

Antihyperglycemic and antioxidant effect of hydroethanolic extract of *Butea monosperma* bark in diabetic mice

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The antihyperglycemic, antihyperlipidemic and antioxidative properties of hydroethanolic extract of *Butea monosperma* bark were investigated in alloxan-induced diabetic mice. Alloxan administration resulted in higher blood glucose level and reduced hepatic glycogen content as compared to normal animals. Besides, serum lipid profile parameters such as total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol were also found to be significantly elevated, whereas the level of high density lipoprotein (HDL) cholesterol was markedly reduced in diabetic animals. Oxidative damage in the tissues of diabetic mice was evidenced by a marked increase in the level of thiobarbituric acid reactive substances (TBARS), distinct decrease in reduced glutathione (GSH) content and declined activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). The daily treatment of diabetic animals with crude extract of *B. monosperma* bark (300 mg kg⁻¹) for 45 days significantly lowered blood glucose level and elevated hepatic glycogen content, bringing the values close to those observed in normal control and glibenclamide-treated diabetic mice. Furthermore, the level of various lipid profile parameters was also reversed towards normal. TBARS and GSH also restored towards normal and the declined activity of antioxidant enzymes in diabetic animals was also normalized in crude extract administered mice, thus indicating the antioxidant efficacy of the drug in diabetes-induced oxidative damage. Significant antihyperglycemic and antioxidant potential of the crude extract of *B. monosperma* bark indicated that it may find use in the management of diabetes and resultant oxidative stress

Keywords: Antidiabetic, Antioxidant, *Butea monosperma*, Diabetes mellitus, Oxidative stress

Diabetes mellitus (DM) refers to a group of diseases that lead to high blood glucose levels due to defects in either insulin secretion (type 1) or insulin action (type 2). Besides hyperglycemia, oxidative stress is also reported to be increased in patients with DM, which plays a major role in the pathogenesis of both types of DM. Oxygen free radicals (OFRs) are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycosylated proteins. Abnormally high levels of free radicals and simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance that in turn can promote the development of complications of DM². In modern medicine, the beneficial effects of synthetic drugs on glycemic levels are well documented,

however, these are associated with one or other side effects. Moreover, synthetic antioxidants are suspected to be carcinogenic and hence are no longer used³. In recent years, popularity of complementary medicine has increased because of their efficacy, lesser side effects and low cost⁴.

Butea monosperma Lam (Leguminosae), known as 'Flame of the Forest' in English and 'Palash' in Hindi has been reported to possess various medicinal properties and the potential to treat a number of ailments such as gout, leprosy and other ailments where the free radicals have been reported to be the major factor contributing to the disorders. Its flowers have also been used as aphrodisiac, antidiabetic, expectorant, tonic, emmenagogue, diuretic and in biliousness³. Antihyperglycemic and antioxidant properties of leaves and flowers have also been investigated in our earlier studies^{5,6}. However, the combined antihyperglycemic, antihyperlipidemic and antioxidative properties of crude ethanolic extract of *B. monosperma* bark have not been investigated so far. In the present study, we have evaluated the effect of 45 days treatment of crude extract of

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B. monosperma bark (BE) on diabetic hyperglycemia and resultant oxidative stress in mice.

Material and Methods

Preparation of extract

Butea monosperma bark was collected from Sanjay Van, Newai (Rajasthan, India) and was taxonomically identified. Dried bark was powdered, soxhlet extracted with 50% aqueous ethanol and vacuum concentrated to dryness under reduced pressure at $60 \pm 1^\circ\text{C}$. After drying in hot air oven ($40\text{--}45^\circ\text{C}$), it was stored in an air-tight container in refrigerator below 10°C . The suspension of ethanolic extract (BE) prepared in 20% tween-20 in normal saline was used for each day of the experiment.

Chemicals

Alloxan monohydrate was purchased from SD Fine Chemicals (Mumbai, India). All other chemicals used for the study were of analytical grade and obtained from HIMEDIA, SRL, CDH and Qualigens.

