Phytochemical screening and Analgesic activity of methanolic extract of *Portulaca grandiflora* on mice

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Abstract
The plant *Portulaca grandiflora* belongs to the family Portulacaceae, a plant native to southern Brazil, Argentina, and Uruguay that is frequently cultivated in gardens. It is also known as Moss rose, Mexican rose and Eleven o'clock. The present study aimed to explore the Analgesict activity is evaluated by by Hot Plate Method, Tail Immersion Test and Acetic Acid Induced Writhing model. The methanolic extracts of *Portulaca grandiflora* on mice (200mg/kg & 400mg/kg) produced dose dependent. Among the two doses, 400 mg/kg showed maximum analgesic activity at reaction time 120 min (7.2±0.44) is slightly lower than the standard drug pentazocine (9.9±0.34) in this analgesic testing model, pentazocine significantly prolonged the reaction time of animals with relatively extended duration of stimulation, confirming centrally active drugs. In the present study, all extracts showed significant ($p< 0.01$) analgesic activity but among the two doses, 400 mg/kg showed highest analgesic activity at reaction time 120 min. Results demonstrated that PGE exhibited a potent dose dependent analgesic activity in all tested models for analgesia.
Keyword: Analgesic, Hot Plate Method, Pentazocine, *Portulaca grandiflora.*

INTRODUCTION

Medicinal plants are the factories of phytochemicals that produce different bioactive compounds in the most selective and precise way.[1] Plants produce both primary and secondary metabolites. Plants use primary metabolites for basic life functions like growth, cell division, storage, respiration, and reproduction; these include the compounds involved in the Krebs cycle, glycolysis, photosynthesis, etc. Phytochemical composition is a key in the usage of medicinal plants for the treatment of various diseases in traditional systems.[2] In the modern medicinal system, natural products provide lead compounds for the production of new drugs in the pharmaceutical industry.[3] There are many secondary metabolites that were investigated and used as major constituents in modern medicine. Various plants are
investigated for the discovery of medicine because plant based drugs are less toxic and more effective. Pain has been defined by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience induced by real or potential tissue injury." The emotional component varies from person to person and from time to time within the same individual. It can be categorized in numerous ways, but in terms of therapeutic application, it is divided into Nociceptive and neuropathic symptoms. In addition to identifying the type of pain, it is required to quantify pain. There are several scoring systems available, such as the

numerical scale, but it is important to remember that the patient is the only person who can quantify his suffering. [4]

The plant *Portulaca grandiflora* belongs to the family Portulacaceae, a plant native to southern Brazil, Argentina, and Uruguay that is frequently cultivated in gardens. It is also known as Moss rose, Mexican rose, and Eleven o'clock. The *Portulaca grandiflora* has been used in various diseases. The plant is used therapeutically as an antioxidant, antibacterial, antidiabetic, sore throat reliever, rash, and detoxifier, a powerful catalyst for the biotransformation of L tyrosine to LDOPA, cytotoxic effect on cancer cells. [5]

The Leaves of *Portulaca grandiflora* have several steroidal saponins, yamogenin glycosides, were isolated. The motive of my present study is to reveal & show the presence of phytoconstituents in leave extract of *Portulaca grandiflora* that will be responsible treatment of analgesia.

MATERIALS AND METHODS

Preliminary work (Selection of Plant)

Gathering sufficient information from vivid articles and journals it was concluded that there is scope to explore some more pharmacological activities in the plant *Portulaca grandiflora* hence it was selected for further studies.

Collection and Authentication of plant

Portulaca grandiflora leaves 2000gm were collected from Sanjeevani Gardan, Bhopal the state of Madhya Pradesh during the month of March. The plant has been identified and authenticated by Dr. Saba Naaz Head of the Department Botany at the Safia college of science, Bhopal (M.P.)

Drying, size reduction and storage of plant material

The plants parts were dried under shade. It was pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve No. 20 to maintain uniformity and packed into airtight container and stored in cool and dry place. This material was used for the further study.

