

Research Article

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Evaluation of antihyperglycemic and antihyperlipidemic activity of *Prosopis cineraria* (Linn.) in wistar rats

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Abstract

The present study was carried out to investigate the antihyperglycemic and antihyperlipidemic properties of the hydroalcoholic extract of *Prosopis cineraria* leaves in Streptozotocin (50 mg/kg intraperitoneal) induced diabetic rats for 12 weeks. The streptozotocin induced diabetic male wistar rats were fed with hydroalcoholic plant extract of *Prosopis cineraria* leaves at the increasing dosage of 250, 500 and 750 mg/kg. Positive control group was receiving metformin 100 mg/kg/day, per oral for 12 weeks. Treatment of streptozotocin induced diabetic wistar rats with the extract caused a significant ($P < 0.05$) reduction in the blood glucose level and significant reduction ($P < 0.05$) in the serum levels of the total cholesterol, triglycerides and a significant increase ($P < 0.05$) in HDL level. The dose of 750mg/kg showed maximum significant decrease ($p < 0.05$) as compared to other two doses. This result suggests that the hydroalcoholic leaf extract of *Prosopis cineraria* possess antidiabetic effect on streptozotocin induced diabetic Wistar rats.

Keywords: *Prosopis cineraria*, Hydro alcoholic extract, Antihyperglycemic, Antihyperlipidemic, Streptozotocin.

Introduction

Diabetes is recognized as one of the leading causes of morbidity and mortality in the world. Currently available antidiabetic drugs have been associated with a number of side effects. Although a range of synthetic drug are available as antidiabetic drugs, many of them do not fulfill all requirement and their numerous side effects and potential interferences with drug metabolism are common. Thus a survey among medicinal herbs is also still important and might provide a useful source for therapy or alternatively as simple dietary adjuncts to existing therapy. It has been reported that traditional systems have immune potential against various diseases.¹ More than thirteen thousand plants have been studied for various pharmacological properties.² The major merits of herbal medicines seem to be their efficacy, low incidence of side effects, and low cost.³

Prosopis cineraria (PC) belongs to family Leguminosae grows in dry and arid regions of Arabia and in India mainly Rajasthan, Haryana, Punjab, Gujarat, Western Uttar Pradesh and drier parts of Deccan and extends as far as South in Tuticorin. It is also known as Khejri, Jand, Janti and Sangri in Rajasthan; Jand in Punjab; Kandi in Sindh; Banni in Karnataka; Vanni or Jambu in Tamilnadu; Sami and Sumri in Gujarat. Since all parts of the tree are useful, it is called 'Kalptaru'.⁴

Prosopis cineraria is used as antihyperlipidemic, antioxidative, anthelmintic, antibacterial, antifungal, antiviral, anticancer, in treatment of dysentery, bronchitis, asthma, leucoderma, piles, leprosy, muscular tremors and wandering of the mind.⁵ It has analgesic and antipyretic activities.⁶ It is also used as a remedy for rheumatism.⁷ Applied on boils and blisters, mouth ulcers in livestock and on open sores on the skin, good for eye, prevent miscarriage, anti-diabetic agent, help in preventing protein calorie malnutrition and iron calcium deficiency in blood.⁸ Numerous bioactive compounds such as flavonoids, alkaloids, diketones, phenolic contents free amino acids, patulitrin, spicigerin, prosogerin A, B, C, D, steroids namely campesterol, cholesterol, β -sitosterol, stigmasterol, alcohols namely octacosanol and triacontan-1-ol and alkanes hentriacontane, lipids, sugars and vitamins have been isolated from various parts of the plant.⁹

Despite progress in conventional chemistry and pharmacology in producing effective drugs, the plant kingdom might provide a useful source of new medicines and may be used in place of existing drugs. Therefore, the present study was carried out to investigate the anti-hyperglycemic and antihyperlipidemic properties of the hydroalcoholic extract of *Prosopis cineraria* leaves in Streptozotocin (STZ) induced diabetic rats.

Materials and Methods

Plant materials

Fresh leaves of plant *Prosopis cineraria* were collected in September 2011 from Sirsa, Haryana, India and authenticated by National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi. The voucher specimen No. was NHCP/NBPGR/2011-61 and the specimen was deposited at herbarium of National Herbarium of Cultivated Plants.

