td>
<td>(- 24.86%)</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>3.8±0.15</td>
<td>3.7±0.14</td>
<td>0.59±0.05</td>
<td>1.9±0.08</td>
<td>2.3±0.09</td>
</tr>
<tr>
<td></td>
<td>(- 2.63%)</td>
<td>(- 84.47%)</td>
<td>(+ 222.03%)</td>
<td>(+ 289.83%)</td>
<td>(+ 289.83%)</td>
</tr>
<tr>
<td>Hepatic glycogen (µg)</td>
<td>354.62±3.85</td>
<td>357.66±2.34</td>
<td>172.81±2.01</td>
<td>235.49±3.21</td>
<td>248.77±3.41</td>
</tr>
<tr>
<td></td>
<td>(+ 0.85%)</td>
<td>(+ 1.97%)</td>
<td>(+ 51.68%)</td>
<td>(+ 36.27%)</td>
<td>(+ 43.95%)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=8). HFD/STZ group showed a significant decrease in insulin and hepatic glycogen with the significant increase in FBG and HbA1c in the HFD/STZ group compared with the control group (P < 0.05 HFD/STZ vs. control group). BM and GL treatment significantly ameliorated these parameters when compared with the HFD/STZ group (P < 0.05 HFD/STZ vs. HFD/STZ + BM or HFD/STZ + GL group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or HFD/STZ group.
Table 9. Effect of BM supplementation on serum lipid profile and FFAs in HFD/STZ induced rat model of diabetes

<table>
<thead>
<tr>
<th>Parameters / groups</th>
<th>Control (C)</th>
<th>C + BM</th>
<th>HFD/STZ</th>
<th>HFD/STZ + BM</th>
<th>HFD/STZ + GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>130.12±1.13</td>
<td>132.33±1.2 (+ 1.69%)</td>
<td>278.92±2.34 (+ 114.35%)</td>
<td>151.64±2.13b (- 45.63%)</td>
<td>170.22±3.22b (- 38.97%)</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>105.27±1.37</td>
<td>106.95±1.35 (+ 1.59%)</td>
<td>203.43±1.82 (+ 93.24 %)</td>
<td>149.56±1.23b (- 26.48%)</td>
<td>152.25±2.02b (- 25.15%)</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>49.36±0.91</td>
<td>48.38±0.88 (- 1.98%)</td>
<td>22.88±0.48 (-53.64%)</td>
<td>29.88±0.60b (+ 30.59%)</td>
<td>36.45±1.1b (+ 59.30%)</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>59.70±0.72</td>
<td>62.54±0.98 (+ 4.76%)</td>
<td>215.35±1.04 (+ 260.71%)</td>
<td>91.84±1.1b (- 57.35%)</td>
<td>103.32±1.1b (- 52.02%)</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>21.05±0.7</td>
<td>21.39±0.8 (+ 1.61%)</td>
<td>40.68±0.56 (+ 93.28%)</td>
<td>29.91±0.40b (- 26.48%)</td>
<td>30.45±0.91b (- 25.14%)</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>2.2±0.1</td>
<td>2.1±0.2 (-4.54%)</td>
<td>3.9±0.3b (+ 77.27%)</td>
<td>2.8±0.2b (- 30.76%)</td>
<td>2.6±0.3b (- 33.33%)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. HFD/STZ group showed a significant increase in TC, TG, LDL-C and VLDL-C, FFAs with decrease in HDL-C in the serum of the HFD/STZ group compared to the control group (P < 0.05 HFD vs. control group). BM and GL treatment significantly ameliorated these parameters in the HFD/STZ + BM or HFD/STZ + GL group compared to the HFD/STZ group (P < 0.05 HFD/STZ vs. HFD/STZ + BM or HFD/STZ + GL group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or HFD/STZ group.