Experimental animals and treatment

Healthy male Swiss albino mice (*Mus musculus*) procured from C. C. S. Haryana Agricultural University (Hissar, Haryana) were housed under standard laboratory conditions of light, temperature ($23 \pm 2^\circ\text{C}$) and relative humidity ($55 \pm 5\%$). The animals were given standard rat pellet feed (Hindustan Liver Ltd.) and tap water *ad libitum*. After one week of acclimatization, mice were randomly divided into 4 experimental groups viz. group I (normal control, NC), II (alloxan-treated diabetic control, DC), III (alloxan-induced diabetic + BE-treated, 300 mg/kg body wt, BE) and IV (alloxan-induced diabetic + glibenclamide-treated, 10 mg/kg body weight, GT) each containing 7 mice ($n = 7$). After over-night fasting, mice of groups II, III and IV were made diabetic by a single intraperitoneal injection of alloxan monohydrate with a dose of 150 mg/kg body weight⁷. One week after alloxan injection, the fasting blood glucose (FBG) concentration was determined by means of One Touch Ultra Glucometer (Johnson & Johnson Co., USA)⁸. Animals with FBG level greater than 140 mg dl^{-1} were considered as diabetic and selected for further study⁹. Maintenance and treatment of animals was done in accordance with the principles of Institutional Animal Ethics Committee constituted as per the directions of the Committee for the Purpose of

Control and Supervision of Experiments on Animals (CPCSEA).

The crude extract and standard antihyperglycemic drug glibenclamide was fed orally for 45 days. Mice of groups I and II received only 20% tween-20 solution orally once a day for 45 days. The experiment was terminated in overnight fasted mice at the end of 45 days.

Biochemical estimations

In blood and serum

Blood samples were obtained from tail tip vein of all experimental animals and FBG concentration was determined at regular time intervals i.e. before alloxan injection, after developing diabetes (0 day) and after 45 days treatment with bark extract.

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

$$\text{VLDL cholesterol} = \frac{\text{Serum triglyceride}}{5}$$

$$\text{LDL cholesterol} = \text{Serum total cholesterol} - \text{VLDL cholesterol} - \text{HDL cholesterol}$$

Results were expressed in mg dl^{-1} . Serum was also used for estimating total protein content¹⁰ and albumin concentration¹¹.

In tissues

Hepatic glycogen was measured according to the anthrone- H_2SO_4 method with glucose as standard¹². Tissue homogenate supernatant of experimental mice was used for studying the antioxidant properties of BE. For this, liver, pancreas and kidney were removed and weighed. Tissues were then homogenized in 0.2 M tris-HCl. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C and the supernatant so obtained was used for estimation of total protein¹⁰, superoxide dismutase (SOD)¹³, catalase (CAT)¹⁴, glutathione peroxidase (GSH-Px)¹⁵, reduced

glutathione (GSH)¹⁶ and lipid peroxidation byproducts (TBARS)¹⁷.

Histopathological examination

Liver, pancreas and kidney of BE-treated groups were studied for histopathological changes. The changes were compared with normal control, diabetic control and glibenclamide-treated groups. For this, a part of liver, pancreas and kidney of sacrificed animals was fixed in 10% formalin. The organs were processed in graded series of alcohol and embedded in paraffin wax. Serial sections of 5 µm were cut using a microtome, mounted on glass slides, stained with hematoxylin-eosin and photomicrographed.

Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis was performed using One-way analysis of variance (ANOVA), followed by Tukey's post-hoc multiple comparison test using SPSS (version 16.0). The values of $P < 0.05$ were considered as statistically significant.

Results

Effect on body weight

Subsequent to alloxan administration, animals of diabetic control group 'II' revealed a significant ($P < 0.05$) loss (29.6%) in body weight, which was persistently observed till the end of the study period (Table 1). In group III, the initial body wt (29.0 ± 1.9 g) which was reduced to 25.6 ± 2.5 g (11.7%) after alloxan administration was regained to 31.6 ± 2.9 g (19% increase) after 45 days of BE-treatment. The increase in body weight subsequent to alloxan administration was comparable to that observed in group IV i.e. glibenclamide-treated group (group IV) where the body wt that was found to

be reduced by 22.3% after alloxan administration was recovered by 32.3% after 45 days of treatment.

Effect on FBG level

In control group I, blood glucose concentration did not change throughout the study. However, alloxan administration in groups II, III and IV produced a significant increase in blood glucose level as compared to normal control group (Table 1). Treatment of group III with BE produced a significant ($P < 0.05$) fall i.e. 43.0% in the FBG level of diabetic mice, which was comparable to glibenclamide, which produced 49.3% reduction in FBG level in group IV after 45 days of treatment.

