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ORIGINAL ARTICLE

ANTIHYPERGLYCEMIC, ANTIHYPERLIPIDEMIC AND ANTIOXIDATIVE POTENTIAL OF *PROSOPIS CINERARIA* BARK

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ABSTRACT

Alloxan administration in male Swiss albino mice, induced diabetes by increasing blood glucose concentration and reducing hepatic glycogen content as compared to normal control group. Besides, serum lipid profile parameters such as total-cholesterol, triglyceride, low-density lipoprotein and very low-density lipoprotein-cholesterol were also elevated, whereas, the level of high-density lipoprotein-cholesterol was reduced significantly ($P<0.05$) in diabetic mice. Treatment of diabetic animals with crude ethanolic extract of bark of Prosopis cineraria (P. cineraria) for 45 days, significantly lowered blood glucose level, elevated hepatic glycogen content and maintained body weight and lipid-profile parameters towards near normal range. Declined activity of antioxidant enzymes and concentration of non-enzymatic antioxidants were also normalized by drug treatment, thereby reducing the oxidative damage in the tissues of diabetic animals and hence indicating the anti-diabetic and antioxidant efficacy of the extract.

KEY WORDS

Prosopis cineraria bark, Diabetes mellitus, Oxidative stress, Antidiabetic, Antioxidant.

INTRODUCTION

Diabetes mellitus (DM) is a group of syndrome characterized by hyperglycemia and altered metabolism of carbohydrates, lipids and proteins. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries (1). Increasing evidences from both experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of DM. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins (2). Abnormally high level of free radicals and the simultaneous decline in antioxidant defense mechanisms may lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance (3). Current drugs used for the treatment of diabetes are associated with several side effects and hence there is need for effective, safe and better oral hypoglycemic agents (4). In the indigenous system of medicine, Ayurveda, a mention has been made on a number of plants for controlling diabetes but only a few have been scientifically evaluated and the active principles are isolated (5).

Prosopis cineraria (Fabaceae), locally known as 'Khejri', has a long history of use in herbal medicine in arid and semi-arid regions in greater parts of India, Burma and Sri Lanka. Since all parts of the tree are useful, it is called 'Kalptaru' (6). Ancient literature has reported the use of *P. cineraria* as folk medicine for various ailments. Its flowers mixed with sugar when administered orally prevent miscarriage (7). With twig, the flowers are also known as an anti-diabetic agent. Dry pods of the plant help in preventing protein calorie malnutrition and iron calcium deficiency in blood. Smoke of leaves is used to cure eye infections (8). Bark of the tree is used in the treatment of asthma, bronchitis, dysentery, leucoderma, leprosy, muscle tremors and piles (9, 10). Numerous bioactive compounds such as flavonoids, alkaloids, diketones, phenolic contents,

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free amino acids, patulitrin, spicigerin, prosogerin A,B,C,D, lipids, b-sitosterol, sugars and vitamins have been isolated from various parts of the plant. Some of these bioactive compounds have been worked out for one or the other medicinal attributes (11, 12). But till date, the anti-diabetic and antioxidant potential of *P. cineraria* has not been scientifically evaluated. Hence, in the present study, the effect of 45 day chronic oral treatment with *P. cineraria* bark extract with a dose of 300 mg/kg body wt on diabetes and resultant oxidative stress was investigated by evaluating its antihyperglycemic, antihyperlipidemic and antioxidative properties in alloxan-induced diabetic mice.

MATERIALS AND METHODS

Chemicals: Alloxan monohydrate was purchased from SD Fine chemicals (Mumbai, India). All other chemicals used for this study were of analytical grade and obtained from HIMEDIA (India), SRL (India), CDH (India) and Qualigens (India/ Germany). Kits for the estimation of total cholesterol, triglyceride and HDL-cholesterol were purchased from Erba Mannheim (Transasia Bio-Medicals Ltd. Daman, India).

