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Antidiabetic and antioxidant potential of ethanolic extract of *Butea monosperma* leaves in alloxan-induced diabetic mice

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The possible protective effect of ethanolic extract of *B. monosperma* leaves (BMEE) on diabetes and diabetes-induced oxidative stress was evaluated in alloxan (ALXN)-induced diabetic male adult mice. Experimental animals were divided into three groups viz., I, II, and III. Diabetes mellitus (DM) was induced in groups II and III mice by a single intraperitoneal injection of alloxan (150 mg/kg body wt). Group I (control mice) received an equal volume of normal saline. Group III mice were further treated with BMEE (300 mg/kg body wt, p.o.) for a period of 45 days. Body weight and fasting blood glucose (FBG) levels were measured at periodic intervals during the test period. At the end of treatment period, blood was collected by cardiac puncture under mild ether anesthesia and serum was isolated to analyze its lipid profile i.e. serum total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). The homogenates of hepatic, pancreatic and renal tissues were also analyzed for both enzymatic and non-enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS) and total protein (TP). Alloxan injection resulted in a significantly (P<0.05) increased concentration of FBG level. Besides, the levels of enzymatic and non-enzymatic antioxidants were decreased and TBARS level increased significantly (P<0.05) in hepatic, pancreatic and renal tissues. Also, serum TC, TG, LDL and VLDL-cholesterol level elevated significantly (P<0.05), whereas HDL-cholesterol reduced significantly (P<0.05) in group II (alloxan-treated diabetic control). The FBG level decreased significantly (P<0.05) after 45 days treatment of BMEE from 172 to 117.143 mg/dl, as compared to normal control (79.286 mg/dl). The activities of antioxidant enzymes (CAT and GSH-Px) and GSH level in hepatic, pancreatic and renal tissues also increased significantly (P<0.05) in BMEE-treated mice, but the activity of SOD was not improved significantly. BMEE treatment also reduced the TBARS levels and lowered serum lipid profile significantly (P<0.05). The findings of the present study indicated significant hypoglycemic and anti-oxidant activity in *B. monosperma* leaves, thus lends credence to its folklore use in the management and/or control of type-2 DM.

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

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by β-cells of pancreas or by the ineffectiveness of the insulin produced, which leads to hyperglycemia and at later stages lipid metabolism is also affected[1,2]. DM in human and experimental animal models also exhibits high oxidative stress, due to persistent and chronic hyperglycemia[3] and protein glycation[4], which thereby depletes the antioxidant defense system[5] and thus promotes *de novo* free radicals generation[6].

Current therapies used for controlling diabetic complications are associated with several side effects[7]. Moreover, as synthetic antioxidants are suspected to be carcinogenic[8], there is a need for effective, safe and better oral hypoglycemic agents. Although, traditional plant remedies have been used in the treatment of diabetes[9] and to manage resultant oxidative stress since a long time, there is a renewed interest in plant-based drugs in the treatment of diabetes and its complications, due to their efficacy, less side effects and low cost[1]. Although a number of plants have been used in the indigenous system of medicine (Ayurveda) for controlling diabetes, only a few have been evaluated and their active principles have been isolated[10,11].

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Abbreviations: ALXN, alloxan; BMEE, ethanolic extract of *Butea monosperma* leaves; BMT, BMEE-treated group; CAT, catalase; DCNT, diabetic control group; DM, diabetes mellitus; FBG, fasting blood sugar level; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; NCNT, normal control group; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TC, total cholesterol; TG, triglyceride; TP, total protein

Butea monosperma Lam (Leguminosae), commonly known as 'Flame of the forest' in English and 'Palash' in Hindi is distributed in arid and semi-arid regions in greater parts of India, Burma and Sri Lanka and has been used in traditional medicine. The plant possesses antifertilty, antimicrobial, antiinflammatory, hepato-protective, antifungal, anthelmintic, anticonvulsant and antidiarrhoeal properties[12-14]. Its flowers show potent free radical scavenging[8], antidiabetic[15], and significant hepatoprotective and antioestrogenic activity[16]. The seeds, root and bark also find uses in various diseases[17-19]. Anti-implantation activity in seeds[20] and antifungal activity[21] in stem bark is also reported. Methanolic extract of leaves shows strong antimicrobial activity against *Bacillus pumilus*[16].