Effect on liver glycogen content

Hepatic glycogen content decreased significantly ($P < 0.05$) by 55.8% in diabetic control (group II) as compared to normal control (group I). BE treatment significantly ($P < 0.05$) increased the depleted glycogen content by 77.7% as compared to diabetic control, which was comparable to glibenclamide (group IV, 82.6% increase) (Table 2).

Effect on serum lipid profile

Compared to the level in normal mice (group I), in untreated diabetic mice (group II), a marked increase was observed in the level of serum TC, TG, LDL and VLDL cholesterol, whereas the level of HDL-cholesterol was significantly ($P < 0.05$) depleted. BE treatment for 45 days caused a significant ($P < 0.05$) reduction in the level of TC and LDL-cholesterol and a simultaneous increase in the level of HDL-cholesterol, as compared to diabetic control mice (Table 2).

Other biochemical parameters

A significant ($P < 0.05$) drop (22.7%) in serum albumin content (from 4.4 ± 0.5 to 3.4 ± 0.4 mg dl⁻¹)

Table 1—Effect of 45 days treatment of BE on body weight and FBG level in alloxan-induced diabetic mice

[Values represent mean ± SEM of 7 observations. Values are significant at $P < 0.05$]

Parameters	Day	Normal control	Diabetic control	Crude extract-treated	Glibenclamide-treated
Body weight (g)	Before diabetes	27.4 ± 2.1	29.7 ± 2.1	29.0 ± 1.9	22.4 ± 1.8
	0	27.6 ± 1.8 ^b	18.4 ± 1.8 ^a	25.6 ± 2.5 ^a	17.4 ± 1.8 ^a
	45	27.9 ± 1.9 ^b	20.9 ± 1.6 ^a	31.6 ± 2.9 ^{b,c}	25.7 ± 1.5 ^{a,c}
FBG Level (mg/dl)	Before diabetes	79.6 ± 7.7	72.7 ± 7.4	96.9 ± 8.9	96.1 ± 9.4
	0	79.6 ± 7.7 ^b	225.7 ± 22.2 ^a	277.0 ± 18.1 ^a	360.6 ± 22.3 ^a
	45	80.7 ± 8.3 ^b	232.9 ± 17.0 ^a	157.9 ± 11.3 ^{a,c}	182.7 ± 14.5 ^{a,c}

^aSignificant ($P < 0.05$) difference, ^bInsignificant difference ($P > 0.05$) compared to basal values;

^cSignificant ($P < 0.05$) difference compared to values obtained after alloxan injection.

Table 2—Effect of 45 days treatment of BE on serum lipid profile parameters and albumin content in alloxan-induced diabetic mice

[Values represent mean \pm SEM of 7 observations. Values are significant at $P < 0.05$]

Parameters (mg/dl)	Normal control	Diabetic control	Crude extract-treated	Glibenclamide-treated
TC	84.9 \pm 2.9	214.7 \pm 3.7 ^a	150.8 \pm 11.6 ^{b,c}	75.0 \pm 3.9 ^b
TG	72.8 \pm 3.1	158.3 \pm 4.4 ^a	160.0 \pm 15.6 ^c	58.1 \pm 1.4 ^b
HDL	26.9 \pm 3.5	15.9 \pm 0.9 ^a	66.6 \pm 3.3 ^{b,c}	46.4 \pm 3.8 ^b
LDL	43.5 \pm 3.6	167.1 \pm 3.3 ^a	52.2 \pm 5.9 ^{b,c}	16.9 \pm 2.4 ^b
VLDL	14.6 \pm 0.6	31.7 \pm 0.9 ^a	32.0 \pm 2.1 ^c	11.6 \pm 1.2 ^b
Albumin	4.4 \pm 0.5	3.4 \pm 0.4 ^a	3.8 \pm 0.5 ^c	4.8 \pm 0.4 ^b

^aSignificant ($P < 0.05$) difference compared to Normal control; ^bSignificant ($P < 0.05$) difference compared to Diabetic control; ^cSignificant ($P < 0.05$) difference compared to Glibenclamide-treated.