Preparation of extract: Bark of *P. cineraria* was collected from the plants available locally at Banasthali University campus (Dist. Tonk, Rajasthan, India). It was taxonomically identified. Dried bark was subjected to size reduction to a coarse powder which was then soxhlet extracted with 50% aqueous 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. The yield of extract was 4.2% w/w (with respect to crude material). The suspension of extract of the bark, prepared in 20% tween 20 in normal saline was used in the experiment.

Animal care and monitoring: Healthy male Swiss albino mice (*Mus musculus*) (4-6 months old, weighing 20-30 g) were procured from C.C.S. Haryana Agricultural University (Hissar, India). They were housed under standard laboratory conditions of light (12:12 h L: D cycle), temperature (23 ± 2°C) and relative humidity (55 ± 5%). The animals were provided standard rat pellet feed and tap water *ad libitum.* Maintenance and treatment of all the 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), India.

Introduction of diabetes and treatment with drugs: For the present study, animals were divided into following 4 groups: NC (normal control), DC (diabetic control), ET (diabetic + *P. cineraria* bark extract treated) and GT (diabetic + glibenclamide treated). After fasting for 18 hours, mice of group DC, ET and GT were made diabetic by a single intra-peritoneal injection of alloxan monohydrate, 150 mg/kg body wt, freshly dissolved in normal saline (13). Subsequent to alloxan administration the mice had free access to food and water and were provided with 50% glucose solution to drink overnight to counter drug-induced hypoglycemic shock. One week after alloxan injection, the fasting blood glucose (FBG) concentration was determined by means of one touch ultra glucometer (Johnson & Johnson Company, USA) and compatible blood glucose strips (14). Mice showing fasting blood level greater than 140 mg/dl were considered diabetic (15, 16) and selected for treatment with drug (10 mg/kg body wt.) or bark extract (300 mg/kg body wt.). The drug and the bark extract were administered orally, once in a day for 45 days.

Biochemical estimations in blood and serum: Fasting blood glucose (FBG) concentration of all the four experimental groups was determined by glucometer during different phases of the experiment by withdrawing blood from the tail vein. Hepatic glycogen content was measured according to the anthrone-H_2SO_4 method, with glucose as standard (17). For estimating serum lipid profile, serum was isolated from the blood collected by cardiac puncture under mild ether anesthesia from overnight fasted mice on day 45^{th} of *P. cineraria* bark extract treatment and serum total cholesterol (TC), triglyceride (TG) and HDL-cholesterol were estimated by using diagnostic kits (Erba Mannheim Cholesterol kit, Transasia Bio-Medicals Ltd., Daman). VLDL and LDL-cholesterol were calculated as per Friedevald's equation. VLDL-cholesterol = Serum triglyceride /5; LDL-cholesterol = Serum total-cholesterol – VLDL-cholesterol – HDL-cholesterol. Results were expressed in mg/dl.

Biochemical estimation in tissue homogenates: Liver, pancreas and kidney were removed, freed from adhering tissues and washed with ice-cold normal saline solution (0.9%). Weight of all the organs was taken only after drying the tissue. 1 g tissue was homogenized in 10 ml of 0.2 M tris-HCl with the help of homogenizer. The homogenate was filtered and then centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant obtained was used for estimation of total protein (18), superoxide dismutase (SOD) (19), catalase (CAT) (20), glutathione peroxidase (GSH-Px) (21), reduced glutathione (GSH) (22) and thiobarbituric acid reactive substances (TBARS) (23).

Statistical Analysis: Results are expressed as mean ± Standard Error of 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) and student's 't'-test using SigmaPlot (version 8.0). The values of $P<0.05$ were considered as statistically significant.

RESULTS

Effect on body weight: All animals treated with alloxan in diabetic control group (DC) revealed a significant (P<0.05) loss in body weight (29.6%), which was persistently observed till the end of the study period (Table 1). Weight reduction induced by diabetes in group ET was cured with a notable 14% increase by *P. cineraria* bark extract treatment. This trend of increasing body weight is comparable to that observed in glibenclamide-treated group (GT) in which 24.4% increase was observed.

