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EVALUATION OF RENAL EFFECTS OF HYDRO-ALCOHOLIC EXTRACT OF *PORTULACA OLERACEA* (WHOLE PLANT) IN ALLOXAN INDUCED DIABETIC RATS

Sabeeha Shafi * and Nahida Tabassum

Department of Pharmaceutical Sciences, University of Kashmir, Hazratbal, Srinagar - 190006, Kashmir, Jammu & Kashmir, India.

Keywords:

Portulaca oleracea, Phytochemical, Renal effects

Correspondence to Author:
Dr. Sabeeha Shafi

Sr. Assistant Professor, Department of Pharmaceutical Sciences, University of Kashmir, Hazratbal, Srinagar - 190006, Kashmir, Jammu & Kashmir, India.

E-mail: sabeehashafi@gmail.com

ABSTRACT: Herbal plants are effective in the treatment of various diseases. More or less they are unscientifically and improperly used. These plants deserve detailed studies in the light of modern medicine. Having detailed investigation and documentation of herbal plants used in local health traditions and after that doing pharmacological evaluation of these plants and their taxonomical classification can lead to the development of valuable plant drugs for many diseases. In the present study, the renal effects of hydro-alcoholic extract of *Portulaca oleracea* (whole plant) were evaluated by performing the kidney function tests. Firstly the hydro-alcoholic extract of the plant was made. Rats were made diabetic by a single dose of 120 mg/kg b.w. After observing them for 72 hours, the blood glucose levels were checked. The rats having blood glucose levels above 200 mg/dl were taken for the study. The extract was first subjected to preliminary phytochemical screening. The doses taken were 100 and 200 mg/kg b.w. Phytochemical screening showed the presence of many plant constituents. The effect of the extracts on kidneys was evaluated, which showed variable results.

INTRODUCTION: Diabetes mellitus is a very common metabolic disease in the world today, affecting at least 15 million people. It has associated with long term complications, including retinopathy, nephropathy, neuropathy and angio-pathy and several others [1]. It is 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 [2]. In India there has been more than 4.00 crore diabetics and the number will increase to be around 9.00 crore by 2030.

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Efforts are taken to understand and manage diabetes mellitus because disease related complications are increasing day by day. In India there are 45,000 plant species and many of them have medicinal properties [3 - 12]. About 800 plant species have shown anti- diabetic activity. People have shown great demand for plant products due to low cost, easy availability and lesser side effects. For this plant materials are continuously scrutinized and explored for their effect as antidiabetic agents.

Portulaca oleracea Linn., belonging to family Portulacaceae (Purslane family) called as common Purslane/Purslane in English, as Kurfa in Mumbai, as Loni, Ghol in Gujrati, as Kursa, Chhota Lunia in Hindi, as Lonak in Punjabi and as Nunar in Kashmiri. It is a cosmopolitan weed. It grows along waste lands and in cultivated gardens in Srinagar. It also contains various chemical constituents like carboxylic acids, gums , fatty acids, beta-carotene and volatile oil and portuloside A, a monoterpene

glucoside, phenolic alkaloids and omega 3 fatty acids. Reported pharmacological activities include antifungal, antibacterial, analgesic, antiinflammatory, gastric anti-ulcerogenic, bronchodilatory, skeletal muscle relaxant, anti-hypertensive, wound healing, neuropharmacological, antioxidant, antifertility and antitumour activities [13 - 30].

Therefore, with the reference to traditional and reported uses, the present study was undertaken to investigate the renal effects of this plant in alloxan induced diabetic rats and give a scientific rational for its use.

MATERIALS AND METHODS:

Plant Material: The whole plant of *Portulaca oleracea* was collected from Shalimar area of the district, Srinagar. The plant was collected during the months of April to June and authenticated by a plant taxonomist in the Centre of Plant Taxonomy, University of Kashmir, Srinagar.

A sample of this plant material was deposited in the herbarium of the Department of Taxonomy, University of Kashmir under voucher specimen number 1012(KASH) for future reference. This plant material of *Portulaca oleracea* was dried, kept in a well ventilated room with outside temperature ranging between 18 – 32 °C .

Preparation of the Extract: The whole plant of *Portulaca oleracea* was coarsely powdered and 500 gm of the material was macerated for 48 hrs with 50 % ethanol, with occasional shaking. After 48 hrs, this extract was filtered through Whatmans filter paper. The plant material was then macerated again with fresh 50 % ethanol. The filtrate obtained was then combined and the solvent was recovered. After the recovery of alcohol, the extract was then evaporated to dryness. The yield was noted. The plant extract was refrigerated at 4°C for future use in experimental studies.