In the present study, the hypoglycemic, hypo-lipidemic and anti-oxidative potential of ethanolic extract of *B. monosperma* leaves (BMEE) has been evaluated in alloxan-induced diabetic mice.

Material and Methods

Animal care and monitoring

Healthy male Swiss albino mice (*Mus musculus*) were procured from C.C.S. Haryana Agricultural University, Hissar (Haryana, India) and housed under standard laboratory conditions of light (12 h light-dark cycle), temperature (23 ± 2°C) and relative humidity (55 ± 5%). The animals were given standard rat pellet feed (Hindustan Liver Ltd., India) and tap water *ad libitum*. After 1 week of acclimatization, mice were randomly divided into 3 experimental groups viz. Group I (Normal control, NCNT), Group II (Alloxan-treated diabetic control, DCNT) and Group III (Alloxan-induced diabetic + BMEE-treated, BMT), each containing 7 mice. 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).

Plant material and preparation of extract

Leaves of *B. monosperma* were collected from Sanjay Van, Newai (Rajasthan) between April and May 2007 and identified taxonomically. Shade-dried leaves were powdered by using grinder and passed through sieve (no. 80) to obtain powder of uniform particle size. The powder was extracted in Soxhlet apparatus with 50% ethanol and concentrated to dryness under reduced pressure at 60 ± 1°C in a vacuum rotatory evaporator. The extract was dried at 40-45°C in hot-air oven till solid to semi-solid mass was obtained and stored in an airtight container in refrigerator below 10°C. The suspension of BMEE prepared in 20% tween-20 in normal saline was used in each day of the experiment.

Chemicals

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

Assessment of extract on alloxan-induced diabetic mice and biochemical assays

Mice of groups II and III were made diabetic by a single intraperitoneal injection of alloxan monohydrate with a dose of 150 mg/kg body wt in overnight fasted mice[22]. Animals were provided with 5% glucose solution to drink overnight to avoid drug-induced hypoglycemia. Two days after alloxan injection, mice with fasting blood glucose (FBG) level >140 mg/dl were considered diabetic and included for further study[23]. Diabetes was allowed to develop and stabilized in the alloxan-treated animals over a period of 3-5 days. All animals in groups I, II and III were kept and maintained under standard laboratory conditions and allowed free access to food and water *ad libitum*. The test compound (BMEE, 300 mg/kg body wt/day p.o.) was administered to group III mice for 45 days. Body weight was measured at regular intervals during experimental period of 45 days.

Biochemical assays

Blood samples were obtained from tail tip vein of all experimental animals and FBG concentration was determined using One-touch ultra glucometer (Johnson & Johnson Co., USA) and compatible blood glucose strips[24] at regular time intervals i.e., 0* (before alloxan injection), 0, 22nd and 45th day (after alloxan injection in groups II and III) of experiment. FBG level of normal control group (Group I) was also measured simultaneously. Hepatic glycogen was measured according to the anthrone-H_2SO_4 method using glucose as standard[25].

For estimating lipid profile, serum was isolated from the blood collected by cardiac puncture under mild ether anesthesia from overnight fasted mice on day 45 of BMEE treatment. Serum total cholesterol (TC), triglycerides (TG), and HDL-cholesterol were estimated by using respective diagnostic kits

(Erba Mannheim Cholesterol Kit, Transasia Bio-Medicals Ltd; Daman). VLDL and LDL- cholesterol were calculated as per Friedevald's equation:

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

LDL-cholesterol = Serum total cholesterol – VLDL-cholesterol – HDL-cholesterol

Results were expressed in mg/dl.

Tissue homogenate supernatant of experimental mice was used to evaluate antioxidant properties of BMEE. For this, liver, pancreas and kidney were removed, freed from adhering tissues, washed with ice-cold normal saline solution (0.9%) until bleached of all the blood and blotted dry. Weight of all the organs was taken only after drying the tissue. After mincing into small pieces, tissues were homogenized in ten times its volume of 0.2 *M* tris HCl with the help of homogenizer. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C and the supernatant was used for estimation of total protein (TP)[26], superoxide dismutase (SOD)[27], catalase (CAT)[28], glutathione peroxidase (GSH-Px)[29], glutathione (GSH)[30] and lipid peroxidation (TBARS)[31].

Statistical analysis

Results were expressed as mean ± S.E.M. Statistical analysis was performed using One-way-Analysis-of-Variance (ANOVA), followed by Tukey's post-hoc multiple comparison test using SPSS (version 16.0) and Student's 't'-test using SigmaPlot (version 8.0). The values of $P<0.01$ and $P<0.05$ were considered as statistically significant.

