PROTECTIVE EFFECT OF *BUTEA MONOSPERMA* ON HIGH-FAT DIET AND STREPTOZOTOCIN-INDUCED NON-GENETIC RAT MODEL OF TYPE 2 DIABETES: BIOCHEMICAL AND HISTOLOGICAL EVIDENCES

KEHKASHAN PARVEEN, WASEEM AHMAD SIDDIQUI*

**Department of Biochemistry, Lipid Metabolism Laboratory, Jamia Hamdard (Hamdard University), New Delhi–110062, INDIA
Email: kehkashan.parveen@gmail.com; was.sid@gmail.com**

ABSTRACT

Naturaceutical antioxidants in the diet have antidiabetic potential and prevent oxidative damage associated with diabetic pathogenesis. The present study explores the beneficial effects of *Butea monosperma* (BM) flower extract on high fat diet (HFD) and streptozotocin (STZ)–induced diabetes in rats. Diabetes was induced by feeding HFD for 2 weeks followed by a single injection of STZ (40 mg/kg body weight, intraperitoneally). BM was given orally at a dose of 300 mg/kg for 4 weeks after diabetes induction. At the end of experiment blood was drawn and their pancreas tissues were dissected. The level of fasting blood glucose (FBG), glycated hemoglobin (HbA1c), total cholesterol (TC), triglycerides (TG), free fatty acids (FFAs), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) increased while insulin and high density lipoprotein cholesterol (HDL-C) level decreased in HFD/STZ group, which were augmented by BM. Moreover, BM significantly decreased levels of thiobarbituric reactive substances (TBARS) and protein carbonyl (PC), and increased level of glutathione (GSH) and antioxidant enzymes [glutathione-s-transferase (GST) and catalase (CAT)] in the pancreas of HFD/STZ group. These results were supplemented by histopathological examination in pancreas. Our study reveals that BM, as a powerful antioxidant, prevents diabetic complications and oxidative damage in non-genetic rat model of type 2 diabetes.

Keywords: *Butea monosperma*, Type 2 diabetes mellitus, Hyperglycemia, Oxidative damage, Hyperlipidemia

INTRODUCTION

Oxidative stress is often defined as an imbalance between reactive oxygen species (ROS) and their removal by antioxidants. This imbalance may originate from an over production of ROS or from a reduction in antioxidant defenses[1]. Endogenous antioxidant systems of our body uses reduced glutathione (GSH) and antioxidant enzymes to combat oxidative damage. Over production of ROS can damage all types of molecules including proteins, lipid, carbohydrates and nucleic acids, leading to the common final pathway of cell death[2]. Increasing evidence suggests that oxidative stress is a prominent and early feature in the pathogenesis of diabetes mellitus (DM) due to persistent hyperglycemia[3]. DM, especially Type 2 diabetes mellitus (T2DM) or non-insulin dependent (NIDDM) is the most common form of diabetes. It is associated with a variety of conditions and characterized by hyperglycemia resulting from a defect in insulin secretion or function, or both[4]. It affects millions of people and has become a major medical and social problem worldwide. Despite the role of genetic predisposition, aging, obesity and dietetic/sedentary life style are major risk factors involved in the development of T2DM. It is 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[5]. While the management of diabetes includes diet, exercise, oral hypoglycemic agents and insulin, these do not effectively prevent its associated complications[6]. In modern medicine, there is still no satisfactory effective therapy available to cure diabetes. A safe and effective therapeutic drug that can not only provide symptomatic relief but also can block or reduce the many harmful effects of diabetes–including hyperglycemia, polyuria, polydipsia and weight loss, is urgently needed.   Many indigenous medicinal plants have been shown a significant therapeutic influence on the management of diabetes[7]. *Butea monosperma* (BM) Lam. (family; Fabaceae) commonly called the Palash, is a well-known traditionally used medicinal plant and possesses a number of pharmacotherapeutic effects including antihepatotoxic, antifungal, estrogenic, anti-inflammatory, antistess and anticonceptive[8]. Most studies of BM as a hypoglycemic and hypolipidemic agent have been done with alloxan model of type 1 diabetes[9-11]. Recently, Bavarva and Narasimhacharya[12] showed the antihyperglycemic and antihyperlipidemic effects of BM in neonatal rat model of T2DM. Despite these pharmacotherapeutic properties of BM, the effects of BM on high fat diet (HFD) and streptozotocin (STZ)–induced diabetes have not been explored previously. HFD/STZ in rats provided a non-genetic model for T2DM, characterized by obesity, insulin resistance, dyslipidemia, diabetes-related alterations and eventually atherosclerotic cardiovascular disease along with oxidative stress[13]. This is particularly relevant clinically since the majority of human diabetic victims suffer from T2DM. In the present study, we evaluated the beneficial effects of BM on hyperglycemia, hyperlipidemia and oxidative damage in pancreas of HFD/STZ rats. Our results support the efficacy of BM in reducing glucose level and improving diabetic complications in non-genetic rat model of T2DM.

