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HEPATOPROTECTIVE AND HYPOLIPIDEMIC ACTIVITIES OF ETHANOLIC EXTRACT OF *PORTULACA OLERACEA* (WHOLE PLANT) IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract:

Objective: The objective of this study was to study the hepatoprotective and hypolipidemic activities of ethanolic extract of *Portulaca oleracea* (whole plant) in streptozotocin induced diabetic rats.

Methods: Preliminary phytochemical screening was carried by following the standard procedures. The animals (rats) were divided into five groups. The first group was Normal Control group which received only the vehicle. The 2nd group was toxic group which included those animals in which diabetes was induced by streptozotocin. The 3rd group were those animals which received streptozotocin and standard antidiabetic drug-glibenclamide. 4th group was diabetic animals which received 50 mg/kg b.w dose of extract of *Portulaca oleracea.* 5th group included those diabetic animals which received 100mg/kg b.w of the plant extract. The biochemical parameters that were evaluated were blood glucose levels, liver function test and lipid profile. At the end, the animals were sacrificed and histopathology of liver was also done.

Results: The phytochemical screening showed that the ethanolic extract of *Portulaca oleracea* revealed the presence of alkaloids, flavanoids, glycosides, terpenes, saponins, carbohydrates, proteins, tannins, phenolics and steroids. The results also showed significant decrease in blood glucose levels, lipid profile and liver function tests in animals treated with different doses of the plant extracts.

Conclusion: This finding indicated that ethanolic extract of whole plant of *Portulaca oleracea* might become interesting candidate for the treatment of those complications associated with Diabetes mellitus.

Key Words: Portulaca oleracea, hepatoprotective, hypolipidemic.

Introduction

Diabetes mellitus is defined as metabolic disease with hyperglycaemia which leads to many complications like diabetic neuropathy, diabetic nephropathy, diabetic retinopathy and many other complications. The disease is taking the shape of an epidemic in the whole world. WHO defines the disease as the 7th cause of death in 2030 (WHO 2016). About 180 million people across the globe have type 2 DM characterized by hyperglycaemia that affects eyes, nerves, kidney and may lead to risk linked with cardiovascular disease. Patients having diabetes can lead to cardiovascular disease which doubles the risk of death. There are number of medicinal plants having a number of pharmacological activities.

Portulaca oleracea is a cosmopolitan weed that is most abundant in Kashmir, commonly called as Nunar. In English, its common name is Common Purslane, in Hindi as Lunia. It mainly grows in warm, temperate, tropical and subtropical regions of the world. This plant has shown many pharmacological activities such as anti-fungal, analgesic, anti-inflammatory, anti-ulcerogenic, bronchodilator and anti-tumour activities. There are very studies regarding its hepatoprotective and hypolipidemic activities related to diabetes. Therefore the present study was to investigate the hepatoprotective and hypolipidemic activities of this plant so as to give scientific rationale for its future use.

Methods

Identification and authentication of Plant Material.

The whole plant of *Portulaca oleracea* was collected from Shalimar area of Srinagar City, collected in the months of April to June. The plant was then authenticated by plant taxonomist in the Centre of Plant Taxonomy in the Centre of Plant Taxonomy in the University of Kashmir, Srinagar. In the herbarium of the Department of Taxonomy, University of Kashmir, a sample of the plant under Voucher number 1012 (KASH) was kept for future reference. The whole plant was dried in shade, kept in well ventilated room with outside temperature from 18-32^{o}C.

Preparation of the extract:-

The plant was coarsely powdered. The material was macerated for 48hrs with 50% ethanol, then filtered, again macerated with 50% ethanol.

The filtrates from two macerations were combined and solvent recovered. The extract was evaporated to dryness. The percentage yield was noted. The extract was refrigerated at 4^0C for experimental studies to be used in future.

Phytochemical Screening

The extract was subjected to qualitative tests for constituents like tannins, alkaloids, saponins, terpenes and many other constituents. This was done by methods described by Trease and Evans.

Pharmacological Study:-

Albino rats of either sex weighing about 180-210gms were used in the pharmacological studies. These animals were procured from Central Animal House, IIIM (Indian Institute of Integrative Medicine) Jammu. They were kept in clean polypropylene cages and acclimatized for a period of 7 days before the experimentation work was done. Standard environmental conditions with temperature ranging from 18-32^0C, relative humidity(70%) and 12 hr dark/light cycle was maintained. Standard rodent pellet diet procured from Ashirwad Industries and water ad libitum was given to animals under strict hygienic conditions. CPCSEA guidelines were followed in all procedures after approval from Institutional Animal and Ethics Committee(IAEC) of the Department of Pharmaceutical Sciences, University of Kashmir [No.F-IAEC(Pharm Sc) Approval].

