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Research Article

STUDY OF ETHANOLIC EXTRACT OF *PORTULACA OLERACEA* (WHOLE PLANT) ON BLOOD GLUCOSE LEVELS AND BODY WEIGHT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Aim: The aim of the present study was to find the effect of ethanolic extract of the whole plant of *Portulaca oleracea* on blood glucose levels and on body weight of streptozotocin induced diabetic rats. **Methods:** Rats were made diabetic by single dose of streptozotocin (50 mg/kg b.w).After checking their blood glucose levels, they were re-grouped into five groups. The 1st group was Normal Control group,2nd group was Diabetic Control group,3rd group received standard drug Glibenclamide,4th group received 50 mg.kg b.w of ethanolic extract of *Portulaca oleracea* and 5th group received 100mg/kg b.w of the ethanolic extract of the plant. Their blood glucose levels and body weights were checked at regular intervals. Histopathology of pancreas of diabetic rats was also done. **Results:** The two doses of the ethanolic extract of the plant showed significant decrease in blood glucose levels and increase in body weights. Histopathological studies also showed significant changes. **Discussion:** Today diabetes mellitus is taking the form of the epidemic in many countries including India. Though conventional treatments are used to cure diabetes mellitus , but due to side effect of these drugs, there is need for alternative ways to cure the disease.**Conclusion:** The ethanolic extract of *Portulaca oleracea* has beneficial effects on blood glucose levels as well as improving other metabolic aberrations.

Key Words: *Portulaca oleracea*, blood glucose levels, body weight

INTRODUCTION

Diabetes mellitus is a metabolic disease in the world today. It is gaining the form of an epidemic in countries like India with 62 million diabetic individuals diagnosed with the disease. India is topping with the highest number of people with diabetes followed by China and United States in second and third place respectively. It has been associated with complications, including retinopathy, nephropathy, neuropathy and angiopathy and several others.[1] Being a multifactorial disease characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes and high oxidative stress induced damage to pancreatic beta cells . In India alone there are more than 4.00 crore diabetics and the number is going to be around 9.00 crore by 2030. Over 7.20 lakh Indians die every year due to diabetes. People with diabetes are 2-4 times more likely to develop heart diseases.[2]

There have been various efforts have been taken to understand and manage diabetes mellitus. India has 45,000 plant species and many of them have medicinal properties.[3-11] About 800 plant species have shown anti- diabetic activity. People nowadays are showing great demand for plant products due to low cost, easy availability and lesser side effects. For this purpose plant materials are continuously scrutinized and explored. India currently faces an uncertain future with respect to the burden that the disease may impose upon this country.

Portulaca oleracea Linn, belongs to Family Portulacaceae (Purslane family). It mainly grows along waste lands and in cultivated gardens in Srinagar. It is commonly called as Common Purslane/Purslane in English, as Lonak in Punjabi ,Kurfa in Mumbai, as Kursa, Chhota Lunia in Hindi, as Loni, Ghol in Gujrati, as and as Nunar in Kashmiri. It is a cosmopolitan weed growing in warm temperate, tropical and subtropical regions of the world. It contains chemical constituents like carboxylic acids, gums , fatty acids, beta-carotene and volatile oil and Portuloside A, a monoterpene glucoside and phenolic alkaloids.[12-16] In folk medicine it is reported that it can be used as a salad, cooked like soups. It is used to treat burns earache, insect stings, inflammation, skin sores, ulcers, pruritis (itching skin) eczema and abscesses It is used in treatment of cardiovascular disorders, dysuria, haematuria, gonorrhoea, dysentery, sore nipples and ulcers of mouth. It is used as blood purifier. Roasted seeds have been reported to be diuretic and anti-dysenteric. Omega 3– fatty acids present are being used in the production of compounds that effect blood pressure clotting, the immune system, lower cholesterol (LDL) and prevent certain cancers and control coronary spasms This plant has positive effect on brain and in such conditions as depression, bipolar disorder, alzhemiers disease, schizophrenia, hyperactivity and migrane. Reported Pharmacological Activities include wound healing, antioxidant, antifertility, antifungal, antibacterial, analgesic, anti-inflammatory, gastric-antiulcerogenic, bronchodilatory, anti hypertensive, neuropharmacological, skeletal muscle relaxant, and antitumour activities.[17-28] Therefore, with the reference to traditional and reported uses, the present study was undertaken to investigate the effect of ethanolic extract of this plant on blood glucose levels and give a scientific rational for its use.