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

Pharmacological Study: [34]

Animals: Albino rats of either sex were used during the study weighing about 180-210 g with good health. The animals were procured from Central Animal House, IIIM (Indian Institute of Integrative Medicine) Jammu. These animals were housed in clean polypropylene cages. Before initiation of experiment, they were acclimatized for a period of 7 days. Standard environmental conditions such as temperature ranging from 18 – 32°C, relative humidity (70%) and 12 hrs dark/light cycle were maintained in the quarantine. The animals were fed with rodent pellet diet (Ashirwad Industries) and water *ad libitum* under strict hygienic conditions.

All 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/2008/4 Dated Oct 23rd, 2008].

Induction of Diabetes: Alloxan monohydrate was used to induce diabetes mellitus. A single dose (120 mg/kg, b.w, i.p) of alloxan monohydrate in sterile saline was used for the induction of diabetes in rats after overnight fasting. After one hour of alloxan administration, the animals were fed standard pellets and water *ad libitum*. After 5 days of alloxan administration, the animals which showed blood glucose levels above 250 mg/dl were used for the study. Hydro-alcoholic extract of the plant *Portulaca oleracea* (PO) was administered at two dose levels 100 and 200 mg/kg b.w.

Experimental Design: These albino rats were fasted overnight for 12 hrs. They were randomly divided into 5 groups of 6 rats per group. The various groups were:

Group I Normal control and received only 0.2 ml of 2 % aqueous gum acacia

Group II Diabetic control and received only alloxan monohydrate and 2% aqueous gum acacia.

Group III Alloxan monohydrate + Glibenclamide (10 mg/kg, p.o) and served as standard antidiabetic drug.

Group IV Alloxan monohydrate + 50 % ethanolic extract of PO (100 mg/kg, p.o)

Group V Alloxan monohydrate + 50 % ethanolic extract of PO (200 mg/kg, p.o)

The treatment with hydro-alcoholic extract of *Portulaca oleracea* was started on the same day except normal control and diabetic control groups which received only 0.2 ml of 2 % aqueous gum acacia for a period of 10 days. The animals in all groups had free access to standard diet and water during this period. Body weight and blood glucose levels were estimated on 1st, 4th, 7th and 10th day of the treatment. The renal effects were observed at the end of the experiment.

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 and Johnson Ltd.) on 1st, 4th, 7th and 10th day.

Estimation of Biochemical Parameters: [35 - 37] On the 10th day, blood of these animals 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, serum urea levels, serum creatinine levels and serum total protein levels.

Body Weight:

Estimation of Glucose:

Principle: Glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of glucose present in the sample.

Calculation:

Total Glucose in mg/dl = Abs.T / Abs.S x100

Esimation of Urea:

Principle: Urease splits urea into ammonia and carbon dioxide. Ammonia released in this reaction reacts with hypochlorite and phenolic chromogen to produce green colour. The absorbance of this green colour at 578 nm. (570 - 620) is directly propotional to the concentration of urea in specimen.

$$\text{Urea in mg \%} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 40$$

Estimation of Creatinine:

Principle: Creatinine in alkaline medium reacts with picrate to produce orange colour. This colour absorbs light at 492 nm. (490 - 510 nm). The rate of increase in absorbance is directly propotional to the concentration of creatinine in specimen.

Calculation: Calculate the average change in absorbance per minute (Abs.) of standard and specimen.

Abs. = Abs. at 90 sec.--- Abs. at 30 sec

$$\text{Serum Creatinine (mg \%)} = \frac{\text{Abs. of Specimen}}{\text{Abs. of Standard}} \times 2$$

Estimation of Total Proteins:

Principle: Proteins, in an alkaline medium, bind with the cupric ions present in the biuret reagent to form a blue-violet coloured complex. The intensity of the colour formed is directly propotional to the amount of proteins present in the sample.

Calculation:

Total Proteins in g/dl = Abs.T / Abs.S x 8

Statistical Analysis: All the values are expressed as mean + SEM. The results were subjected to statistical analysis using one-way ANOVA followed by students t-test. $p<0.001$ was considered very highly significant.

RESULTS: Hydro-alcoholic extract of *Portulaca oleracea* (whole plant)

Weight of the dried whole plant taken = 2750 gm

Weight of the extract obtained = 385 gms

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

% age yield of the hydro-alcoholic extract = 14%

TABLE 1: HYDRO-ALCOHOLIC EXTRACT OF *PORTULACA OLERACEA*

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.