Preparation of extract

The leaves were washed in tap water, shade dried for 10 days and then homogenized to fine powder of 40 mesh size using the electric blender and stored in airtight bottles. For extract preparation, 100g of the powder was filled in the thimble and extracted using 500 ml of distilled ethanol in soxhlet apparatus for 2 hours. The extract was filtered through Whatman No.1 filter paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and lyophilized.⁸

Preliminary phytochemical screening

The extracts were preliminary investigated for various phytochemical constituents such as Alkaloids, Carbohydrates, Steroids, Proteins, Phenols, Tannins, Flavonoids, Glycosides and Saponins.¹⁰

Animal care and monitoring

The study was carried out on mixed sex of Wistar albino rats (150–200 g). Animals were obtained from the C.C.S. Haryana Agricultural University, Hissar, India. They were housed at a temperature of $24 \pm 2^\circ\text{C}$ and at relative humidity of 50 % maintained on 12 h light/dark cycle and allowed food and water *ad libitum*. Permission for the use of animal and animal protocol was obtained from the Institutional Animal Ethical Committee (IAEC) (No. 800/03/C/CPCSEA) as per the requirement of Committee for the Purpose of Control and Supervision on Animals (CPCSEA), New Delhi.

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling techniques were employed in this study. The animal were kept fasting for overnight providing only water. Then the extracts (bark and leaves) were administered orally at the dose of 2000 mg/kg by intra-gastric tube and observed for 2 days for the gross behavioral changes and mortality.¹¹

Anti-diabetic activity

Experimental Induction of diabetes

After fasting for 18hrs 40 rats were injected by intraperitoneally with a single dose of 50 mg/kg streptozotocin after dissolving it in freshly prepared ice cold citrate buffer (PH 4.5).¹² After the injection they had free access to feed and water and were given 5% glucose solution to drink over night to counter the hypoglycemic shock.¹³ The development of diabetes was confirmed after 48hrs of the Streptozotocin injection. The animal having fasting blood glucose levels more than 200mg/dl were selected for the experimentation. Out of 40 animals 3 died before grouping and one was omitted from the study because of mild hyperglycemia. Remaining 36 diabetic animals were divided into 6 groups each having 6 rats.

Experimental protocol

36 animals are divided into four groups as follows:

Group 1: Control (n=6): received distilled water *ad libitum* for the period of 12 weeks.

Group 2: Diabetic rats (HFD: High Fat Diet, n=6): administered distilled water *ad libitum* for period of 12 weeks.

Group 3: *P. cineraria* treated group:

Diabetic rats were administered hydroalcoholic extract of *P. cineraria* (PC) at different doses of 250, 500 and 750 mg/kg/day¹⁴ and further divided in subgroups. Diabetic animals were treated with *P. cineraria* orally for a period of 12 weeks.

Group3a: Rats treated with 250mg/kg/d (PC1) of aqueous extract of *P. cineraria* leaves.

Group 3b: Rats treated with 500mg/kg/d (PC2) of aqueous extract of *P. cineraria* leaves.

Group 3c: Rats treated with 750mg/kg/d (PC 3) of aqueous extract of *P. cineraria* leaves.

Group 4: Metformin treated groups:

Diabetic rats were administered metformin 100 mg/kg/day, p.o for 12 weeks.

Measurement of Body weight & Blood Glucose Level:

All the group of animals received the treatment for 12 weeks. The body weight and blood glucose level were measured at about every 5 days interval. Blood samples were collected one hr after the drug administration to determine the blood glucose level by electronic glucometer. Blood samples were obtained from retro orbital plexus under light ether anaesthesia using in capillary tubes (Micro Hemocrit capillary, Mucaps) into eppendorf tubes containing EDTA and serum was separated within 30 mins after collection using centrifuge at 2000 rpm for 2 min.

Measurement of Serum lipid profile

Total cholesterol (TC), Triglyceride (TG), and Serum HDL-c were estimated by enzymatic methods by using diagnostic kit. (Transasia Bio-Medicals Ltd. Daman, India).

LDL-cholesterol levels were calculated using the formula of Friedewald et al, formula.¹⁵

$$\text{LDL-c} = \{ \text{TC} - (\text{HDL-c}) + (\text{TG}/5) \}$$

Statistically analysis

Data obtained from pharmacological experiments are expressed as mean \pm SD (Difference between the treatments in this experiment was tested for significance using Paired t-test). P value < 0.05 considered as significant.