MATERIALS AND METHODS

Plant Material

The plant was collected from Shalimar area of the district, Srinagar, was collected during the months of April to June. It was authenticated by a plant taxonomist in the Centre of Plant Taxonomy, University of Kashmir, Srinagar. A sample of the plant material was then deposited in the herbarium of the Department of Taxonomy, University of Kashmir under voucher specimen number 1012(KASH) and was kept for future reference. The plant material was allowed to dry. It was then kept in a well ventilated room. The outside temperature was ranging between 18 to 32^0 C .

Preparation of the extract

The whole plant of *Portulaca oleracea* Linn was coarsely powdered. 500 gm of the material was macerated for 48 hrs with 50% ethanol. Occasional shaking was done. After 48 hrs, the ethanolic extract was filtered through Whatmans filter paper. The plant material was then macerated again with fresh 50% ethanol. The filtrate obtained from the first and the second maceration were then combined and the solvent was recovered . After alcohol was recovered, the extract was then evaporated to dryness. The percentage yield was noted. The extract was refrigerated at 4^0 C for future use in experimental studies.

Phytochemical Screening [29-31]

The ethanolic extract obtained was subjected to qualitative tests for identification of different constituents like tannins, alkaloids, saponins, glycosides, terpenes, phenolics, flavonoids, carbohydrates, proteins and steroids. This was done by using simple and standard qualitative methods described by Trease and Evans.[31-33]

Pharmacological Study [32]

Animals

Albino rats preferably healthy of either sex were used during the study weighing about 180-210 g. The animals were procured from Central Animal House, IIIM (Indian Institute of Integrative Medicine) Jammu .These animals were housed in clean polypropylene cages. The rats were acclimatized for a period of 7 days, before initiation of experiment. In the quarantine standard environmental conditions such as temperature ranging from 18 to 32^o C, relative humidity (70%) and 12 hrs dark/light cycle were maintained. Rodent pellet diet (Ashirwad Industries) and water *ad-libitum* under strict hygienic conditions was given to the animals. All these procedures were performed in accordance to CPCSEA guidelines after approval from the Institutional Animal and Ethics Committee (IAEC) of the Department of Pharmaceutical Sciences, University of Kashmir[No. F-IAEC (Pharm.Sc) Approval]

Induction of diabetes

Ethanolic extract of *Portulaca oleracea* Linn (whole plant) PO (50 and 100 mg/kg b.w) were evaluated against streptozotocin induced diabetes mellitus in rats. Rats were divided into five groups consisting of six rats each. Initially sixty rats were taken to account for any mortality. The rats were acclimatized for a period of 7 days before starting the experiment. After an overnight fasting, hyperglycaemia was induced by administering a single dose of streptozotocin.(50 mg/kg b.w) to all rats excepting group I which served as normal control. Streptozotocin(STZ) was freshly dissolved in 0.1 M citrate buffer (pH=4.5) and injected intraperitioneally within 15 min of dissolution in a vehicle volume of 0.4 mL with 1 mL of tuberculin syringe fitted with 24 gauge needle. Diabetes mellitus was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin. The animals were given free access to water during this period. After 3rd day of STZ administration, fasting blood glucose levels of rats were checked by glucose strips. The animals having blood glucose levels > 250 mg/dl were separated and selected for further studies and then re-grouping of these diabetic rats was done as per the following protocol.

Rats were given the following treatment in this study.
Group I Normal Control (2% of gum acacia).
Group II Diabetic Control. Received STZ (50 mg/kg b.w single dose i.p)
Group III STZ + Glibenclamide (3 mg/kg,)
Group IV STZ + PO (50 mg/kg b.w)
Group V STZ + PO (100mg/kg b.w)

The treatment was started from the same day except normal control and diabetic control groups for a period of 15 days orally. Animals in all groups had free access to standard diet and water during this period. Blood glucose levels were estimated on 1st, 4th, 9th and 15th day of the treatment. Besides this during this study the body weight of the rats were recorded on 1st , 4th 9th and 15th day of the treatment. On day 16th, blood samples were collected from overnight fasted rats by cardiac puncture. The animals were anaethesized by mild ether anaesthesia before cardiac puncture. Blood was collected and allowed to stand for one hour, serum was separated by centrifuging and evaluated for blood glucose levels. The animals were killed and pancreas was taken out. Histopathology of pancreas was also done.

Sample Collection

The blood samples were collected by pricking the tail from overnight fasted rats and blood glucose levels were estimated using One Touch Ultra glucose strips (Johnson & Johnson Ltd) on 1st, 4th, and 9th day.