Antidiabetic Activity: The blood glucose levels showed a highly significant decrease in groups III, IV and V ($p < 0.01$) when it was compared to group II (Diabetic control). There was a highly significant increase in blood glucose levels seen in diabetic group as compared to normal control group I ($p< 0.01$). **(Table 2**, **Fig. 1)**

Kidney Function Tests: (Table 3, **Fig. 2)**

Serum Urea Levels (mg/ dl): The rats of Group I showed a level of serum urea level of (22.32 ± 3.75 mg/dl). Group II rats which received only alloxan monohydrate showed a non significant increase ($p>0.05$) in serum urea levels (23.06 ± 0.69 mg/dl). The rats of group IV receiving 100 mg/kg b.w of 50 % ethanolic extract of *Portulaca oleracea* showed a non significant level ($p>0.05$)of (26.47 ± 2.95 mg/dl) increase in serum urea levels. Rats of group V which received 200 mg/kg b.w of ethanolic extract showed a non-significant level ($p>0.05$) of (25.95 ± 2.88 mg/dl).

Serum Creatinine Levels (mg/ dl): There was non-significant rise ($p>0.05$), in serum creatinine levels in rats in the diabetic group (Group II) (1.14 ± 0.12 mg/dl) as compared to normal control (Group I) (0.85 ± 0.07). *Portulaca oleracea* in the dose levels of 100 and 200 mg/kg b.w showed a non-significant decrease in the serum creatinine levels. A dose of 100 mg/kg b.w administered to rats of Group IV showed a non-significant level ($p>0.05$), of (1.65 ±0.24 mg/dl) and a dose of 200 mg/kg b.w administered to rats of Group V also showed a non-significant level ($p>0.05$), of (1.38 ± 0.14 mg/dl).

Serum Total Proteins Levels (g/ dl): As compared to group I (4.95 ± 0.61), the levels in the group II rats which had received only alloxan mono-hydrate, there was non-significant change ($p>0.05$). In the serum total protein levels (3.18 ± 0.26 g/ dl). When 50 % ethanolic extract of *Portulaca oleracea* (100 mg/kg b.w) was administered to rats of Group IV it showed a non significant ($p>0.05$). Increase (4.66±0.77 g/ dl) while a dose of 200 mg/kg b.w administered to rats of Group V showed a significant increase ($p<0.05$). In the serum total proteins levels (5.20 ± 0.66 g/ dl). (Besides recording the effect on biochemical parameters, effect on body weight of rats revealed the following results.

TABLE 2: EFFECT OF 50 % ETHANOLIC EXTRACT OF *PORTULACA OLERACEA* (WHOLE PLANT) PO ON BLOOD GLUCOSE LEVELS (mg/dl) AGAINST ALLOXAN INDUCED DIABETES MELLITUS IN RATS (10 DAYS STUDY)

Groups	Treatment	Blood Glucose Levels (mg/dl)			
		Day 1	Day 4	Day 7	Day 10
I	Normal control 0.2 ml of 2% gum acacia	85.07 ± 4.35	86.16 ± 4.43	84.82 ± 5.96(NS)	84.71 ±6.11(NS)
II	Diabetic control Alloxan monohydrate	261.47 ± 8.37	264.28 ± 8.29	268.03 ± 8.48(NS)	271.33 ± 8.18(NS)
III	Alloxan monohydrate + Std drug - Glibenclamide (10 mg/kg b.w)	200.37 ± 5.25	141.18 ± 2.43	124.52 ± 2.00**	114.84 ±3.20***
IV	Alloxan monohydrate + PO(100 mg/kg b.w)	203.38 ± 4.04	147.87 ± 2.30	144.56 ± 2.56**	142.82 ± 2.76***
V	Alloxan monohydrate + PO(200 mg/kg b.w)	201.24 ± 4.90	146.29 ± 2.06	130.44 ± 1.87**	111.57 ± 2.67***

Alloxan monohydrate (120 mg/kg) was administered i.p, in sterile saline, single dose, 5 days before the administration of different ethanolic extracts. Standard drug, Glibenclamide and the plant given in two doses as hydro-alcoholic extracts in 2 % gum acacia were administered orally for 10 days, in a single dose daily five days after confirmation of hyperglycaemia.
n=6 (No of animals in each group)
Day 10 compared with day 1; ***$p< 0.001$ very highly significant; $p < 0.01$ Highly Significant; $p> 0.05$ non-significant (NS)

