Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING, RENAL AND HAEMATOLOGICAL EFFECTS OF *PORTULACA OLERACEA* (WHOLE PLANT) IN SWISS ALBINO MICE

Sabeeha Shafi*, Nahida Tabassum

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

*Corresponding Author Email: saabeeha_shafi@yahoo.com

Article Received on: 17/04/15 Revised on: 07/05/15 Approved for publication: 16/05/15

DOI: 10.7897/2230-8407.06672

ABSTRACT

Portulaca oleracea (Family Portulacaceae) has been used all over the world for its nutritional and medicinal properties. It shows a number of pharmacological activities but very little information is known about its toxicity profile. The present study was undertaken to find its acute and subacute oral toxicity. In acute oral toxicity, four doses 500mg, 1000mg, 1500mg and 2000mg/kg b.w were administered orally to swiss albino mice with the help of oral feeding needle. In subacute oral toxicity, the doses of 200mg and 400mg/kg b.w were given. A total of 28 swiss albino mice, divided in different groups were taken. Behavioural changes and LD₅₀ were recorded in acute oral toxicity. In subacute oral toxicity studies, kidney function tests, hematological and histopathological changes were recorded. The results showed that LD₅₀ was 500mg/kg. There were behavioural and biochemical variations with no toxicity on kidney and spleen.

Keywords: Acute oral toxicity, subacute toxicity, renal and haematological activity

INTRODUCTION

Since the beginning of civilization, herbal plants have been used in the treatment of various diseases in India and all over the world. These herbal plants have been the source of synthetic drugs as well as traditional herbal medicine¹⁻⁸. India has a rich wealth of medicinal plants thousands of which have been scientifically proved to be beneficial, while a lot is still to be done to prove if these plants have any toxicity on different organs. These plants are pharmacologically active. Different parts of the herbal plants have been used e.g stem, leaves, fruits, roots etc.

Major pharmaceutical companies are nowadays conducting extensive research on herbal plants. World Health Organization (WHO) estimates that 80% of the total world population use herbal medicine for some aspect of primary health care because of wider acceptability and lesser side effects. To ensure the safety of any herbal drug, its toxicity evaluation is necessary. Three types of toxicity evaluation are usually carried out viz., acute, subacute and chronic type^{9,10}. Acute toxicity gives information about the LD₅₀ the minimum lethal dose that kills 50% of the animal population. Subacute and chronic type gives information on the cumulative toxicity at low dose on prolonged period of time.

Portulaca oleracea L (Portulacaceae) is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term "Global Panacea". Its common name is Nunar in Kashmir, common Purslane in English. It occurs as a cosmopolitan weed^{11,12}, along waste lands and in cultivated gardens. Some of the chemical constituents include gums, fatty acids, betacarotene, volatile oil, carboxylic acids and omega-3-fatty acids¹³⁻¹⁵. The major pharmacological activities include bronchodilatory, antihypertensive, neuropharmacological, wound healing, antifungal, antibacterial and many others.¹⁶⁻²⁸ Popularity of this plant in both traditional medicinal system as well as in various research studies focused the attention on its toxicity profile. Very

little literature is known about its toxicity profile on different biochemical parameters and organs.

Objective: The present study was undertaken to find its LD₅₀ (minimum lethal dose) acute oral toxicity studies for 72 hours and subacute oral toxicity on kidney and blood parameters with histopathological studies on kidney and spleen.

MATERIALS AND METHODS

Plant Material

Portulaca oleracea (whole plant) was collected in the months of April to June from Nishat area of Srinagar city. It was authenticated by Mr Naqshi, plant taxonomist in the Centre of Plant Taxonomy, University of Kashmir, Srinagar. The plant material under Voucher specimen number 1011(KASH) dated 15-09-2008 was deposited in the herbarium of the Department of Taxonomy, University of Kashmir for future reference. It was dried under shade with outside temperature ranging between 18 to 32°C in a well ventilated room.

Preparation of the Plant Extract

Whole plant was collected, shade dried and coarsely powdered separately by using pulverizer. With occasional shaking, 500 gm of the material was allowed to macerate for 48 hrs with 50% ethanol by cold percolation process to yield the extract. After 48 hrs, the 50% ethanolic extract was filtered through Whitman's filter paper. It was then macerated again with fresh 50% ethanol and the filtrate obtained from the first and the second maceration was then combined. The solvent was recovered. The extract was then evaporated to dryness, after the recovery of ethanol. This process was repeated several times. At the end, the yield was noted. The 50% ethanolic extract was refrigerated at 4°C for future use in experimental studies.