MATERIALS AND METHODS

Chemicals and reagents

Trichloroacetic acid (TCA), Glutathione reduced (GSH), 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), ethylene diamine tetra acetic acid (EDTA) and 2, 4-dinitrophenyhydrazine (DNPH), streptozotocin (STZ) were purchased from Sigma–Aldrich, Chemicals Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

Animals

Male Wistar rats weighing 160–200g were used in the study. They were kept in the Central Animal House of Jamia Hamdard (Hamdard University) in colony cages at an ambient temperature of $25 \pm 2°C$ and relative humidity of 45–55% with 12 h light/dark cycles. They had free access to standard rodent pelleted diet (Hindustan Lever Ltd., Bombay, India) and water ad libitum, prior to the dietary manipulation. All procedures using animals were reviewed and approved by the Institutional Animal Ethical Committee that is fully accredited by the Committee for Purpose of Control and Supervision on Experiments on Animals (173/CPCSEA, 28th Jan 2000) Chennai, India.

Plant material

Butea monosperma Lam., belonging to the family Fabaceae, holds a reputed position in both Ayurvedic and Unani systems of medicine.

The *B. monosperma* flowers were procured from Saiba industries, Mumbai, INDIA.

Preparation of the active phytochemical constituents of *Butea monosperma*

The powdered flower material was extracted exhaustively in a Soxhlet apparatus. Briefly, 500 g dried powdered plant material was Soxlet extracted with methanol for 20 h. The extract was filtered and then solvent was removed under reduced pressure in rotatory evaporator, to obtain reddish orange powder (yield 25%). The dried extract was dissolved in normal saline and used for experimental work. A phytochemical screening[14] of the powder revealed the presence of flavonoids, phytosterols, glycosides, saponins, phenolics compounds and fat. Tests for tannins, alkaloids and proteins were negative.

Experimental design and development of HFD/STZ model of type 2 diabetes

Thirty two rats were divided into four groups of eight animals each: group I (control group) rats were fed standard diet (12% calories as fat) throughout the experiment; group II (control + BM) rats were fed standard diet throughout the experiment and given BM (300 mg/kg body weight (wt)., orally; in saline) for 4 weeks; group III (HFD/STZ group) rats were fed HFD (40% fat as a percentage of total kcal) for 2 weeks[13] and then injected with STZ (40 mg/kg body wt); intraperitoneally (IP); in citrate buffer; pH 4.5); group IV (HFD/STZ + BM) rats were fed HFD for 2 weeks and then injected with STZ and then supplemented with BM (300 mg/kg body wt., orally) for 4 weeks. The development of hyperglycemia in rats was confirmed by fasting blood glucose (FBG) estimation after 6 days of STZ injection. The animals that maintained FBG higher than 140 mg/dl were considered diabetic and selected for studies. The BM treatment was started after diabetic confirmation and dose was determined from previous studies[10, 12].

Oral glucose tolerance test (OGTT)

Two days before termination of experiment, the OGTT was performed to assess the glucose tolerance. For this purpose, overnight fasted rats were fed orally 2 gm/kg body wt glucose. Blood was collected at 0, 30, 60 and 120 min intervals from orbital sinus for glucose estimation. Animals were not anesthetized for this procedure.

Tissue preparation

At the end of experiment, rats were anesthetized by ether inhalation and blood was collected from the dorsal aorta. Approximately 0.2 ml of whole blood was taken in EDTA containing microtubes from the total blood of each animal and immediately preserved in the refrigerator for subsequent analysis of glycated hemoglobin (HbA1c). Serum was separated by centrifugation at 1200 x g for 10 min and stored at – 80°C before analysis. Rats were then sacrificed and their pancreas was excised immediately and perfused with ice-cold saline. For biochemical estimations, pancreas tissues were homogenized at 4°C with 10 times (w/v) 0.1 M phosphate-buffer (pH 7.4) containing protease inhibitors in a polytron homogenizer (Kinematica A.G.). The homogenate was centrifuged at 800 x *g* for 5 min at 4°C to separate the nuclear debris and was used for estimation of thiobarbituric reactive substances (TBARS). The supernatant was further centrifuged at 10,000 x *g* for 20 min at 4°C to get the post-mitochondrial supernatant (PMS), which was used for various biochemical assays.

Analytical procedures

Measurement of FBG

Fasting blood glucose was measured by glucose oxidase method using a commercial diagnostic kit from Span diagnostic Ltd. Surat, India.

Determination of HbA1c level

HbA1c was assayed by cation-exchange method using a diagnostic kit from Crest Biosystem, Goa, India.

Determination of serum insulin content

Serum insulin content was determined by ELISA kit using rat insulin as standard (Ultra sensitive Rat Insulin ELISA kit, Crystal Chem INC. USA) and was expressed as ng/ml.

Assay for lipid profile

Lipid Profile (total-cholesterol (TC), triglycerides (TG), and high density lipoprotein cholesterol (HDL-C)) were estimated by using enzymatic kits procured from SPAN Diagnostics Ltd. Surat, India. Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were calculated by using Friedewald's equation.

Free fatty acids (FFAs) determination

The method of Falhot et al.,[15] was used for FFAs determination. 50μl serum was added with 1.0 ml phosphate buffer and 6.0 ml chloroform-heptane-methanol (CHM) solution in a test tube. This was shaken vigorously for go set, left to stand for 15 min and then centrifuged (4000 x g, 10 min). The buffer was removed carefully by suction and 5.0 ml of the CHM phase was shaken with 2.0 ml Cu-TEA in 5 min on a Vortex mixer. After centrifugation (4000 x g, 5 min) 3.0 ml of the upper phase was transferred to a test tube containing 0.5 ml DPC solution and mixed carefully. Absorbance was taken after 15 min at 550 nm.