Effect on fasting blood glucose level: Alloxan injection to DC, ET and GT groups resulted in a significant (P<0.05) increase in blood glucose level as compared to normal control group A. Treatment with bark extract significantly reduced the glucose level by 27.3% (i.e. from 207.0 ± 10.9 mg/dl to 150.5 ± 12.1 mg/dl). This FBG lowering trend of *P. cineraria* bark extract was comparable to a certain extent with that of glibenclamide, which produced 49.3% reduction (from 360.6 ± 31.9 mg/dl to 182.7 ± 21.8 mg/dl) in blood glucose level of group D after 45 days of treatment (Table 1).

Effect on liver glycogen content: Hepatic glycogen content decreased significantly by 58.7% in diabetic control group (DC) compared to normal control group (NC). However, treatment with bark extract significantly (P<0.05) increased the glycogen content in ET by 72.3% as compared to DC Similarly, treatment of group GT with glibenclamide increased the hepatic glycogen content by 81.5% (Table 2).

Table 2: Effect of 45 days treatment of *P. cineraria* bark extract on hepatic glycogen content in alloxan-induced diabetic mice. Values are mean ± SEM of 7 observations

Parameters	NC	DC	ET	GT
Hepatic Glycogen (mg/g)	14.3 ± 1.1	5.9 ± 0.6[a]	21.3 ± 1.9[b c]	31.9 ± 3.0[b]

Student's 't'-test is significant at $P<0.05$. [a]significant ($P<0.05$) difference, [a]insignificant ($P>0.05$) difference compared to NC; [b]significant ($P<0.05$) difference, [b]insignificant ($P>0.05$) difference compared to DC; [c]significant ($P<0.05$) difference, [c]insignificant ($P>0.05$) difference compared to GT. ANOVA between groups: F = 122.9, $P<0.05$

Effect on serum lipid profile: Compared to normal control group (NC), the level of serum TC, TG, LDL and VLDL-cholesterol was significantly (P<0.05) increased by 60.5%, 54.0%, 73.9% and 53.9% respectively, whereas the level of HDL-cholesterol was significantly (P<0.05) reduced by 40.9% in untreated diabetic mice (DC) (Table 3). After treatment of group ET with *P. cineraria* bark extract, the level of serum TC, TG, LDL and VLDL-cholesterol reduced significantly (P<0.05) by 68.2%, 36.8%, 96.9% and 36.9% whereas, the level of serum HDL-cholesterol was significantly increased by 63.1%. Glibenclamide treatment reduced the level of TC, TG, LDL and VLDL-cholesterol by 65.1%, 36.8%, 89.9% and 36.9% and enhanced the level of HDL-cholesterol by 65.7%.

Antioxidative status: It was observed that after inducing diabetes, the level of hepatic, pancreatic and renal GSH content decreased significantly in diabetic control group (DC)

Table 1: Effect of 45 days treatment of *P. cineraria* bark extract on body weight and FBG level in alloxan-induced diabetic mice. Values are mean ± SEM of 7 observations

Parameters	Day	NC	DC	ET	GT
Body Weight (g)	BD*	27.4 ± 3.2	29.7 ± 3.3	30.7 ± 3.0	22.4 ± 1.8
	0	27.4 ± 3.2[b]	18.4 ± 3.5[a]	27.7 ± 3.5[a]	19.4 ± 1.8[a]
	45	27.9± 3.1[b]	20.9 ± 4.5[a]	32.1 ± 3.7[b c]	24.3 ± 3.0[a c]
FBG Level (mg/dl)	BD*	79.6 ± 7.7	72.7 ± 7.4	81.7 ± 8.8	96.1 ± 9.4
	0	79.6 ± 7.7[b]	244.3 ± 11.6[a]	207.0 ± 10.9[a]	360.6 ± 37.6[a]
	45	80.7 ± 8.3[b]	232.9 ± 34.9[a]	150.4 ± 12.1[a c]	182.7 ± 14.5[a c]