Estimation of biochemical parameters

On the 15th day of the experiment , blood was collected from overnight fasted rats under ether anesthesia by cardiac puncture. It was kept aside for 30 min for clotting. By centrifuging the same sample at 6000 rpm for 20 min, the serum was separated and was analyzed for blood glucose.

Statistical Analysis

The data obtained from the different studies and the biochemical estimation done is expressed as Mean ± SEM for each group. After this, the statistical analysis was carried out using one way analysis of variance (ANOVA) followed by students t-test. Values $p > 0.05$ were considered non significant; $p < 0.05$ as significant, $p<0.01$ as highly significant and $p<0.001$ as very highly significant respectively.

Biochemical Estimation done

Estimation of Glucose was done according to standard procedure [33]

Histopathological studies

Pancreas: The organs were taken out, preserved in 10% formalin and sent for histopathological studies.

RESULTS

Physical Characteristics and Percentage Yeild of the ethanolic extract of *Portulaca oleracea* (whole plant)

Weight of the dried whole plant taken = 2750 gms
Weight of the extract obtained = 385 gms

% yield = Weight of the extract obtained / Weight of the dried whole plant taken x 100

% age yield of the ethanolic extract = 14 %

Extract	Colour	Odour	% Extractive value
50% Ethanolic	Dark Brown	Characteristic	14%

Phytochemical analysis

The phytochemical analysis of the extract showed the presence of alkaloids, flavonoids, glycosides, carbohydrates, tannins, terpenes, steroids, Proteins, saponins and phenolics
During the course of these studies blood glucose level and average body weight were recorded on day 1, day 4, day 9 and day 15.

Effect on Blood Glucose Levels

Table 1: Effect of ethanolic extract of *Portulaca oleracea*(PO) whole plant, on Blood Glucose Levels (mg/dl) against Streptozotocin induced diabetes mellitus in rats

Groups	Treatment	Blood Glucose Levels(mg/dl)			
		DAY 1	DAY 4	DAY 9	DAY15
I	Normal control 0.2 ml of 2% gum acacia	80.83±3.63	79.58±3.37	80.26±3.96(NS)	77.62±4.96(NS)
II	Diabetic control 0.2 ml of 2% gum acacia	200.48±2.89	200.24±3.67	206.76±3.23(NS)	207.50±2.97(NS)
III	STZ+ Std drug Glibenclamide (3mg/kg. b.w)	220.85±2.37	201.98±6.58	158.71±4.04**	129.56±12.97**
IV	STZ + P.O (50 mg/kg b.w)	210.52±2.29	186.10±2.44	176.99±1.73**	166.99±3.29***
V	STZ + P.O(100 mg/kg b.w)	220.84±1.70	193.01±3.47	167.87±3.67**	145.96±1.95***

Effect on Average Body Weight

Table 2: Effect of 50% ethanolic extract of *Portulaca oleracea*(PO) whole plant, on Average Body Weight (gms) against Streptozotocin induced diabetes mellitus in rats

Groups	Treatment	Average Body weight (in gms)			
		DAY 1	DAY 4	DAY 9	DAY 15
I	Normal control 0.2 ml of 2% gum acacia	250.58±8.14	253.90±9.93	262.12±10.47(NS)	267.23±13.09 (NS)
II	Diabetic control 0.2 ml of 2% gum acacia	205.83±7.64	202.16±6.61	175.56±6.77**	158.8±7.51**
III	STZ + Std drug Glibenclamide (3mg/kg. b.w)	203.17±4.91	200.67±4.94	180.92±7.63*	160.00±8.69**
IV	STZ + P.O (50 mg/kg b.w)	220.82±9.49	222.17±10.75	212.30±10.82*	207.18±11.12**
V	STZ + P.O (100 mg/kg b.w)	210.17±12.18	223.98±12.03	232.20±11.14*	238.78±10.12**

STZ Dissolved in 0.1M citrate buffer at a. dose of 50mg/kg b..w and injected i.p. single dose Diabetes confirmed on third day post administration of streptozotocin. Standard drug Glibenclamide & three plants given as 50% ethanolic extracts were administered orally for 15 days, in a single dose daily after confirmation of hyperglycaemia.
n = 6 (Number of animals in each group)
DAY 1 compared with DAY 15
*p< 0.05 significant; **p<0.01 highly significant; ***p< 0.001 very highly significant; p> 0.05 non-significant (NS)

Histopathology of Pancreas in Diabetic rats induced by streptozotocin (STZ)

