

Antidiabetic Activity of Methanolic Fruit Extracts of *Prunus Armeniaca* Linn in Streptozotocin - Induced Diabetic Rats

Deepak Shrivastav¹, Gagandeep Kaur², Dr. Suman Lata³

Amar Shaheed Baba AjitSingh Jujhar Singh Memorial College of Pharmacy, Bela, Ropar, Punjab, India, 140111

Abstract: To characterize and screening of diabetes preventive effect of methanolic extract of *Prunus armeniaca* Linn after exposure to streptozotocin. Methanolic extract of *Prunus armeniaca* Linn was prepared using soxhlet apparatus and was characterized using ultraviolet spectroscopy, infra-red spectroscopy, nuclear magnetic resonance spectroscopy and mass spectroscopy. Diabetes preventive activity of methanolic extract was evaluated by giving extract to healthy Wistar rats for 18 days followed by administering streptozotocin on 19th day by intra peritoneal route along with oral administration of extract at selected doses for additional 72 hours. Glycaemic parameters were evaluated to check the glycaemic controlling effect of extracts at selected doses. In dose dependent manner methanolic extract of *Prunus armeniaca* Linn results in statistically significant reduction in fasting plasma glucose, glycated haemoglobin and urinary glucose levels. Dose dependent glucose lowering effect was also observed in Oral Glucose Tolerance Test. Methanolic extract of *Prunus armeniaca* Linn offers significant protection against streptozotocin induced diabetes.

Keywords: Anti-diabetic activity, Methanolic extract, *Prunus armeniaca*, Streptozotocin

1. Introduction

The term "diabetes mellitus" describes a disorder of chronic hyperglycemia with disturbances of fat and macromolecule metabolism ensuing from defects in hormone action, hormone secretion or both the consequences of Diabetes mellitus embody long-term harm, pathology and failure of varied organs (WHO 1999). The chronic hyperglycemia of diabetes is associated with dysfunction, Long term damage and failure of different organs especially the eyes, nerves, heart, kidneys and blood vessels. These range from autoimmune damage of the pancreatic b-cells with consequent insulin deficiency to defects that result in resistance to insulin action [1], [2], [3].

Apricot is botanically known as *Prunus armeniaca* Linn belongs to family rosaceae, subfamily- *rosidae* and order *rosales*. In latin word it is known as "precocious" or "early ripening. It is also called as khubani. The fruit ripens in the end of July to mid of august. Large quantity of apricot fruit is exported from France. The genus *Prunus* includes about 430 species which occur naturally in temperate regions. Due to their anaesthetic and organoleptic properties the stone of apricot fruit plays a very important role. Apricot also includes sugars and acids due to which it contains phytochemicals in a very low concentration. The literature survey indicates that no work has previously been done to evaluate the antidiabetic effect of *Prunus armeniaca* Linn fruits. Therefore, the present study evaluates the antidiabetic activity of the methanolic extract of *Prunus armeniaca* Linn. fruit in the different models of rats to ascertain the scientific basis for the use of these plants in the treatment of diabetes [4], [5], [6].

2. Material and Methods

2.1 Collection and authentication of plant material

The fruit of *Prunus armeniaca* Linn. was purchased during November- December 2018 from Bela chowk, (Punjab, India). The fruits were identified and authenticated by Dr. Sunita Garg, Head of raw material, Herbarium and museum at NISCAIR CSIR (National Institute of Science Communication and Information Resources), New Delhi 110067. A voucher specimen no. (Ref. No. NISCAIR/RHMD/Consult/2018/3229-30) was deposited in the same herbarium.

2.2 Extraction

The dried fruit material of (*Prunus armeniaca* Linn.) was made coarsely powdered after slicing and drying under shade. Bymaceration method, the powdered fruit extract (100 gm) was added into 200 ml of *n*-Hexane solvent in RBF (500 ml) and allowed to stand with occasional shaking for 24 hrs. Filtered it and filtrate was discarded. No material was found. By soxhlet apparatus method, Marc was dried and then it was packed into soxhlet apparatus and extracted successively with 200 ml of methanol as solvent on water bath. The methanol filtrate was concentrated and dried with distillation apparatus. The yield was found 1.21 gm. The extract was stored at cool temperature and used for subsequent experiments [7], [8].

2.3 Preliminary phytochemical screening

Preliminary phytochemical screening was revealed about presence of the active constituents in methanolic fruit extract such as, Carbohydrates, Minerals, flavonoids, Phenols, Polyphenols, Volatile compound, and Glycosides,

B-Carotene, Vitamin B1. Some compositional properties of main Malatya apricot (*Prunus armeniaca* L.) varieties [9], [10].