Induction of Diabetes

Hyperglycaemia was induced by administering a single dose of streptozotocin(STZ) 50mg/kg b.w. It was freshly dissolved in 0.1 M citrate buffer (pH.4.5) and injected intraperitoneally within 15 minutes of dissolution in a vehicle volume of 0.4 ml with 1 ml of tuberculin syringe fitted with 24 gauge needle. Diabetes is confirmed on 3rd day post administration of streptozotocin by estimating the fasting blood glucose concentration. During this period the animals are given free access to water. Fasting blood glucose concentration is checked by glucostrips. The rats having blood glucose levels > 250 mg/dl are separated and selected for further studies. The animals are given the following treatment in the study.

Group I. Normal Control receiving 2% of gum acacia.

Group II. Diabetic Control which received STZ 50mg/kg b.w single dose i.p

Group III. STZ + Glibenclamide (3 mg/kg)

Group IV. STZ+ Portulaca oleracea (PO) [50mg/kg.b.w]

Group V. STZ+ PO [100mg/kg.b.w]

The treatment was started on the same day except normal control and diabetic control rats for a period of 15 days orally. The rats were given free access to standard diet and water during this period. Fasting blood glucose levels were estimated on 1^{st}, 4^{th} , 9^{th} and 15^{th} day of the treatment. On the 16^{th} day, blood samples were collected from overnight fasting animals by cardiac puncture. The rats were anaesthetized by mild ether anaesthesia before cardiac puncture. The blood sample was kept aside for 30 minutes for clotting. By centrifuging the sample at 6000 r.p.m for 20 minutes, the serum was separated and analyzed for various biochemical parameters. At the end of the experiment, the animals were sacrificed and liver was taken out. Histopathology of the liver was also done.

Statistical Analysis.

The data obtained from the biochemical estimations 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 student 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.

The biochemical parameters were estimated as per the following methods

i. **Serum glucose levels (Recorded on day 1,day 4,day 9 and day 15)**

ii. **Lipid Profile**

   a) Serum Total Cholesterol Levels

   b) Serum Triglycerides Levels

   c) Serum HDL Cholesterol Levels

   d) Serum LDL Cholesterol Levels

iii. **Liver function tests**

   a) Serum bilirubin levels

   b) Serum SGOT

c) Serum SGPT levels

d) Serum total proteins

e) Serum albumin

f.) Serum alkaline phosphatase

iv. Histopathology studies: The liver was taken out, preserved in 10% formalin and sent for histopathological studies.

Results

a) Physical Characteristics and Percentage Yeild of the Extract

i) Ethanolic extract of *Portulaca oleracea* (whole plant)

Weight of the dried whole plant taken = 2750gms

Weight of the extract obtained = 385gms

$$\% \text{ yield} = \frac{\text{Weight of the extract obtained}}{\text{Weight of the dried whole plant taken}} \times 100$$

% age yield of the ethanolic extract = 14 %

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

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***

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<0.01 highly significant; ***p< 0.001 very highly significant; p> 0.05 non significant (NS)

Table-2. Effect of ethanolic extract of Portulaca oleracea (whole plant) on lipid profile in streptozotocin induced diabetic rats.

Group	Treatment	Serum total Cholesterol mg/dl	Serum triglyceride mg/dl	Serum HDL Cholesterol mg/dl	Serum LDL Cholesterol mg/dl
I	Normal Control (0.2 ml of 2% gum acacia)	88.22±2.01	76.71±3.45	33.07±2.15	42.51±2.35
II	Diabetic control (STZ)	195.18***±3.54	193.01***±4.84	18.88***±2.62	87.02***±3.07
III	STZ + Std Antidiabetic drug Glibenclamide (3mg/kg)	193.34±5.69	186.96±4.31	19.75±1.95	86.26±3.02
IV	STZ +PO (50mg/kg)	180.56*±1.56	162.44**±4.44	25.91±4.64	82.36±3.91
V	STZ+PO (100mg/kg)	171.09**±1.20	149.09***±2.38	29.59**±2.86	74.04*±3.76

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 & plant given as

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)

Group II is compared with Group I and all other groups are compared with group II

*** p < 0..001 Very highly significant; ** P< 0.01; Highly significant; *p< 0.05 significant

Table-3. Effect of ethanolic extract of *Portulaca oleracea* (whole plant) on liver function tests in streptozotocin induced diabetic rats.

