EFFECT OF ALCHOLIC EXTRACT OF PORTULACA OLERACEA LINN FROM PULWA DISTRICT OF KASHMIR VALLEY ON ALLOXAN-INDUCED DIABETIC RATS

Prince Ahad Mir¹, Neha Sharma² and G. N. Bader*¹

¹Department of Pharmaceutical Science, University of Kashmir, Harzatbal, Srinagar-190006.
²Department of Pharmaceutical Science, Guru Nanak Dev University, Amritsar -143001.

ABSTRACT
Portulaca oleracea Linn belongs to the family Portulacaceae. Traditionally it is used in conditions of diarrhea, dysentery, leprosy, ulcers, asthma, piles, to reduce small tumors and inflammations. The aim of this work was to study the effect of ethanolic extract of this herb on pancreas in the alloxan induced diabetic rats and to see whether there exists any geographical variation as per its pharmacological effect is concerned. The alcoholic extract of Portulaca oleracea Linn from Pulwama district of Kashmir valley at the dose level of 400 mg/kg b.w showed the maximum protection against degeneration of acinar cells caused by alloxan (150 mg/kg b.w). Glibenclamide was used as standard. The extract showed dose dependent protection which was revealed by hematoxylin and eosin stained pancreatic tissue.

KEYWORDS: Pancreas, Portulaca oleracea, diabetes mellitus, albino rats.

INTRODUCTION
Portulaca oleracea (Purslane) belonging to family Portulacaceae is an herbaceous plant widely distributed throughout the world. It has an extensive old-world distribution extending from North Africa to the Middle East and Malaysia to the Indian Subcontinent and Australasia. Purslane has a taproot with fibrous secondary roots and is able to tolerate poor, compact soils and drought.¹ In traditional system it has been claimed to cure conditions as leprosy, diarrhea, ulcers, dysentery, asthma, and piles. Also, thought to reduce small tumors and inflammations.² The herb is considered to possess refrigerant, vulnerary, antiscorbutic, aperient and diuretic properties.³ It has been reported to possess potent pharmacological

*Correspondence for Author
G. N. Bader
Department of Pharmaceutical Science, University of Kashmir, Harzatbal, Srinagar-190006.
actions as analgesic, hepatoprotective, anti-inflammatory, wound healing, neuropharmacological, bronchodilatory, antioxidant, antihypertensive and many other biological actions.[4] Phytochemical screening of the herb revealed the presence of secondary metabolites like Alkaloids, Proteins & Amino acids, Tannins, Fats, Fixed oils, Saponins, Flavonoids, Steroids and Phenols. It is reasonably safe with LD$_{50}$ of 2.99g/kg (ethanolic extract). It is one of the herbs found useful in traditional practice in the management of diabetes, probably due to increase in the concentration of serum insulin.[5] Pulwama region is mostly plain in otherwise mostly mountainous Kashmir valley.

Diabetes represents a spectrum of metabolic disorders, which has become a major health challenge worldwide.[6] Diabetes is pandemic in both developed and developing countries. In 2000, there were an estimated 175 million people with diabetes worldwide and by 2030, the projected estimate of diabetes is 354 million.[7] The greatest relative rise is predicted in the developing countries of the Middle Eastern Crescent, Sub-Saharan Africa and the Indian subcontinent. By the year 2030, over 85 percent of the world’s diabetic patients will be in developing countries.[8] In India alone, the prevalence of diabetes is expected to increase from 31.7 million in 2000 to 79.4 million in 2030.[9] These estimates are valid only if the prevalence of obesity remains the same. In the past 20 years, the rates of obesity have tripled in developing countries as they have adopted a Western lifestyle involving decreased physical activity and over consumption of cheap, energy dense food.[10]

Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. In the laboratory, experimental diabetes is commonly induced by using Alloxan or Streptozotocin which selectively destroys the pancreatic β cells via production of reactive oxygen species. Alloxan induces type 1 diabetes mellitus while streptozotocin induces both type 1 and type 2 diabetes mellitus.[11] Insulin, whose absolute or relative lack leads to diabetes, is produced by the β cells of pancreatic islets of Langerhans. The islets which represent the endocrine part of the pancreas contain two main cell types, the alpha (α) cells and the beta (β) cells. The α cells which produce glucagon make up about 20% of the islet cells and have a characteristic peripheral distribution within the islet. The β cells which produce insulin are numerous forming about 70% of the islet cells and occupy the interior of the islet.[12,13] The present study was undertaken to validate or reject the claims of Portulaca oleracea Linn in the management of
diabetes mellitus and to show whether there was any effect of geographical distribution on the activity of this herb.

MATERIALS AND METHODS
Portulaca Oleracea Linn was collected from Pulwama district of Kashmir in the month of August. The herb was identified and authenticated at the Centre for Biodiversity and Taxonomy (CBT), Department of Botany, University of Kashmir under specimen reference number 123 KASH.

Preparation of Extract
The plants were washed with tap water to remove debris and dust particles, then were air dried at room temperature for two weeks, powdered and subjected to soxhlet extraction with ethanol. The extract was filtered and the filtrate dried Using vaccum rotary pump. The yield was calculated to be 30%. The extract was then stored at 0 - 4 °C for subsequent experimentation.

