



INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

EVALUATION OF ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC ACTIVITY OF *PROSOPIS CINERARIA* (LINN.)

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Accepted Date: 23/06/2015; Published Date: 27/06/2015

Abstract: The present study was carried out to investigate the Antihyperglycemic Potential of *Prosopis cineraria* (Linn.) Druce. Phytosomes with Crude Extract in Streptozotocin (50 mg/kg intraperitoneal) induced diabetic rats for 12 weeks. The streptozotocin induced diabetic male wistar rats were fed with (PCM) of methanolic extract of *Prosopis cineraria* leaves at the increasing dosage of 200, 300 and 400 mg/kg. Positive control group was receiving pioglitazone 100 mg/kg/day, per oral for 12 weeks. Treatment of streptozotocin induced diabetic wistar rats with the extract caused a significant ($P < 0.05$) in the serum levels of the total cholesterol, triglycerides and a significant increase ($P < 0.05$) in HDL level. The dose of 300 mg/kg showed maximum significant decrease ($P < 0.05$) as compared to other two doses. This result suggests that the PCM possess antidiabetic effect on streptozotocin induced diabetic Wistar rats.

Keywords: *Prosopis cineraria*, methanolic extract, Antihyperglycemic, Antihyperlipidemic, Streptozotocin



PAPER-QR CODE

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Access Online On:

www.ijprbs.com

How to Cite This Article:

Sangeeta Asija, IJPRBS, 2015; Volume 4(3): 458-466

INTRODUCTION

Medicinal plants play a key role in the human health care. About 80% of the world population relies on the use of traditional medicine which is predominantly based on plant materials^{1,2}. Novel drug delivery system is a novel approach to drug delivery that addresses the limitations of the traditional drug delivery systems. Our country has a vast knowledge base of Ayurveda whose potential is only being realized in the recent years³⁻⁴.

Diabetes mellitus is a manifestation of metabolic disturbances that affect human body in terms of physical, psychological and social health due to improper regulation of homeostasis of carbohydrate and lipid metabolism by insulin⁵.

The increasing burden of diabetes worldwide is well-known, and its occurrence and consequences are highly observed in the developed as well as developing countries. The International Diabetes Federation (IDF) estimates that in 2013, 382 million people suffered from diabetes while and this prevalence will rise to 592 million by 2035. Diabetes caused 5.1 million deaths in 2013 and every six seconds a person dies from diabetes.

Prosopis is a genus of flowering plants in the pea family, Fabaceae. It contains around 45 species of spiny trees and shrubs found in subtropical and tropical regions of the Americas, Africa, Western Asia, and South Asia.

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'.

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. It has analgesic and antipyretic activities. It is also used as a remedy for rheumatism. 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. Numerous bioactive compounds such as flavonoids, alkaloids, diketones, phenolic contents free amino acids, patulitricin, 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 plant⁶⁻¹⁰.

Materials and Methods

The whole plant was collected from Jhotwara, Jaipur (Rajasthan, India) in the month of March 2010. The identity of the collected plant was confirmed by Dr. Narendra K Patel, Plant taxonomist and ethnobotanist in Hemchandracharya North Gujarat University, Patan (Gujarat, India). The Herbarium of the plant was deposited in the BSI against voucher specimen no. **MNSC/Bot/Idnt/Spec/2001**.

Preparation of extract

About 100 gm of the coarsely powdered, air-dried plant material was extracted successively with n-hexane, ethyl acetate and methanol using Soxhlet apparatus. Finally, the marc left was extracted with water under reflux. The solvent was removed by concentrated in a rotary evaporator and water bath. The dried extracts were stored in refrigerator until further studies. The color and the percentage yield of the extracts are presented in results section.

Preliminary phytochemical screening

The extracts were preliminary investigated for various phytochemical constituents such as Alkaloids, Carbohydrates, Steroids, Proteins, Phenols, Tannins, Flavonoids, Glycosides and Saponins¹¹⁻¹³.

Animals

Albino Wistar rats weighing 150-200 g were procured. They were maintained in essential condition of controlled temperature (<30° C) and humidity (< 70%) with 12 h day and night cycle according to the norms of CPCSEA. Each rat was housed in plastic box cage individually and had free access to autoclaved, untreated tap water and standard rat chow. Animals were used as per the protocol approved by the Institute Animal Ethics Committee. Animals described as fasted were deprived of food for 16 h but had free access to water.