Table 3—Effect of 45 days treatment of BE on other biochemical parameters in alloxan-induced diabetic mice

[Data represent mean \pm SEM of 7 observations. Values are significant at $P < 0.05$]

Parameters	Normal control	Diabetic control	Crude extract-treated	Glibenclamide-treated
Hepatic				
GSH ¹	6.1 \pm 0.6	3.9 \pm 0.2 ^a	10.4 \pm 0.4 ^{b,c}	12.3 \pm 1.0 ^b
TBARS ²	43.9 \pm 6.3	1425 \pm 122 ^a	317.5 \pm 27.3 ^{b,c}	1001 \pm 85.6 ^b
SOD ⁵	308.6 \pm 9.2	238.1 \pm 22.3 ^a	178.8 \pm 13.7 ^{b,c}	192.2 \pm 6.2 ^b
CAT ³	233.0 \pm 12.7	176.7 \pm 19.9 ^a	693.7 \pm 29.5 ^{b,c}	197.6 \pm 12.0 ^b
GSH-Px ⁴	689.5 \pm 27	453.1 \pm 20.7 ^a	473.7 \pm 22.9 ^{b,c}	287.5 \pm 27.9 ^b
Glycogen ⁶	4.3 \pm 0.4	1.9 \pm 0.2 ^a	8.5 \pm 0.8 ^{b,c}	10.9 \pm 1.0 ^b
Pancreatic				
GSH ¹	5.6 \pm 0.7	3.9 \pm 0.1 ^a	11.0 \pm 0.5 ^{b,c}	12.2 \pm 0.3 ^b
TBARS ²	9.6 \pm 1.3	419.6 \pm 25.8 ^a	72.2 \pm 10.3 ^{b,c}	345.6 \pm 30.5 ^b
SOD ⁵	321.7 \pm 20.4	317.5 \pm 19.6 ^a	307.6 \pm 26.3 ^{b,c}	358.0 \pm 46.8 ^b
CAT ³	266.6 \pm 18.4	167.5 \pm 6.7 ^a	369.3 \pm 24.7 ^{b,c}	277.1 \pm 15.6 ^b
GSH-Px ⁴	721.0 \pm 71.7	481.7 \pm 20.9 ^a	638.3 \pm 55.1 ^{b,c}	534.1 \pm 19.1 ^b
Renal				
GSH ¹	5.9 \pm 0.7	4.1 \pm 0.2 ^a	10.9 \pm 0.5 ^{b,c}	12.3 \pm 0.3 ^b
TBARS ²	110.1 \pm 4.9	964 \pm 119 ^a	603.5 \pm 30.6 ^{b,c}	638.8 \pm 67.7 ^b
SOD ⁵	333.1 \pm 29.2	299.6 \pm 28.8 ^a	213.4 \pm 19.2 ^{b,c}	294.6 \pm 25.2 ^b
CAT ³	272.1 \pm 21.5	161.9 \pm 10.6 ^a	460.2 \pm 20.9 ^{b,c}	245.8 \pm 16.4 ^b
GSH-Px ⁴	723.2 \pm 57.2	377.7 \pm 42.9 ^a	432.9 \pm 31.3 ^{b,c}	420.2 \pm 16.6

¹mg/g tissue, ²nM TBARS/mg protein, ³μmoles H₂O₂ decomposed/min/mg protein, ⁴μg GSH consumed/min/mg protein, ⁵Units/min/mg protein, ⁶mg/g tissue

^aSignificant ($P < 0.05$) difference, ^ainsignificant ($P > 0.05$) difference compared to Normal control; ^bsignificant ($P < 0.05$) difference, ^binsignificant ($P > 0.05$) difference compared to Diabetic control; ^csignificant ($P < 0.05$) difference, ^cinsignificant ($P > 0.05$) difference compared to Glibenclamide-treated.

was observed in diabetic control (group II), in comparison to normal control. However, 45 days treatment of glibenclamide in group III produced a significant ($P < 0.05$) increment in albumin level by 29.2%. Unlike glibenclamide, BE alleviated the albumin content insignificantly ($P > 0.05$) i.e. only by 10%, as compared to diabetic animals (Table 2).

The level of reduced GSH in hepatic, pancreatic and renal tissues of diabetic mice (group II) was significantly ($P < 0.05$) lowered as compared to normal control (group I, Table 3). Administration of BE for 45 days significantly ($P < 0.05$) increased the GSH content in all the three tissues, similar to glibenclamide in group IV ($P < 0.05$).

