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Research Article

ACUTE ORAL TOXICITY AND HYPOGLYCAEMIC STUDY OF ETHANOLIC EXTRACT OF *PORTULACA OLERACEA* (WHOLE PLANT) IN SWISS ALBINO MICE

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ABSTRACT

Objective: To carry out a preliminary phytochemical screening, acute oral toxicity and hypoglycemic study of 50% ethanolic extract of *Portulaca oleracea* (whole plant) in swiss albino mice.

Methods: Preliminary phytochemical screening was done as per standard procedures. Acute oral toxicity study was conducted as per OECD 423 guidelines, and method of Babu. The doses taken were 500 mg/kg,1000 mg/kg,1500 mg/kg and 2000 mg/kg b.w. The doses were given only once and the observations were made for 72 hours. Different behavioural parameters like grooming, hyperactivity, sedation, respiratory arrest, convulsions, increased and decreased motor activity and mortality were observed. The hypoglycaemic activity of the 50% ethanolic extract was also evaluated. In this activity the doses taken were 200 and 400mg/kg b.w. The doses were given once daily for 14 days. The hypoglycaemic activity was assessed by estimating the serum glucose levels. Both the studies were compared with the Normal Control groups.

Result: Phytochemical screening revealed that the whole plant extract of *Portulaca oleracea* contain alkaloids, tannins, glycosides carbohydrates, flavonoids, terpenes, phenolics, saponins, proteins and steroids. In acute oral toxicity study, the animals showed behavioural changes also.50% of the animals died at the dose level of 500 mg/kg b.w and 100% animals died at the dose levels of 1000,1500 and 2000mg/kg b.w, thereby indicating that the dose below than 500mg.kg b.w is safe for further studies. In hypoglycemic activity the dose of 400mg/kg b.w showed a highly significant reduction of serum glucose levels.

Conclusion: These Preliminary tests indicate that the 50% ethanolic extract of *Portulaca oleracea* (whole plant) at the dose level of 500 mg/kg can be used for further pharmacological research and at the dose level of 400mg/kg has significant hypoglycemic activity.

Keywords: Ethanolic extract, Acute oral toxicity, Phytochemical screening, Hypoglycemic activity.

INTRODUCTION

Diabetes is silent killer that kills one person every 10 seconds. India is having the highest number of diabetics in the world. It is a multifactorial disease which is 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 It is associated with long term complications, including retinopathy, nephropathy, neuropathy and angiopathy and several others[1]. People with diabetes are 2-4 times more likely to develop heart diseases[2]. Efforts are ongoing to understand and manage diabetes mellitus because the disease and disease related complications are increasing day by day. In spite of presence of large number of medicines in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease. There has been 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 and hypoglycaemic activity. The acute oral toxicity test aims at establishing the *therapeutic index*, i.e. the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species (LD50/ED50). The greater the index the safer the compound and *vice versa.* However, the term acute oral toxicity is most often used in connection to lethality and LD determinations [3][4]. The evaluation of the toxic action of the plant extracts is important in order to consider a treatment safe.

Portulaca oleracea, belonging to Family Portulacaceae (Purslane family) is commonly called as Common 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 in warm temperate, tropical and subtropical regions of the world[5][6]. In Srinagar it grows along waste lands and in cultivated gardens. It contains carboxylic acids, some gums, fatty acids[7] beta-carotene and volatile oil and Portuloside A, a monoterpene glucoside and phenolic alkaloids[8]. In folk medicine it is reported that it can be used as a salad and cooked like soups. It is used to treat burns earache, insect stings, inflammation, skin sores, ulcers, pruritis (itching skin) eczema and abscesses, in treatment of cardiovascular disorders, dysuria, haematuria, gonorrhoea, dysentery, sore nipples and ulcers of mouth. It is used as blood purifier. Roasted seeds are reported to be diuretic and anti-dysenteric. Omega 3– fatty acids [9] present are 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. They have positive effect on brain and in such conditions as depression, bipolar disorder, Alzhemiers disease, schizophrenia, hyperactivity and migrane. Reported Pharmacological Activities include antifungal[10], antibacterial[11], analgesic, anti-inflammatory [12][13], gastric antiulcerogenic [14], bronchodilatory [15], skeletal muscle relaxant [16],antihypertensive [17], neuropharmacological [18], wound healing [19], antioxidant [20], antifertility [21] and antitumour activities [22].Therefore, with reference to traditional and reported uses, the present study was undertaken to investigate the hypoglycaemic activity of this plant and give a scientific rational for its use.

