

Full Length Research Paper

Evaluation of ethanolic extract of *Portulaca tuberosa* Roxb leaves for antipyretic activity

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Portulaca tuberosa (*P. tuberosa*) Roxb belonging to the family Portulacaceae, is traditionally used internally in dysuria as well as applied externally to erysipelas by the natives. The aerial parts of the plant contain diterpenoids, pilosanone A and pilosanone B. Previous studies showed that *P. tuberosa* has good diuretic and analgesic effects when used by the people traditionally, however, *in vivo* antipyretic activity was still lacking for established scientific evidences; hence the present study was carried out to investigate and report the antipyretic activity of ethanolic leaf extract of *P. tuberosa* (ELPT) using aspirin as standard drug against pyrexia induced by subcutaneous injection of 20% w/v aqueous yeast suspension in normal saline at the dose of 10 ml/kg body weight. Swiss albino mice were used for the study and divided into 5 groups containing 6 mice in each group. Three doses of ELPT that is, 200, 400 and 800 mg/kg B/w were used for activity. After 4 h of extract administration, the rectal temperatures were recorded by digital thermometer (Holden medical B.V, Netherlands). The result showed that *P. tuberosa* leaf extract at a dose of 800 mg/kg possessed more potent significant ($p < 0.01$) antipyretic activity in comparison to the other doses that is, 200 and 400 mg/kg when compared to the standard.

Key words: *Portulaca tuberosa* Roxb, yeast-induced pyrexia, antipyretic, aspirin.

INTRODUCTION

The plant "*Portulaca tuberosa* Roxb" commonly known as "Bichhu-buuti", belongs to the family Portulacaceae. The plant is native to peninsular India, Sind in West Pakistan and to the Srilanka (Ceylon) near sea-coasts. A number of active chemical compounds such as tannins, phenol, terpenoids and saponins have been found in the genus and exhibits antidiabetic, antimicrobial and neuroprotective properties. The plant is an herb perennial, prostrate, diffusely branched, fleshy and glabrous. Branches are straggling, 4 to 15 cm long with 2

to 3 mm long internodes. Roots are tuberous, somewhat fusiform and 5 to 8 cm long. Leaves are alternate, sessile to subsessile, linear or linear-lanceolate, 8 to 14 mm long and 1 to 1.5 mm broad, fleshy, acute; stipular hairs dense, 4 to 8 mm long, somewhat brownish. Inflorescence a small, sessile cluster of 2 to 3 flowers subtended by 6 to 8 leaved involucre. Flowers are 10 mm across, yellow, surrounded by ring of bracteate hairs akin to stipular hairs. In Ayurvedic system of medicine, various parts of plant are traditionally used including analgesic, diuretic

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and in fever (Khare, 2007).

MATERIALS AND METHODS

Chemicals

Acetyl salicylic acid (CDH Ltd, New Delhi), Yeast *Saccharomyces cerevisiae* (CDH Ltd New Delhi), digital thermometer (Dr. Morepen Digital Hard Tip Thermometer (MT 101)), digital weighing balance (Scientech Zeta Analytical Balances), absolute ethanol.

Test animals

The animals used in the study were Swiss albino mice (20 to 30 g), within age 3 to 4 week of either sex. The animals were kept in cages at animal house, and maintained at room temperature of $25 \pm 2^\circ\text{C}$ with relative humidity ($60 \pm 10\%$) under 12 h night and light cycle. All the animals were kept at overnight fasting before every experiment. The animals used were approved according to animal ethics committee Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad, U.P.

Collection and extraction of plan

The whole plant of *P. tuberosa* Roxb was collected from District Gaya Bihar, and was authenticated after washing the leaves, covered with cloth and dried in shade for 20 days at room temperature. The leaves were grinded through mechanical grinder to coarse powder. The powdered plant material (250 g) was extracted through maceration technique using 80% ethanol (1:5) as a solvent for 72 h at room temperature with occasional manual shaking. After maceration, the mixture was then filtered through Whatman filter No.1 paper in a flask and tightly capped. The extract was then concentrated under reduced pressure through rotary evaporator and then air dried (Singh et al., 2013).