Table 10. Effect of BM supplementation on renal function markers (BUN, Scr and ALP) in serum of the HFD/STZ induced rat model of diabetes

<table>
<thead>
<tr>
<th>Parameters / Groups</th>
<th>Control (C)</th>
<th>C + BM</th>
<th>HFD/STZ</th>
<th>HFD/STZ + BM</th>
<th>HFD/STZ + GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mg/dl)</td>
<td>18.91±1.5</td>
<td>20.27±1.6 (+ 7.19%)</td>
<td>51.77±2.1b (+ 173.77%)</td>
<td>31.34±1.3b (- 39.46%)</td>
<td>26.76±1.4b (- 30.46%)</td>
</tr>
<tr>
<td>Scr (mg/dl)</td>
<td>1.07±0.004</td>
<td>1.11±0.018 (+ 3.73%)</td>
<td>2.69±0.009b (+ 142.34%)</td>
<td>1.94±0.018b (- 27.88%)</td>
<td>1.76±0.017b (- 34.57%)</td>
</tr>
<tr>
<td>ALP (units/dl)</td>
<td>25.87±0.92</td>
<td>26.06±0.80 (+ 0.73%)</td>
<td>43.16±0.98 (+ 68.83%)</td>
<td>33.89±0.91b (- 23.79%)</td>
<td>30.75±0.88b (- 28.75%)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (n=8). HFD/STZ group showed a significant increase in renal function markers (BUN, Scr and ALP) in serum of the HFD/STZ group compared with the control group (P < 0.05 HFD/STZ vs. control group). BM and GL treatment significantly ameliorated these parameters in the supplemented groups compared with the HFD/STZ group (P < 0.05 HFD/STZ vs. HFD/STZ + BM or HFD/STZ + GL group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or HFD/STZ group.
**Effects on TNF-α, IL-β and NO levels in the HFD/STZ-induced rat model of diabetes**

Significant ($P < 0.05$) increased in the levels of serum TNF-α, IL-β and NO was observed in HFD/STZ group compared to control group. BM and GL administration in HFD/STZ + BM or HFD/STZ + GL group however decreased significantly ($P < 0.05$) the TNF-α, IL-β and NO levels when compared to HFD/STZ group. Only BM treatment did not show any significant difference in the levels of TNF-α, IL-β and NO when compared to the control rats.

**Effect on oxidative damage in the liver and pancreas**

**Effect of BM on TBARS and MDA contents in the HFD/STZ-induced rat model of diabetes**

Effects of BM on TBARS and MDA contents were measured to demonstrate the end products and rate of LPO in the liver and pancreas of the HFD/STZ group. There were no significant changes in TBARS and MDA contents in control + BM treated group compared with control group. These parameters were significantly ($P < 0.05$) increased in the HFD/STZ group compared with the control group. Levels of TBARS and MDA in the HFD/STZ group decreased significantly ($P < 0.05$) with BM and GL supplementation. (Fig. 14 A and B).

**Effect of BM on PC in the HFD/STZ-induced rat model of diabetes**

PC content did not change by BM supplementation in the control + BM group compared with the control group. PC content was significantly ($P < 0.05$) increased in the HFD/STZ group compared with the control alone. BM and GL supplementation significantly ($P < 0.05$) attenuated PC content in the HFD/STZ + BM and HFD/STZ + GL group respectively, compared with the HFD/STZ group alone (Fig. 15).