Results

Effect on body weight

All animals treated with alloxan in diabetic control group II showed a significant ($P<0.05$) loss in body weight (from 29.714 ± 3.326 g to 20.857 ± 4.518 g) which was persistently observed till the end of the study period i.e. 45 days (Table 1). In group III, the initial body weight was reduced (from 30.413 ± 3.796 g to 24.000 ± 4.243 g) after alloxan treatment and regained to almost its near initial values (i.e. 29.000 ± 3.546 g) after 45 days of BMEE treatment ($P>0.05$).

Effect on fasting blood sugar (FBG) level

Fig. 1 shows that in normal control group (group I), treatment with normal saline alone did not affect the normal blood glucose concentration. Alloxan treatment resulted in a significant ($P<0.05$) increase in blood glucose level in groups II and III from 74.143 ± 7.511 to 225.714 ± 64.236 mg/dl and 87.286 ± 7.478 to 172.000 ± 18.593 mg/dl, respectively. These values were considerably higher than that of group I, in which normal FBG concentration ranged between 78.857 ± 6.916 and 80.714 ± 8.276 mg/dl persistently. The significant ($P<0.05$) reduction of

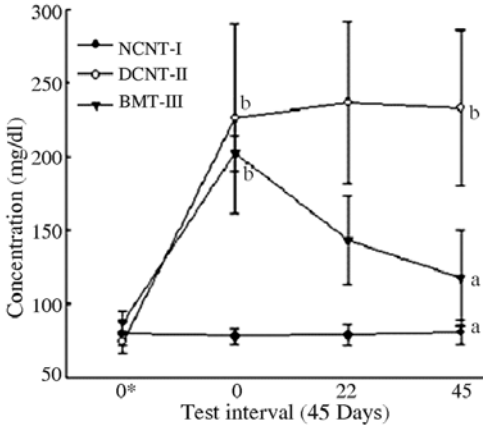

Fig. 1—Effect of 45 days treatment of BMEE on FBG level in alloxan-induced diabetic mice [Values represent mean ± S.E.M. (n = 7 in each group), with standard error indicated by vertical bars. Student's 't'-test was significant at $P<0.05$. [a]Insignificant difference ($P>0.05$) compared with basal values; [b]significant difference ($P<0.05$) compared with basal values. 0* day (before alloxan treatment); 0, 22nd and 45th day (after alloxan treatment). ANOVA between treatments: F = 22.792, $P<0.01$]

Table 1—Effect of ethanolic extract of *B. monosperma* leaves (BMEE) on body weight (g) in alloxan-induced diabetic mice after 45 days of treatment

[Values represent mean ± S.E.M. (n = 7)]

Parameters	Days	Control	ALXN-Treated	ALXN + BMEE-Treated
Body weight (g)	Before diabetes	27.428 ± 3.201	29.714 ± 3.326	30.413 ± 3.796
	0	27.428 ± 3.201[a]	18.429 ± 3.458[b]	24.000 ± 4.243[b]
	45	27.857 ± 3.136[a]	20.857 ± 4.518[b]	29.000 ± 3.546[a]

Student's 't'-test was significant at $P<0.05$. [a]Insignificant difference ($P>0.05$) compared with basal values; [b]Significant difference compared with basal values. ANOVA – Between treatments: F = 4.271; $P<0.05$

FBG concentration (from 172.000 ± 18.593 to 117.143 ± 32.502 mg/dl) in BMEE-treated group after 45 days of treatment indicated anti-diabetic potential of the extract.

Effect on liver glycogen content

Hepatic glycogen content decreased significantly (P<0.05) by 33.58% in diabetic control group (group II) as compared to normal control group (group I), whereas it increased significantly (P<0.05) by 28.99% after 45 days treatment of BMEE (group III) as compared to diabetic group (Fig. 2).