Results

The preliminary phytochemical studies indicated the presence of carbohydrate, protein, tannins, phenol, flavonoids, terpenes, saponins and gums in the hydroalcoholic extract of the leaf.

Acute toxicity study shows that the hydroalcoholic extract of *Prosopis cineraria* leaf did not produced lethality up to the dose level of 2000 mg/kg. Induction of diabetes in the experimental rats was confirmed by the presence of high blood glucose level. Administration of graded doses of hydroalcoholic extract for a 12 weeks experimental period produced a statistically significant decrease in blood glucose concentration when compared with the diabetic control ($p < 0.05$). Treatment with the hydroalcoholic extracts of *Prosopis Cineraria* at all the three dose levels was found to be dose dependant, with the higher dose showing more significant activity. The effects of the hydroalcoholic leaf extract on fasting blood glucose levels in diabetic animals are presented in (Table 1). The difference between the fasting blood glucose level in diabetic and control rats were found to be statistically significant. Significant ($p < 0.05$) difference was observed in body weight changes (Table 2), serum lipid profiles (Table 4) of extract-treated diabetic animals when compared with the diabetic control. The lipid profile such as TC, TG and LDL levels were significantly increased in diabetic control animals (DC) where as HDL levels were decreased when compared to the control rats.¹⁶

Table 1: Effect of hydroalcoholic extract of *P. cineraria* and metformin on blood glucose

Groups	Blood glucose (mg/dl)	
	Initial	Final
Normal Control	214.04±5.6	220.04±5.6
Diabetic Control	259.86±8.0	276.86±8.0
Diabetic + PC1	251.26±8.5	220.26±8.5
Diabetic + PC2	254.53±8.57	206.53±8.57
Diabetic + PC3	252.21±10.3	175.21±10.3
Diabetic+Metformin	267.98±4.2	120.98±4.2

Values are expressed as means ± SD (n=6 rats)

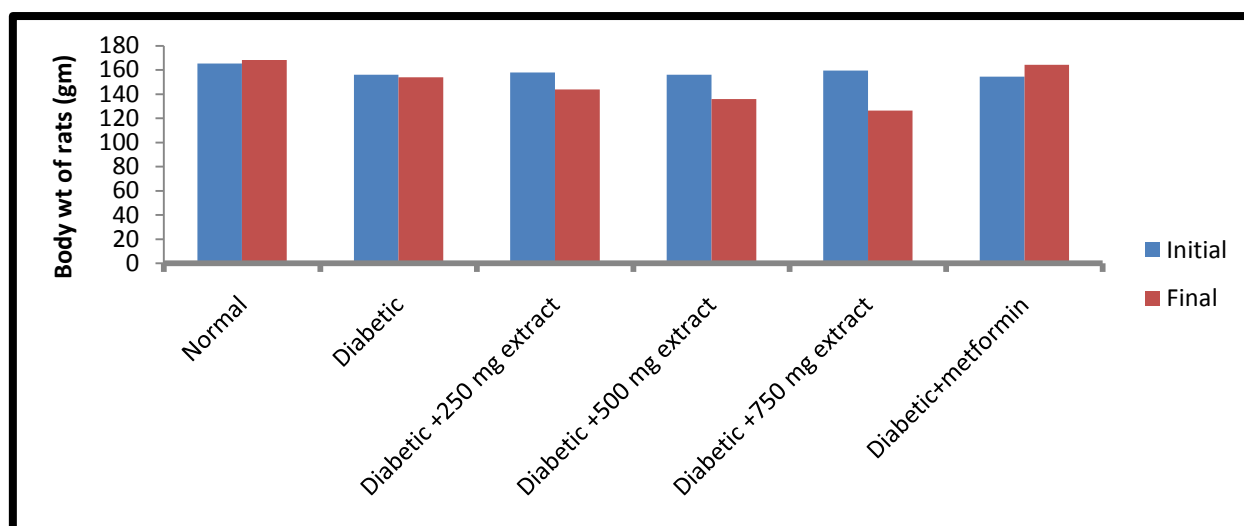


Figure1: Bar chart depicting comparative changes in blood glucose of rats before and after treatment with hydroalcoholic leaf extract of *P. cineraria* and metformin

Table 2: Effect of Hydroalcoholic extract of *P. cineraria* on body weight of HFD-STZ induced diabetic rats after a prolonged treatment