TBARS level also significantly ($P < 0.05$) increased in hepatic, pancreatic and renal tissues of diabetic mice. Glibenclamide treatment for 45 days diminished the TBARS levels in group IV. BE treatment for 45 days caused a marked reduction in the level of TBARS in group III (Table 3).

Alloxan administration resulted in markedly diminished activity of SOD in liver, but the decrease was statistically insignificant ($P > 0.05$) in pancreas and kidney. Treatment with BE 45 days did not improve the activity of SOD in any of the tissues. CAT activity was significantly ($P < 0.05$) decreased in liver, pancreas and kidney of diabetic animals (group II), as compared to normal mice (group I). Diabetic mice treated with BE (group III) showed significantly ($P < 0.05$) increased CAT activity and the increase was even greater than that observed in glibenclamide-

treated animals (group IV). GSH-Px activity was also significantly ($P < 0.05$) depleted in diabetic animals as compared to normal ones; however, it was significantly ($P < 0.05$) improved in pancreas of BE-treated group. Although insignificantly ($P > 0.05$), the GSH-Px activity was also increased in the liver and kidney of this group. The effect of glibenclamide was also insignificant ($P > 0.05$) in improving the GSH-Px activity in all the studied tissues (Table 3).

Histopathological examination

Autopsy revealed remarkable damage in the liver, pancreas and kidney of persistently diabetic animals. However, the photomicrograph of vehicle-treated mice of normal control (group I) showed normal histological details of these tissues (Fig. 1a, e and i). Liver of diabetic mice (group II) revealed dilated