Preparation of *Portulaca grandiflora* leaves extract

Extraction of *Portulaca grandiflora* was done by Soxhlet extraction method.

Soxhlet Extraction: The coarse powder 500gm was packed tightly in the soxhlet apparatus and extracted with 500ml 70% methanol for 72 hours with occasional shaking maintained at 78°C throughout the extraction process. The extract was concentrated to ¼ of its original volume by evaporation. The resulting extract of *Portulaca grandiflora* was subjected to phytochemical study.

Phytochemical Analysis of Crude Extracts

The crude extracts of plants obtained by solvent extraction were subjected to various qualitative tests to detect the presence of common chemical constituents as: alkaloid, glycoside, carbohydrate, phytosterols, saponins, tannin, flavonoids and protein etc. [6,7]

Acute Toxicity Studies

The acute toxicity was performed according to OECD guidelines 423. The selected albino mice were used for toxicity studies. The animals were divided into five groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Extract was given

orally to mice at the graded doses like 5, 50, 100, 1000 & 2000 mg/ kg body weight. Immediately, after dosing, the animals were observed continuously for first four hours for behavioural changes were closely observed for hyperactivity, ataxia, convulsion, salivation, tremors, diarrhoea, lethargy, sleep. They were then kept under observation up to 14 days after drug administration to determine the mortality, if any. One-tenth and one-fifth of the maximum tolerated dose (200 and 400 mg/ kg, body weight) of methanol leaf extract of *Portulaca grandiflora* selected to evaluate analgesic activity studies in mice. [8]

ANALGESIC ACTIVITY

HOT PLATE METHOD IN MICE

The hot plate assay method was employed for the purpose of preferential assessment of possible centrally medicated analgesic effects of Methanolic extract of *Portulaca grandiflora*. The central analgesic drug pentazocine was used for positive control group. In this experiment, four groups (n=6) of Swiss albino mice (20-25 g) were placed on a hot plate maintained at room temperature for 15 min. Food was withdrawn on the preceding night of the experiment. Group I- Normal Control received CMC (0.5%), and Group II- standard treated with pentazocine (3 mg/kg i.p), whereas group III and IV- animals were treated orally with Methanolic extract of *Portulaca grandiflora* (200 and 400 mg/kg respectively). [9]

Group I : Normal control (CMC)

Group II : Standard (Pentazocine 3 mg/kg)

Group III : Test Drug I (Methanolic extract of *Portulaca grandiflora*)

Group IV : Test Drug II (Methanolic extract of *Portulaca grandiflora*)

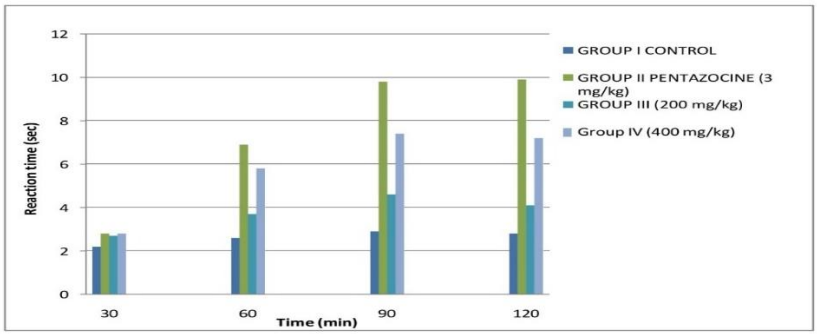
Each animal was then individually placed gently on Eddy's hot plate at 55ºC. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate were determined at 30, 60, 90 and 120 min after administration of the drugs or vehicle.