Oxidative damage parameters in pancreas

Assay for TBARS content

The method of Utley et al.,[16] with some modification was used to estimate the rate of LPO. Homogenate (0.25 ml) was pipetted into 15×100 mm test tubes and incubated at 37°C in a metabolic shaker for 1 h. An equal volume of homogenate was pipetted into a centrifuge tube, placed at 0°C and marked at 0 h incubation. After 1 h of incubation, 0.5 ml of 5% (w/v) chilled trichloroacetic acid (TCA), followed by 0.5 ml of 0.67% TBA (w/v) was added to each test tube and centrifuge tube, and centrifuged at 1000 × g for 15 min. Thereafter, the supernatant was transferred to other test tubes and was placed in a boiling water bath for 10 min. The absorbance of pink color produced was measured at 535 nm in a spectrophotometer (Shimadzu-1601, Japan). The TBARS content was calculated by using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nmol of TBARS formed min^{-1} mg^{-1} of protein.

Assay for protein carbonyl (PC)

PC level was measured by the method of Levine et al.,[17]. The PMS (0.5 ml) was treated with an equal volume of 20% TCA for protein precipitation. After centrifugation, the pellet was resuspended in 0.5 ml of 10 mM DNPH in 2 M HCl and vortexed repeatedly at 10 min intervals for 1 h in dark. This mixture was treated with 0.5 ml of 20% TCA. After centrifugation at 10, 000 × g at 4°C for 3 min, the precipitate was extracted three times with 0.5 ml of 10% TCA and dissolved in 2.0 ml of NaOH at 37°C. Absorbance was recorded at 360 nm in a spectrophotometer (Shimadzu-1601, Japan). PC level was expressed as nmol carbonyl mg^{-1} protein, using a molar extinction coefficient of 22×10^4 M^{-1} cm^{-1}.

Assay for GSH

GSH content was determined by the method of Jollow et al.,[18] with slight modification. PMS was mixed with 4.0% sulfosalisylic acid (w/v) in a 1:1 ratio (v/v). The samples were incubated at 4°C for 1 h, and later centrifuged at 1200 × g for 15 min at 4°C. The assay mixture contained 0.1 ml of supernatant, 1.0 mM DTNB, and 0.1M PB (pH 7.4) in a total volume of 1.0 ml. The yellow color that developed was read immediately at 412 nm in a spectrophotometer (Shimadzu-1601, Japan). The GSH content was calculated as nmol GSH mg^{-1} of protein, using a molar extinction coefficient of 13.6×10^3 M^{-1} cm^{-1}.

Assay for glutathione-s-transferase (GST)

The activity of GST was measured by the method of Habig et al.,[19]. The reaction mixture consisted of 1.0 mM GSH, 1.0 mM 1-chloro-2,4-

dinitrobenzene (CDNB), 0.1 M phosphate buffer (pH 7.4) and 0.1 ml of PMS in a total volume of 3.0 ml. The change in absorbance was recorded at 340 nm by using Shimadzu spectrophotometer UV-1601 and enzyme activity was calculated as nmol of CDNB conjugate formed min^{-1} mg^{-1} protein using molar extinction coefficient of 9.6 x 10^3 M^{-1} cm^{-1}.

Assay for catalase (CAT)

CAT activity was assayed by the method of Claiborne[20]. Briefly, the assay mixture consisted of 0.05 M phosphate buffer (pH 7.0), 0.019 M hydrogen peroxide (H_2O_2), and 0.05 ml PMS in a total volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was expressed as nmol H_2O_2 consumed min^{-1} mg^{-1} protein.

Protein content

Protein content was determined by the method of Lowry et al.,[21] using bovine serum albumin (BSA) as a standard.

Histological examinations

For histological examinations, pancreas from different groups was stained with hematoxylin and eosin (H and E). Briefly, at the end of experiment, the rats were anesthetized with ether and perfused transcardially with saline. Pancreas were removed quickly and postfixed in buffered formalin (10%) for 24 h. After completion of fixation, slices (3-4 mm) of these tissues were dehydrated and embedded in paraffin. At least four cross-sections were taken from each tissue in 5-μm thickness and stained with H and E. Following two changes xylene washes of 2 min each tissue sections were mounted with DPX mountant. The slides were observed for histopathological changes and microphotographs were taken using an Olympus BX50 microscope system (Olympus, Japan).

Statistical analysis

Results are expressed as mean ± S.E.M. (n = 8). Statistical analysis of the data was obtained via analysis of variance (ANOVA), followed by Tukey's test. $p < 0.05$ was considered as statistical significant.