Phytochemical Screening²⁹⁻³¹

The powdered whole plant of *Portulaca oleracea* PO was subjected to preliminary phytochemical screening. The presence of various phytoconstituents was determined by the standard qualitative methods²⁹⁻³¹

Tannins: To 2 ml of aqueous extract 2 ml of 5% FeCl₃ was added. Formation of yellow brown precipitate indicates that tannins are present

Alkaloids: To 2 ml methanolic filtrate, 1.5 ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Mayors reagents/ Wagner's reagent / Dragendorff's reagent was added. Formation of orange precipitate indicates the presence of alkaloids

Saponins: Aqueous extract of 2 g powder was made and subjected to frothing test. Persistence of the froth indicated presence of saponins. Latter the froth was mixed with few drops of olive oil. Formation of emulsion indicates presence of saponins.

Glycosides: To 2 ml alcoholic filtrate, 1 ml glacial acetic acid and 1-2 drops of FeCl₃ was added followed by 1 ml of concentrated H₂SO₄. Appearance of brown ring at the interface indicates presence of glycosides. A violet ring may also appear below the brown ring.

Terpenes: To 2 ml of aqueous extract, 5 ml chloroform, 2 ml acetic anhydride and concentrated H₂SO₄ was added carefully to form layer. Reddish brown coloration of interface indicates terpenes.

Flavonoids: 2 g plant material was extracted in 10 ml alcohol of water. To 2 ml filtrate few drops of concentrated HCl followed by 0.5 g of zinc or magnesium turnings was added. After 3 minutes magenta red or pink colour indicated the presence of flavonoids.

Phenolics: To 2 ml of alcoholic or aqueous extract, 1 ml of 1% ferric chlorohide solution was added. Blue or green colour indicates phenols.

Carbohydrates 200 mg of the powdered drug was extracted in 10 ml of water. To 5 ml of this solution were added few drops of alcoholic α naphthol and then 0.2 ml of concentrated H₂SO₄ was added slowly along the sides of the test tube, any purple colour appearance at the junction confirms the presence of carbohydrates.

Proteins 20 mg of powdered drug was boiled with 3 ml of 0.2% solution of Ninhydrin (Indane 1,2,3 trionehydrate). Appearance of violet colour indicates the presence of proteins

Steroids: To 0.5 gms of ethanolic extract, 2 ml of acetic anhydride was added and then 2 ml of H₂SO₄ were carefully added. Any colour change from violet to green indicates the presence of steroids

Pharmacological Study³²⁻³⁴**Animals and Exposure conditions**

Swiss albino mice (20-25 gm) procured from Central Animal House, IIM (Indian Institute of Integrative Medicine) Jammu were taken for conducting acute and subacute oral toxicity studies. The animals were housed in clean polypropylene cages. Standard environmental conditions such as temperature ranging from 18 to 32° C, relative humidity (70%) and 12 hrs dark/light cycle were maintained in the quarantine. The mice were acclimatized for a period of 7 days, before initiation of experiment. All the animals were fed with rodent pellet diet (Ashirwad Industries) and water *ad-libitum* under strict hygienic conditions. 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

Portulaca oleracea PO (50% ethanolic extract of whole plant) was screened for acute oral toxicity study. The animals were divided into five groups.

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

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

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

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

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

All extracts were given in 2% gum acacia as vehicle. Food was withheld for 2 hours after the extract administration. The extracts were administered by using specially designed mice oral feeding needle. The animals were observed continuously for the first four hours and then for 72 hours for behavioural changes like grooming, hyperactivity, sedation, respiratory arrest, convulsions, increased and decreased motor activity and mortality.

Subacute Toxicity study (14 days)

Portulaca oleracea PO (50 % ethanolic extract of whole plant was administered orally once daily to mice. Animals of either sex (20-25 g body weight) were divided into three groups of six mice each. The treatment was given as per the following protocol.