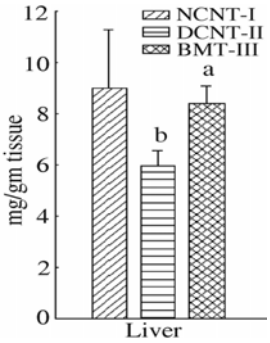

Fig. 2—Effect of 45 days treatment of BMEE on liver glycogen content in alloxan-induced diabetic mice [Values represent mean ± S.E.M. (n = 7 in each group) with standard error indicated by vertical bars. Student's 't'-test was significant at P<0.05. [b]Significant difference (P<0.05) compared with NCNT; [a]significant difference (P<0.05) compared with DCNT. ANOVA between treatments: F = 49.862, P<0.01]

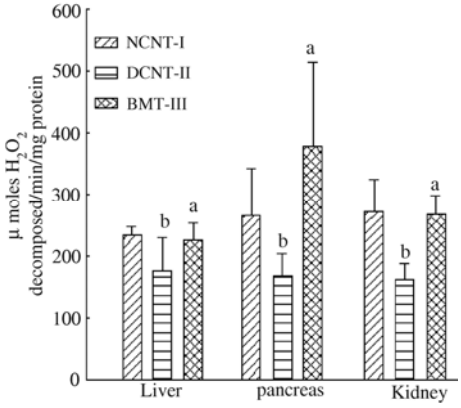

Fig. 4—Effect of 45 days treatment of BMEE on CAT in alloxan-induced diabetic mice [Values represent mean ± S.E.M. (n = 7 in each group) with standard error indicated by vertical bars. Student's 't'-test was significant at P<0.05. [b]Significant difference (P<0.05) compared with NCNT; [a]significant difference (P<0.05) compared with DCNT. ANOVA between treatments: Liver-F = 4.443, P<0.05; Pancreas-F = 4.445, P<0.027; Kidney-F =31.5, P>0.001]

Other biochemical assays

The effect of BMEE on biochemical variables in liver, pancreas and kidney is shown in Figs 3, 4, 5, 6 and 7. Results showed that the hepatic, pancreatic and renal activity of SOD, CAT, GSH-Px and the level of GSH decreased significantly (P<0.05), while TBARS increased significantly (P<0.05) in the alloxan-treated diabetic group (group II). The normal control group (group I) maintained optimal value activity of the antioxidants. BMEE treatment significantly (P<0.05) decreased the TBARS and significantly (P<0.05)

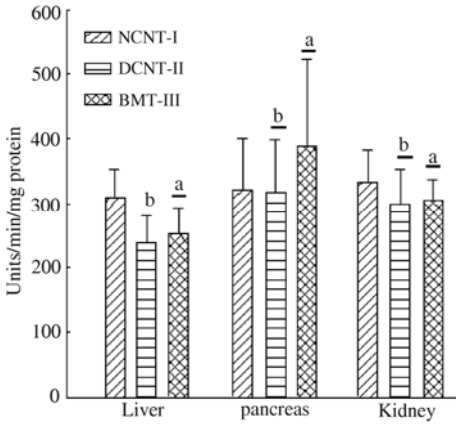

Fig. 3—Effect of 45 days treatment of BMEE on SOD in alloxan-induced diabetic mice [Values represent mean ± S.E.M. (n = 7 in each group) with standard error indicated by vertical bars. Student's 't'-test was significant at P<0.05. [b]Significant difference (P<0.05) and [b]insignificant difference (P>0.05) compared with NCNT; [a]insignificant difference (P>0.05) compared with DCNT. ANOVA between treatments: Liver-F = 0.910, P>0.05; Pancreas-F = 0.907, P>0.05; Kidney-F = 0.158, P>0.05]

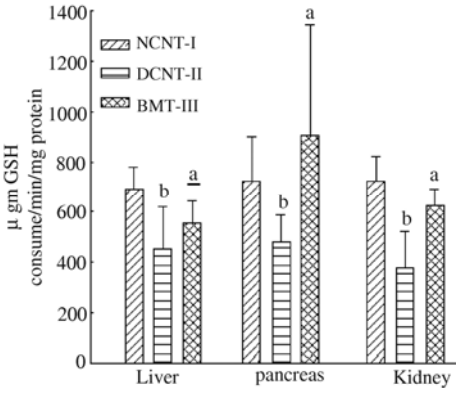

Fig. 5—Effect of 45 days treatment of BMEE on GSH-Px in alloxan-induced diabetic mice [Values represent mean ± S.E.M. (n = 7 in each group) with standard error indicated by vertical bars. Student's 't'-test was significant at P<0.05. [b]significant difference (P<0.05) compared with NCNT; [a]Significant difference (P<0.05) and [a]insignificant difference (P>0.05) compared with DCNT. ANOVA between treatments: Liver-F = 5.557, P<0.05; Pancreas-F = 3.892, P<0.05; Kidney-F = 30.757, P<0.01]