RESULTS

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 Figure 1. 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 treatment in the HFD/STZ + BM group showed significant ($p < 0.05$) decrease in blood glucose level at 60 and 120 min when compared to the HFD/STZ group.
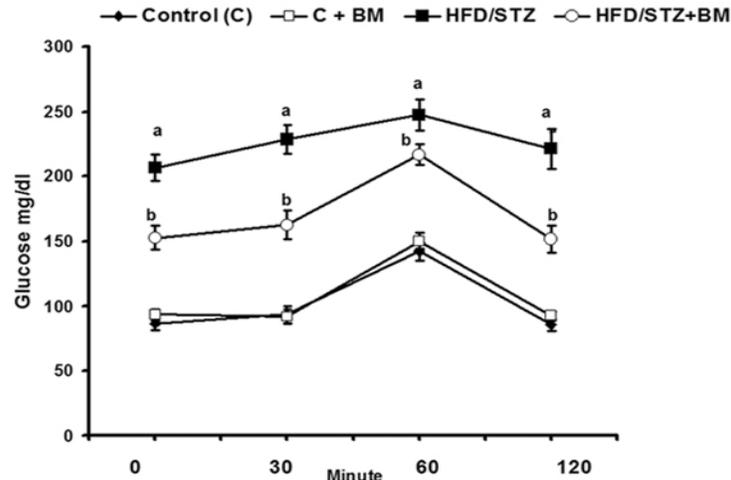
Fig. 1: Effect of BM supplementation on oral glucose tolerance. Values are expressed as mean ± S.E.M. (n = 8). ([a]$p < 0.05$ HFD/STZ vs. control group; [b]$p < 0.05$ HFD/STZ + BM vs. HFD/STZ group).

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 1). Administration of BM significantly ($p < 0.05$) reduced blood glucose level in the HFD/STZ + BM 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. The four-week treatment with BM resulted in significant hypoglycemic effect in the HFD/STZ group.

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 1). BM treatment in the HFD/STZ + BM 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 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 1). 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 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 2). 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. BM treatment did not show any significant changes in lipid profile in the control + BM group compared to the control.

Table 1: Effect of BM supplementation on FBG, HbA1c and insulin

Groups/Parameters	Control (C)	C + BM	HFD/STZ	HFD/STZ + BM
FBG (mg/dl)	106.37±4.5	108.47±4.3 (+ 1.97%)	332.80±5.4[a] (+ 212.87%)	217.85±5.1[b] (- 52.76%)
HbA1c (%)	5.47±0.07	5.62±0.07 (+ 2.74%)	10.94 ±0.08[a] (+ 100%)	8.97 ±0.03[b] (- 18.00%)
Insulin (ng/ml)	3.8±0.15	3.7±0.14 (- 2.63%)	0.59±0.05[a] (- 84.47%)	1.9±0.08[b] (+ 222.03%)

Values are expressed as mean ± S.E.M. (n=8). ([a]$p < 0.05$ HFDSTZ vs. control group, [b]$p < 0.05$ HFD/STZ vs. HFD/STZ + BM group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or HFD/STZ group.

Table 2: Effect of BM supplementation on serum lipid profile and FFAs

Parameters/groups	Control (C)	C + BM	HFD/STZ	HFD/STZ + BM
TC (mg/dl)	130.12±1.13	132.33±1.2 (+ 1.6%)	278.92±2.34[a] (+114.35%)	151.64±2.12[b] (- 45.63%)
TG (mg/dl)	105.27±1.37	106.95 ±1.35 (+ 1.59%)	203.43±1.82[a] (+ 93.23%)	149.56±1.23[b] (- 26.48%)
HDL-C (mg/dl)	49.36±0.91	48.38±0.88 (- 1.98%)	22.88±0.48[a] (-53.64%)	29.88 ±.60[b] (+ 30.59%)
LDL-C (mg/dl)	59.70±0.72	62.54±0.98 (+ 4.76%)	215.35±1.64[a] (+ 260.71%)	91.84±1.10[b] (- 57.35%)
VLDL-C (mg/dl)	21.05 ±0.7	21.39 ±0.8 (+ 1.61%)	40.68±0.56[a] (+ 93.28%)	29.912±0.40[b] (- 26.48%)
FFA (mmol/l)	2.2±0.1	2.1±0.2 (- 4.54%)	3.9±0.3[a] (+ 77.27%)	2.8±0.2[b] (-30.76%)

Values are expressed as mean ± S.E.M. ([a]$P < 0.05$ HFD vs. control group, [b]$P < 0.05$ HFD/STZ vs. HFD/STZ + BM group). Values in parentheses indicate percentage increase (+) or decrease (-) as compared with the control or HFD/STZ group.

Effect on oxidative damage in the pancreas

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

Effect of BM on TBARS contents was measured to demonstrate the end products and rate of LPO in the pancreas of the HFD/STZ group. There were no significant changes in TBARS contents in control + BM treated group compared with control group. This parameter was significantly ($p < 0.05$) increased in the HFD/STZ group compared with the control group. Levels of TBARS in the HFD/STZ group decreased significantly ($p < 0.05$) with BM supplementation in the HFD/STZ + BM group (Figure 2. A).

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 supplementation significantly ($p < 0.05$) attenuated PC content in the HFD/STZ + BM group compared with the HFD/STZ group alone (Figure 2. B).

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 supplementation significantly ($p < 0.05$) augmented GSH level in the HFD/STZ + BM group compared with the HFD/STZ group (Figure 3).

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 has restored the activity of these enzymes in the HFD/STZ + BM group significantly ($p < 0.05$) as compared to the HFD/STZ group (Figure 4).