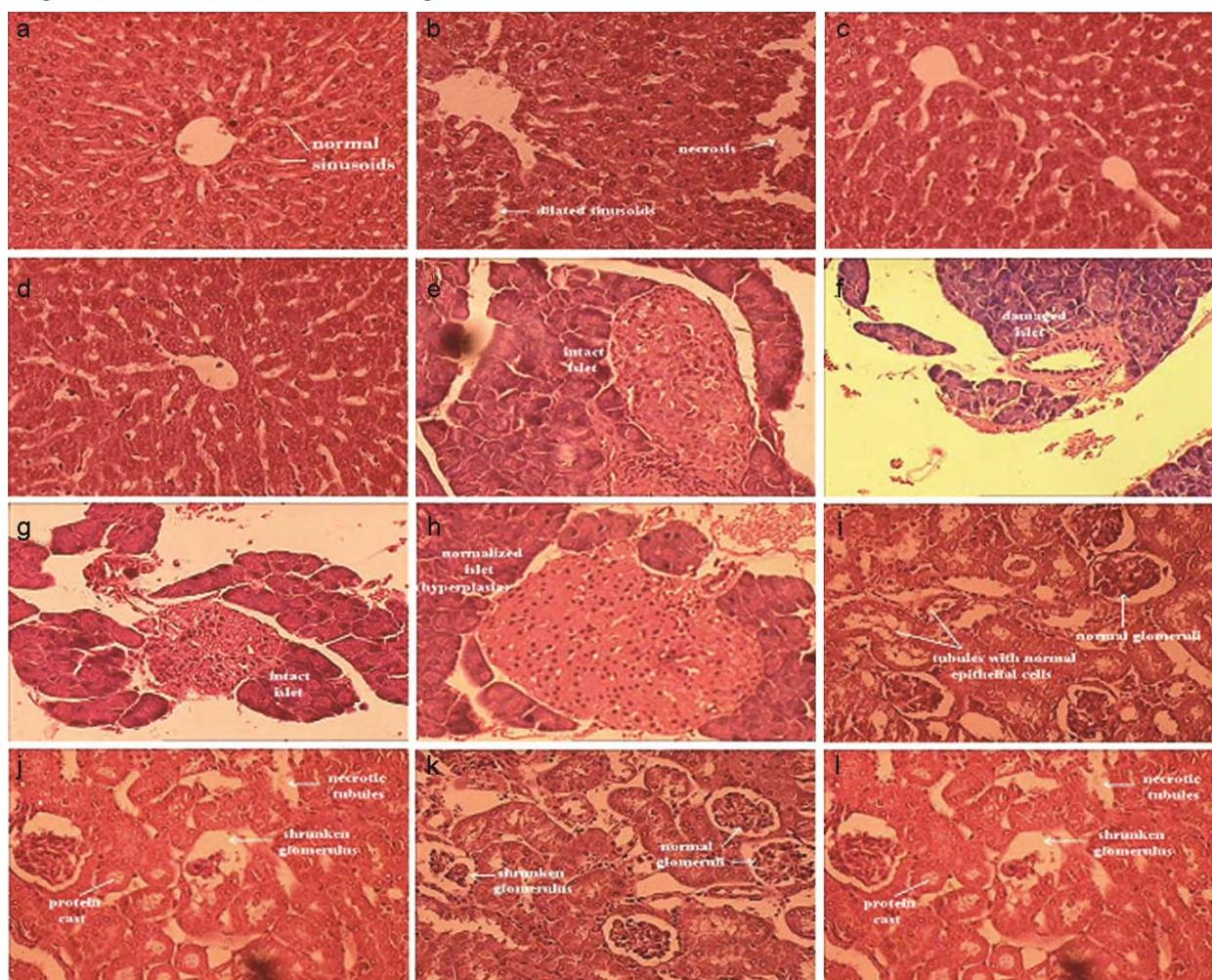


Fig. 1—Photomicrographs showing histological details of liver, pancreas and kidney (H & E, 40X) [a. Normal liver; b. diabetic liver (showing atrophic hepatocytes and necrosis); c. BE-treated liver; d. glibenclamide-treated liver; e. normal pancreas; f. diabetic pancreas (showing damaged islets); g. BE-treated pancreas; h. glibenclamide pancreas; i. normal kidney; j. diabetic kidney (showing protein cast and shrunken glomeruli); k. BE-treated kidney; and l. glibenclamide-treated kidney]

sinusoids, atrophic hepatocytes and some necrotic regions (Fig. 1b). However, the above deformities were not witnessed in BE-treated (group III, Plate 1c) and glibenclamide-treated (group IV, Fig. 1d) animals. The alleviating response might be due to protective effect of the provided treatments.

Histopathological details of pancreas of alloxan-administered diabetic mice showed damaged islets. Atrophic islets of langerhans were the most striking feature in these animals (Fig. 1f). No discernible islets were found in severely and persistently diabetic animals. However, in BE (Fig. 1g) and glibenclamide-treated (Fig. 1h) mice, the islets were comparable to normal ones, except for few traces of degenerative changes. Besides, rare evidence of hyperplasia marked by increase in the cellular components was also noticed in the glibenclamide-treated group. Thus, histological examination of pancreas revealed that BE administration restored the activity of islets of langerhans of diabetic animals.

Histopathological observation of kidney of diabetic control (Fig. 1j) indicated the presence of protein casts in some of the tubular lamina as compared to animals of normal control (Fig. 1i). Besides, shrinkage of glomeruli was the most striking feature observed in renal tissue of diabetic mice (Fig. 1j). Although, treatment with BE (Fig. 1k) and glibenclamide (Fig. 1l) subsequent to alloxan administration reverted the normal structure of kidney to a certain extent.

Thus, histopathological study of liver, pancreas and kidney revealed that alloxan administration severely deteriorated the histology of these tissues, but BE and glibenclamide treatment to a certain extent restored the detected deformities.

Discussion

Alloxan causes a massive reduction of insulin-producing β -cells of islets of langerhans, thus leading to insulin deficiency and ultimately hyperglycemia¹⁸. The increased blood glucose level in diabetic mice as compared to normal ones might be due to glycogenolysis and/or gluconeogenesis in the former¹⁹. However, treatment of diabetic mice with BE caused a significant reduction (43%) in FBG levels, which might be due to increased peripheral glucose utilization or by potentiating the insulin effect via stimulation of the undamaged or residual pancreatic islets to release insulin. Moreover, glibenclamide also exerts its hypoglycemic effect by

