Research Article

Evaluation of Antidiabetic Activity of *Prosopis cineraria* Bark Extract on Albino Wistar Rats

Anshika Gupta[1], Gunjan Sharma[1], Md. Umama Ashhar[1], Kanika Sharma[2], S.S. Agrawal[3]
[1]Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh, India.
[2]Department of Pharmacology, Faculty of Pharmacy, Jamia Hamdard, Delhi, India.
[3]Delhi Pharmaceutical Sciences and Research University, Delhi, India.
***Corresponding author's E-mail:** delhigunjan@gmail.com

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ABSTRACT

Diabetes mellitus is a major public health problem prevailing all over the globe. The global increase in the prevalence of diabetes is due to population growth, aging, urbanisation and an increase of obesity and physical inactivity. In the present study, the potential roles of hydroalcoholic bark extract of *Prosopis cineraria* (khejri) in reducing the Blood Glucose level on Streptozotocin-induced diabetic albino Wistar rats. Thirty-six male albino rats weighing 125-200 gms body weight (BW) which was divided into six groups (A, B, C, D, E and F). Group A served as Normal control, Group B as Diabetic control, Group C as Standard control, Group D is treated with crude *Prosopis cineraria* bark extract at dose 200 mg/kg, 400 mg/kg and 600 mg/kg body weight daily per orally for 30 days. Diabetes is induced with Streptozotocin 60 mg/kg (IP) and Glimepiride is used as the standard at dose 2 mg/kg. Hypoglycemic activity of the extracts were studied and compared with a reference antidiabetic drug Glimepiride. The results were also compared with normal control and diabetic control group. Different parameters like body weight, blood glucose, cholesterol and lipid profile was measured in all six groups.

Keywords: Anti-diabetic activity, *Prosopis cineraria*, Streptozotocin, Blood glucose level, Diabetes mellitus.

INTRODUCTION

Diabetes Mellitus

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. It is a major public health problem now-a-days[1]. The prevalence of diabetes is rising rapidly worldwide due to increased food consumption, decreased physical activity and widespread embrace of a western lifestyle[2]. In the whole world, 150 million people are suffering from diabetes. The International Diabetes Federation (IDF) estimates the total number of people in India with diabetes to be around 50.8 million in 2010, rising to 87.0 million by 2030. India is declared as "Diabetes capital of the world". According to the World Health Organization (WHO) criteria, the prevalence of known diabetes was 5.6% and 2.7% among urban and rural areas, respectively in india[3].

Animals used in biomedical research helps us to understand how our bodies work to find cures and treatments for diseases, to test new drugs for safety and to evaluate medical procedures before they are used on people.

Rodents are mainly used for many studies because they are small in size, easy to handle, relatively inexpensive to buy and keep and produce many offspring in a short period of time[4].

Experimental induction of diabetes mellitus in animal models is essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure.

Diabetes is induced in albino *wistar* rats using Alloxan or Streptozotocin. In the present study, Streptozotocin (STZ) which is [2-deoxy-2-(3-methyl-3-nitrosourea) 1-D-glucopyranose] is a broad-spectrum antibiotic, which is produced from *Streptomyces achromogens*.

Using 60mg/kg Streptozotocin dose can begin an autoimmune process that results in the destruction of the Langerhans islets beta cells and results in the toxicity of beta cells with emergence of clinical diabetes within 2-4 days[5].

Hence, the present study was aimed to evaluate the antidiabetic potential of *Prosopis cineraria* on streptozotocin-induced diabetic albino *Wistar* rats.

Prosopis cineraria (KHEJRI)

Prosopis cineraria belong to family Fabaceae. It is small to moderate sized tree commonly called as "Queen of Desert".

It is an important tree (Khejri- a local name in Rajasthan) for the Thar Desert with hard climatic adaptation and one of the lifelines in desert habitat as mentioned in ancient literature.

It is distributed in the arid and semi-arid regions of India, Afghanistan, Pakistan, Iran and Arabia. Since all parts of the tree are useful, it is called 'Kalptaru'. The plant contains patuletin glycoside patulitrin, 10 sitosterol, spicigerine, Flavone derivatives ProsogerinA, Prosogerin

B, Prosogerin C, Prosogerin D, Prosogerin E, 3-benzyl-2-hydroxy-urs-12-en-28-oic acid.