Groups	Body weight (g)	
	Initial	Final
Normal Control	163.28±8.40	172.38±9.85
Diabetic Control	148.05±9.70	142.07±12.72
Diabetic + PC1	154.6±11.72	139.01±10.32
Diabetic + PC2	152.2±13.50	137.07±15.80
Diabetic + PC3	159.07±11.52	128.57±13.50
Diabetic+Metformin	143.4±10.56	157.45±13.89

Values are expressed as means ± SD (n=6 rats)

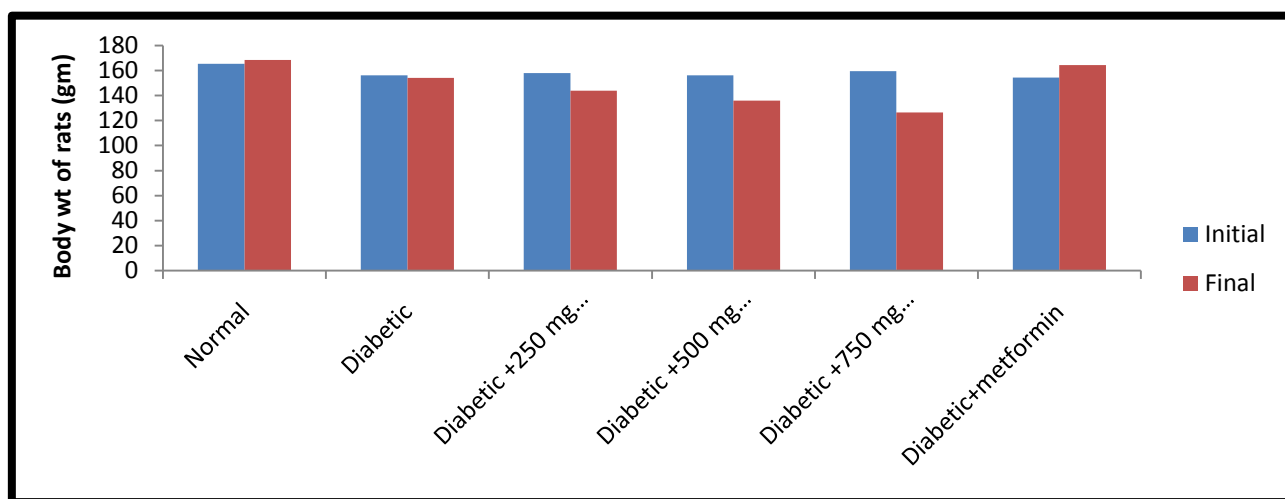


Figure 2: Bar chart depicting comparative changes in body weight of rats before and after treatment with leaf extracts of *P. cineraria* and metformin

Table 3: Effect of Hydroalcoholic extract of *P. cineraria* on food intake of HFD-STZ induced diabetic rats.

Groups (g/day)	Food intake(g)	
	Initial	Final
Normal Control	16.0 ± 0.5	18.3 ± 0.7
Diabetic Control	15.42 ± 1.62	16.12 ± 1.09
Diabetic + PC1	15.0 ± 0.2	14.71 ± 1.88
Diabetic + PC2	15.71 ± 1.39	13.52 ± 1.48
Diabetic + PC3	16.0 ± 0.5	13.14 ± 0.82
Diabetic Metformin	15.92 ± 1.22	18.25 ± 1.43

Values are expressed as means ± SD (n=6 rats)