Acute toxicity study

Acute toxicity study of the extract was determined on Swiss albino mice of either sex. The dose of extract was increased one to three folds to determine the safety level of the extract. The mice were divided into three groups each containing two mice. The first group received only normal saline and the second and third groups received i.p injection of tested drug at doses of 1000 and 3000 mg/kg. After administration of doses, mice were observed for 72 h for any toxic effect.

Antipyretic activity

The antipyretic effect of the ethanolic leaf extract of *P. tuberosa* (ELPT) was determined on Swiss albino mice (20 to 30 g). The animals were divided into five groups containing six mice in each. The normal body temperature of each mouse was recorded by using digital thermometer, inserted in rectum at predetermined interval. Fever was induced by subcutaneous injection of yeast 20% w/v in normal saline at the rate of 10 ml/kg body weight. 15 h after the injection of yeast, the rectal temperature of each animal was again recorded by digital thermometer. Only those animals that show a minimum increased of 0.7°C in temperature after injection of yeast were taken for experiments. Aspirin (100 mg/kg, i.p) was used as reference drug. Group 1 received only (10 ml/kg) normal saline i.p, group 2 received Aspirin (100 mg/kg) as a reference

drug, while groups 3, 4 and 5 received 200, 400 and 800 mg/kg B/w of ELPT, respectively. After drug administration rectal temperature of each animal were then recorded following 0, 1, 2, 3 and 4 h by digital thermometer. Significant decrease in fever in tested animals was compared to control group (Adams et al., 1968).

RESULTS

The present study was performed to find out the antipyretic effect of the ELPT. The result of the present study showed that the ELPT has significant antipyretic effect with a reasonable safe profile.

Statistical analysis

Results were expressed as mean \pm standard error of mean (SEM). The statistical significance between control and treated groups were performed using analysis of variance (ANOVA) test. For multiple comparisons among the groups Bonferroni test was performed. A probability level of $p < 0.05$, $p < 0.01$ and $p < 0.001$ was accepted statistically significant.

Acute toxicity study

The ELPT was tested at two doses, 1000 and 3000 mg/kg for toxicity and then compared with control (normal saline group). No major behavioral changes or mortality were noted post administration of the extract at dose of 1000 mg/kg for 72 h. While mortality was observed at the dose of 3000 mg/kg of extract for 72 h.

Antipyretic effect against yeast induced pyrexia

The antipyretic activity of the ELPT was determined by yeast induced pyrexia in mice. The result showed that tested drug at different doses caused lowering of the body temperature up to 4 h following its administration (Table 1). Seventeen hours after s/c injection of 20% of yeast, a significant increase in rectal temperature was observed in all animals. The effect of ethanolic extract on yeast induced pyrexia shows that rectal temperature was 38.10°C , 15 h after the s/c injection of yeast suspension, which further decreased to 37.31°C within 1 h by the treatment of extract (800 mg/kg), and subside after 4 h showing a sizeable reduction in rectal temperature and was comparable to reference drug Aspirin (Figure 1). The extract at a dose of 200 mg/kg body weight showed reduction of pyrexia induced by yeast but it was not significant statistically $p > 0.05$ up to 3 h and showed significant $p < 0.001$ at 4 h when compared to control. Treatments with ethanolic extract of *P. tuberosa* at a dose of 400 and 800 mg/kg body weight decreased rectal temperature significantly ($p < 0.05$, $p < 0.001$, respectively)

Table 1. Effect of *P. tuberosa* (200, 400 and 800 mg/kg) i.p on yeast induced pyrexia in mice.