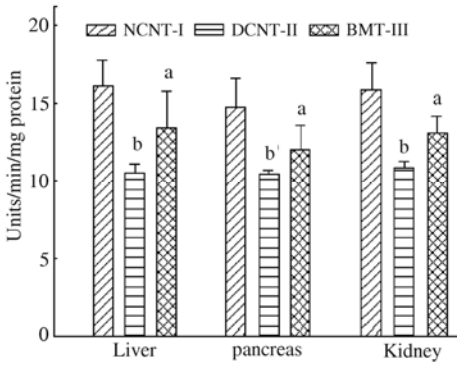

Fig. 6—Effect of 45 days treatment of BMEE on GSH in alloxan-induced diabetic mice [Values represent mean ± S.E.M. (n=7 in each group) with standard error indicated by vertical bars. Student's 't'-test was significant at $P<0.05$. [b]Significant difference ($P<0.05$) compared with NCNT; [a]significant difference ($P<0.05$) compared with DCNT. ANOVA between treatments: Liver-F = 16.172, $P<0.01$; Pancreas-F = 15.192, $P<0.01$; Kidney-F = 25.204, $P<0.01$]

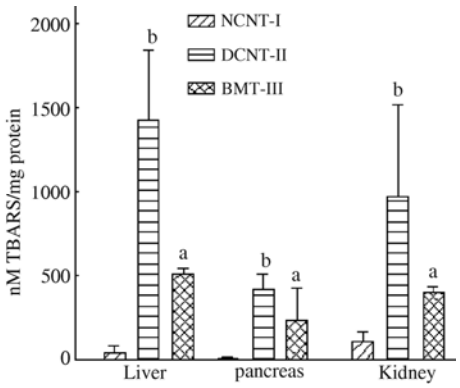

Fig. 7—Effect of 45 days treatment of BMEE on lipid peroxidation in alloxan-induced diabetic mice [Values represent mean ± S.E.M. (n = 7 in each group), with standard error indicated by vertical bars. Student's 't'-test was significant at $P<0.05$. [b]Significant difference ($P<0.05$) compared with NCNT; [a]significant difference ($P<0.05$) compared with DCNT. ANOVA between treatments: Liver-F = 194.549, $P<0.01$; Pancreas-F = 52.609, $P<0.01$; Kidney-F = 11.003, $P<0.01$]

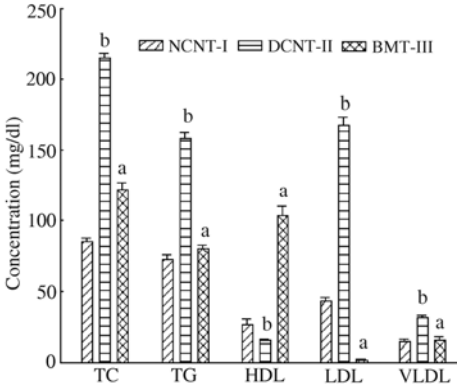

Fig. 8—Effect of 45 days treatment of BMEE on serum lipid profile in alloxan-induced diabetic mice. Values represent mean ± S.E.M. (n = 7 in each group) with standard error indicated by vertical bars. Student's 't'-test was significant at $P<0.05$. [b]Significant difference ($P<0.05$) compared with NCNT; [a]significant difference ($P<0.05$) compared with DCNT. ANOVA between treatments: TC-F = 1.350, $P<0.01$; TG-F = 1.121, $P<0.01$; HDL-F = 737.432, $P<0.01$; LDL-F = 2.254, $P<0.01$; VLDL-F = 1.117, $P<0.01$]

increased the antioxidant enzyme activities (except SOD, where the increase was not significant, $P>0.05$) and reversed to their near normal values.

Effect on serum lipid profile

Fig. 8 shows the serum TC, TG, LDL and VLDL-cholesterol levels elevated significantly ($P<0.05$) in diabetic control group (group II) as compared to normal control group (group I), whereas HDL-cholesterol reduced significantly ($P<0.05$) in untreated diabetic control group. All serum lipid profile parameters improved towards their near normal values after 45 days treatment with BMEE.