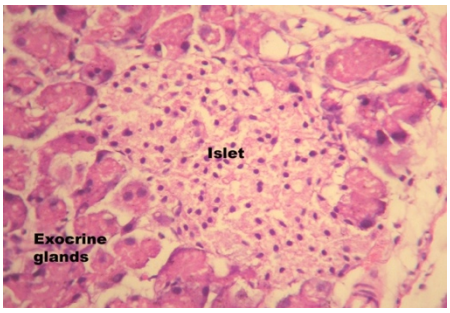

Fig 1: Group –I Normal Control
Pancreas of rats showing a large islet structure surrounded by exocrine gland tissue. No inflammatory cells are seen in the islet (H&E x 40X)

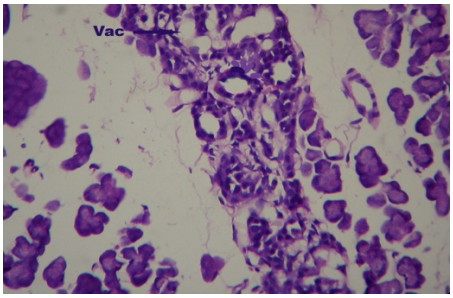

Fig 2 (a):Group-II – Diabetic Control
Pancreas from diabetic rats showing a islet structure surrounded by exocrine gland tissue. There is vacuolation of the islet cells and lymphocytic infiltration into the islet. (H&E x 40X)

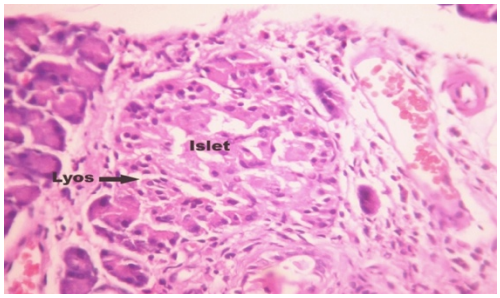

Fig 2 (b): Group-II – Diabetic Control
Pancreas from diabetic rats showing a islet structure surrounded by exocrine gland tissue. There is vacuolation of the islet cells and lymphocytic infiltration into the islet. (H&E x 40X)

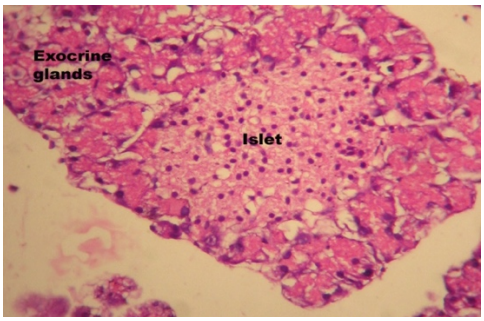

Fig 3: Group III–STZ*+Standard anti diabetic drug Glibenclamide (3 mg/kg b.w)
Pancreas from diabetic rats showing a large islet structure surrounded by exocrine gland tissue with no vacuolation. No inflammatory cells are seen in the islet (H&E x 40X)

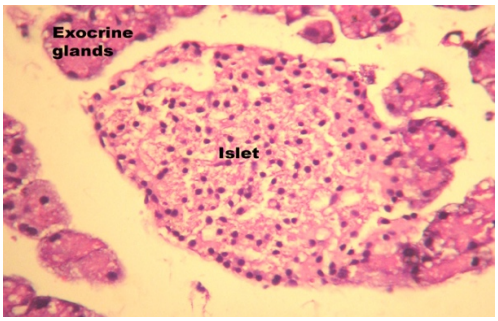

Fig 4 ::Group IV- STZ*+Portulaca oleracea (50mg/ kg b. w)
Pancreas from diabetic rats showing a large islet structure with exocrine gland tissue seen at upper edge. Few inflammatory cells are seen in the islet. (H&E x 40X)

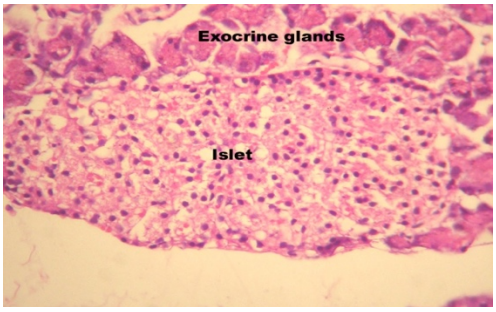

Fig 5: Group V- STZ*+ *Portulaca oleracea* (100 mg / kg b.w)
Pancreas from diabetic rats showing a large islet structure with exocrine gland tissue seen at upper edge. No inflammatory cells are seen in the islet. (H&E x 40X) *Streptozotocin (STZ) (50mg/kg) b.w. given once i.p

DISCUSSION

Diabetes mellitus is a metabolic disorder characterized by resistance in the action of insulin, insufficient insulin secretion or both. It is becoming one of the most common diseases of the world. Type II diabetes in young has increased 30 fold over the last 20 years concomitant with increase in obesity. Studies have revealed that all incidences of diabetes in this young age group is 2.5% and alarmingly 25% of their young adults have abnormalities of blood glucose.