2.4 Animals

Albino *Wistar* Rats of either sex between 2 and 3 months of age, with weight ranging 220- 250 gm were used for the study, which were obtained from central animal house, ASBASJSM College of Pharmacy, Bela (Ropar)- 140111 (Punjab). They were housed in polypropylene cages which were large enough and rats were feed with standard diet and water in *ad libitum*. The animals were exposed under controlled room temperature ($24\pm 2^{\circ}\text{C}$) for 12 h under light and dark cycles with relative humidity (44-55 %). The animals were allowed to familiarize to the environment for 7 days and supplied with standard rodent pellet diet and water to animals according to their *ad libitum*. All the experimental procedure and protocols involving in this animal study were approved by the Institutional Animal Ethics Committee with CPCSEA (Registration number: ASCB/IAEC/12/18/133) and according to the guidelines of CPCSEA.

2.5 Acute toxicity study

The methanolic extract of *Prunus armeniaca* Linn. was administered orally in dose of 200 mg/kg and 400 mg/kg to groups of rats (n=6). After administration of the extract the rats were perceived for toxic effects for 48-78 h. If mortality was observed in 1-3 animals, then the dose administered was consigned as LD₅₀. If mortality was observed in one animal then the same dose was repeated to confirm the toxic dose. If no mortality was observed at higher dose then there is no toxic effect in any of the groups [11], [12].

2.6 Anti-Diabetic activity

- 1) Selection of animals: Healthy adult rats of either sex having weight of 180-220gms were selected for the study. The experimental protocol was subjected to the scrutiny of the Institutional Animals ethics panel, and was cleared by same before starting the experiment [13].
- 2) Maintenance of animals: The animal house was well ventilated and the temperature was unbroken between 19-20°C. The animals were housed in giant large healthful cages throughout the course of the experimental amount. The animals were fed with rat feed and pure filter water because the injured animals were prone to infection healthful conditions were maintained [17].
- 3) Induction of diabetes: After overnight fasting, Diabetes was induced by freshly prepared intraperitoneal injection of streptozotocin dissolved in 0.1M cold sodium citrate buffer, pH 4.5 at a dose of 60mg/kg. After a week for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycaemia were considered as diabetic rats and used for the experiment [14].

2.7 Experimental design

The rats were divided into six groups of six animals in each group as follows.

Group 1: normal control administered with 0.9% sodium chloride.

Group 2: STZ induced diabetes mellitus

Group 3: STZ + Standard (10mg/kg)

Group 4: Diabetic rats administered with *Prunus armeniaca* Linn (400 mg/kg)

2.8 Biochemical estimation

At different time intervals the hyperglycemic state of rats fasting glucose was measured. Under fasting conditions, the blood samples were collected at the end of 21 days study period. After centrifuge the serum obtained was used for the determination of glucose levels. The serum total cholesterol measurement, Low density lipoproteins (LDL) and High-density lipoproteins (HDL) was determined by using kit method [15].

2.9 Statistical analysis

The results are conveyed as mean±S.E.M Statistical difference was tested by using one-way analysis of variance (ANOVA) followed by turkey's test. A difference in the mean *P* value <0.05 was significant.

3. Results

3.1 Percentage yield

The powdered drug of apricot 200gm was extracted with methanol in water bath at a temperature of 60° C by using Soxhlet extraction apparatus. The percentage yield of methanol was found to be 36.7%.

3.2 Phytochemical tests

Preliminary phytochemical screening of the methanolic extract of fruit *Prunus armeniaca* Linn. Discovered the presence of Flavanoids, Phenols, Polyphenols, B-Carotene, Glycosides, Volatile compounds, Vitamins, Carbohydrates and Minerals.

3.3 Acute toxicity study

The acute oral administration result of methanolic extract of fruit *Prunus armeniaca* Linn in doses of 200mg/kg and 400 mg/kg indicate no mortality upto 7 days after treatment.

3.4 Oral glucose tolerance test

The glucose administration (2.5gm/kg) produced substantial change in blood glucose level of normal rats. At two different doses (200mg/kg, p.o, 400mg/kg, p.o.) of methanolic extract of fruit of *Prunus armeniaca* Linn. when treated it shows significantly reduced serum glucose level in normal fasting, at

0 min, 30 min, 60 min, 90 min and 120 min compared to normal control group (Table 1).