Values are mean ± SEM of 7 observations. *Before diabetes (Basal values); Student's 't'-test is significant at $P<0.05$. [a]significant ($P<0.05$) difference, [b]insignificant difference ($P>0.05$) compared to basal values; [c]significant ($P<0.05$) difference compared to values obtained after alloxan injection. ANOVA between treatments: Body weight - F = 2.7, $P>0.05$; FBG level – F = 207.6, $P<0.05$

Table 3: Effect of 45 days treatment of *P. cineraria* bark extract on serum lipid profile in alloxan-induced diabetic mice. Values are mean ± SEM of 7 observations

Parameters (mg/dl)	NC	DC	ET	GT
TC	84.9 ± 2.9	214.7 ± 3.7[a]	68.3 ± 2.2[b c]	75.0 ± 3.9[b]
TG	72.8 ± 3.1	158.3 ± 4.4[a]	100.1 ± 3.1[b c]	58.1 ± 5.2[b]
HDL	26.9 ± 3.5	15.9 ± 0.9[a]	43.1 ± 2.2[b c]	46.4 ± 3.8[b]
LDL	43.5 ± 3.6	167.1 ± 3.3[a]	5.2 ± 1.4[b c]	16.9 ± 1.9[b]
VLDL	14.6 ± 0.5	31.7 ± 0.9[a]	20.0 ± 0.6[b c]	11.6 ± 1.2[b]

Student's 't'-test is significant at $P<0.05$. [a]significant ($P<0.05$) difference compared to NC; [b]significant ($P<0.05$) difference compared to DC; [c]significant ($P<0.05$) difference and [c]insignificant ($P>0.05$) difference compared to GT. ANOVA between groups: TC – F = 2.8, $P<0.05$; TG – F = 635.5, $P<0.05$; HDL – F = 151.4, $P<0.05$; LDL – F = 3.6, $P<0.05$; VLDL – F = 635.4, $P<0.05$.

as compared to normal control group (NC). Treatment of group ET with *P. cineraria* bark extract for 45 days significantly (P<0.05) increased the tissue's GSH level as compared to DC. Glibenclamide treatment also increased the GSH level significantly in GT (Table 4).

Data of the present study also revealed a significantly (P<0.05) increased concentration of TBARS, a measure of lipid peroxidation, in the hepatic, pancreatic and renal tissues of diabetic mice. However, a significant reduction in the level of TBARS was observed in the liver of *P. cineraria* bark extract treated group (ET) but the recession observed in pancreatic and renal tissue of this group was statistically insignificant. The decreased level of TBARS was also observed in glibenclamide treated group (GT) (Table 4). Induction of diabetes significantly (P<0.05) reduced the activity of catalase and GSH-Px in liver, pancreas and kidney of DC as compared to NC. However, 45 days treatment of diabetic animals with *P. cineraria* bark extract (ET) increased the activity of these enzymes in all the tissues and the elevation was convincingly higher than that observed in glibenclamide treated group (Table

Table 4: Effect of 45 days treatment of *P. cineraria* bark extract on antioxidative status in alloxan-induced diabetic mice. Values are mean ± SEM of 7 observations