Group I- Normal Control animals which received 2% aqueous gum acacia

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

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

This treatment was continued for 14 days. After 14 days of the treatment, animals were fasted overnight and blood was collected by cardiac puncture. The blood samples were evaluated for kidney function tests and haematological parameters. The blood was allowed to clot for one hour and serum was separated by centrifuging. The animals were sacrificed, after taking the blood samples. Kidney and spleen were excised from the animals. They were preserved in 10% formalin and sent for histopathological studies. The following biochemical parameters were evaluated in the subacute oral toxicity studies.

A) Kidney Function Tests

- i. Serum Urea Levels
- ii. Serum Creatinine Levels
- iii. Serum total protein levels.
- iv. Serum albumin levels.

B) Blood Function Tests

- i. Hemoglobin Value
- ii. WBC Count

Statistical analysis

Results were presented as mean $\pm$ 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

<i>Portulaca oleracea</i>	
Phytochemical Constituents	Results
Tannins	+
Alkaloids	+
Saponins	+
Glycosides	+
Terpenes	+
Phenolics	+
Flavonoids	+
Carbohydrates	+
Proteins	+
Steroids	+

Acute Oral Toxicity Tests (72 hour study)

At four dose levels (500, 1000, 1500 and 2000 mg/kg b.w) *Portulaca oleracea* administered as 50% ethanolic extract revealed the following effects. 50% of the animals died at the dose level of 500 mg/kg b.w. At the dose levels of 1000, 1500 and 2000 mg/kg b.w, 100% of the animals died thereby indicating that the dose below than 500 mg/kg b.w is safe for further studies. Mice which were treated as control group and which had received 2% of gum acacia showed normal behavior. No grooming was observed at all the four dose levels after 48 and 72 hours. The plant extract did not show effect on the activity of mice which remained normal. In the dose range of 1000, 1500 and 2000 mg/kg b.w 100 % animals showed sedation, respiratory arrest, convulsions, decreased motor activity and mortality.

Subacute Oral Toxicity Tests (14 day study)

Group	Treatment	Serum urea levels (mg/dl)	Serum creatinine levels (mg/dl)	Serum total protein levels (g/dl)	Serum albumin levels (g/dl)	Haemoglobin levels (g/dl)	WBC Count Per cubic mm
I	Normal Control	16.74	0.83	6.81	3.45	7.40	1900
II	PO 200mg/kg	21.17	0.52	6.49	3.64	7.36	1866
III	PO 400mg/kg	22.64*	0.65	6.93	4.00*	7.53	1500*

The observations are mean $\pm$ 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). n= 6

Figure 1: Effect of 50% ethanolic extract of whole plant of *Portulaca oleracea* on kidney function tests and hemoglobin in swiss albino mice

Figure 2: Effect of 50% ethanolic extract of whole plant of *Portulaca oleracea* on WBC Count in swiss albino mice

Figure 3: Histopathology of spleen of mice of Group I –Normal Control showing normal red and white pulp areas in the splenic parenchyma. (H&E x 40X) WP= White Pulp, RP= Red Pulp.

Figure 4: Histopathology of spleen of mice of Group –II *Portulaca oleracea* whole plant (200 mg/ kg b.w) showing the normal red and white pulp areas in the splenic parenchyma. (H&E x 40X) WP=White Pulp, RP=Red Pulp.

Figure 5: Histopathology of spleen of mice of Group –III –*Portulaca oleracea* whole plant (400mg/kg b.w) showing the normal red and white pulp areas in the splenic parenchyma. (H&E x 40X) WP= White Pulp, RP= Red Pulp

Figure 6: Histopathology of kidney of mice of Group I- Normal Control showing renal parenchyma. Glomerulus of normal size and cellularity. Tubules are within normal limits (H&E x 40X) G = Glomerulus, T= Tubules

Figure 7: Histopathology of kidney of mice of Group II *Portulaca oleracea* whole plant (200mg/kg) showing renal parenchyma. The Glomerulus is of normal size and cellularity. Tubules are within normal limits. (H&E x 40X) G = Glomerulus T= Tubules

Figure 8: Histopathology of kidney of mice of Group III *Portulaca oleracea* wholeplant(400mg/kg.w) showing renal parenchyma. Glomerulus of normal size and cellularity. Tubules within normal limits. (H&E x 40X) G = Glomerulus T= Tubules

CONCLUSION

The present study concludes that the 50% ethanolic extract of whole plant of *Portulaca oleracea*, based on acute oral toxicity study is safe at the dose of 500mg/kg or below this dose. The extract showed changes in behaviour also in acute oral toxicity when observed for 72 hours. In subacute oral toxicity, there were variable changes in renal and hematological biochemical parameters (Figures

1 & 2) but these levels were not that much toxic on tissues. Histopathological studies showed the extract when administered at the dose level of 200 and 400mg/kg did not cause any toxicity to kidneys and spleen (Figures 3-8). From the literature survey, the reported phytoconstituents are responsible for the protective function. Further biochemical and pharmacological investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as therapeutic target in research³⁵.