It is used to treat various diseases. The flowers are pounded mixed with sugar and used during pregnancy as a safeguard against miscarriage[6]. The bark is dry, acrid, bitter, with sharp taste; cooling anthelmintic, tonic; cures leprosy dysentery, asthma, leucoderma, piles tremors of the muscle, wandering of the mind. Extract of crushed pods is used for earache, toothache, pain relief from fractured bones[7]. Aqueous extract of bark and leaves applied externally to treat skin disease disinfects wounds and promotes healing[8]. Extensive research has revealed that the plant has a wide range of pharmacological activities. Antibacterial[9], anti-depressant and skeletal muscle relaxant[10], hypercholesterolemic, hypolipidemic and antiatherosclerotic[11], spasmodic and bronchodilator[12], nootropic activities[13], antitumour[14].

***Prosopis cineraria* in Diabetes**

A study reports that *Prosopis cineraria* bark extract exert hypoglycaemic activity in normal and alloxan induced diabetic rats orally and prevents glucose absorption from the gut. This ant hyperglycemic action may be due to insulin potentiating effect via stimulation of the undamaged or residual pancreatic islets to release insulin. Apart from controlling hyperglycemia it is also beneficial in the alleviation of associated diabetic complications including the prevention of the development of atherosclerosis and other coronary artery diseases[15].

One more study reports that *Prosopis cineraria* leaves extract exert antihyperglycemic and antihyperlipidemic activity in streptozotocin-induced diabetic rats.

Administration of the hydroalcoholic extract of test drug 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.

It can be concluded that hydroalcoholic extract of *Prosopis cineraria* has dose dependant effect with higher dose showing the significant decrease in the elevated blood glucose, cholesterol, TG's and thereby increasing the HDL levels in diabetic rats.[16]

Aim and Objective

The aim of the present study is to evaluate and compare the anti-diabetic activity of hydroalcoholic extract of the bark of *Prosopis cineraria* against streptozotocin-induced diabetic rats.

- Preparation of hydroalcoholic extract of *Prosopis cineraria* crude bark.
- Induction of diabetes in albino wistar rats by administration of streptozotocin (60 mg/kg i.p.)
- Evaluation of antidiabetic activity of Prosopis cineraria bark extract against streptozotocin induced diabetic rats by parameters like blood glucose level, body weight, food intake and lipid profile.

MATERIALS AND METHODS

Plant Material

- The bark of *Prosopis cineraria* (Khejri) were obtained from Sikar, Rajasthan, India for research work.

Drugs

- Streptozotocin (1g) purchased from Santa cruz biotechnology, STZ- (U9-9889), stored at -20°C.
- Marketed preparation of Glimiperide was purchased from Ajay medical store, sec- 18, Noida.

Experimental Design

Male albino wistar rats weighing 180-200 gms were procured from animal house of Amity Institute of Pharmacy (AIP); Amity University Noida, U.P. India. The protocol was approved by the Animal Ethical Committee (Protocol No. CPCSEA/AIP/2013/02/003). All animals were housed in polypropylene cages lined with husk. It was renewed every 24 hours under a 12:12 hour light: dark cycle at room temperature. They were fed with standard animal feed pellets (M/s Ashirwaad feed; Chandigarh, India) and water *ad libitum.*

Preparation of Prosopis Cineraria Bark Extract

The bark was dried in hot air oven at 60°C for approximately 3 hours, broken into small pieces and then grinded into coarse powder. The coarse powder of bark of *Prosopis cineraria* was kept in thimble and extracted with 50% ethanol in a soxhlet apparatus. The hot extraction process was continued for about 24 hrs at a temperature not exceeding 60 degrees. The hydroalcoholic extract was then concentrated using water bath at 60°C and complete drying was done in hot air oven. The extract was then stored in air-tight container at room temperature.

Induction of Diabetes Mellitus

In the present study, a total of 36 rats (n=6) were taken.

Group I: Normal control rats

Group II: Diabetic control rats

Group III: Diabetic rats were given standard drug Glimepiride (2 mg/kg body weight)

Group IV: Diabetic rats were given extract of Prosopis cineraria (200 mg/kg body weight)

Group V: Diabetic rats were given extract of Prosopis cineraria (400 mg/kg body weight)

Group VI: Diabetic rats were given extract of Prosopis cineraria (600 mg/kg body weight)

Albino wistar rats were fasted for 18 hours before the experiment and their weight and blood glucose level was measured. Rats were then made diabetic by the administration of single intraperitoneal injection of streptozotocin (60 mg/kg). Streptozotocin was first weighed individually for each animal group and then

dissolved in 0.1 M freshly prepared ice cold citrate buffer prior to injection. 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. The development of diabetes was confirmed after 48hrs of the Streptozotocin injection. The animal having fasting blood glucose levels more than 250mg/dl were selected for the experimentation.