Preparation of Experimental Animals
Albino rats of either sex (150 -200g) were employed for evaluating the antidiabetic activity. Animals were procured from the central animal house of University of Kashmir under approval number: 801/03/CA/CPCSEA. Animals were maintained in well-ventilated room with humidity 75(±5) % and temperature 25(±2)°C. The experimental protocol was approved by the Institutional Animal Ethics Committee constituted for the purpose of control and supervision of experiment on animals, and complied with the NIH guidelines for care and use of experimental animals.

Induction of Diabetes
Diabetes was induced in overnight fasted rats by an intraperitonial injection of Alloxan monohydrate (150 mg/kg body weight). After fortnight, rats with marked hyperglycemia were selected and used for the study. All the animals were allowed free excess to water, pellet diet and maintained at room temperature (25±2)°C.

Experimental Design
Thirty six albino rats were randomly divided into 6 groups (A, B, C, D, E& F) each group comprising of six animals. Group A served as normal control and received 0.5ml of normal saline daily. In other groups (B,C,D,&E) Diabetes was induced by a single intraperitoneal
injection of Alloxan monohydrate (200 mg/kg body weight). After seven days of diabetes induction, fasting blood glucose (FBG) was determined and animals with FBG 250 mg/dl and above were selected for the study. Group B served as the diabetic control and received only 0.5ml of normal saline daily. Group C served as standard drug treated, group D and E received herbal extract (200mg/kg and 400mg/kg respectively) via orogastric intubation. The administration was continued for 28 days. On 29th day animals were anaesthetized using diethyl ether inhalation and sacrificed by decapitation as per OECD guidelines 2011. The pancreas were immediately dissected and washed with ice cold saline. The tissues were immediately transferred and preserved in 10% formalin solution for histopathological studies.

RESULTS

Fig: 1 Group A: Normal Control (received 0.5ml of Normal saline) Hematoxylin and eosin stained pancreatic tissue showing the acinar cells stained strongly and arranged in lobules with prominent nuclei. The islet cells are seen embedded within the acinar cells and surrounded by fine capsule.

Fig: 2 Group B(Diabetic control) received 0.5ml of Normal saline: The acinar cells around the islets though seem to be in normal proportion do not look classical. The islets are largely occupied by a uniform eosinophilic material which also surrounds the blood vessels.
Fig: 3 Group C: Standard drug treated (received 200mg/kg Glibenclamide): Hematoxylin and eosin of pancreatic tissue showing acinar cells in normal arrangement and the islet cells in small groups in low concentration.

Fig: 4 Group D: Portulaca extract treated; 200mg/kg): Hematoxylin and eosin treated pancreatic tissue showing acinar cells in normal arrangement and islet cells in groups in low concentration but higher than std. drug treated group.

Fig: 5 Group E Portulaca extract treated: 400 mg/kg): Hematoxylin and eosin treated pancreatic tissue shows the acinar cells in normal arrangement and the islet cells less degenerated in high concentration.
DISCUSSION
Insulin-dependent diabetes mellitus (IDDM) is believed to be an autoimmune disease that results from autoimmune destruction of the insulin-secreting β-cells of the pancreas which are responsible for producing insulin.\textsuperscript{[15]} Depletion of these β cells results in insulin deficiency which leads to disorder in carbohydrate, protein and fat metabolism resulting in hyperglycemia. In this study, Alloxan, a urea derivative which selectively destroys β cells of the pancreatic islet was used to induce type 1 diabetes mellitus.\textsuperscript{[16]} Insulinitis and loss of β cells were observed which are seen in type 1 DM. Insulinitis is evidenced by heavy lymphocytic infiltration in and around the islet. This is commonly seen in islets containing residual β cells and it supports the possibility of a specific, immunologically mediated destruction of β cells as the cause of type 1 DM.\textsuperscript{[17]} Insulinitis was seen in the group D (200mg/kg bw of plant extract) showing that some of the scanty cells seen in the islet are β cells (fig.4). Large deposits of a homogenous eosinophilic material occupying the islet and around blood vessels are seen in the diabetic control group (Fig.2). Islet cells of group E treated with 400mg/kg bw of plant extract has regenerated considerably. It may be due to the presence of stable cells in the islets with the ability of regeneration (Fig.5). This also suggests that the plant extract at this dose has the ability of inducing the quiescent cells to proliferate and replace the lost cells. The exact mechanism is not known but it has been proved that the polysaccharide and flavonoids are usually anti-diabetic compounds in plants.\textsuperscript{[18]} It is obvious that alcoholic extract of Portulaca oleracea Linn collected from Pulwama region of Kashmir is similar in constitution and there are no geographical or other differences as per its antidiabetic property is concerned. Portulaca oleracea Linn is able to cause regeneration of pancreatic β cells at a dose 400mg/kg bw either by its active constituents (polysaccharides, flavonoids etc) acting singly or synergistically. Further studies to isolate, identify and characterize the active principle(s) are underway to substantiate the present findings.

CONCLUSION
From the study it can be concluded that alcoholic extract of Portulaca olearacea is able to regenerate pancreatic β cells at a dose level of 400mg/kg bw. However at 200mg/kg bw the effect is not so pronounced. Further geographical variation is not there as per the antidiabetic effect is concerned.
REFERENCE