**Effect of BM on GSH in the HFD/STZ-induced rat model of diabetes**

Level of GSH did not affect by BM supplementation in the control + BM treated group compared with the control group. However, a significant ($P < 0.05$) depletion in GSH was observed in the HFD/STZ group compared with the control group. BM and GL supplementation significantly ($P < 0.05$) augmented GSH level in the treatment groups compared with the HFD/STZ group (Fig. 16).
Fig 13. HFD/STZ group showed significant increase in TNF-α, IL-1β and NO levels compared with the control group (P < 0.05 HFD/STZ vs. control group). BM and GL supplementation significantly decreased these parameters in the supplemented groups compared with the HFD/STZ induced diabetic group (P < 0.05 HFD/STZ + BM or HFD/STZ + GL vs. HFD/STZ group). Values are expressed as mean ± S.E.M. (n = 8).
Fig 14. (A). HFD/STZ group showed significant increase in TBARS levels compared with the control group ($P < 0.05$ HFD/STZ vs. control group). BM and GL significantly decreased this marker in the supplemented groups when compared with the HFD/STZ-induced diabetic group ($P < 0.05$ HFD/STZ + BM or HFD/STZ + GL vs. HFD/STZ group). (B). The HFD/STZ group showed a significant increase in MDA levels compared to the control group ($P < 0.05$ HFD/STZ vs. control group). BM and GL treatment significantly decreased MDA when compared to the HFD/STZ group ($P < 0.05$ HFD/STZ + BM or HFD/STZ + GL vs. HFD/STZ group). Values are expressed as mean ± S.E.M. (n = 8).
Fig 15. HFD/STZ group showed significant increase in PC content compared with the control group ($P < 0.05$ HFD/STZ vs. control group). BM and GL supplementation significantly decreased PC content when compared with the HFD/STZ induced diabetic group ($P < 0.05$ HFD/STZ + BM or HFD/STZ + GL vs. HFD/STZ group). Values are expressed as mean ± S.E.M. (n = 8).

Fig 16. HFD/STZ group showed significant decrease in GSH content compared with the control group ($P < 0.05$ HFD/STZ vs. control group). BM and GL supplementation significantly increased GSH content in the treatment groups compared with the HFD/STZ induced diabetic group ($P < 0.05$ HFD/STZ + BM or HFD/STZ + GL vs. HFD/STZ group). Values are expressed as mean ± S.E.M. (n = 8).
Chapter-1

Butea monosperma

Effect of BM on GST and CAT activity in the HFD/STZ-induced rat model of diabetes

The activity of GST and CAT in the control + BM group was attenuated but the elevation was not significant when compared to the control group. On the other hand, the activity of GST and CAT was depleted significantly (P < 0.05) in the HFD/STZ group when compared to the control group. The BM and GL has restored the activity of these enzymes in the HFD/STZ + BM or HFD/STZ + GL group significantly (P < 0.05) as compared to the HFD/STZ group (Fig. 17 A and B).

HISTOPATHOLOGICAL FINDINGS

Effect of BM on histopathological changes in the HFD/STZ-induced rat model of diabetes

H and E staining is used to visualize and differentiate between tissue components in normal and pathological conditions (Fig. 18). The histological examination of the H and E-stained control liver tissues showed normal architecture of hepatocytes. Liver section of HFD/STZ-induced diabetic group showed vacuolization of hepatocytes in the centrizonal area with remarkable variation of nuclear size of hepatocytes and dilated sinusoidal spaces. The HFD/STZ + BM group that received BM treatment showed normal hepatic parenchyma. The severity of degenerative changes was lessened by BM supplementation in the HFD/STZ + BM group compared with the HFD/STZ group, indicating a partial protective effect of BM in diabetic animals. Photomicrograph from GL treated group showing regularly arranged liver lobules and portal triad with moderate hyalinization of hepatic arterial wall.