The treatment for diabetes mellitus would be a drug that not only controls the glycemic level but also prevents the development of atherosclerosis and other complications of diabetics. New drugs and new drug delivery systems for insulin have also been introduced

The Indian indigenous drugs have great importance both from professional and economic point of view. A large number of plants have been reported to possess anti-diabetic activity e.g., *Aconitum napeilus, Aloe vera, Carum carvi, Cichorium intybus, Allium cepa, Aralia cachemirica, Allium sativum, Momordia charantia etc*

In the present study , *Portulaca oleracea* whole plant was evaluated for antidiabetic activity as it has been reported to have hypoglycaemic activity in the traditional system of medicine.

Different model systems like alloxan, streptozotocin[34] , viruses, insulin antibodies, hormones like dexamethasone, adrenaline and dithizone are available to screen the anti-diabetic activity of a given substance In the present study chemical like streptozotocin was used to produce marked diabetic effects in animals. Streptozotocin induced diabetic animals inhibit reduced response to insulin in hepatic and peripheral tissues. Rats treated with streptozotocin display many of the features seen in human with uncontrolled diabetes mellitus In the present study, streptozotocin was administered (i.p) at the dose of 50 mg/kg, b.w, for inducing diabetes.

Albino rats (Wistar strain) of both sexes, weighing 125-250g were procured from IIIM Jammu and kept in clean polypropylene cages under uniform conditions of food, water, temperature and degree of nursing care. It was ensured that the animals were in good health and free from any infectious diseases. Male and female animals were kept in separate cages so that there was no interference in evaluation of biochemical parameters during the period of study. The temperature and the humidity of the room in which the animals were housed were in the range of 15-25^{o}C and 70-75 % respectively.

In the present study, preliminary phytochemical screening, antidiabetic studies using streptozotocin for inducing diabetes, was carried out.

Studies have reported that *Portulaca oleracea* whole plant (PO) have revealed the presence of alkaloids, saponins, glycosides, terpenes, phenolics flavonoids, carbohydrates, proteins and steroids.

Portulaca oleracea whole plant (PO) in streptozotocin induced diabetic model given at the dose levels of 50 and 100 mg/kg b.w showed the following effects in biochemical parameters. At the dose level of 100 mg/kg b.w , serum glucose level ($p<0.001$) showed a significant decrease (Table 1).

During the course of 15 days streptozotocin induced diabetes mellitus, blood glucose levels were estimated on day 1, day 4, day 9, day 15. Day 1 was compared with day 15. There was non-significant decrease in blood glucose level in normal control group while diabetic control group revealed non significant rise in blood glucose levels on day 15. Glibenclamide, revealed significant reduction of blood glucose levels after 9th day and on day 15. Rats treated with *Portulaca oleracea*, (50 and 100mg/kg b.w) revealed significant decrease in blood glucose levels on day 9 and on day 15 at dose of 100mg/kg b.w. This shows that *Portulaca oleracea* has a significant antidiabetic activity. Increase in body weight shows that it has a protective action(Table2) Histopathological studies also showed protective action of *Portulaca oleracea* on pancreas of diabetic rats.

The literature reports reveal that flavonoids and tannins present in the plant extract known to possess antidiabetic activity. The observed effects of the plant extract in diabetic rats makes *Portulaca oleracea* quite important in the management of diabetes. Since there is a strong well-established link between diabetes mellitus, dyslipidemia, obesity, hypertension and ischemic heart disease, effect of the plant extract on weight loss/gain needs to be explored on scientific base.

CONCLUSION

The ethanolic extract of *Portulaca oleracea* has beneficial effects on blood glucose levels as well as improving other metabolic aberrations. Further studies on pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will help in projecting this plant as an therapeutic target in diabetics research. The level of morbidity and mortality because of this disease and its potential complications which are enormous, pose significant healthcare burdens on the families and society in India.These days diabetes mellitus has shown tremendous increase in younger people than in an elderly people. There is an urgent need to change the lifestyle of people and inclusion of fruits and vegetables that will reduce the frequency of taking medicines in near future.

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