Parameters	NC	DC	CT	GT
Hepatic GSH[1]	16.0 ± 1.7	10.5 ± 0.6[a]	22.7 ± 1.8[b c]	32.5 ± 2.7[b]
Hepatic TBARS[2]	43.9 ± 6.3	1425 ± 122[a]	565.9 ± 32.6[b c]	1001 ± 85.6[b]
Hepatic CAT[3]	233.0 ± 12.7	1 76.7 ± 19.9[a]	221.4 ± 15.7[b c]	157.4 ± 12.0[b]
Hepatic GSH-Px[4]	689.5 ± 27	453.1 ± 20.7[a]	466.9 ± 27.2[b c]	287.5 ± 27.9[b]
Hepatic SOD[5]	308.6 ± 9.2	238.1 ± 22.3[a]	201.9 ± 18.3[b c]	192.2 ± 6.2[b]
Pancreatic GSH[1]	14.8 ± 1.8	10.4 ± 0.3[a]	19.8 ± 0.6[b c]	32.2 ± 0.9[b]
Pancreatic TBARS[2]	9.6 ± 1.2	419.6 ± 25.8[a]	319.1 ± 4.4[b c]	345.6 ± 30.5[b]
Pancreatic CAT[3]	266.6 ± 18.4	167.5 ± 6.7[a]	381.4 ± 21.6[b c]	277.1 ± 15.6[b]
Pancreatic GSH-Px[4]	721.0 ± 71.7	478.0 ± 20.9[a]	674.7 ± 68.4[b c]	534.1 ± 19.1[b]
Pancreatic SOD[5]	321.7 ± 20.4	317.6 ± 19.6[a]	335.2 ± 22.3[b c]	358.0 ± 46.8[b]
Renal GSH[1]	15.8 ± 1.8	10.8 ± 0.4[a]	19.8 ± 2.9[b c]	32.6 ± 0.8[b]
Renal TBARS[2]	110.1 ± 4.9	964 ± 119[a]	650.6 ± 37.5[b c]	638.8 ± 61.7[b]
Renal CAT[3]	272.1 ± 21.5	161.9 ± 10.6[a]	314.8 ± 28.1[b c]	245.8 ± 16.4[b]
Renal GSH-Px[4]	723.2 ± 57.2	377.7 ± 42.9[a]	629.8 ± 65.7[b c]	420.2 ± 16.6[b]
Renal SOD[5]	333.1 ± 29.2	299.6 ± 28.8[a]	293.9 ± 24.8[b c]	294.6 ± 26.8[b]

[1]mg/gm tissue, [2]nM TBARS/mg protein, [3]μ moles H_2O_2 decomposed/min/mg protein, [4]μg GSH consumed/min/mg protein, [5]Units/min/mg protein, Student's 't'-test is significant at $P<0.05$. [a]significant ($P<0.05$) difference, [a]insignificant ($P>0.05$) difference compared to NC; [b]significant ($P<0.05$) difference, [b]insignificant ($P>0.05$) difference compared to DC; [c]significant ($P<0.05$) difference, [c]insignificant ($P>0.05$) difference compared to GT.
ANOVA between groups: Hepatic GSH – F = 159.1, $P<0.05$; Pancreatic GSH – F = 294.6, $P<0.05$; Renal GSH – F = 344.1, $P<0.05$;
Hepatic TBARS – F = 108.2, $P<0.05$; Pancreatic TBARS – F = 20.3, $P<0.05$; Renal TBARS – F = 9.5, $P<0.05$;
Hepatic CAT – F = 3.8, $P<0.05$; Pancreatic CAT – F = 18.7, $P<0.05$; Renal CAT – F = 17.9, $P<0.05$;
Hepatic GSH-Px – F = 14.9, $P<0.05$; Pancreatic GSH-Px – F = 6.4, $P<0.05$; Renal GSH-Px – F = 30.3, $P<0.05$;
Hepatic SOD – F = 13.9, $P<0.05$; Pancreatic SOD – F = 1.8, $P>0.05$; Renal SOD – F = 1.2, $P>0.05$.

4). Alloxan injection significantly ($P<0.05$) decreased the activity of SOD in liver but the reduction in pancreas and kidney was insignificant. 45 days treatment with *P. cineraria* bark extract did not significantly improved the activity of SOD in any of the tissues in ET (Table 4). Likewise, glibenclamide treatment also proved inefficient in alleviating the activity of SOD.