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

The histological examination of the H and E-stained control pancreatic sections showed normal architecture of islet cells (Figure 5). 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).
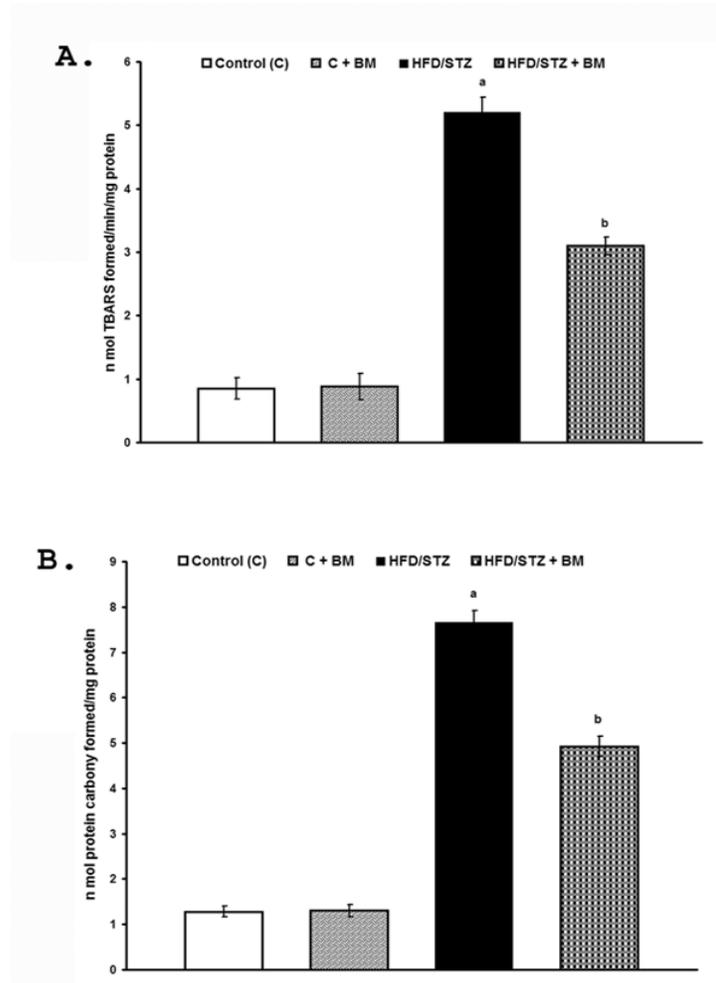
Fig. 2: (A). Effect of BM supplementation on TBARS contents; (B) Effect of BM supplementation on PC content

Values are expressed as mean ± S.E.M. (n = 8). ([a]*p* < 0.05 HFD/STZ *vs.* control group, [b]*p* < 0.05 HFD/STZ + BM *vs.* HFD/STZ group). Values are expressed as mean ± S.E.M. (n = 8). ([a]*p* < 0.05 HFD/STZ *vs.* control group, [b]*p* < 0.05 HFD/STZ + BM *vs.* HFD/STZ group).
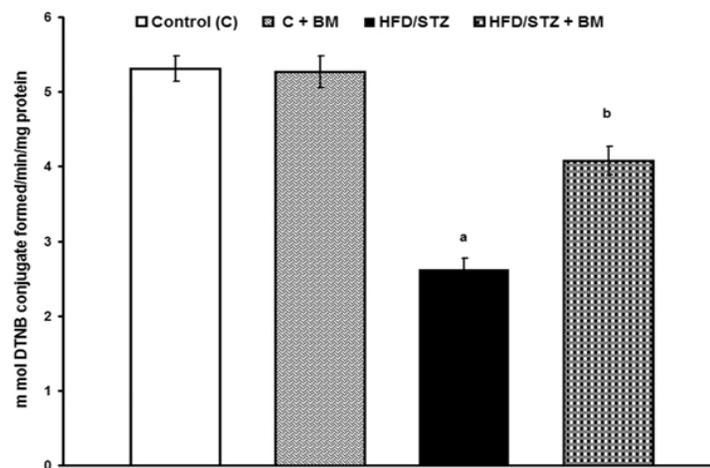
Fig. 3: Effect of BM supplementation on GSH level.

Values are expressed as mean ± S.E.M. (n = 8). ([a]*p* < 0.05 HFD/STZ *vs.* control group, [b]*p* < 0.05 HFD/STZ + BM *vs.* HFD/STZ group)
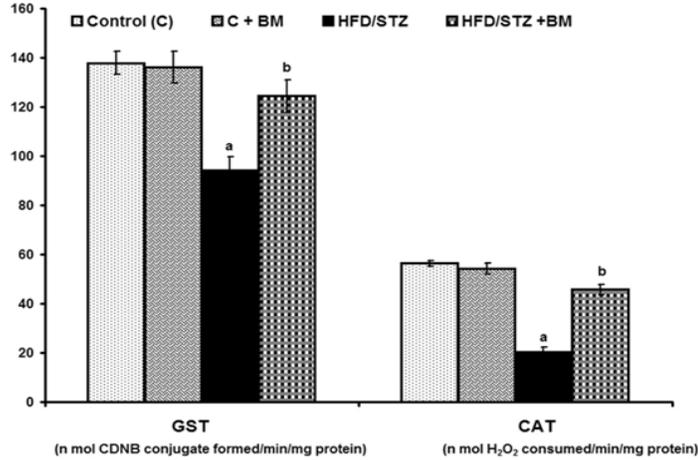
Fig. 4: Effect of BM supplementation on GST and CAT activity