Treatment	Dose mg/kg	Normal (X)	After15 h (Y)	Rectal temperature (°C)				
				After administration of drug				
				0 h	1 h	2 h	3 h	4 h
Normal Sali ± Std	10	35.73±0.178	37.88±0.111	37.63±0.148	37.46±0.133	37.36±0.136	37.30±0.132	37.46±0.141
Aspirin ± Std	100	35.70±0.225	37.96±0.133	37.85±0.148	37.35±0.135	36.65±0.177	36.03±0.136	35.40±0.121
	200	35.66±0.254	37.73±0.199	37.66±0.150	36.23±0.176	36.96±0.131	36.37±0.145	36.53±0.145***
Extract± Std	400	35.31±0.248	37.78±0.158	37.70±0.211	37.33±0.165	36.71±0.083 *	36.35±0.131 ***	36.15±0.145 ***
	800	35.80±0.188	38.10±0.086	37.93±0.067	37.31±0.101	36.70±0.093*	35.93±0.117***	35.63±0.0136***

Values are expressed as mean ± SEM, (number of mice N = 6), significant *P < 0.05, ** P< 0.01 and *** P < 0.001, when compared to control.

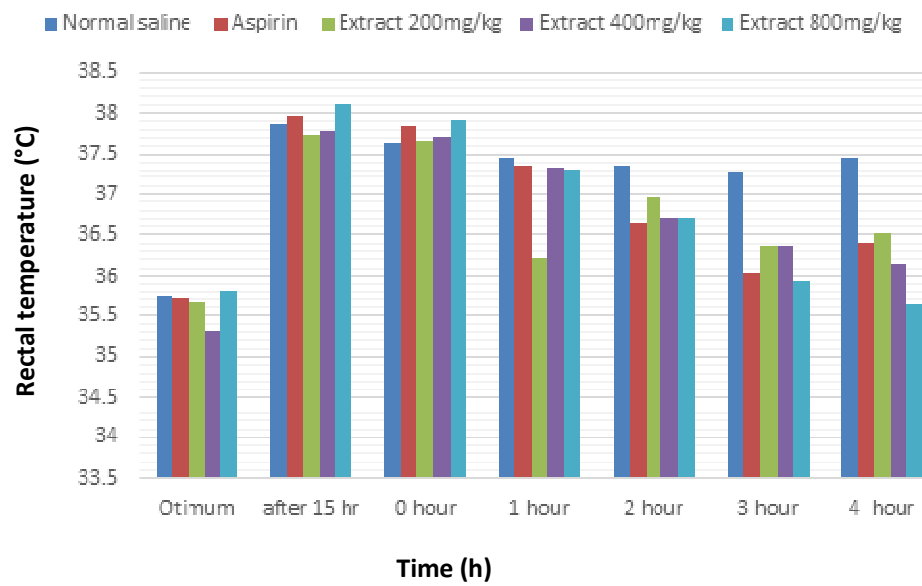


Figure 1. Effect of HEOM on yeast induced pyrexia. The data are expressed as mean ±SEM (N = 6)

at 2, 3 and 4 h after administration compared to normal control. Treatment with Aspirin at a dose of 100 mg/kg significantly p < 0.001 reduced

pyrexia induced by yeast at 3 h after administration. Treatment with ethanolic extract of *P. tuberosa* at dose of 400 and 800 mg/kg was nearly equally potent to reference compound Aspirin (Buffum and Buffum, 2000; Cao and Prescott, 2002; Fadeyi et al., 2005).

DISCUSSION

The ELPT exhibited significant antipyretic activity when compared to standard. The possible mechanism of antipyretic action may be the inhibition of prostaglandin's synthesis. The aim of the present research was to validate the traditional uses of the ELPT for antipyretic action (Olajide et al., 2000; Panthong et al., 2007). Fever or pyrexia is a common medical sign associated primarily with abrupt increase in body temperature above normal and caused by certain illness related behavioral features like fatigue, depression, lethargy, anorexia, hyperalgesia (Fadeyi et al., 2005). The elevation of body temperature occurs as a result of the release of certain chemical substances by immune system (Fung and Kirschenbaum, 1999). Infected or injured tissue enhances the formation of pro-inflammatory mediator that is, cytokines like interleukin-1beta, alpha, beta and TNF- alpha which increase the synthesis of prostaglandin E2 (PGE2) and thereby stimulating hypothalamus to raise body temperature (Fadeyi et al., 2005; Flier et al., 1994).