TABLE 3: EFFECT OF 50 % ETHANOLIC EXTRACT OF *PORTULACA OLERACEA* (WHOLE PLANT) ON KIDNEY FUNCTION TESTS IN ALLOXAN INDUCED DIABETIC RATS

Group	Treatment	Serum urea levels mg/dl	Serum creatinine mg/dl	Serum total protein levels g/dl
I	Normal control 0.2 ml of 2% aqueous gum acacia	22.32 ± 3.75	0.85 ± 0.07	4.95 ± 0.61
II	diabetic control 0.2 ml of 2% aqueous gum acacia	23.06 ± 0.69	1.14 ± 0.12	3.18 ± 0.26
III	(Alloxan monohydrate +standard drug glibenclamide 10 mg/kg)	20.82 ± 1.32	0.76 ± 0.08	4.14 ± 0.88
IV	Alloxan monohydrate + ethanolic extract (PO, 100 mg/kg)	26.47 ± 2.95	1.65 ± 0.24	4.66 ± 0.77
V	Alloxan monohydrate +ethanolic extract (PO, 200 mg/kg)	25.95 ± 2.88	1.38 ± 0.14	5.20 ± 0.66

Animal: Albino rats, Alloxan: 120 mg/kg. i.p and extract: p.o. Value are mean ± S.E.M: n=6 ; * p> 0.05 non significant, **p< 0.05 significant , ***P< 0.01 highly significant;
Groups III, IV, V *vs* Diabetic control (Group II) and Group I *vs* Group II on 10th day

Average Body Weight (g): (Table 4, Fig. 3) The rats of Group I showed a body weight of (222.05 ± 4.74 g) in grams and the rats of Group II showed a very highly significant decrease (p<0.001). In average body weight (124.76 ± 2.35 g) in grams. The rats receiving 50 % ethanolic extract of *Portulaca oleracea* showed a dose dependent increase in average body weight indicating significant reduction in average body weight. A dose of 100 mg/kg b.w administered to rats of Group IV showed a significant increase (p<0.05) of (145.50 ± 8.35 g) and a dose of 200 mg/kg b.w administered to rats of Group V showed a highly significant increase (p<0.01) of (150.61 ± 3.62 g). During the course of these studies, blood glucose levels and average body weight were observed on day 1, day 4, day, 7 and day 10.

TABLE 4: EFFECT OF 50 % ETHANOLIC EXTRACT *OF PORTULACA OLERACEA* (WHOLE PLANT) ON AVERAGE BODY WEIGHT (G) AGAINST ALLOXAN INDUCED DIABETES MELLITUS IN RATS (10 DAYS STUDY)

Groups	Treatment	Average body weight (g)			
		Day 1	Day 4	Day 7	Day 10
I	Normal control 0.2 ml of 2 % gum acacia	228.85	229.88	233.00(NS)	222.05(NS)
II	Diabetic control alloxan monohydrate	180.58	163.86	152.21***	124.76***
III	Alloxan monohydrate + Std drug -Glibenclamide (10 mg/kg b.w)	183.83	173.85	152.98(NS)	137.50**
IV	Alloxan monohydrate + PO (100 mg/kg b.w)	167.20	167.00	163.12(NS)	145.50*
V	Alloxan monohydrate + PO (200 mg/kg b.w)	156.37	153.98	148.15(NS)	150.61(NS)

Alloxan monohydrate (120 mg/kg), was administered i.p, in sterile saline, single dose, 5 days before the administration of different ethanolic extracts. Standard drug, glibenclamide and three plants given as 50 % ethanolic extracts in 2 % gum acacia were administered orally for 10 days, in a single dose daily five days after confirmation of hyperglycaemia.
n=6 (No of animals in each group); Day 10 compared with day 1 *p< 0.05 significant; **p<0.01 highly significant; ***p< 0.001 very highly significant; p> 0.05 non-significant

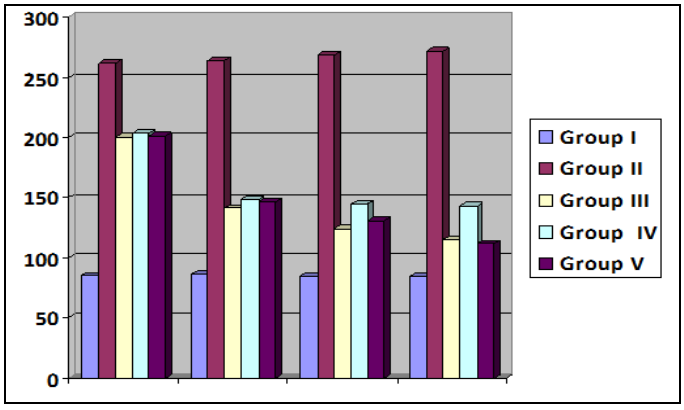

FIG. 1: EFFECT OF HYDRO-ALCOHOLIC EXTRACT OF *PORTULACA OLERACEA* (WHOLE PLANT) PO ON BLOOD GLUCOSE LEVELS mg/dl) AGAINST ALLOXAN INDUCED DIABETES MELLITUS IN RATS