Group	Treatment	Serum Bilirubin Levels (mg/dl)	Serum SGOT (IU/L)	Serum SGPT (IU/L)	Serum Total Proteins Levels (gm/dl)	Serum Albumin Levels (gm/dl)	Serum Alkaline Phosphatase Levels (U/l)
I	Normal Control (0.2 ml of 2% gum acacia)	0.55±0.05	21.61±1.31	26.06±1.78	7.13±0.31	2.65±0.09	78.2±4.6
II	Diabetic control (STZ)	3.11***±0.23	32.12**±4.23	40.72**±2.28	4.79*±0.33	1.16*±0.19	92.88**±2.58
III	STZ + Std Antidiabetic drug Glibenclamide (3mg/kg)	2.54±0.15	31.77±1.46	38.13±1.97	5.41±0.26	1.57±0.13	86.92±1.59
IV	STZ +PO (50mg/kg)	1.61*±0.17	28.58±1.6	29.69±2.27	4.55±0.34	1.34±0.07	92.28±2.06
V	STZ+PO (100mg/kg)	0.75**±0.11	18.17*±1.02	23.02*±1.76	5.99±0.17	2.08±0.21	81.39*±6.85

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& plant given as 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)

Group II is compared with Group I and all other groups are compared with group II

*** p < 0..001 Very highly significant; ** P< 0.01; Highly significant; *p< 0.05 significant p>0.05 Non significant

Histopathology Results

Effect of 50% Ethanolic extract of *Portulaca oleracea* (PO) whole plant, on histopathology of liver against streptozotocin induced diabetes mellitus.

Liver

Histopathological examination of the liver slides of rats of Group I (Normal control) showed the portal triad area with no abnormality **(Fig 1)**

Livers of the rats of diabetic group II also showed sinusoidal dilatation and necrosis with inflammatory cell infiltration and haemorrhage (**Fig 2a,2b).**

Glibenclamide (Standard antidiabetic drug) when administered at the dose level of 3 mg/kg b.w to rats f Group III showed the portal triad area with no abnormality **(Fig 3).**

Portulaca oleracea whole plant when administered at the dose level of 50 mg/kg b.w to rats of Group IV showed the portal triad area with no abnormality **(Fig 4).**

Portulaca oleracea whole plant when administered at the dose level of 100 mg/kg b.w to rats of Group V showed the portal triad area with no abnormality **(Fig 5).**

HISTOPATHOLOGY OF LIVER IN RATS

DIABETES INDUCED BY STREPTOZOTOCIN (STZ)

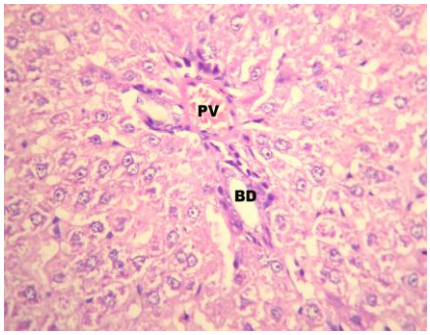

Fig 1: Normal Control
Liver of rats showing the portal triad area. No. abnormality seen. (H&E x 40X)
BD= Bile Duct, PV= Portal Vein

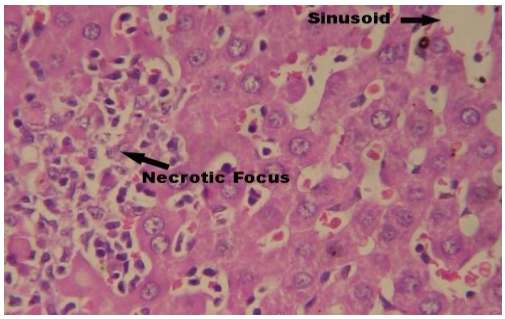

Fig 2(a): Diabetic Control liver of Diabetic rats Showing the sinusoidal dilatation and necrosis with Inflammatory cell infiltration and haemorrhage (H&E x 40X).