The histological examination of the H and E-stained control pancreatic sections showed normal architecture of islet cells (Fig. 19). Pancreatic section of HFD/STZ-induced diabetic group showed vacuolization and lymphocytic infiltration into the degenerated islet cells and dilated sinusoidal spaces. The severity of degenerative changes was lessened by BM supplementation in the HFD/STZ + BM group compared to the HFD/STZ group. BM supplementation did not show any remarkable effects in the group treated with BM alone compared to the vehicle control group (data not shown). Pancreas of GL treated animal showing a small islet with normal exocrine glands surrounding it. A cluster of lymphocytes on the edge of the islet and numerous beta cells with abundant basophilic cytoplasm in the centre were seen in GL supplemented group.
Fig 17. (A). HFD/STZ group showed significant decrease in GST activity compared with the control group (\(p < 0.05\) HFD/STZ vs. control group), BM and GL supplementation significantly increased enzyme activity in the treatment groups compared with the HFD/STZ induced diabetic group (\(p < 0.05\) HFD/STZ + BM or HFD/STZ + GL vs. HFD/STZ group). (B). The HFD/STZ group showed a significant decrease in CAT activity compared to the control group (\(p < 0.05\) HFD/STZ vs. control group). BM and GL treatment significantly increased enzyme activity in the supplemented groups compared to the HFD/STZ group (\(p < 0.05\) HFD/STZ + BM or HFD/STZ + GL vs. HFD/STZ group). Values are expressed as mean ± S.E.M. (n = 8).
Figure 18. Photograph from the control group at low power -100 x. showing normal liver architecture. PT = Portal Triad, CV = Central Vein, at high power-400 x. showing details with normal portal triad. PV = Portal Vein, BD = Bile Duct, HA = Hepatic Artery. HFD/STZ group at low power- 100 x. showing mild sinusoidal dilatation in the centrizonal area, at high power-400 x. showing vacuolated hepatocytes, variation in nuclear size and mild sinusoidal dilatation in the centrizonal area. BM supplementation in the HFD/STZ + BM group at low power-100 x. showing normal hepatic parenchyma with normal PT and CV, at high power-400 x. showing showing a single vacuolated hepatocytes with the hepatocytes all around are within normal limits. Vac = Vacuoles in hepatocytes. Photograph from the HFD/STZ + GL group at low power -100 x. showing regularly arranged liver lobules. PT=Portal Triad, CV = Central Vein, at high power-400 x. showing a portal triad with moderate hyalinization of Hepatic arterial wall. BD = Bile Duct, HA = Hepatic Artery.
Fig 19. Photograph from the control group at low power -100 x. showing normal appearance of pancreatic tissue with a single islet of langerhans seen in the centre. The islet size is also within the normal range of 200 to 400 microns, at high power-400 x. showing details of cells in the islet. HFD/STZ group at low power-100 x. showing changes in islet of langerhans. The exocrine tissue is within normal limits, at high power-400 x. degenerated islet cells with vacuolization is seen. There is also significant lymphocytic infiltration into the islet and the sinusoidal spaces are dilated. BM supplementation in the HFD/STZ + BM group at low power-100 x. showing pancreatic tissue with a small sized islet of langerhans seen, at high power-400 x. showing the islet cells appear normal. GL in the HFD/STZ + GL group at low power-100 x. showing a small islet with normal exocrine glands surrounding it, at high power-400 x. Islet showing a cluster of lymphocytes on the edge of the islet and numerous beta cells with abundant basophilic cytoplasm in the centre.
DISCUSSION
In the present study we demonstrated that both STZ (type 1 diabetic model) and HFD/STZ (type 2 diabetic model) induced model in rats causes hyperglycemia, hyperlipidemia, altered renal function, inflammatory markers, accompanied by the presence of oxidative damage in liver and pancreas. Moreover, treatment with BM, by virtue of its antioxidant potential significantly ameliorated these alterations and also morphological changes in rat liver and pancreas. The beneficial effects of BM possess a vast ethnomedical history and represent a phytochemical reservoir of heuristic therapeutic value [Burlia and Khadeb, 2007] and exhibit hypoglycemic and high antioxidant potential [Seth et al., 2006; Somani et al., 2006; Bavaria and Narasimhacharya, 2008; Sharma and Garg, 2009; Panda et al., 2009].

Preliminary phytochemical screening of the extract of BM revealed the presence of various bioactive components of which phenolic, flavonoids and saponin were the most prominent (Table 1). These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator [Rice-Evans et al., 1997; Jorgensen et al., 1999]. In this present study the antioxidant activity of the methanolic extract of the flowers of BM was investigated by using reducing power, \( \text{H}_2\text{O}_2 \) scavenging and DPPH scavenging assay of the extract. All the three methods have proven the effectiveness of the methanol extract compared to the reference standard antioxidant ascorbic acid (Fig. 1 a, b & c). The result showed that BM consists of hydrophilic poly phenolic compounds that cause the greater reducing power. The decomposition of \( \text{H}_2\text{O}_2 \) by BM may at least partly result from its antioxidant and free radical scavenging activity. The results obtained in the present study indicate that BM extract exhibited free radical scavenging, reducing power and chelating activity.