Values are expressed as mean ± S.E.M. (n = 8). ([a]$p < 0.05$ HFD/STZ *vs.* control group, [b]$p < 0.05$ HFD/STZ + BM *vs.* HFD/STZ group).
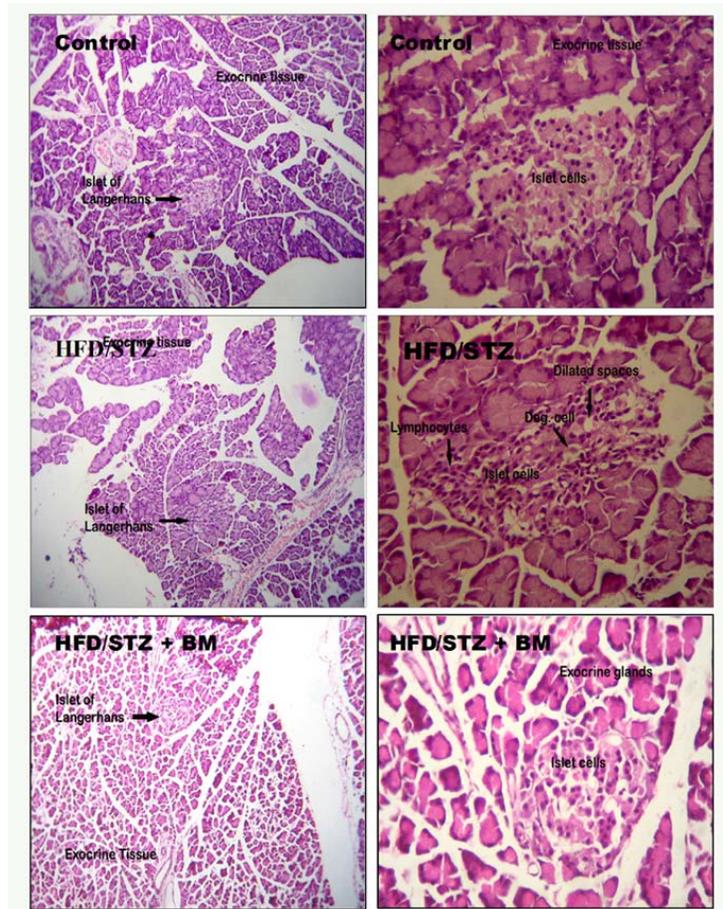
Fig. 5: Effect of BM supplementation on H and E staining in pancreas sections of control and experimental groups.

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, at high power-400x. showing the islet cells appear normal.

DISCUSSION

In the present study we demonstrated that HFD/STZ in rats causes hyperglycemia, hyperlipidemia accompanied by the presence of oxidative damage in the pancreas. Moreover, treatment with BM, by virtue of its antioxidant potential significantly ameliorated HFD/STZ-induced alterations and also morphological changes in rat pancreas. The beneficial effects of BM possess a vast ethnomedical history and represent a phytochemical reservoir of heuristic therapeutic value[8] and exhibit hypoglycemic and high antioxidant potential[9-12, 22].

Preliminary phytochemical screening of the BM flower extract revealed the presence of various bioactive components of which phenolic, flavonoids and saponin were the most prominent. Phenolic and flavonoids compounds have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals[23, 24].

Increased generation of free radicals or impaired antioxidant defenses causes oxidative damage to membrane lipids, proteins, carbohydrates, glucose and nucleic acids. These effects are regarded as an important risk factor in the acceleration of chronic diseases including diabetes[3]. Overproduction of free radicals in diabetes could be due to increase in blood glucose levels[3]. Moreover, when animals were subjected to OGTT, blood glucose level was found to be increased with time and was maintained until 120 min in diabetic rats. The rate of glucose disposal was found to be significantly decreased in HFD/STZ group. Treatment with BM significantly improved glucose tolerance, as indicated by reduction in peak blood glucose level at 60 and 120 min in HFD/STZ + BM group during OGTT. From the results obtained, it is evident HFD/STZ-induced diabetic rats had much higher glucose levels and decreased insulin level than of control rats. Oral administration of BM extract 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[25]. 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[9]. A number of other plants have also been observed to exert hypoglycemic activity through insulin release stimulatory effects[26, 27]. In diabetes, there is an increased glycosylation of a number of proteins including hemoglobin and β-crystalline of lens. Measurement of HbA1c has proven to be particularly useful in monitoring the effectiveness of therapy in diabetes[28]. HbA1c level increased in HFD/STZ group 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 HFD/STZ + BM group reduced the glycosylation of hemoglobin by virtue of its free radical scavenging property and thus decreased the level of HbA1c. Decreased blood glucose level might also contribute to decrease level of glycated hemoglobin in BM treated HFD/STZ group.

It has been demonstrated that insulin deficiency in DM leads to a variety of derangements in metabolic and regulatory processes, which leads to accumulation of TC and TG in diabetic patients[29]. Hyperglycemia itself leads to higher levels of circulating FFAs. An overabundance of FFAs may result in endothelial dysfunction, enhanced coagulation, insulin resistance, increased lipids deposits in various organs and also affect cholesterol components[30]. The normalization of serum lipids may contribute in part due to the beneficial effects of BM on β-cell function and islet morphology observed herein. In the present study, HFD/STZ treated diabetic rats demonstrated 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 for four-weeks was sufficient to significantly reduce TC, TG, LDL-C, VLDL-C and significantly increased HDL-C levels in diabetic rats. These results indicate that BM has hypoglycemic and hypolipidemic effects on the diabetic rats, consistent with the previous reports[9-12].