Fever or pyrexia is a normal response against invading microorganisms to provide defense against infections (Tonks and Watson, 2003). Antipyretic drugs inhibit COX-2 enzyme expression and thus inhibiting biosynthesis of PGE2 to reduce high body temperature (Cao and Prescott, 2002). Cyclooxygenase (COX) also known as prostaglandin (PG) synthetase catalyzes the conversion of arachidonic acid into prostaglandin H2 (Seibert et al., 1995). The common therapy for management and control of fever is nonsteroidal anti-inflammatory drugs (NSAIDs). They are used for relief of inflammation, headache, anti arthritis pain, heart attacks and stroke. NSAIDs drugs inhibit prostaglandin and its derivatives produced through cyclooxygenase enzyme that cause inflammation, fever, pain and related diseases (Fung and Kirschenbaum, 1999). However, NSAIDs produces a number of side effects like gastrointestinal bleeding, mucosal erosion, hepatotoxicity, renal toxicity and nephropathy (Greisman and Mackowiak, 2002). Meanwhile, in order to avoid side effect, there are development and introduction of new antipyretic agents that compete with NSAIDs.

The use of natural remedies for the treatment of inflammatory and painful condition has long history starting with Ayurvedic treatment, extends to the Europe. Plant drugs are known to play a vital role in the management of inflammatory diseases. Intraperitoneal administrations of the yeast to mice significantly increase rectal temperature and the tested drug significantly reduced rectal temperature. Thus it can be hypothesized

that the extract contained pharmacologically compounds that interfere with the release of prostaglandin.

The present results shows that extract possess significant as well as dose dependent antipyretic effect in yeast induced pyrexia which is comparable to that of standard drug. The effect of ethanolic extract on yeast induced pyrexia shows decrease in rectal temperature within 1 h of the extract (800 mg/kg) treatment, and at 4 h a sizeable reduction in rectal temperature which was comparable to reference drug aspirin. The tested drug at a dose of 400 and 800 mg/kg body weight decreased rectal temperature significantly ($p < 0.001$), respectively after administration as compared to negative control. Treatment of the tested drug at a dose of 400 and 800 mg/kg produced a decrease in rectal temperature that was comparable to reference compound aspirin. The evaluated body temperature intensified the process of lipid per oxidation, which indicates that increase of oxidative stress causes pyrexia. The supplementation of antioxidant decreased the lipid per oxidation processes (Sehgal et al., 2011). The flavonoids have antioxidant activity. Thus, antioxidant activity of *P. tuberosa* may be one of the possible mechanisms to reduce the elevated body temperature. The lowering of body temperature observed can be recognized to the presence of flavonoids in *P. tuberosa* that might be responsible for lowered body temperature (Nwafor et al., 2012). The extract may reduce PGE2 by its action on cyclooxygenase (COX-2) or by increasing the production of the body's own antipyretic substances like vasopressin and arginine (Olowokudejo et al., 2008). Various studies show that formulation containing tannins, alkaloids, flavonoids and carbohydrates has been reported for their antipyretic potential (Qadrie et al., 2009).

Conclusion

The study concludes the scientific basis in support of its traditional use in fever by the native peoples. The results of the present study showed that plant extract significantly reduced elevated body temperature in dose dependent manners. These results validate the basis for the traditional use of *P. tuberosa* against fever. The preliminarily analysis shows the presence of phenols, flavanoids, alkaloids, saponins, tannins and terpenoids. The pharmacological activity of the plant extract may be due to presence of these phytochemicals. However, further detail studies are necessary for isolation of pure secondary metabolites from the plant to understand the exact mechanism responsible for pharmacological activities of the plant (Singh et al., 2013; Tonks and Watson, 2003; Olajide et al., 2000).

Conflict of interest

Authors have none to declare.

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