Table 01- Analgesic effect of Methanolic extract of *Portulaca grandiflora* on hot plate test in Swiss albino mice

GROUP	Paw licking or jumping in seconds			
	30 min	60 min	90 min	120 min
Group-I Control	2.2±0.22	2.3±0.12	2.4±0.21	2.3±0.10
Group-II Pentazocine (3mg/kg)	2.8±0.18	6.9±0.62**	9.8±0.64**	9.9±0.34**
Group-III (200mg/kg)	2.7±0.20	3.7±0.15*	4.6±0.21**	4.1±0.41**
Group-IV (400mg/kg)	2.8±0.14	5.8±0.37**	7.4±0.39**	7.2±0.44**

Values were mean ± SEM, (n=6), *P<0.05 **P<0.01 Vs control.

Data were analyzed by using One-way ANOVA followed by Dunnett's test.



Graph 01: Analgesic effect of Methanolic extract of *Portulaca grandiflora* on hot plate method in mice.

TAIL IMMERSION TEST

This method assessment was used to evaluate the centrally medicated analgesic effects of Methanolic extract of *Portulaca grandiflora*. The mice were divided into four groups each consists of six animals. They were placed into individual restraining cages leaving the tail hanging out freely. The lower 5cm portion of the tail is marked and this part of the tail was immersed in a water bath containing water at a temperature of 55± 0.5 ºC. Withdrawing the tail from the hot water showed the analgesic effect. The reaction time was noted on a stop-watch. Each animal served as control. The average of the two values was the initial reaction time. Group –II served as standard and received pentazocine (3 mg/kg, i.p) The Group III and IV were treated orally with Methanolic extract of *Portulaca grandiflora* (200 mg/kg and 400mg/kg) respectively. [10-12]

Group I : Normal Control

Group II : Pentazocine (3 mg/kg)

Group III : Test Drug I (Methanolic extract of *Portulaca grandiflora*)

Group IV : Test Drug II (Methanolic extract of *Portulaca grandiflora*)

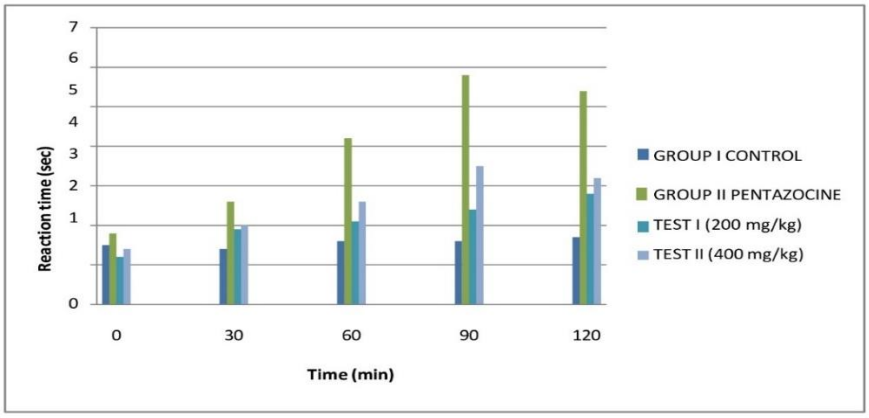
The reaction time of the groups were taken at 0, 30, 60, 90 and 120min. The cut off time of the immersion was 15seconds. The reaction time was measured.

Table 02- Analgesic effect Methanolic extract of *Portulaca grandiflora* on tail immersion method in rats

GROUP	Mean latency to tail immersion in seconds				
	0 min	30 min	60 min	90 min	120 min
Group-I Control	1.5±0.04	1.4±0.02	1.6±0.01	1.6±0.03	1.7±0.04
Group II Pentazocine (3mg/kg)	1.8±0.06	2.6±0.04**	4.2±0.02**	5.8±0.06**	5.4±0.02**
Group III (200mg/kg)	1.2±0.02	1.9±0.01*	2.1±0.04*	2.4±0.02	2.8±0.04*
Group IV (400mg/kg)	1.4±0.01	2.0±0.04*	2.6±0.01**	3.5±0.04**	3.2±0.01**

Values were mean ± SEM, (n=6), *P<0.05 **P<0.01 Vs control.

Data were analyzed by using One-way ANOVA followed by Dunnett's test.