All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages. All the animals were regularly observed for their general behaviour.

Preparation of Test Materials

In order to administer the crude extract, 200, 400 & 600 mg/kg of the extract was measured and was triturated unidirectional way by proper mixing of extract with normal saline (0.1%) in 20% tween 80 and then slowly added normal saline to make up the volume. To prepare the standard, Glimepiride 2 mg/kg was dissolved in distilled water and made up the volume. Normal saline (0.1%) in 20% tween 80 was given in control group.

Sampling

The Blood sample was collected from tail vein (10 µl) to determine blood glucose level on 0, 4, 7, 14, 21 and 28 days. A standard Accu-check glucometer was used to determine the glucose in blood sample. Body weight was measured using the weighing balance on 0, 4, 8, 12, 16, 20, 24, 28, 32 days of the study. The blood sample collected from retro-orbital puncture was used to estimate the lipid profile before and after the treatment.

Data Analysis

Results were expressed as Mean ± SEM (Standard error mean) for six rats in each experimental group. Data was statistically evaluated by use of one-way analysis of variance (ANOVA), followed by group mean comparison by student's t-test using Sigma plot software. The values were considered to be significant if $p<0.05$ was obtained.

RESULTS

Changes in Blood Glucose Level

The effect of Prosopis cineraria on blood glucose level is reported in Table 7. In normal control group only 0.1 M citrate buffer was given but no streptozotocin. But, still there was little increase in blood glucose level that may be due to increase in food consumption. In Diabetic control group blood glucose level increased day by day and raised above 400 after the 4th day of STZ injection. But in all other groups blood glucose level rise up to 4th day and then decreased because of continuous treatment. When compared to diabetic control, all the treated groups show significant results. Results were given as Mean ± SEM of six rats in each group.

The results indicate that after 30 days of treatment with glimepiride shows 42.63% significant reduction in while *Prosopis cineraria* 200, 400 and 600 mg/kg shows 18.24%, 27.76% and 30% significant reduction in blood glucose level when compared with the diabetic group.

Changes in Body Weight

The results of changes in body weight with the effect of *Prosopis cineraria* and glimepiride are reported in Table 8. % Change 1 shows changes in body weight between day 0 and day 4. In this diabetic control group and standard group shows -19% and -4.02% decreases in body weight. While other groups show increase in body weight after treatment with Prosopis cineraria at different doses. % Change 2 shows changes in body weight between day 4 and day 35. During this period, diabetes control group shows more reduction in body weight. Figure shows the graphs of changes in body weight from day 0-35.

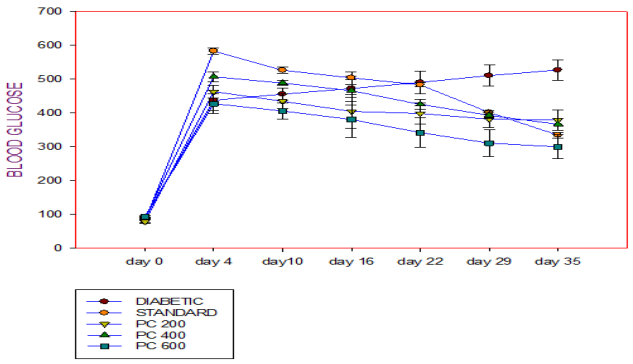

Figure 1: Blood glucose level comparison of rats in diabetic and standard and test drug treated rats

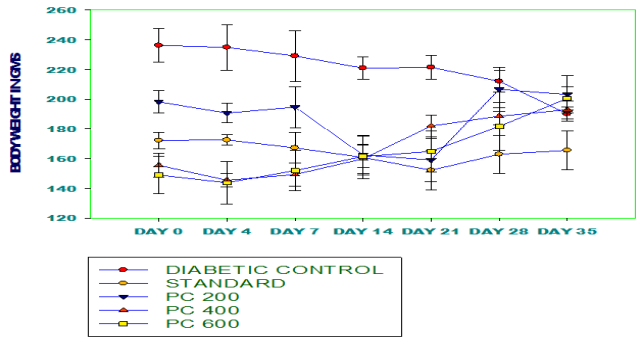

Figure 2: Body weight of comparison of rats in diabetic, standard and drug treated rats.