Increased generation of free radical-induced oxidative damage to membrane lipids, proteins, carbohydrates, glucose and nucleic acids is regarded an important risk factor in the acceleration of chronic diseases including diabetes [Paolisso and Giugliano, 1996; Feillet-Coudray et al., 1999]. Overproduction of free radicals in diabetes could be due to increases in blood glucose levels [Baynes and Thorpe, 1999]. Moreover, when animals were subjected to OGTT, increased blood glucose level was found increased with time and was maintained until 120 min in diabetic rats (both TIDM and T2DM). The rate of glucose disposal was found to be significantly decreased in diabetic groups. Treatment with BM significantly improved glucose tolerance, as indicated by reduction in peak blood glucose level at 60 and 120 min in treatments (STZ + BM and HFD/STZ + BM) groups during OGTT. From the results obtained, it is evident STZ and HFD/STZ-induced diabetic rats had much higher glucose levels and decreased insulin level than of control rats. Oral administration of BM
extract and GI decreased the blood glucose level in diabetic rats. Anti-hyperglycemic effect of medicinal plant extracts is generally dependent upon the degree of β-cell destruction [Grover et al., 2000]. In the present study, fasting blood glucose level was found to increase in both diabetic groups. Treatment with BM significantly improved glucose level, as indicated by a reduction in blood glucose level in the treatment groups. BM extract may bring about its anti-hyperglycemic effect through insulin secretion from the remnant β-cells and from regenerated β-cells or due to increased peripheral glucose utilization [Somani et al., 2006]. A number of other plants have also been observed to exert hypoglycemic activity through insulin release stimulatory effects [Ravi et al., 2004; Sathishsekhar and Subramanian, 2005]. In diabetes, there is an increased glycosylation of a number of proteins including haemoglobin and β-crystalline of lens. Measurement of HbA1c has proven to be particularly useful in monitoring the effectiveness of therapy in diabetes [Goldstein, 1995]. HbA1c level increased in diabetic groups (STZ and HFD/STZ) when compared to control rats. Agents with antioxidant or free radical scavenging property may inhibit oxidative reactions associated with protein glycation. Administration of BM to STZ + BM and HFD/STZ + BM group reduced the glycosylation of haemoglobin by virtue of its free radical scavenging property and thus decreased the level of HbA1c. A decreased in blood glucose level might also contribute to decrease level of glycated haemoglobin in BM treated group. In our study, the hepatic glycogen content was decreased significantly in the STZ and HFD/STZ group and BM supplementation increased its content in the STZ + BM and HFD/STZ + BM treated group. BM might enhance glucose utilization by peripheral tissues and increase the glycogen stores in the liver due to restoration of delayed insulin response because it significantly decreased the blood glucose level in glucose supplemented rats and was comparable to standard.

In patients with TIDM exocrine pancreatic functions have been a subject of studies in recent years [Hardt et al., 2000; Lernmark, 2000]. Several clinical studies in diabetic patients have shown that the impairment of pancreatic exocrine functions is due to insulin deficiency during TIDM [Taniyama et al., 1999; Hardt et al., 2000; Lernmark, 2000]. Insulin promotes the synthesis of pancreatic amylase and under certain circumstances, has been shown to control mRNA content for this enzyme [Korc et al., 1981]. Qualitatively similar changes have been in our studies, STZ-induced diabetic group exhibited decreased insulin and so forth amylase activity while BM treatment (STZ plus BM group) increased its level thereby consistent with the previous reports.
Dyslipidemia is one of the major cardiovascular risk factors. Diabetic patients have problems in packaging cholesterol and tend to have higher serum TG and FFAs levels. Hyperglycemia itself leads to higher levels of circulating FFAs. An overabundance of FFAs may result in endothelial dysfunction, enhanced coagulation, insulin resistance, increased lipid deposits in various organs, and inflammation, and also affect cholesterol components (Choy et al., 2008). Diabetic patients have problems packaging cholesterol and tend to have higher serum TG levels. Our data were in line with this notion as the STZ models of diabetes exhibited clear-cut abnormalities in lipid metabolism as evidenced from the significant elevation of serum TC, TG, LDL-C, VLDL-C and reduction of HDL-C levels. Treatment with BM and GL for 30 days was sufficient to produce a significant reduction in the TC, TG, LDL-C, VLDL-C and significant increase in HDL-C levels in diabetic rats. These results indicate that BM has a lipid-lowering effect on the diabetic rats. These findings are consistent with the previous report of BM [Somani et al., 2006; Bavaria and Narasimhacharya, 2008; Sharma and Garg, 2009; Panda et al., 2009].