Hydroxyradicals (H_2O_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_2O_2 can penetrate all biological membranes and can therefore cause cellular damage[1]. The antioxidant system uses GSH, the most abundant non-protein thiol, which buffers free radicals[31]. 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 catalyses the conjugation of reduced glutathione with a variety of electrophilic compounds[32]. Glutathione can thus directly scavenge free radicals or act as a substrate for GST during the detoxification of H_2O_2. A reduction in the level of GSH may impair H_2O_2 clearance and promote formation of OH•, the most toxic molecule, leading to more oxidant load. CAT, another antioxidant enzyme, removes H_2O_2 in the form of oxygen and water. The increase in H_2O_2 might have induced the peroxidation of polyunsaturated fatty acids (lipid peroxidation) and lead to the formation of byproducts of lipid such as TBARS. These oxyradicals can also lead to the formation of protein/protein cross-linkages, oxidation of protein backbone resulting in protein fragmentation and modification of amino acid side chains, which includes oxidation of sulphydryl moieties and formation of PC[33]. Consistent with previous studies,[34, 35] in the present study, HFD/STZ causes cellular damage by generating overproduction of free radicals, which might have caused oxidative damage to membrane lipids and proteins and ultimately led to increased TBARS, PC contents and a decreased GSH level and antioxidant enzymes (GST and CAT) in the pancreas. Following treatment with BM, as a powerful antioxidant, significantly abrogated all the oxidative alterations induced by HFD/STZ in the pancreas of rat, corroborating previous studies of BM[10, 22].

The beneficial effects of BM in diabetic rats were also confirmed by histopathological observation in pancreas tissues. Following examination of pancreatic sections of 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 HFD/STZ + BM group, indicating a partial protective effect of BM in diabetic animals.

It is now widely accepted that the activation of inflammatory mediators such as NF-κB, is the common cause of insulin resistant T2D[36, 37]. Furthermore studies have suggested hyperglycemia and oxidative stress is linked to nuclear factor kappa-B (NF-κB) activation[38, 39]. 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 shows glucose lowering effect is due to presence of its constituent butrin, isobutrin and butein, which acts through inhibiting activation of NF-κB[40].

The exact mechanism of action of the protective effects of BM against HFD/STZ-induced diabetes in rats is not completely known. However, its antidiabetic and antioxidative effects may be due to the presence of its active constituents, viz., sterols, poly-phenols, flavanoids and saponins. These diverse groups of compounds have received much attention as potential natural antioxidants in terms of their ability to act as both efficient free radical scavengers and metal chelator. Preliminary studies have shown that saponins are useful in treatment of diabetes, phytosterols have lipid lowering effects on hyperlipidemia and poly-phenols and flavanoids have potential antioxidant properties[41, 42].

In the present study, it was concluded that the mechanism of action of BM is through its antioxidant potential. Thus, our data suggest that BM is a potent antidiabetic agent and beneficial in the control of diabetes related abnormalities such as hyperglycemia, lipids abnormalities, oxidative damage and histopathological changes in pancreas of HFD/STZ-induced non-genetic rat model of T2DM. Further studies are needed to find out novel pharmacological approaches and exact mechanistic pathways of BM that can be used for the management of diabetes.