DISCUSSION

Alloxan has been reported to cause a massive reduction of β-cells of the islets of langerhans and induce hyperglycemia (13). In our study also, a marked hike in the level of fasting blood glucose in DC, ET and GT groups as compared to NC was monitored subsequent to alloxan administration. The observed hyperglycemia may be due to glycogenolysis or gluconeogenesis (24). However, continuous treatment of diabetic animals (ET) with *P. cineraria* bark extract for 45 days caused a significant reduction in FBG level. This antihyperglycemic action may be due to insulin potentiating effect via stimulation of the undamaged or residual pancreatic islets to release insulin. Moreover, significant reduction in blood glucose level in glibenclamide treated group (GT) strengthens the above explanation, since it also exerts its hypoglycemic effect by increasing insulin secretion (25, 26). Further, level of glycogen, the primary intracellular storable form of glucose in various tissues is a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. The observed depletion of liver glycogen stores in diabetic mice is consistent with an earlier study (27), indicating that it could be due to the loss of glycogen-synthase activating system (28) and/or increased activity of glycogen phosphorylase (27). Since alloxan causes selective destruction of β-cells of the islets of langerhans resulting in a marked decrease of insulin levels, it is rational to believe that the glycogen level in liver of diabetic animals will decrease, as synthesis of glycogen depends on insulin for the influx of glucose. However, previous studies show that the reduced hepatic glycogen content was normalized by insulin treatment (29). Administration of *P. cineraria* bark extract for 45 days also showed a trend towards a significant increase in glycogen content when compared to diabetic mice, thus confirming its insulin potentiating effect to a certain extent, which is comparable to that of glibenclamide. The antihyperglycemic efficacy of this herbal drug can be correlated with the findings of Osinubi et al (30). Similarly, alloxan caused body weight loss was also regained to its above-initial values by *P. cineraria* bark extract treatment, which reflects an improved health of bark extract treated animals.

Altered carbohydrate metabolism during diabetes is also accompanied by disordered fat and protein metabolism (31). The characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, low HDL-cholesterol concentration and increased concentration of small dense LDL-cholesterol particles (32). Faulty glucose utilization causes hyperglycemia and mobilization of fatty acids from adipose tissue for energy purpose (33). The lipid changes associated with diabetes mellitus are attributed to increased flux of free fatty acids into the liver secondary to insulin deficiency/ resistance (34,35). This results in excess fatty acid accumulation in the liver, which are converted to triglycerides (32,33). The impaired ability of insulin to inhibit free fatty-acid release leads to elevated hepatic VLDL-cholesterol production (36). The increased VLDL-cholesterol and triglyceride levels decrease the level of HDL-cholesterol and increase the concentration of small dense LDL-cholesterol particles by activation of lipoprotein lipase and lecithin acyl-cholesterol transferase (37). In our study, elevated levels of serum TC, TG, LDL and VLDL-cholesterol and decreased HDL-cholesterol concentration in alloxan-induced diabetic mice are in accordance with the previous research findings (38). However, treatment with *P. cineraria* bark extract normalized all the lipid profile parameters. This antihyperlipidemic attribute of bark extract may also be attributed to insulin potentiating effect and can be correlated with a previous report (39)

Earlier, it has been explored that oxidative stress induced by hyperglycemia is a key factor in the pathogenesis of diabetic complications (40). However, biological systems have evolved an effective and complicated network of defense mechanisms, which enable cells to cope with lethal oxidative environment. These defense mechanisms involve both non-enzymatic and enzymatic antioxidants.

Among various non-enzymatic antioxidants total thiols play a vital role in the structure, activity and transport function of proteins, membrane and enzymes and have been found to decrease the oxidative stress (41). The protective effects of thiols can be brought about directly by scavenging free radicals or indirectly by elevating the level of GSH that has a multifaceted role in antioxidant defense (42). In our study, the level of hepatic, pancreatic and renal GSH was reduced in diabetic mice, which is consistent with an earlier report (43). The decrease in tissue's GSH content could be the result of decreased synthesis or increased degradation of GSH by oxidative stress that prevails during diabetes (44). Furthermore, lipid peroxidation is one of the characteristic features of chronic diabetes (45). In the present study, along with decreased level of reduced GSH, a marked increase in

the concentration of TBARS was also observed in liver, pancreas and kidney of diabetic mice; however, treatment of these animals with *P. cineraria* bark extract decreased the elevated level of TBARS. Simultaneously, reduced GSH content was also increased significantly which indicates that the *P. cineraria* bark extract can either increase the biosynthesis of GSH and/or reduce the oxidative stress that ultimately reduces the degradation of GSH. Similar results were also witnessed in an earlier study (29).