Graphs 02: Analgesic Effect of Methanolic extract of *Portulaca grandiflora* On Tail Immersion Method in mice.

ACETIC ACID INDUCED WRITHING RESPONSE IN MICE

This method was used to preferentially evaluate possible peripheral analgesic effects of Methanolic extract of *Portulaca grandiflora.* Four groups of Swiss albino male mice (n=6) were fasted overnight prior to start the experiment with free access to water. The peripheral analgesic drug Diclofenac sodium (10 mg/kg) was used as a positive control. Group-I Normal Control received CMC (0.5%) Group-II was treated with Diclofenac Sodium (10mg/kg), whereas Group III *grandiflora* at a dose of 200 mg/kg and 400mg/kg respectively. [13,14]

Group I : Normal Control

Group II : Diclofenac Sodium (10mg/kg)

Group III : Test Drug I (Methanolic extract of *Portulaca grandiflora*.)

Group IV : Test Drug II (Methanolic extract of *Portulaca grandiflora*.)

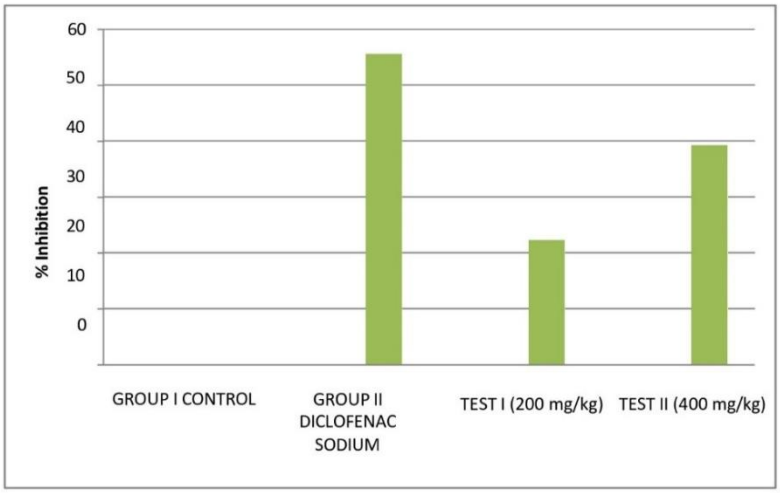
After 30 min of treatment, the mice were injected intra peritoneally with 0.1 ml of 1% acetic acid solution to induce the characteristic writhings. The mice were then placed in an observation box and the numbers of writhing were counted in a 5min period. The response of the extract and Diclofenac sodium treated groups was compared with those of animals in the and IV were treated orally with the Methanolic extract of *Portulaca* control group.

Table 03- Analgesic effects of Methanolic extract of *Portulaca grandiflora* (on acetic acid writhing test in Swiss albino mice

GROUP	Number of writhes	% Inhibition
Group-I Control	51.4±6.4	--
Group-II Diclofenac Sodium (10mg/kg)	22.8±1.9**	55.64
Group-III (200mg/kg)	39.4±2.4**	23.34
Group-IV (400mg/kg)	31.2±2.1**	39.29

Values were mean ± SEM, (n=6), ***P*<0.01 Vs control.

Data were analyzed by using One-way ANOVA followed by Dunnett's test.



Graph 03: Analgesic effect of Methanolic extract of *Portulaca grandiflora* on acetic acid induced writhing response in mice. Results are expressed as a percentage of inhibition.

RESULTS AND DISCUSSION

The results obtained from the preliminary phytochemical screening of *Portulaca grandiflora* extract showed the presence of flavonoids, alkaloids, tannins etc. It was reported that the flavonoids frequently found in plants posses analgesic activity. The study of the plant of *Portulaca grandiflora* was done by using mice with the oral doses of 5, 50, 100, 1000 & 2000 mg/ kg body weight of extract and no mortality was observed for 24 hours. Thus dose was identified as per OECD 423 Guidelines.