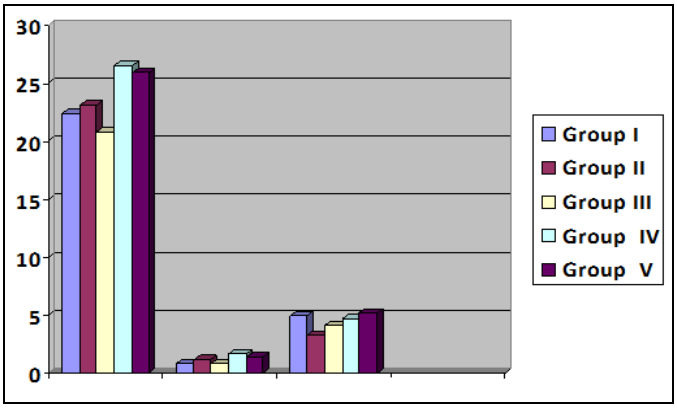

FIG. 2: EFFECT OF 50 % ETHANOLIC EXTRACT OF *PORTULACA OLERACEA* (WHOLE PLANT) ON KIDNEY FUNCTION TESTS IN ALLOXAN INDUCED DIABETIC RATS

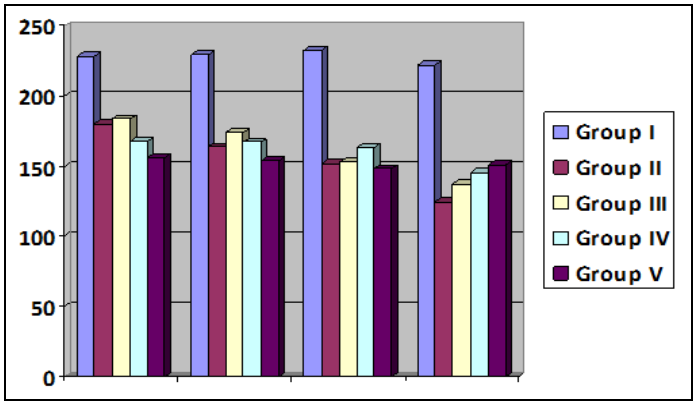

FIG. 3: EFFECT OF 50 % ETHANOLIC EXTRAC OF *PORTULACA OLERACEA* (WHOLE PLANT) ON AVERAGE BODY WEIGHT (G) AGAINST ALLOXAN INDUCED DIABETES MELLITUS IN RATS (10 DAYS STUDY)

DISCUSSION: Pancreas being the primary organ of the body is 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. There are different chemicals for inducing diabetes like alloxan and streptozotocin [38]. Alloxan is used for the induction of diabetes mellitus apart from streptozotocin. Having a destructive effect on the beta cells of the pancreas, alloxan 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 disorder characterized by resistance in the action of insulin, insufficient insulin recreation or both. 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 which weighed in the range of 180 - 210 g were procured from IIIM Jammu. They were 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. 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 °C and 70 - 75 % respectively. The phytochemical investigation of hydro-alcoholic 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 hydro-alcoholic extract of *Portulaca oleracea* reduced the glucose level in animals made diabetic with alloxan. Alloxan has been shown to induce free radical production and cause tissue injury. Pancreas is especially susceptible to the action of alloxan induced free radical damage. In the present study, hydroalcoholic extract of *Portulaca oleracea* demonstrated the significant anti-diabetic but variable renal activity [39 - 47]. The antidiabetic effect of the hydro-alcoholic 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 effect of the hydro-alcoholic 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 enhan-cement of insulin sensitivity. The literature reports reveal that flavonoids and total phenolic content present in the plant extract known to possess antidiabetic activity.

CONCLUSION: It has been concluded that the hydro-alcoholic extract of *Portulaca oleracea* has beneficial effects on blood glucose levels. Further studies on pharmacological and biochemical investtigations will clearly elucidate the mechanism of action and will help in projecting this plant as a therapeutic target in diabetics research and its effect on various complications of diabetes mellitus.

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CONFLICT OF INTEREST: Nil

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