Previous research has reported activity of renal function markers such as Scr and urea is increased in diabetic rats [Eliza et al., 2009; Liu et al., 2008]. Increased urea nitrogen production in diabetes may be accounted for by enhanced catabolism of both liver and plasma proteins [Jorda et al., 1982]. Our work clearly shows increased levels of renal function markers in serum (viz. BUN, Scr, and ALP) in the both diabetic model of rats. In contrast, the STZ + BM and HFD/STZ + BM treated rats showed a significant reduction in these markers, thus showing BM ability to protect against diabetes induced renal damage. GL supplementation group also showed a significant reduction in these markers.

One of the important pathogenetic mechanisms of pancreatic β-cell damage during diabetes is associated with increased expression of proinflammatory cytokines TNF-α, IL-1β, increased expression of the inducible NO synthase (iNOS) gene and increased NO production in the pancreatic islets [Haluzik and Nedvidkova, 2000; Tuck et al., 1993]. There is evidence that the release of TNF-α induced by high glucose in vitro may be mediated by reactive oxygen species and that oxidative stress might be implicated in promoting a low-grade systemic inflammation in diabetes mellitus [Giugliano and Ceriello, 2001; Zhang et al., 2003; Fernandez-Real and Ricart, 2003]. In the present study, NO, IL-1β and TNF-α level increased significantly in the serum of the diabetic group. Treatment with BM and GL significantly ameliorated these parameters.
LPO is often used as an index of oxidative tissue damage which causes free radical damage to membrane components of the cell and resulting cell necrosis and inflammation (Salminen and Vihko, 1983). The increase oxidative stress might have induced the peroxidation of polyunsaturated fatty acids and lead to the formation of TBARS and MDA, as byproducts of LPO. Increased TBARS and MDA production played an important role in the progression of diabetes [Iihan et al., 2001]. Free radicals can also lead to the formation of carbonyl groups which are the end products of protein oxidation. Their levels in tissues serve as relatively stable markers of oxidative damage [Chevion et al., 2000]. In the present study, TBARS, MDA and PC formation increased significantly in the liver and pancreas of diabetic groups. Treatment with BM and GL significantly modulated these parameters.