Experimental design

As per the objective, it is required to produce the type 2 diabetes by administering the STZ in obese animals. The animals which are selected for the induction of type 2 diabetes shall possess high level of cholesterol and triglycerides. For achieving the high lipid profile level the rats were fed on high fat diet. After 30 days of high fat diet, blood glucose and lipid profile measurement were carried out. Those found with sufficient rise in cholesterol and triglyceride level were selected for the STZ administration. Forty eight hours after STZ administration blood samples were withdrawn by retro-orbital puncture and glucose levels determined by using a one touch Glucometer to confirm diabetes. The rats exhibiting blood glucose levels in the range of 275

and 350 mg/dl were considered diabetic and were selected for the studies. The diabetic rats were divided randomly into groups for treatment as per study protocol. The dose of PC extracts and were determined on the basis of toxicity studies and glucose tolerance test while the dose of pioglitazone was calculated from human dose.

Composition and preparation of high fat diet

High fat diet prepared by mixing the following ingredients- Powdered NPD (600 gm/kg), Coconut oil (200 gm/kg), Casein (40 gm/kg), Cholesterol (5 gm/kg), Vitamin and mineral mix (50 gm/kg), Fructose (25 gm/kg), Sucrose (25 gm/kg), dl-Methionine (03 gm/kg), Sodium chloride (02 gm/kg). Diets used in this study are given ad libitum throughout the experiment. Food was withdrawn 5 hours before blood sampling on day 40. Rats had free access to water throughout the study. Rats were weighed at the beginning of the study and then weekly till the end of the study.

Acute toxicity studies

Healthy adult Wistar albino rats of either sex, starved overnight were divided into six groups (n = 6), four groups of extract in increasing doses of 100, 500, 1000 and 3000 mg/kg body weight¹⁴. The rats were observed continuously for 2 h and periodically for 72 h for behavioral profile (alertness, restlessness, irritability, and fearfulness), Autonomic profile (defecation and urination), neurologic profile (locomotion, reactivity, touch & pain response), physical state and any lethality or death.

Anti-diabetic activity

Experimental Induction of diabetes

Streptozotocin (STZ) is a synthetic antineoplastic agent classified as an anti-tumor antibiotic and chemically is related to other nitrosoureas used in cancer chemotherapy. Streptozotocin sterile powders are provided and prepared as a chemotherapy agent. Each vial of sterilized Streptozotocin powder contains 1 gram of Streptozotocin active ingredient with the chemical name, 2-Deoxy-2- [(methylnitrosoamino) - carbonyl] amino]-D-glucofuranose and 200 mg. citric acid. Streptozotocin is available for intravenous use as a dry frozen, pale yellow, sterilized product. Pure Streptozotocin has alkaline pH¹⁵⁻¹⁷.

Rats were kept on fasting prior to STZ injection. On the day of administration, STZ was freshly dissolved in 50 mM sodium citrate (pH 4.5) solution containing 150 mM NaCl and given as subcutaneous injection at the dose of 35 mg/kg body weight.

Experimental protocol

Group 1: Normal control rats administered drinking water.

Group 2: Diabetes control rats.

Group 3: Treated with PCM (200 mg/kg).

Group 4: Treated with PCM (300 mg/kg).

Group 5: Treated with PCM (400 mg/kg).

Group 6: Treated with 75% dose of Pioglitazone + PCM (200 mg/kg).

Group 7: Treated with 50% dose of Pioglitazone +PCM (200 mg/kg).

Group 8: Treated with 25% dose of Pioglitazone + PCM (200 mg/kg).

Group 9: Treated with 100% dose of Pioglitazone (5 mg/kg).

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 min¹⁸.

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, formula¹⁹.

Statistical analysis

Data were statistically evaluated using one way ANOVA, expressed as mean \pm S.E.M. followed by post Dennett test using 5.04 trial version of Graph pad prism computer software. $P \leq 0.05$ was considered to be significant.

Results & discussion

The preliminary phytochemical studies indicated the presence of flavonoids, phenolic compound & saponins in the methanolic extract of the. Acute toxicity study shows that the

methanolic 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.

In order to choose the optimum effective dose for the diabetic animals, different doses of PCM (200, 300 and 400 mg/kg) were evaluated on glucose tolerance in normal rats along with the standard drug pioglitazone (5 mg/kg). The rats were treated with the extract and improvement in OGTT was assessed by comparing the blood glucose level (BGL) before and after the treatment. In OGTT, PCM produced dose dependent reduction of BGL. The maximum BGL reduction (32.5%) was observed for 300 mg/kg dose after 120 min. which is comparable to 5 mg/kg PGL (27.9%) (Table 1.1).