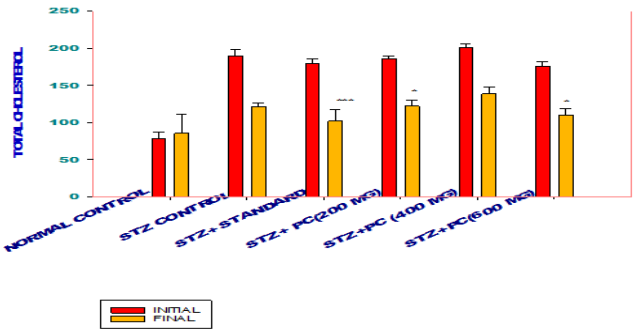

Figure 3: Total Cholesterol Level

Values are expressed as Mean ± SEM (n=6). Data was analysed by one-way ANOVA followed by Dunnett's test; *$p<0.05$ is considered significant; ***$p<0.001$ is considered significant

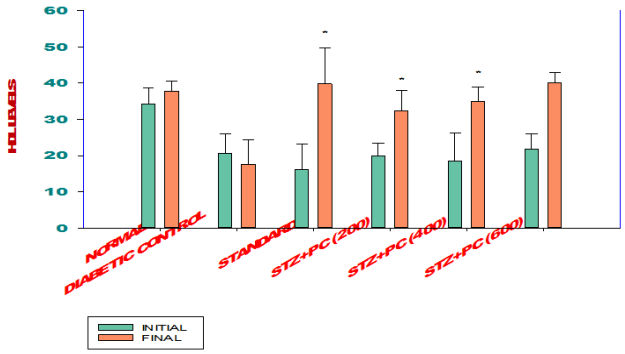

Figure 5: HDL Level

Values are expressed as Mean ± SEM (n=6); Data was analysed by one-way ANOVA followed by Dunnett's test; *p<0.05 is considered significant

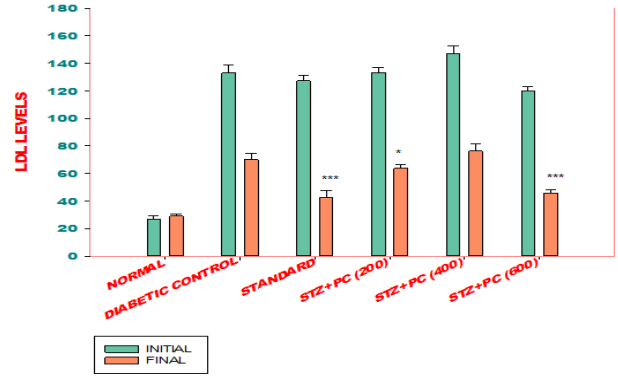

Figure 6: Total LDL Level

Values are expressed as Mean ± SEM (n=6); Data was analysed by one-way ANOVA followed by Dunnett's test; *p<0.05 is considered significant; ***p<0.001 is considered significant.

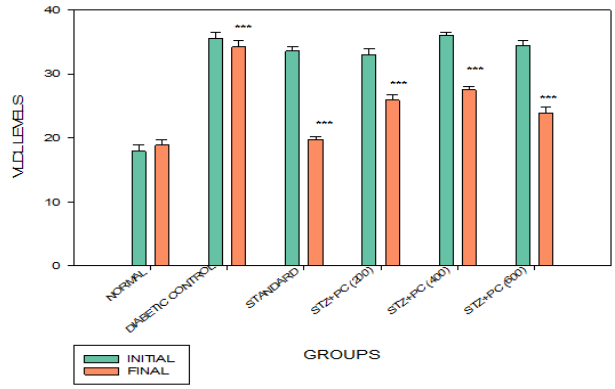

Figure 7: Total VLDL Level

Values are expressed as Mean ± SEM (n=6), Data was analysed by one-way ANOVA followed by Dunnett's test,***p<0.001 is considered significant

Lipid Profile

The results of changes in lipid profile with the effect of *Prosopis cineraria* were reported in Table 9-13. After the induction of diabetes, there was an increase in total cholesterol, triglycerides, LDL and VLDL level while there was a decrease in HDL level. The lipid changes associated with diabetes mellitus are attributed to increased flux of free fatty acids into the liver secondary to insulin deficiency/resistance[17]. This results in excess fatty acid accumulation in the liver, which is converted to triglycerides[18].