MATERIALS AND METHODS

Plant Material

The whole plant of *Portulaca oleracea* (Family Portulacaceae) were collected from Nishat area of the district, Srinagar, during the months of April to June and authenticated by a plant taxonomist in the Centre of Plant Taxonomy, University of Kashmir, Srinagar. The identification was done on the basis of the characters described by Kirtikar and Basu, 1935. A sample of the plant material was deposited in the herbarium of the Department of Taxonomy, University of Kashmir under voucher specimen number 1011(KASH) dated 15-09-2008 for future reference. The plant material was dried in a well ventilated room with outside temperature ranging between 18 to 32^0 C.

Preparation of the extract

The dried whole plant was coarsely powdered and 500 gm of the material was allowed to macerate for 48 hrs with 50% ethanol, with occasional shaking. After 48 hrs, the ethanolic extract was filtered through Whatmans filter paper. The plant material was then macerated again with fresh 50% ethanol and the filtrate obtained from the first and the second maceration was then combined and the solvent was recovered. After the recovery of alcohol, the extract was then evaporated to dryness. The process was repeated several times and the yield was noted. The extract was refrigerated at 4^0 C for future use in experimental studies.

Phytochemical Screening

The 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. [23][24][25].

Pharmacological Study[26][27]28]

Animals and Exposure conditions

Swiss albino mice weighing about 20-25 gm were taken for conducting acute oral toxicity study and for hypoglycaemic activity. The animals were procured from Central Animal House, IIIM (Indian Institute of Integrative Medicine) Jammu & were housed in clean polypropylene cages. Before initiation of experiment, the mice were acclimatized for a period of 7 days. Standard environmental conditions such as temperature ranging from 18 to 32^o C, relative humidity (70%) and 12 hrs dark/light cycle were maintained in the quarantine. All 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].

Acute Oral Toxicity study

50% ethanolic extract of ***Portulaca oleracea*** (whole plant) (PO) was screened for acute oral toxicity study. The animals were distributed into five groups. Group I, II, III, IV and V.

Group I served as Normal Control and received 2% gum acacia.

Group II received 500 mg/kg b.w (PO),

Group III received 1000 mg/kg b.w (PO)

Group IV received 1500 mg/kg b.w (PO)

Group V received 2000 mg/kg b.w (PO).

All extracts were given in 2% gum acacia. After the extract administration, food was withheld for 2 hours. The extracts were administered in a single dose by using specially designed mice oral feeding needle. The observations that were recorded during 72 hours were grooming, hyperactivity, sedation, respiratory arrest, convulsions, increased and decreased motor activity and mortality if any.

Observations made during acute oral toxicity studies

Grooming- Clearing the fur and skin of itself or another animal

Hyperactivity- Abnormality and excessive activity; unable to relax

Sedation- Calm and composed animals without any stress

Respiratory arrest- Rising of head

Convulsions- Tremor in the tail or paddling of the feet

Increased motor activity

Decreased motor activity

Mortality.

Hypoglycaemic activity

The 50 % ethanolic extract of *Portulaca oleracea* whole plant (PO) was administered orally once daily to mice at the dose levels of 200mg/kg b.w and 400 mg/kg b.w respectively and compared with Normal Control group. Before initiation of experiment, the mice were acclimatized for a period of seven days. To study hypoglycaemic activity, animals of either sex (20-25 g b w) were divided into three groups of six mice each. The treatment was given as per the following protocol.

Group I- Normal Control (2% aqueous gum acacia)

Group II PO (200 mg/kg b.w)

Group III PO (400 mg/kg b.w)

The treatment was continued for 14 days. During this period, mice of Normal Control group received only 2% gum acacia.

Assessment of hypoglycaemic activity

After 14 days of treatment, the mice were fasted overnight and on the 15th day the mice were anaethesized with diethyl ether and blood sample from each animal was collected by cardiac puncture, in sterilized centrifuge tubes. The blood samples were allowed to coagulate at 30^oC. Serum was separated by centrifugation at 2500 r/min at 30^oC for 15 min and subjected to biochemical investigation using standard test kit to assess serum glucose level [29][30]. Glucose kit was obtained from Crest Biosystems, Goa, India.

Statistical analysis

All the results were expressed as mean ± SEM. One way analysis of variance (ANOVA) was used for the statistical analysis of data. Students "t" test was used for determining the significance. A probability value of $p > 0.05$ was considered as non significant, *$p< 0.05$ – significant, **$p< 0.01$- highly significant and ***$p<0.001$ as very highly significant.