It therefore appears that 400 mg/kg of the PCM is the effective dose on BGL and OGTT of STZ-induced diabetic rats. Therefore, this dose was assessed by plant extract in STZ-induced diabetic rats.

Effect on elevated blood glucose level

Table 1.1 The effect of pioglitazone in combination with PCM in elevated blood glucose level

Gr. No.	Groups	0 day	15th day	% reduction	30th day	% reduction	40th day	% reduction
1	NC	138.5±5.63*	135.83±4.66**	1.92%	134±1.78**	3.20%	140.32±2.11**	-1.3%
2	DC	578.5±13.53*	613.5±8.29***	-6.05%	610±6.43***	-5.40%	552.45±14.53**	4.5%
3	PCM (200)	411±12.32	388.17±13.23**	5.60%	335.3±8.03***	18.40%	292.17±7.16***	28.90%
4	PCM (300)	521.33±8.7	498.17±1.93**	4.40%	429.5±0.79***	17.60%	379.17±1.13***	27.20%
5	PCM (400)	451.33±8.40	401.83±10.65**	10.90%	360.5±7.48***	20.10%	284.5±11.24***	36.90%
6	PCM+75%PGL	499.17±9.95	457.33±9.03***	8.30%	375.83±9.20***	24.70%	187.50±7.37***	62.40%
7	PCM+50%PGL	499±14.83	407.17±16.52**	18.40%	297.33±6.50***	40.40%	212.83±4.35***	57.30%
8	PCM+25%PGL	517.67±12.08**	416±16.03***	19.60%	336.83±15.41**	34.90%	271.67±12.02**	47.50%
9	PGL-100%	440.33±19.17**	354±19.42***	19.60%	273.33±11.67**	37.90%	160.32±9.65***	63.50%

Each value represents the Mean ± SEM for each group (n=6)

p<0.001 Normal control Vs Diabetic control ***p<0.001 Diabetic control Vs. Treated group
 **p<0.01 Diabetic control Vs Treated group *p<.05 Diabetic control Vs Treated group
 # p<0.05 Normal control Vs Diabetic control

Values without superscript signs are not significant when comparing with Diabetic control.

Effect on elevated lipids

Significant increase in the values of TC (Total cholesterol) & TG (Triglycerides) observed in the diabetic control group ($p < 0.001$) as compared to the normal control, contrary to this HDL (High density lipoprotein) values are significantly decreased in the diabetic group compared to normal animals. Results are expressed in table 3.21.

Table 1.2: The effect on elevated Lipids.

sr. no	Groups	Total Cholesterol	Triglyceride	HDL
1	NC	179.83±3.70	138.50±5.13	52.17±0.83
2	DC	319.67±4.52 ^{###}	236.33±5.23 ^{###}	38.17±0.76
3	PCM (200 mg/kg)	175.17±1.12 ^{***}	124.33±2.38 ^{***}	40.17±0.96
4	PCM (300 mg/kg)	167.00±1.19 ^{***}	118.17±1.44 ^{***}	43.83±0.56
5	PCM (400 mg/kg)	161.00±1.24 ^{***}	104.67±2.18 ^{***}	46.83±1.12
6	PCM+75%PGL	138.67±2.56 ^{***}	78.33±2.11 ^{***}	52.17±0.78
7	PCM+50%PGL	144.00±2.71 ^{***}	93.67±1.21 ^{***}	48.17±0.69
8	PCM+25%PGL	168.00±1.51 ^{***}	103.33±1.70 ^{***}	45.17±0.92
9	PGL-100%	142.17±1.13 ^{***}	98.17±1.44 ^{***}	56.67±0.83*

Each value represents the Mean ± SEM for each group (n=6)

$p < 0.001$ Normal control Vs. Diabetic control *** $p < 0.001$ Diabetic control Vs. Treated group
* $p < .05$ Diabetic control Vs. Treated group ** $p < 0.01$ Diabetic control Vs. Treated group

CONCLUSION

In conclusion, results from the present investigations tend to indicate that PCM inhibits the uptake of d-glucose, and l-tyrosine. It is likely that PCM possess bioactive phytochemicals capable of lowering blood glucose levels by inhibiting the uptake of glucose across the intestine and at same time increasing metabolism of glucose in cells. Our study tends to validate the ethnobotanical use of PCM in traditional medicines and by adding the pioglitazone strengthened the use of herbal therapy in diabetes by minimizing side effects of synthetic drugs as well as the cost of the therapy and ensuring the better results for longer duration.

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