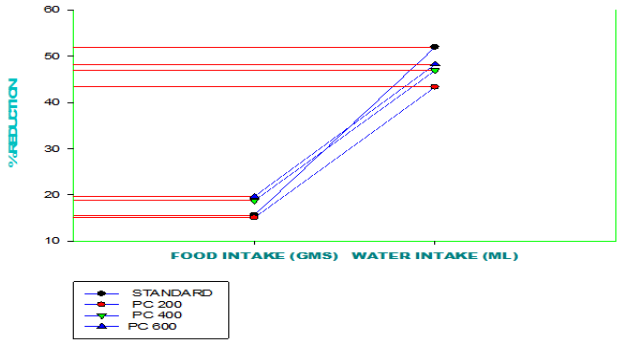

Figure 8: Total Food and Water Intake

The impaired ability of insulin to inhibit free fatty-acid release leads to elevated hepatic VLDL-cholesterol production[19]. 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[20].

After the treatment with standard and test drug, there was decrease in total cholesterol, triglycerides, LDL and VLDL levels while there was increase in HDL levels.

The study conducted for evaluation of the potential of plant extracts, clearly demonstrate that the test drugs *Prosopis cineraria* may have beneficial effects for the prevention and management of diabetes.

The reasons may be attributed to their antihyperglycemic, anti-inflammatory, antioxidant and antihyperlipidaemic properties. Physical and biochemical results confirmed that test drug was most potential and possessed synergistic actions and dose-dependent effect for prevention and management of diabetes against streptozotocin-induced diabetes.

Food and Water Consumption

Polyphagia, polydipsia and polyuria are the important indications of diabetes. In our study average food and water consumption per day per rat was measured along with the body weight of the animals. High consumption was observed in the diabetic control group but their body weight significantly reduced as compared with other groups. Induction of diabetes by streptozotocin leads to body weight loss due to increased muscle wasting and loss of tissue protein. Also in the diabetic condition the body is either not able to produce insulin or is resistant to it, so the glucose cannot be processed. In either case, the cells do not get enough glucose and become exhausted. Essentially body expresses its need for glucose hunger and thus polyphagia.

Glucose build up in the blood stream is expelled in the form of frequent urination dehydrating the body, which leads to polydipsia.

DISCUSSION

The present study demonstrated that Hydroalcohlic extract of the bark of *Prosopis cineraria* holds significant anti-diabetic activity. Diabetes is described as the increase in blood glucose level while chronic elevation of blood glucose level leads to damage of blood vessels causing various serious complications. Therefore, this plant has been considered for investigation on different parameters related to diabetes including body weight, food & water intake, blood glucose level, total cholesterol, triglycerides, HDL, LDL and VLDL levels.

Induction of Diabetes Mellitus

Experimental induction of diabetes mellitus in animal models is essential for the advancement of our knowledge and understanding of various aspects of its pathogenesis and ultimately finding new therapies and cure. Several methods have been used to induce diabetes in laboratory animals. In the present studies, diabetes is induced in rats using Streptozotocin which is a synthetic anti-neoplastic agent and induces “chemical diabetes” in a wide variety of animal species thus it is an effective diabetes induction agent. It has been widely used to induce diabetes mellitus in experimental animals models allowing investigation of hypoglycemic agents in the treatment of diabetes.

In the present study, Albino wistar rats were fasted for 18 hours before start of paste experiment. Their body weight and blood glucose level was measured and then single intraperitoneal injection of streptozotocin was given at dose of 60 mg/kg dissolved 0.1 M citrate buffer[21], except in normal rats. The blood glucose levels of rats were raised due to permanent destruction of β-cells. Changes in blood glucose level were recorded for all the groups on 4th day, which shows that the blood glucose level rise in all animals as compared to normal control. This indicated induction of diabetes in streptozotocin-induced animals.

CONCLUSION

From the present preliminary study, it can be concluded that the extract of bark of *Prosopis cineraria* possessed dose-dependent anti-hyperglycemic and anti-hyperlipidaemic effect in the experimental rodent models demonstrating its prominent action on the β-cells, 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. Moreover, additional parameters such as assay of insulin, HbA1c etc should also be studied. 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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