Table 1: Effect of methanolic extract of fruit *Prunus armeniaca* Linn. on Oral glucose tolerance test

Treatment	Fasting plasma glucose concentration (mg/dl)					
	Normal	0 min.	30	60	90	120
Normal control	126.75±1.89	127.75±1.79	128.78±1.70	130.35±1.73	135.78±1.69	128.85±1.82
Diabetic control	130.96±1.77	131.79±1.87	134.55±1.84	136.51±1.84	137.77±1.65	135.35±1.43
Standard (10 mg/kg)	94.68±0.77	110.54±1.37	120.81±1.82	122.16±1.79	110.92±1.54	108.65±1.28
STZ+200mg/kg	121.68±1.10	122.98±1.03	124.67±1.11	126.84±0.89	125.64±0.80	123.92±0.89
STZ+400mg/kg	112.9±1.79	113.85±1.74	115.23±1.71	117.08±1.96	115.97±1.932	113.93±1.90

3.5 Changes in body weight

At the end of 21 days, the body weight of albino wistar rats changes normally when the methanolic extract of *Prunus armeniaca* Linn were given to the treatment group and compare with control group. The body weight of diabetic control group decreased whereas the treatment with methanolic extract of fruit *Prunus armeniaca* Linn. (200mg/kg, p.o, 400mg/kg, p.o.)was significantly enhanced the body weight toward normal level (Table 2).

Table 2: Effect of methanolic extract of fruit of *Prunus armeniaca* Linn. on body weight

Treatment	Body weight (Day 21)
Normal control	201.9±4.32
Diabetes control	276.88±0.65
STZ + Standard (10 mg/kg)	206.22± 1.45
STZ + 200 mg/kg	212.45±14.22
STZ + 400 mg/kg	225.5±4.94

3.6 Urine glucose estimation

After 21 days of treatment period, it was observed that the animals treated with methanolic extract of fruit *Prunus armeniaca* Linn. (200mg/kg, p.o, 400mg/kg, p.o.) Shows substantial reduction in diabetes induced urine glucose levels (Table 3).

Table 3: Effect of methanolic extract of fruit of *Prunus armeniaca* Linn. on urine glucose estimation

Treatment	Urine glucose estimation			
	Day 1	Day 7	Day 14	Day21
Normal control	NIL	NIL	NIL	NIL
Diabetic control	+	++	+++	++++
STZ + Standard (10 mg/kg)	+++	++	+	+
STZ+ 200 mg/kg	++	++	+	+
STZ+ 400 mg/kg	++	+	NIL	NIL

3.7 Cholesterol estimation

After oral administration of methanolic extract of fruit *Prunus armeniaca*Linn. resulted in a significant reduction in total cholesterol level and LDL (Low density lipoproteins). HDL

(High density lipoproteins)is increases in rats as compared to the diabetic rats (Table 4).

Table 4: Effect of methanolic extract of fruit of *Prunus armeniaca* Linn. on cholesterol

Treatment	Cholesterol	HDL	LDL
Normal control	125.58±6.87	59.87±5.21	70.69±4.66
STZ	187.84±7.99	62.53±5.76	109.02±3.58
STZ + Standard (10mg/kg)	128.89±4.11	60.09±2.30	73.34±4.01
STZ+200mg/kg	170.13±4.91	60.36±2.83	140.64±3.28
STZ+400mg/kg	110.38±2.32	54.12±2.02	91.17±5.76

3.8 Anti-hyperglycemic activity

The present study shows clear result which indicates that the STZ induced diabetes mellitus group showed significant increase in blood glucose level from 70mg/dl to 120 mg/dl in rats. After treatment with methanolic extract of fruit of *Prunus armeniaca* Linn. at different doses of 200 mg/kg and 400mg/kg reduced the blood glucose level when compare with control and diabetic group rats (Table 5).

Table 5: Effect of methanolic extract of fruit of *Prunus armeniaca* Linn. on Blood glucose levels

Treatment	Blood glucose concentration (mg/dl)			
	Day 1	Day 7	Day 14	Day 21
Normal control	112.45±1.26	102.38±1.58	108.16±1.53	116.39±0.99
Diabetic control	254.02±1.39	278.32±1.37	280.5±1.37	264.96±2.69
STZ + Standard (10 mg/kg)	251.1±1.28	262.55±1.31	197.34±1.12	132.69±1.01
STZ+ 200 mg/kg (MEFPA)	221.86±1.34	233.45±1.67	257.31±1.85	243.72±1.92
STZ+ 400 mg/kg (MEFPA)	186.42±1.53	180.63±1.86	169.04±1.77	137.49±1.48

3.9 HbA1c

HbA1c level in STZ diabetic control rats was moderately higher after 8 weeks of intraperitoneal administration of STZ as compared to normal rats. Administration of methanolic

extract of fruit of *Prunus armeniaca* Linn (200 mg/kg and 400 mg/kg) to rats for 3 weeks daily (21 days) prevented the escalating HbA1c level. However, the treated group did not show any significant change in the HbA1c level (Table 6).