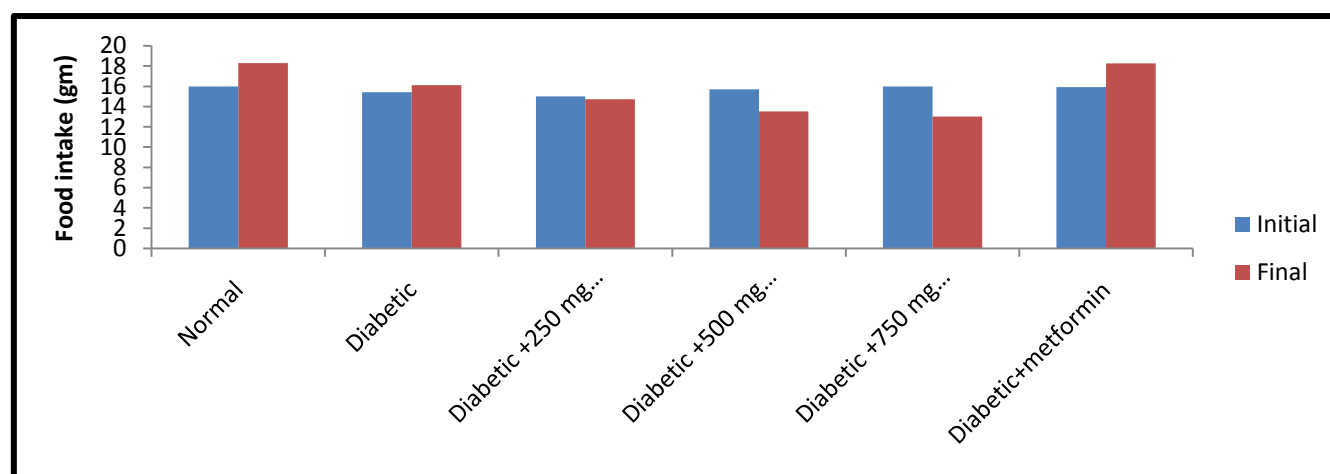


Figure 3: Bar chart depicting comparative changes in food intake of rats before and after treatment with leaf extract of *P. cineraria* and Metformin

Table 4: Effect of Hydroalcoholic extract of *P. cineraria* on lipid profile of HFD-STZ induced diabetic rats after a prolonged treatment

Lipid profile	Groups					
	Normal Control	Diabetic Control	Diabetic+ PC1	Diabetic + PC2	Diabetic + PC3	Diabetic+ Metformin
TC	31.9±3.21	52.8±2.75	48.03±2.10	35.22±3.25	24.28±1.07	72.3 ± 1.56
TG	64.9±4.24	105.6±6.84	94.12±3.16	89.23±2.19	53.53±2.04	78.08± 1.13
HDL	26.8±2.89	20.6±2.5	22.21±1.26	29.37±1.79	35.67±3.1	26.7±0.8
LDL	11.6±3.98	19.1±2.58	18.07±2.61	16.26±2.28	13.15±2.75	17.11±0.19
VLDL	13.0±1.25	18.2±2.84	17.41±2.74	15.14±2.16	13.26±2.64	15.21±1.38

Values are expressed as means ± SD (n=6 rats)

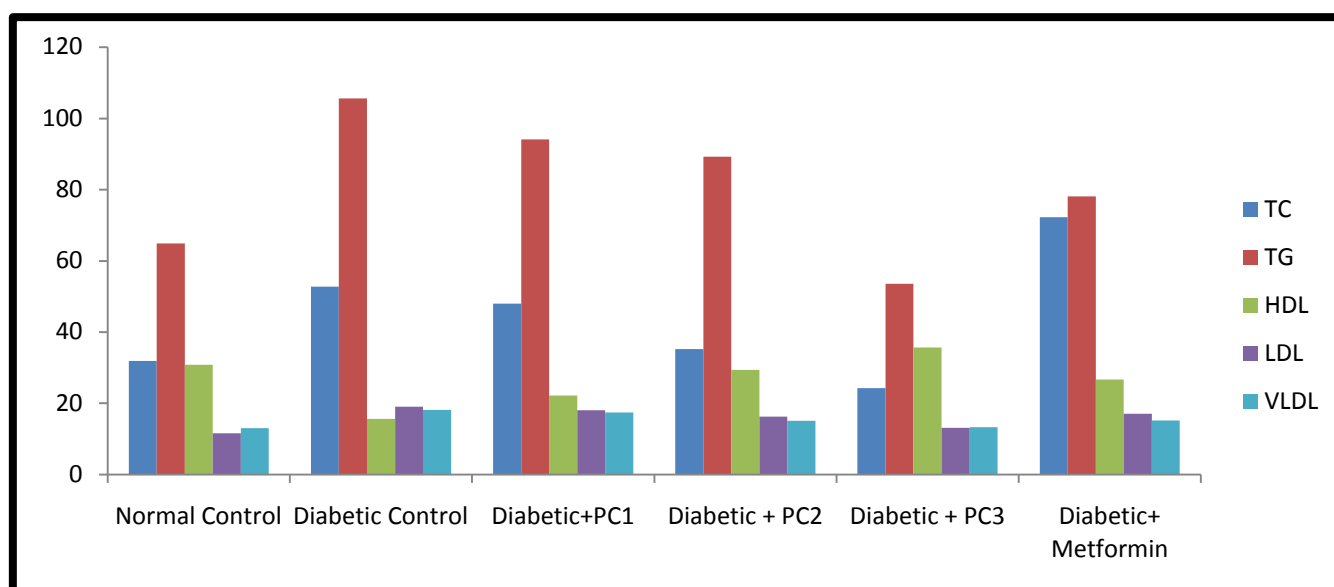


Figure 4: Bar chart depicting comparative changes in serum lipid profile of rats with hydroalcoholic extract of *P. cineraria* on serum lipid profile