RESULTS

Phytochemical screening (Table 1)

The phytochemical screening of 50% ethanolic extract of ***Portulaca oleracea*** **(PO)** carried out by standard procedures revealed the presence of tannins, alkaloids, saponins, glycosides, terpenes, phenolics, flavonoids, carbohydrates, proteins and steroids.

The results obtained were comparable and satisfied the standard literature.

Table 1: Results of Phytochemical screening of *Portulaca oleracea* (whole plant)

	Phytoconstituents	Results
1	Tannins	+
2	Alkaloids	+
3	Saponins	+
4	Glycosides	+
5	Terpenes	+
6	Phenolics	+
7	Flavonoids	+
8	Carbohydrates	+
9	Proteins	+
10	Steroids	+

Acute Oral Toxicity Tests (72 hour study) (Table 2)

Portulaca oleracea administered at four dose levels (500, 1000, 1500 and 2000 mg/kg b.w) revealed the following effects during the acute toxicity studies conducted in mice for 72 hours. Control mice which had received 2% of gum acacia showed normal behaviour

i) Grooming: After, 48 and 72 hours, no grooming was observed at all the four dose levels.

ii) Hyperactivity: PO extract at the administered dose levels had no effect on the activity of mice which remained normal.

iii) **Sedation:** After 48 hours, sedation was seen in 50 % of animals at the dose of 500 mg/kg b.w while 100 % of animals in the dose range of 1000, 1500 and 2000 mg/kg b.w showed sedation,as the animals remained calm and composed without any stress.

iv) **Respiratory arrest:** After 48 hours, 50 % of animals in the dose range of 500 mg/kg b.w and 100% animals in the dose range of 1000, 1500 and 2000 mg/kg b.w showed respiratory arrest indicated by raising of head in both animals of these groups.

v) **Convulsions:** After 48 and 72 hours 50 % of animals in the dose range of 500 mg/kg b.w and 100% animals in the dose range of 1000,1500 and 2000 mg/kg b.w showed convulsions as tremor in the tail and the paddling of the feet.

vi) **Motor Activity:** After 48 hours, 50 % of animals at the dose of 500 mg/kg b.w and. 100 % of animals in the dose range of 1000,1500 and 2000 mg/kg b.w showed decreased motor activity.

vii) **Mortality:** 50 % of animals died at the dose of 500 mg/kg b.w after 72 hours while 100 % of animals in the dose range of 1000, 1500 and 2000 mg/kg b.w died after 24 hours.

Table 2: Observations during acute toxicity studies of different doses of 50% Ethanolic extract of *Portulaca oleracea* (whole plant)

S. No.	Treatment	Observations (Hours)														
		Grooming							Hyperactivity							
		1	2	3	4	24	48	72	1	2	3	4	24	48	72	
1	Normal control 2% aqueous gum acacia	+	+	+	+	+	+	+	-	-	-	-	-	-	-	
2		+	+	+	+	+	+	+	-	-	-	-	-	-	-	
1	500 mg/kg b.w	+	+	+	+	-	-	-	-	-	-	-	-	-		
2		+	+	+	+	-	-	-	-	-	-	-	-			
1	1000mg/kg b.w	+	+	+	+	-			-	-	-	-	-			
2		+	+	+	+	-			-	-	-	-	-			
1	1500mg/kg b.w	+	+	+	-	-			-	-	-	-	-			
2		+	+	+	-	-			-	-	-	-	-			
1	2000mg/kg b.w	+	+	+	-	-			-	-	-	-	-			
2		+	+	+	-	-			-	-	-	-	-			

S. No	Treatment	Observation (Hours)														
		Sedation							Respiratory Arrest							
		1	2	3	4	24	48	72	1	2	3	4	24	48	72	
1	Normal control 2% aqueous gum acacia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1	500mg/kg b.w	-	-	-	-	-	-	-	-	-	-	-	_	-		
2		-	-	-	-	+			-	-	-	-	+	-		
1	1000mg/kg b.w	-	-	-	+	+			-	-	-	-	+	-		
2		-	-	-	+	+			-	-	-	-	+	-		
1	1500mg/kg b.w	-	-	-	+	+			-	-	-	-	+	-		
2		-	-	-	+	+			-	-	-	-	+	-		
1	2000mg/kg b.w	-	-	-	+	+			-	-	-	-	+	-		
2		-	-	-	+	+			-	-	-	-	+	-		