ACKNOWLEDGEMENT

We are highly thankful to University Grants Commission, New Delhi for financial assistance. The facilities provided by the Department of Pharmaceutical Sciences University of Kashmir for carrying out this work also need appreciation.

REFERENCES

- Kirtikar and Basu. Indian Medicinal Plants. Dehra Dun, Uttaranchal, India, 2001; 2: 333-35.
- Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Lalit Mohan Basu, Allahabad, 1933;2: 1478-81.
- Kirtikar KR, Basu BD. Illustrated Indian Medicinal Plants, India Sri Satguru Publications, Delhi, 2000a; 2: 330-33.
- Kirtikar KR, Basu BD. Illustrated Indian Medicinal Plants, India, Sri Satguru Publications, Delhi, 2000b;7: 2241.
- Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants Publications and Information's Directorate (CSIR), New Delhi, 1999; I: 326, 398.
- Rastogi RP, Mehrotra BN. Compendium of India Medicinal Plants Publications and Information Directorate (CSIR), New Delhi, 1990; II: 398.
- Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants Publications and Information Directorate (CSIR), New Delhi, 1991; II: 660.
- Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants India Publications and Information Directorate (CSIR), New Delhi, 1995; V: 405.
- Ghosh MN. Fundamentals of experimental Pharmacology 2nd Edition, Scientific Book Agency Calcutta, 1984: 154-57.
- Ghosh MN. Fundamentals of Experimental Pharmacology, 3rd Edition. S.K. Ghosh & others publications; 2005: 190-97.
- Banerjee, Gautam and Mukherjee. *Portulaca oleracea* L: A gem of aliens in India. J of Phytochem Res, 1996; 9(2): 111-15.
- Mitish, Larry W. Common purslane (*Portulaca oleracea*). Weed Technology, 1997;11(2): 394-97
- Liu L. Fatty acids and β carotene in Australian Purslane (*Portulaca oleracea*) varieties. J Chromatogr, 2000; 893: 207-13. [http://dx.doi.org/10.1016/S0021-9673\(00\)00747-0](http://dx.doi.org/10.1016/S0021-9673(00)00747-0)
- Zijuan Y, Cejia L, Lan X, Yinan Z. Phenolic alkaloids as a new class of antioxidants in *Portulaca oleracea*, *Phytotherapy Res*, 2009; 23(7): 1032-35. <http://dx.doi.org/10.1002/ptr.2742>
- Simopoulos AP, Norman HA, Gillaspay JE, Duke JA. Common Purslane: a source of omega-3 fatty acids and anti-oxidants. J Amer College Nutr, 1992; 11 (4): 374-82. <http://dx.doi.org/10.1080/07315724.1992.10718240>
- Banerjee G, Mukherjee A. Biological activity of a common weed: *Portulaca oleracea* L.-II. Antifungal activity. Acta Botan Hungarica, 2002; 44(3-4): 205-08. <http://dx.doi.org/10.1556/ABot.44.2002.3-4.1>
- Banerjee G, Mukherjee A. Antibacterial activity of a common weed, *Portulaca oleracea* L. Geobios (Jowhpur), 2003; 30(2-3): 143-44.
- Chan.K, Islam MW, Kamil M, Radhakrishnan R, Zakaria MNM. The analgesic and anti-inflammatory effects of *Portulaca oleracea* L., sub sp. *Sativa*. J of Ethnopharmacol 2000; 73 (3): 445-51. [http://dx.doi.org/10.1016/S0378-8741\(00\)00318-4](http://dx.doi.org/10.1016/S0378-8741(00)00318-4)
- Islam MW, Zakaria MNM, Radhakrishnan R. Evaluation of analgesic activity of the aerial parts of *Portulaca oleracea* v. *sativa* and its comparison with two related spices. J of Pharm and Pharmacol, 1998 ;50: 226-30 <http://dx.doi.org/10.1111/j.2042-7158.1998.tb02426.x>
- Karimi G, Hosseinzadeh H, Ettehad N. Evaluation of the gastric antilucerogenic effects of *Portulaca oleracea* L. extracts in mice. *Phytother Res*, 2004; 18(6): 484-87. <http://dx.doi.org/10.1002/ptr.1463>