Hydroxyradicals (H$_2$O$_2$) accumulation can lead to reduction in cleavage of bonds between oxygen atoms leading to the production of hydroxyl radical, which is a very reactive and unstable oxidizing species that reacts instantaneously with any biological molecule. H$_2$O$_2$ can penetrate all biological membranes and can therefore cause cellular damage [Halliwell and Gutteridge, 1984]. The antioxidant system uses GSH, the most abundant non-protein thiol, which buffers free radicals [Meister and Anderson, 1983]. It is well known that GSH is involved in the protection of normal cell structure and function by maintaining the redox homeostasis, quenching free radicals and participating in detoxification reactions. GST, a phase II drug-metabolizing enzymes that plays a significant role in the detoxification of foreign compounds, which catalyze the conjugation of reduced glutathione with a variety of electrophilic compounds [Landi, 2000]. Glutathione can thus directly scavenge free radicals or act as a substrate for GST during the detoxification of H$_2$O$_2$. It has been described that low glutathione levels in diabetes is an indicator of increased oxidative stress [Ugochukwu et al., 2004]. A reduction in the level of GSH may impair H$_2$O$_2$ clearance and promote formation of OH$, the most toxic molecule, leading to more oxidant load. CAT, an antioxidant enzyme, removes H$_2$O$_2$ in the form of oxygen and water. SOD, another antioxidant enzyme, can catalyze dismutation of O$_2$ to H$_2$O$_2$, which is then deactivated to H$_2$O by CAT or GPx. Both antioxidant enzymes (CAT and GPx) scavenges H$_2$O$_2$ and lipid peroxides and terminate oxidative tissue damage reactions. GR regenerates reduced glutathione (GSH) from oxidized glutathione, which has been formed by oxidation and maintained the GSH pool in tissues. These antioxidant enzymes have a complementary catalytic activity leading to reduced TBARS, MDA and PC concentrations in the tissues. Consistent with previous studies [Tana et al., 2005; Kaviarasan and Pugalendi, 2009] in the present study, STZ and HFD/STZ causes cellular damage by generating overproduction of free radicals, which might have caused oxidative damage to membrane lipid and protein and ultimately led to decreased GSH level and antioxidant enzymes (GPx, GR, GST, SOD and CAT) in the liver and pancreas. Following treatment with BM as a powerful antioxidant significantly abrogated all the oxidative alterations liver and pancreas of rats, corroborating
previous studies of BM [Schrawat et al., 2006; Sharma and Garg, 2009]. Antioxidant effects of BM also comparable with the standard diabetic drug.

The beneficial effects of BM in diabetic rats were also confirmed by histopathological observation in liver and pancreas tissues. Sections from liver tissue of the STZ and HFD/STZ group showed vacuolization of hepatocytes in the centrilobular area with significant variation of nuclear size of hepatocytes and dilated sinusoidal spaces. All of these degenerative changes were ameliorated with BM supplementation, indicating a partial protective effect of BM as a potent anti-oxidant. GL treatment brought degenerative changes back to normal level. Following examination of pancreatic sections of STZ and HFD/STZ group, it was noted that degenerated islet cells with vacuolization with lymphocytic infiltration into the islet and dilated sinusoidal spaces. BM supplementation ameliorated the severity of degenerative changes in the STZ + BM and HFD/STZ + BM groups, indicating beneficial effect of BM on pancreas of diabetic animals. An increased number of islets were also observed in the pancreatic sections taken from diabetic rats supplemented with glibenclamide.

It is now widely accepted that the activation of inflammatory mediators such as nuclear factor kappa-B (NF-κB) is the common cause of insulin resistant T2D [Yoon and Jun, 2005; Ortiz et al., 2006]. Furthermore studies have suggested hyperglycemia and oxidative stress is linked to NF-κB activation [Schreck et al., 1992; Mohamed et al., 1999]. Thus, novel strategy that can counteract inflammatory mediators would be useful in the prevention and management of T2D. One possible mechanism of action by which BM show glucose lowering effect is due to presence of its constituent butrin, isobutrin, and butein, which acts through inhibiting activation of NF-κB [Rasheed et al., 2010]. The exact mechanism action by which BM provides protection in diabetes has not been completely known. However, its anti-diabetic and anti-oxidative effects are suggested due to the presence of its active constituents, including sterols, poly-phenols, flavanoids, ascorbic acid and saponins [Wagner et al., 1986; Chokchaisiri et al., 2009]. Preliminary studies have shown that saponins are useful in the treatment of diabetes, phytosterols have lipid lowering effects on hyperlipidemia, and poly-phenols and flavanoids have potential anti-oxidant properties [George et al., 2002; Augustin et al., 2005].

In the present study, it is concluded that BM acts through its anti-oxidant potential. Thus, our data suggest that BM is a potent anti-diabetic agent and beneficial in the control of diabetes related abnormalities in metabolic markers, renal function, inflammatory markers and oxidative damage and histopathological changes in both non-genetic model of DM (T1DM and T2DM). Further studies are needed to determine novel pharmacological approaches and exact mechanistic pathways of BM that can be used for the management of diabetes.