ACKNOWLEDGMENT

REFERENCES

1. Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 1984; 219: 1–14.
2. Gutteridge JMC. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995; 41: 1819–1828.
3. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 1999; 48: 1–9.
4. Green J, Feinglos M. Update on type 2 diabetes mellitus: understanding changes in the diabetes treatment paradigm. Int J Clin Pract Suppl 2007; 61: 3-11.
5. Ahmed I, Goldstein B. Diabetes mellitus. Clin Dermatol 2006; 24: 237-246.
6. Plumbo PJ. Glycemic control, mealtime glucose excursions and diabetic complications in type 2 diabetes mellitus. Mayo Clin Proc 2001; 76: 609-618.
7. Modak M, Dixit P, Londhe J, Ghaskadbi S, Paul A, Devasagayam T. Indian herbs and herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr 2007; 40: 163-73.
8. Burlia DA, Khadeb AB. A Comprehensive review on Butea monosperma (Lam.) Kuntze. Pharmacognosy Rev 2007; 1: 333-337.
9. Somani R, Kasture S, Singhai AK, Antidiabetic potential of Butea monosperma in rats. Fitoterapia, 77 (2): 86-90, (2006).
10. Sharmna N, Garg V. Antihyperglycemic and antioxidative potential of hydroalcoholic extract of Butea monosperma Lam flowers in alloxan-induced diabetic mice. Indian J Exp Biol 2009; 47: 571-576.
11. Panda S, Jafri M, Kar A, Mehet BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from Butea monosperma. Fitoterapia 2009; 80: 123-126.
12. Bavarva JH, Narasimhacharya AV. Preliminary study on antihyperglycemic and antihyperlipaemic effects of Butea monosperma in NIDDM rats. Fitoterapia 2008; 79: 328-331.
13. Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM, Reaven GM. A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metabolism 2000; 49: 1390–1394.
14. Kokate CK, Purohit CK, Gokhale SB. Phytochemical tests. In: pharmacognosy, Nirali Prakashan; Pune, India, 1996. p.510–2.
15. Falholt K, Falholt WN, Lund B. An easy colorimetric method for routine determination of free fatty acids in plasma. Clin Chim Acta 1973; 46: 105–111.
16. Utley HC, Bernheim F, Hochslein P. Effect of sulfhydryl reagent on peroxidation in microsome. Arch Biochem Biophys 1967; 118: 521–531.
17. Levine RL, Garland D, Oliver CN et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990; 186: 464-478.
18. Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene-induced liver necrosis: Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 1974; 11: 151-169.
19. Habig WH, Pabst M, Jakoby WB. Glutathion–S-transferase: The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-7139.
20. Claiborne A. Catalase activity. In: Green Wald RA, editors. CRC handbook of methods for oxygen radical research. CRC Press Boca Raton: FL; 1985. p.283-4.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-275.
22. Sehrawat A, Khan TH, Prasad L, Sultana S. Butea monosperma and chemomodulation: protective role against thioacetamide-mediated hepatic alterations in Wistar rats. Phytomedicine 2006; 13: 157-163.
23. Rice-Evans C, Sampson J, Bramley PM, Holloway DE. Why do we expect carotenoids to be antioxidants in vivo. Free Radic Res 1997; 26: 381-398.
24. Jorgensen LV, Madsen HL, Thomsen MK, Dragsted LO, Skibsted LH. Regulation of phenolic antioxidants from phenoxyl radicals: An ESR and electrochemical study of antioxidant hierarchy. Free Radic Res 1999; 30: 207-220.
25. Grover JK, Vats V, Rathi SS. Anti-hyperglycemic effect of Eugenia jambolana and Tinospora cordifolia in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. J Ethnopharmacol 2000; 73: 461–470.
26. Ravi K, Ramachandran B, Subramanian S. Effect of Eugenia jambolana seed kernel on antioxidant defense system in streptozotocin-induced diabetes in rats. Life Sci 2004; 75: 2717–2731.
27. Sathishsekar D, Subramanian S. Beneficial effects of Momordica charantia seeds in the treatment of STZ-induced diabetes in experimental rats. Biol Pharm Bull 2005; 28: 978–983.
28. Goldstein DE. How much do you know about glycated hemoglobin testing. Clin Diabet 1995; 13: 60–63.
29. Goldberg RB. Lipid disorders in diabetes. Diabetes care 1981; 4: 561–572.
30. Choy CK, Rodgers JE, Nappi JM, Haines ST. Type 2 diabetes mellitus and heart failure. Pharmacotherapy 2008; 28: 170–192.
31. Meister A, Anderson ME. Glutathione. Ann Rev Biochem 1983; 52: 711–760.
32. Landi S. Mammalian class theta GST and differential susceptibility to carcinogens: a review. Mutat Res 2000; 463: 247–283.
33. Berlett BS, Stadtman ER, Protein oxidation in ageing, disease and oxidative stress. J Biol Chem 1997; 272: 20313–20316.
34. Tana BKH, Tanb CH, Pushparaj PN. Anti–diabetic activity of the semi–purified fractions of Averrhoa bilimbi in high fat diet fed–streptozotocin–induced diabetic rats. Life Sci 2005; 76: 2827–239.
35. Kaviarasan K, Pugalendi KV. Influence of flavonoid-rich fraction from Spermacoce hispida seed on PPAR-alpha gene expression, antioxidant redox status, protein metabolism and marker enzymes in high-fat-diet fed STZ diabetic rats. J Basic Clin Physiol Pharmacol 2009; 20: 141-158.
36. Yoon JW, Jun HS. Autoimmune destruction of pancreatic beta-cells. Am J Ther 2005; 12: 580-591.
37. Ortis F, Cardozo AK, Crispim D, Storling J, Mandrup-Poulsen T, Eizirik DL. Cytokine-induced proapoptotic gene expression in insulin-producing cells is related to rapid, sustained, and nonoscillatory nuclear factor-kappaB activation. Mol Endocrinol 2006; 20: 1867-79.
38. Schreck S, Albermann K, Baeuerle PA. Nuclear factor kappa B: an oxidative stress responsive transcription factor of eukaryotic cells. Free Radic Res Commun 1992; 17: 221–237.
39. Mohamed AK, Bierhaus A, Schiekofer S, Tritschler H, Ziegler R, Nawroth PP. The role of oxidative stress and NF-B activation in late diabetic complications. Biofactors 1999; 10 (2-3): 157–167.
40. Rasheed Z, Akhtar N, Khan A, Khan KA, Haqqi TM. Butrin, isobutrin, and butein from medicinal plant Butea monosperma selectively inhibit nuclear factor-kappaB in activated human mast cells: suppression of tumor necrosis factor-alpha, interleukin (IL)-6, and IL-8. J Pharmacol Exp Ther 2010; 333: 354-63.
41. George F, Zohar K, Harinder PS, Makkar Klaus B. The biological action of saponins in animal systems: a review". Brit J Nutr 2002; 88: 587-605.
42. Augustin S, Ian TJ, Mike S. Polyphenols: antioxidants and beyond. Am J Clin Nutr 2005; 81: 215S-217S.