Enzymatic antioxidants include SOD, which catalyzes the dismutation of O_2^- to H_2O_2 and O_2 (46), catalase and peroxidases, which remove hydrogen peroxide and hydroperoxides (47). Earlier reports have demonstrated that oxidative processes result in the loss of key antioxidant enzymes. The damage brought about by oxidative stress is expected to be exacerbated if the antioxidant enzymes themselves are damaged and inactivated by glycation-induced oxidative stress ultimately resulting in the perturbation of cellular redox status (48). Data of the present study also revealed a declined content of antioxidant marker enzymes such as CAT, GSH-Px and SOD in hepatic, pancreatic and renal tissues of diabetic animals. Glucose that forms schiff's base with proteins has been reported to have high affinity for proteins specially those containing transition metal ions (49) thus reducing their activities. Since, glycation mediated reaction with proteins, including antioxidant enzymes is inevitable under diabetic conditions; therefore, formation of ROS is also unavoidable (48). It has been shown that copper, zinc superoxide dismutase (50) and catalase (51) are inactivated by glycation. It has also been worked out that lens cells exposed to 50-100 µM glucose for three days displayed significantly decreased activity of antioxidant enzymes thus confirming the glycation mediated alteration of antioxidant enzymes in diabetes (48). However, *P. cineraria* bark extract therapy increased the activity of CAT and GSH-Px in hepatic, pancreatic and renal tissues of treated diabetic animals. These findings can be complemented with the study of Hussain (52). The antioxidant activity of the test drug might have been due to the inhibition of glycation of antioxidant enzymes. The increased activity of CAT and GSH-Px suggest a compensatory response to oxidative stress as it reduces the endogenous H_2O_2 produced thus diminishing the toxic effects due to this radical or other free radicals derived from secondary reactions (53, 54, 55). However, the level of SOD, in any of the tissues of diabetic mice was not improved by *P. cineraria* bark extract treatment. This perhaps is due to different metabolic actions of the tissues and their different responses to oxidative stress (55). Moreover, the observed antioxidative effect of *P. cineraria* bark extract was greater than the reference drug glibenclamide. This may be because of synergistic action of various compounds present in the extract in contrast to the purified compound of the drug.

Results of this study do not allow definite conclusion to be drawn on the mechanism of action of *P. cineraria* bark extract in the experimental animal paradigms used. However, a number of investigators have shown that a host of various plant secondary metabolites possess hypoglycemic, hypotensive, anti-inflammatory and other pharmacological properties (56). As mentioned earlier, *P. cineraria* is also known to contain various secondary metabolites. Therefore, it is not unreasonable to speculate that some of these chemical compounds are presumably responsible for imparting the antihyperglycemic, antihyperlipidemic and antioxidative properties to the *P. cineraria* bark extract.

From the results obtained, it can be concluded that *P. cineraria* bark possess significant antihyperglycemic, antihyperlipidemic and antioxidative properties. Hence, apart from controlling hyperglycemia it would also be beneficial in the alleviation of associated diabetic complications including the prevention of the development of atherosclerosis and other coronary artery diseases. However, further studies are needed to investigate and elucidate the possible mechanism of action of the active ingredients, establish complete safety profiles and evaluate the potential value of *P. cineraria* bark extract for the management of diabetes and hyperlipidemia in the clinic. Moreover, additional parameters such as assay of insulin, HbA_{1c} etc should also be studied. This may prove helpful for developing new drugs from this plant for managing diabetes and associated complications.

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