The hot plate model was selected to investigate central antinociceptive activity because it has several advantages particularly the sensitivity to strong antinociceptive and limited tissue damage. Prostaglandins and bradykinins were suggested to play an important role in pain. Phenolic compounds are reported to inhibits prostaglandin synthesis. A number of phenolic compounds have been reported to produce analgesic activity. Other studies have demonstrated that various flavonoids such as rutin, quercetin, luteolin, bioflavonoids and triterpenoids produced significant antinociceptive effect. As phytochemical test showed presence of flavonoids and tannins in Methanolic extract of *Portulaca grandiflora,* they might suppress the formation of prostaglandin and bradykinins.

The centrally acting analgesic activity of the extract was also corroborated in our study by tail immersion test results. The fact that in thermal stimuli (hot plate & Tail immersion tests), the antinociceptive effect should be shown by acting centrally on opioid receptors. Since the drugs had shown the analgesic activity in tail immersion test, it seems that the methanolic extract can act centrally.

Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response. The effect of the extract against the noxious stimulus may be an indication that it depressed the production of irritants and thereby reduction in number of writhes in the animals. The writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting anti- nociceptive. This response is

thought to involve local peritoneal receptors. This result indicates that the analgesic effect of Methanolic extract of *Portulaca grandiflora,* might be mediated by inhibiting the synthesis or action of prostaglandins.

The Analgesic activity is evaluated by by Hot Plate Method, Tail Immersion Test and Acetic Acid Induced Writhing model. The methanolic extracts of *Portulaca grandiflora* on mice (200mg/kg & 400mg/kg) produced dose dependent. Among the two doses, 400 mg/kg showed maximum analgesic activity at reaction time 120 min (7.2±0.44) is slightly lower than the standard drug pentazocine (9.9±0.34) in this analgesic testing model, pentazocine significantly prolonged the reaction time of animals with relatively extended duration of stimulation, confirming centrally active drugs. In the present study, all extracts showed significant ($p< 0.01$) analgesic activity but among the two doses, 400 mg/kg showed highest analgesic activity at reaction time 120 min.

There was a significant reduction of pain full sensation due to tail immersion in warm water. The maximum inhibitory effect of Methanolic extract of *Portulaca grandiflora* showed significant ($p< 0.01$) at 90 min post dose in 400 mg/kg. The maximum anti- nociceptive properties of the plant extract (3.5±0.04) were not as effective as that of pentazocine, 3 mg/kg (5.8±0.06).

Injection of acetic acid into control mice produced 51.4±6.4 writhes. Pre- treatment with Methanolic extract of *Portulaca grandiflora* at doses of 200 and 400 mg/kg reduced the number of writhes 39.4±2.4 (23.34 % protection) and 31.2±2.1 (39.29 % protection) respectively. Among the two doses 200, 400 mg/kg showed the slightly lower analgesic activity than standard drug Diclofenac Sodium 22.8±1.9 (55.64 % protection) it was observed that the onset of writhing was delayed and duration of writhing was shortened.

The Present study showed that the Methanolic extract of *Portulaca grandiflora* posses peripheral and central analgesic activity in animal model. Flavonoids and tannins are the major constituents of Methanolic extract of *Portulaca grandiflora*, which may be responsible for its Analgesic activity. Further detailed study on *Portulaca grandiflora* plant using different agents in this area will enable us to understand the mechanism of action underline the above mention activity.

CONCLUSION

Phytochemical screening and Analgesic activity of methanolic extract of *Portulaca grandiflora* on mice" in experimental animal models was found effective in the analgesic activity due to the presence of phytoconstituents. In conclusion we can say that extract dose (400 mg/kg) of *Portulaca grandiflora* leaf show significantly reducing the peripheral and central pain with the help of flavonoids, saponin, alkaloid, tannins, phenols and some other phyto-constituents. The plant extract was also found to have optimal safety margin based on the limit test at 2000 mg/kg dose level acute toxicity test. Therefore, the plant is potentially useful to develop plant based products after further studies to identify the active principle and the mechanism of action.

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