Discussion

In the present study, the anti-diabetic and antioxidant attributes of ethanolic extract of *B. monosperma* leaves were evaluated in alloxan-induced diabetic adult male Swiss albino mice. Alloxan was reported to cause a significant reduction of insulin-producing β-cells of islets of langerhans, thus inducing hyperglycemia[32]. The increased blood glucose level in diabetic mice (group II) as compared to normal ones (group I) might be due to glycogenolysis and/or gluconeogenesis[33].

A dose of alloxan up to 140-200 mg/kg body wt was found to be non-lethal[7]. In accordance with the earlier reports, in this study, dose of 150 mg/kg body wt[34] alloxan was selected. Under these conditions, insulin was secreted, but not sufficiently to regulate the blood glucose level, thus leading to the significant increase of FBG level in alloxan-induced diabetic mice. However, treatment of diabetic mice with BMEE (300 mg/kg body wt) for 45 days caused a significant reduction in FBG level, which might be due to increased peripheral glucose utilization or potentiating of the insulin effect. Moreover, glycogen level, the primary intracellular storable form of glucose in various tissues is a direct reflection of insulin activity, as insulin promotes its deposition by stimulating glycogen synthetase and inhibiting glycogen phosphorylase. The observed depletion of liver glycogen level in diabetic mice was consistent

with the earlier results[35], indicating that it was possibly be due to loss of glycogen synthetase-activating system[36] and/or increased activity of glycogen phosphorylase[35]. As alloxan caused selective destruction of β-cells of the islets of langerhans, resulting in a marked decrease of insulin levels[32], it was pertinent to believe that the liver glycogen level of alloxan-treated animals would decrease, as the synthesis of glycogen depends on insulin for the influx of glucose. Furthermore, earlier studies also showed that the reduced hepatic glycogen content was normalized by insulin treatment[37]. Moreover, administration of BMEE for 45 days showed a trend towards the significant increase in glycogen content towards its near initial values, when compared to diabetic control group (group II), thus confirming its insulin potentiating effect to a certain extent. The hypoglycemic efficacy of BMEE was in agreement with the previous findings[1].

The elevated TG, TC, LDL and VLDL level and decreased HDL level in alloxan-induced diabetic mice was in agreement with the previous reports regarding alteration of these parameters under diabetic condition[23,38]. The diabetes-induced hyperlipidemia might be due to excess mobilization of fat from the adipose tissue because of underutilization of glucose[39]. BMEE treatment for 45 days not only decreased the serum TC, TG, LDL and VLDL, but also increased HDL level significantly. The hypolipidemic action of BMEE might be due to inhibition of lipid peroxidation and was consistent with an earlier study[40], wherein increase in lipid peroxides and a decrease in antioxidant enzymes in DM was indicated.

The ROS are a contributory factor in the development of diabetes complications[41]. Furthermore, alterations in the antioxidant parameters during diabetes-induced oxidative stress have also been reported[42]. GSH and uric acid are important antioxidants that remove ROS[43]. In our study, alloxan was used for inducing diabetes. After being absorbed by the β-cells of pancreas and liver, alloxan results in the formation of ROS[44] and the resultant oxidative stress is mainly responsible for the pathogenesis of diabetes and its late complications[45].

In the present study, antioxidant variables during diabetes-induced oxidative stress showed a significant increase in the level of MDA (lipid peroxidation) and decrease in the GSH content and antioxidant enzymes, such as SOD, CAT, and GSH-Px in hepatic, pancreatic and renal tissues. These observations were in accordance with the earlier reports[42,46], indicating depletion in tissue's GSH content on alloxan induction in mice, due to higher levels of free radicals generation that convert GSH to its oxidized form. But, treatment of diabetic mice for 45 days with BMEE decreased the MDA levels (TBARS). Simultaneously, GSH content also increased significantly, indicating that BMEE could either increase the biosynthesis of GSH and/or reduce the oxidative stress, which ultimately reduced the degradation of GSH. Earlier, similar results were reported[37], when alloxan-treated diabetic mice were treated with ethanolic leaf extract of *Cleome droserifolia* for 30 days. Besides, the activities of antioxidant enzymes were also significantly increased and in some cases, for instance, GSH-Px and CAT activity in pancreatic tissue of BMEE-treated group, the elevation even exceeded the values observed in normal control group (group I), indicating the significant efficacy of the drug in providing antioxidant defense.

In conclusion, the present study demonstrated that BMEE showed significant antidiabetic and antioxidant potential in alloxan-induced diabetes-related oxidative stress and hence may find use for the management and/or control of diabetes.

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