increasing insulin secretion^{20,21}. Furthermore, glycogen content in various tissues is a direct indicator of insulin activity. This is because insulin stimulates glycogen synthetase and inhibits glycogen phosphorylase, which in turn promotes intracellular glycogen deposition. Diminished liver glycogen content in diabetic mice was consistent with the results of earlier study²², indicating the probable cause to be the loss of glycogen synthetase-activating system²³ and/or increased activity of glycogen phosphorylase²². Alloxan causes selective destruction of β -cells, thus leading to insulin deficiency, which in turn is responsible for reduced level of hepatic glycogen, as glycogen synthesis is dependent on insulin for the influx of glucose. Moreover, normalization of reduced hepatic glycogen content subsequent to insulin treatment has also been reported²⁴. Likewise, administration of BE produced a significant increase in glycogen content, thus confirming its insulin potentiating effect. Furthermore, antihyperglycemic potential of BE was comparable with that of glibenclamide and was in agreement with the previous findings^{5,6}. Similarly, body weight loss caused by alloxan was also attenuated in BE-treated animals, reflecting the improved health.

Besides altered carbohydrate metabolism, diabetes is also accompanied by disordered fat and lipid metabolism²⁵. Diabetes-induced dyslipidemia may be due to excess mobilization of fat from adipose tissue because of underutilization of glucose²⁶. BE treatment for 45 days not only decreased the elevated levels of TC and LDL, but also significantly increased the HDL-cholesterol level. These results were consistent with several earlier studies^{23,24,27}. The anti-dyslipidemic action of BE might be due to inhibition of lipid peroxidation, since DM is associated with an increase in lipid peroxides and a decrease in antioxidant enzymes²⁸.

There are many reports indicating alterations in the antioxidant parameters during diabetes-induced oxidative stress²⁹ that make β -cells susceptible to damage by free radicals. Furthermore, diabetogenic action of alloxan also involves the formation of ROS and hence altered levels of antioxidant defence system³⁰.

Non-protein thiols like GSH are among the important primary defences that counteract the oxidative stress by reacting with peroxides and hydroperoxides³¹. In the present study, reduced level

of tissue GSH of diabetic mice was consistent with the earlier report³². Apart from the susceptibility of -SH group of glutathione to the action of alloxan, the observed decrease might also be due to utilization of non-protein thiols by increased OFRs produced in hyperglycemic condition associated with DM. These free radicals convert reduced GSH to its oxidized form²⁹. Furthermore, lipid peroxidation is one of the characteristic features of chronic diabetes³³. In the present study, treatment with BE decreased the elevated levels of TBARS (an index of lipid peroxidation) in tissues of diabetic mice. Simultaneously, GSH content was also increased significantly indicating that BE could either increase the biosynthesis of GSH and/or reduce the oxidative stress that in turn reduces the degradation of GSH. Lipid peroxides in the presence of GSH are converted to alcohol derivatives and not to malondialdehyde (MDA) and hence in the presence of increased availability of GSH (due to BE), the level of TBARS decreased in the test group (III). This conclusion was consistent with an earlier study²².

Higher levels of lipid peroxides along with reduction in antioxidants (as observed in this study) indicated oxidative stress in diabetic animals. BE treatment significantly ameliorated the declined activity of CAT and GSH-Px, which suggests a compensatory response of BE against oxidative stress, as it reduces the endogenous H₂O₂ produced, thus diminishing the toxic effects due to H₂O₂ and other free radicals derived from secondary reactions. The antioxidant activity of BE might be due to the inhibition of glycation of the antioxidant enzymes³⁴.

Histopathological examination of pancreatic sections of diabetic mice (group II), revealed that the islets were less in number and destroyed as compared to normal ones, which might be due to infiltration of lymphocytes. Pancreas from BE and glibenclamide treated group were comparable to those of normal control ones. Similar observations have also been reported in earlier studies^{35,36}. In the liver of alloxan-diabetic mice, granular cytoplasm, dilated sinusoids and inflammation were noticed. However, the observed degenerative changes in the liver of diabetic animals were restored in BE and glibenclamide-treated groups, indicating their beneficial and protective effect on the liver of diabetic mice. Glomerulosclerosis is the most common histological problem in diabetic individuals. However, most of the notable changes (except persistence of few shrunken

glomeruli along with normalized ones) witnessed in kidney sections subsequent to alloxan-administration were normalized in BE and glibenclamide-treated groups. These observations were consistent with the report of Noor *et al*³⁶.

In conclusion, the present study demonstrated that the crude extract of *B. monosperma* bark possess significant antihyperglycemic and antioxidant potential and hence may find use in the management of diabetes and resultant oxidative stress.

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