- Malek F, Oskabady MH, Borushaki MT, Tohidi M. Bronchodilatory effect of *Portulaca oleracea* in airways of asthmatic patients. J Ethnopharmacol, 2004; 93(1): 57-62. <http://dx.doi.org/10.1016/j.jep.2004.03.015>
- Okwuasaba FC, Ejibe, Parry O. Skeletal muscle relaxant properties of the aqueous extract of the *Portulaca oleracea*. J Ethnopharmacol, 1986; 17: 139-60. [http://dx.doi.org/10.1016/0378-8741\(86\)90054-1](http://dx.doi.org/10.1016/0378-8741(86)90054-1)
- Parry O, Okwuasaba F, Ejibe C. Effect of an aqueous extract of *Portulaca oleracea* leaves on smooth muscle and rat blood pressure. J Ethnopharmacol, 1988; 22: 33-44. [http://dx.doi.org/10.1016/0378-8741\(88\)90228-0](http://dx.doi.org/10.1016/0378-8741(88)90228-0)
- Radhakrishnan, R, Zakaria MNM, Islam MW, Ismail A, Habibullah M, Chan K. Neuropharmacological actions of *Portulaca oleracea* v. *sativa*. J of Pharm and Pharmacol, 1998;50: 225. <http://dx.doi.org/10.1111/j.2042-7158.1998.tb02425.x>
- Rashed AN, Afifi FU and Disi AM. Simple evaluation of the wound healing activity of a crude extract of *Portulaca oleracea* L. in *Mus musculus*. J Ethnopharmacol, 2003; 88(2-3): 131-36. [http://dx.doi.org/10.1016/S0378-8741\(03\)00194-6](http://dx.doi.org/10.1016/S0378-8741(03)00194-6)
- Sanja SD, Sheth NR, Patel NK. Characterization and evaluation of anti-oxidant activity of *Portulaca oleracea*. Inter J of Pharm and Pharmaceutical Sci, 2009; 1 (1):74-84
- Verma OP, Kumar S, Chatterjee SN. Anti-fertility effects of common edible *Portulaca oleracea* on the reproductive organs of male albino mice. Ind J of Med Res, 1982;75: 301-10.
- Yoon JW, Ham SS, Jun HS. *Portulaca oleracea* and tumour cell growth. Official Gazette of the United States Patent and Trademark Office Patents, 1999; 1219(2): 1472, 585
- Harborne JB *Phytochemical methods*. Chapman and Hall Ltd., London, 1973: 49-188.
- Rafia Rasool, Bashir A Ganai, Seema Akbar, Azra Kamili. Phyto chemical screening of *Prunella vulgaris* L. An important Medicinal Plant of Kashmir Pak. J. Pharm Sci, 2010; 23(4): 399-402.
- Trease GE and Evans WC. *Pharmacognosy*, 11th edn., Brailliar Tiridel Macmillian Publishers;1989.
- OECD. Guidance document on the recognition, assessment and use of clinical signs as humane endpoints for experimental animals used in safety evaluation. Series on Testing and Assessment No. 19, ENV/ JM/MOMO; 2000: 7.
- OECD. Acute oral toxicity. Acute and toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the OECD guidelines for the testing of chemicals organization for economical co-operation and development Paris, June, 2002.
- Babu V, Gangadevi T, Subramanian A. Antidiabetic activity of ethanol extract of *Cassia Klenii* leaf in streptozotocin-induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. Ind. J of Pharmacol 2003; 35: 290-296.
- Shorinwa OA, Aghanva JE. Evaluation of the anti-diabetic activity of ethanol extract of *Anchomanes difformis* leaves in albino rats. Int Res J Pharm.2015; 6(2):90-93. <http://dx.doi.org/10.7897/2230-8407.06221>

Cite this article as:

Sabeeha Shafi, Nahida Tabassum. Preliminary phytochemical screening, renal and haematological effects of *Portulaca oleracea* (whole plant) in swiss albino mice. Int. Res. J. Pharm. 2015; 6(6):349-353 <http://dx.doi.org/10.7897/2230-8407.06672>