Table 1 shows the effect of different concentrations of hydroalcoholic extract of *P. cineraria* and metformin on blood glucose of HFD-STZ induced diabetic rats. As evident from Table 1 and Figure 1 significant fall in blood glucose levels has been observed in case of Group 3c i.e. in case of rats treated with 750/kg/day of hydroalcoholic extract of *P. cineraria*. Table 2 and Figure 2 clearly depicts that weight loss was found to be maximum in case of Group 3c i.e. in case of rats treated with 750/kg/day of hydroalcoholic extract of *P. cineraria*. Table 3 shows the Effect of Hydroalcoholic extract of *P. cineraria* and metformin on food intake of HFD-STZ induced diabetic rats. As evident from Table 3 and Figure 3 maximum food intake in Group 3c i.e. in case of 750 mg/kg/day. Table 4 shows the effect of hydroalcoholic extract of *P. cineraria*

and metformin on lipid profile viz., total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, very low density lipoprotein of HFD-STZ induced diabetic rats. Table 4 and Figure 4 clearly depict the levels of TC, TG, HDL, LDL and VLDL in the experimental rats. There was significant increase in lipids except HDL in diabetic control rats ($p < 0.05$). *P. cineraria* leaf extract and metformin significantly reduced the lipid levels whereas HDL was significantly increased ($p < 0.05$).

Discussion

The present results indicated significant decrease in body weight and raise in blood glucose levels in diabetic rats and they became normal when treated with the plant

extract. This suggests that the plant *Prosopis cineraria* has protective role in reducing glucose levels as well as in increasing body weight. Administration of the hydroalcoholic extract of PC of dose 750 mg/kg caused statistically highly significant decrease in the blood glucose levels of STZ induced diabetic rats as compared to the normal control. Chronic administration of the aqueous extract of PC for 12 weeks in diabetic rats caused significant increase in the serum insulin levels of all groups, indicating that these fractions may probably activate the surviving β -cells of the islets of Langerhans and revert them to the normal state i.e. an insulinogenic effect. The decrease in body weight observed in the diabetic control group in our study may be attributed to due to increase in muscle glucose uptake which results in preventing tissue loss.¹⁷ A significant increase was observed in the body weight of rats treated with the aqueous extract of PC after the completion of 12 weeks as compared to the standard drug metformin.¹⁸ Hence, it can be postulated that PC does not have any effect on the degradation of depot fat but it probably maintains the body weight in the type II diabetic state by its protective effect in controlling muscle wasting.

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.¹⁹ Faulty glucose utilization causes hyperglycemia and mobilization of fatty acids from adipose tissue for energy purpose.²⁰ The lipid changes associated with diabetes mellitus are attributed to increased flux of free fatty acids into the liver secondary to insulin deficiency/ resistance.^{21, 22} This results in excess fatty acid accumulation in the liver, which is converted to triglycerides.^{19, 21} The impaired ability of insulin to inhibit free fatty-acid release leads to elevated hepatic VLDL-cholesterol production.²³ 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.²⁴ In our study, elevated levels of serum TC, TG, LDL and VLDL-cholesterol and decreased HDL cholesterol concentration in streptozotocin-induced diabetic mice are in accordance with the previous research findings.²⁵ However, treatment with *P. cineraria* bark extract normalized all the lipid profile parameters. This antihyperlipidemic attribute of leaf extract may also be attributed to insulin potentiating effect and can be correlated with a previous report.²⁶ From the above results, it can be concluded that hydroalcoholic extract of *Prosopis cineraria* has dose dependant effect

with higher dose showing significant decrease in the elevated blood glucose, cholesterol, TG's and thereby increasing the HDL levels in diabetic rats, and hence *Prosopis cineraria* may be effectively active against Diabetes mellitus. However, more studies such as isolating the active constituents of the compound are needed to understand the exact mechanism of *Prosopis cineraria* on diabetes mellitus disease.

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