Table 6: Effect of methanolic extract of fruit of *Prunus armeniaca* Linn. on HbA1c

Groups	Treatment	HbA1c
Normal control	Normal saline treatment	12.15±0.032
Diabetic control	STZ 60 mg/kg	14.41±0.039
STZ+Standard(10 mg/kg)	Glibenclamide (10 mg/kg)	12.79±1.23
STZ+200mg/kg	STZ 60 mg/kg+MEFPA200 mg/kg	13.21±0.053
STZ+400mg/kg	STZ 60 mg/kg+MEFPA400 mg/kg	12.4±0.022

Table 7: Effect of methanolic extract of fruit of *Prunus armeniaca* Linn. on water and feed intake of STZ- induced diabetic rats

Treatment	Water intake	Feed intake
	Day 21	
Normal control	11.91±1.42	8.68±0.41
Diabetic control	15.67±0.75	9.66±0.61
STZ + Standard	12.01±1.01	8.65±0.51
TZ + MEFPA (200 mg/kg)	11.04±1.44	8.53±0.44
TZ + MEFPA (400 mg/kg)	16.75±1.00	9.79±0.60

4. Discussion

In our day to day life most of the people take stress and decrease in neutering dietary conditions that causes lack of endocrine ultimately results in the hyper glycaemic condition called polygenic disorder. Ancient Indian system of drugs has several plants with excellent medicinal properties that need detailed investigation for effective drug development. The main advantage of natural and herbal products is that they are economical and less toxicity as compared to artificial agents which exhibit more toxicity.

The aim of this present study is to evaluate the anti-diabetic effect of the methanolic extract of fruit *Prunus armeniaca* Linn. on normal and STZ induced diabetes mellitus mediated various metabolic alterations in albino wistar rats. Latterly there was no any other toxic substance was found in the dose. The acute toxicity results no mortality which describes the non-toxic nature of the extract.

At the end of the study the result shows that the methanolic extract of fruit of *prunus armeniaca* Linn. at a dose of 400 mg/kg per body weight shows significant improvement in oral glucose tolerance test in glucose fed hyperglycaemic normal rats and due to this lowering in the rate of glucose absorption in STZ-induced diabetic rats.

When STZ is induced into the normal rat results in characteristic loss of body weight due to which it shows loss of tissue proteins and increased muscle wasting. After 21 days

of treatment with methanolic extract, it was observed that the body weight increases in diabetic rats as compared to normal control and STZ-induced diabetes mellitus group.

5. Conclusion

Diabetes is a chronic disease which cause various complications like Diabetic retinopathy, Neuropathy, Nephropathy etc. Various studies have been done on the methanolic extract of the common seeds and oiland its anti-diabetic properties were studied in vivo models of Albino wistar rats.

However, Anti-diabetic activity of this fruit extract has not been tested by normal ways. Our results indicate that the methanolic extract of fruit of *Prunus armeniaca* Linn. decreases the effect of diabetes and shows anti-diabetic activity. The conclusion comes from the decrease in the glucose levels with the help of common fruit extract compared to regulate or untreated polygenic disease.

The methanolic extract of fruit of *prunus armeniaca* Linn. shows various medicinal activities like Anti-oxidant, Anti-microbial, Anti-cancer and Hepatoprotective activity. The extract of methanolic extract of fruit of *Prunus armeniaca* Linn. decrease the level of blood glucose and shows no toxicity. Hence the methanolic extract of this fruit is safe to use as compared to other allopathy medicines as they have a lot of side effects.

The extract of this fruit has amazing potential benefits and may be used as a natural and safe to the standard medication treatment.

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Author Profile



Dr. Suman Lata received D. Pharmacy from Govt. Medical College Patiala, B. Pharmacy from Institute of Pharmaceutical Science and Drug Research from Punjabi University, Patiala, M. Pharmacy (Specialization in Pharmaceutical Chemistry) from Institute of Pharmaceutical Sciences and Drug Research (Punjabi University, Patiala), PhD From IKG PTU, Kapurthala, Jalandhar (Punjab). She is now working as Associate Professor in Department of Pharmaceutical Chemistry in ASBASJSM College of Pharmacy with teaching experience of 17 years and 4 months. She is now residing in Ropar (Punjab) with +91 9463909206.