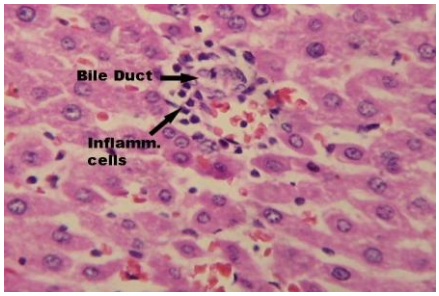

Fig 2(b): Liver of Diabetic rats showing inflammatory cell infiltration and haemorrhage around a bile duct area (H&E × 40X)

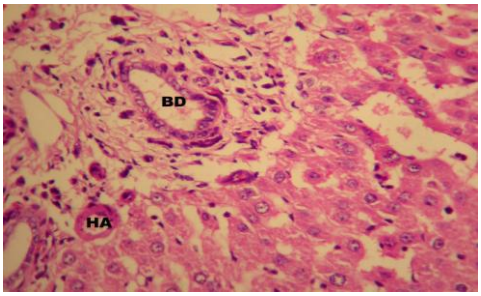

Fig 3: Liver of Diabetic rats receiving Standard anti-diabetic drug Glibenclamide (3mg/kg.b.w) No abnormality seen in portal HA=Hepatic artery BD=Bile duct

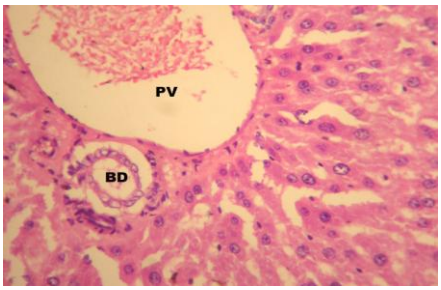

Fig 4:Liver of Diabetic rats showing the portal area in rats Receiving extract of ***Portulaca oleracea*** (50mg/kg.b.w)

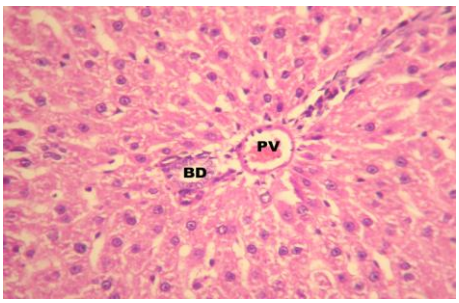

Fig 5: Liver of Diabetic rats showing the portal area Receiving extract of ***Portulaca oleracea*** (100mg/kg)

Discussion

The pancreas is the primary organ of the body involved in sensing the organism's dietary and energetic states through glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted. Streptozotocin has been the usual substance used for the induction of diabetes mellitus apart from alloxan. It has a destructive effect of the beta cells of the pancreas It causes a massive reduction in insulin release by the destruction of beta-cells of the islets of Langerhans thereby inducing hyperglycemia. Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose and increased lipid profile. Diabetes

mellitus is a metabolic disorder characterized by resistance in the action of insulin, insufficient insulin recreation or both. It is 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.[1]

Herbal plants have received greater attention as an alternative to conventional therapy. The demand for these remedies has currently increased. Experimental screening method is imperative in order to establish the safety and efficacy of traditional and herbal products and also to set up the active components of the herbal products.

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.*

Rats weighing in the range of 180-210g were procured from IIIM Jammu and kept in polypropylene cages under uniform conditions of food, water, temperature and degree of nursing care. It was ensured that the animals were in good health. These animals were free from 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 were in the range of 15-25^{O}C and 70-75 % respectively.

The phytochemical investigation of ethanolic extract of whole plant of *Portulaca oleracea* carried out by standard procedures revealed the presence of alkaloids, flavanoids, glycosides, terpenes, saponins, carbohydrates, proteins, tannins, phenolics and steroids.

The results of the present study found that ethanolic extract of *Portulaca oleracea* reduced the glucose level in animals made diabetic with streptozotocin. Streptozotocin has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of streptozotocin induced free radical damage. In the present investigation, ethanolic extract of *Portulaca oleracea* demonstrated the significant anti-diabetic, hepatoprotective and hypolipidemic activity. The results from the present study also indicate that ethanolic extract can reduce the levels of serum lipids. The antidiabetic effect of the ethanolic extract may be due to the enhanced secretion of insulin from the beta cells of pancreas or may be due to

increased tissue uptake of glucose by enhancement of insulin sensitivity. The literature reports reveal that flavonoids and tannins present in the plant extract known to possess antidiabetic and hypolipidemic activity. Since many antidiabetic drugs do not correct dyslipidemia, the observed hypolipidemic 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

It has been concluded that the ethanolic extract of *Portulaca oleracea* has beneficial effects on blood glucose levels as well as improving hyperlipidemia and 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. It 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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Conflicts of Interest: NONE.

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