S. No	Treatment	Observations (Hours)														
		Convulsions							Increased motor activity							
		1	2	3	4	24	48	72	1	2	3	4	24	48	72	
1	Normal control 2% aqueous gum acacia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1	500mg/kg b.w	-	-	-	-	-	-		-	-	-	-	-	-		
2		-	-	-	-	+			-	-	-	-	-	-		
1	1000mg/kg b.w	-	-	-	-	+			-	-	-	-	-			
2		-	-	-	-	+			-	-	-	-	-			
1	1500mg/kg b.w	-	-	-	+	+			-	-	-	-	-			
2		-	-	-	+	+			-	-	-	-	-			
1	2000mg/kg b.w	-	-	-	+	+			-	-	-	-	-			
2		-	-	-	+	+			-	-	-	-	-			

S. No	Treatment	Observation (Hours)														
		Decreased Motor Activity							Death							
		1	2	3	4	24	48	72	1	2	3	4	24	48	72	
1	Normal control 2% aqueous gum acacia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1	500mg/kg b.w	-	-	-	-	-	-		-	-	-	-	-	-	-	
2		-	-	-	-	+			-	-	-	-	-	-	Dead	
1	1000mg/kg b.w	-	-	-	-	+			-	-	-	-	Dead			
2		-	-	-	-	+			-	-	-	-	Dead			
1	1500mg/kg b.w	-	-	-	+	+			-	-	-	-	Dead			
2		-	-	-	+	+			-	-	-	-	Dead			
1	2000mg/kg b.w	-	-	-	+	+			-	-	-	-	Dead			
2		-	-	-	+	+			-	-	-	-	Dead			

Table 3: Results of hypoglycaemic activity of different doses of 50% ethanolic extract of *Portulaca oleracea* (whole plant)

Group	Treatment	Serum Glucose Levels (mg/dl)
I	Normal Control	91.50± 6.80
II	PO 200 mg/kg	60.00± 9.83*
III	PO 400 mg/kg	46.83± 5.84**

The observations are mean ± SEM of 6 animals,*p<0.05 and **p<0.01 as compared to that of Normal Control group (One way ANOVA followed by students "t "test).

*p < 0.05 – Significant, **p < 0.01 – Highly Significant

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. Today 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. India has 45,000 plant species and several thousand have medicinal properties. More than 800-1000 plant species have anti- diabetic activity. Herbal medicines have received greater attention as an alternative to clinical therapy and the demand for these remedies has currently increased.

The Indian indigenous drugs have great importance both from professional and economic point of view[31-40]. 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.*.An acute toxicity test can give details about the biologic properties and the mechanism of action of a chemical compound than any other single test. Long-term studies usually start with a dose finding exercise under acute conditions. The importance of this plant in folk medicine as well as promoting pharmacological properties, make studies about its toxicity very important.

The phytochemical screening of 50% ethanolic extract of whole plant of *Portulaca oleracea* carried out by standard procedures revealed the presence of alkaloids, flavonoids, glycosides, terpenes, saponins, carbohydrates, proteins, tannins, phenolics and steroids.

The oral administration of 50% ethanolic extract of whole plant in doses of 500-2000 mg/kg b.w showed that 50% of animals died in the dose range of 500 mg/kg b.w and 100 % of animals died in the dose range of 1000, 1500 and 2000 mg/kg b.w. w after 72 hours.

The behavioural observations showed that there was effect on grooming, sedation, respiratory arrest, convulsions. There was decreased motor activity before death. It can be concluded that LD50 of the plant is 500mg/kg b.w which means that doses below 500 mg/kg b.w are safe for oral administration. Since LD50 of the ethanolic extract was 500mg/kg b.w, so the present studies were carried at the dose levels of 200 and 400 mg/kg b.w/day of the 50% ethanolic extract of the plant.

In hypoglycaemic study, the 50 % ethanolic extract of the whole plant when administered for 14 days showed reduction in serum glucose levels. The dose of 400mg/kg was found to be more effective. This shows that *Portulaca oleracea* has significant hypoglycemic effect. The literature and the studies reveal that flavonoids and tannins present in the plant extract might be responsible for showing this activity.

CONCLUSION

From the study, it can be concluded that the 50% ethanolic extract of whole plant of *Portulaca oleracea* has beneficial effects on serum glucose levels by showing hypoglycaemic activity. Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as an therapeutic target in diabetics research.

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