



EVALUATION OF ANALGESIC AND ANTI INFLAMMATORY ACTIVITIES OF *PORANA PANICULATA* WHOLE PLANT

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ABSTRACT

Porana paniculata is an ever green creeper belongs to family *Convolvulaceae* which is widely grown in tropical countries like India. *Porana paniculata* whole plant is used in ayurveda and folklore for treatment of various disorders including pain and inflammations. Present study was aimed to evaluate preliminary phytochemical studies and analgesic and anti-inflammatory activities of *Porana paniculata* whole plant. Plant material was subjected to extraction by maceration by using ethanol and water mixture as solvent and subjected to preliminary phytochemical screening. For Analgesic activity, hot plate, tail immersion and acetic acid induced writhing models were used where as for anti inflammatory activity, carrageenan and histamine induced model were employed. A thick green viscous matter about 28.9 gm was obtained from 1000 gm of plant material and the percentage was found to be 2.89% w/w. Preliminary phytochemical screening of whole plant of *Porana paniculata* revealed the presence of alkaloids, carbohydrates, saponins, tannins and flavonoids. In hot plate and tail immersion methods, plant extract showed significant increase in reaction time and it showed 50.09 % inhibition of the writhing caused by acetic acid ($p < 0.05$). In anti inflammatory activity, plant extract showed 25.86, 43.10 % inhibition in carrageenan induced model and 13.41, 54.87 % inhibition in histamine induced model at its lower and higher dose levels respectively. From the above findings, it can be concluded that *Porana paniculata* whole plant possesses significant analgesic and anti inflammatory activities. Present study supports the folklore claim of the plant for pain and inflammation.

KEY WORDS: *Porana paniculata*, Hot plate, Tail immersion, Acetic acid, Carrageenan and Histamine



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INTRODUCTION

The use of plant parts and their extracts in the treatment of various diseases is known as herbalism and the medicine is said to be herbal medicine. Plants that occur in the nature are the potential sources of medicines. Herbal approaches usually have been pursued because of the perception that many of these therapies produce fewer side effects than pharmaceuticals¹. The use of traditional medicine is expanding to newer horizons and plants still remain as the novel source of structurally important compounds that lead to the development of innovative and the anti-inflammatory and analgesic properties of plants cited in the traditional literature². Many herbal preparations are being prescribed as anti inflammatory and analgesic in the traditional literature. The search for new anti-inflammatory and analgesic agents from the huge array of medicinal plant resources is intensifying. *Porana paniculata* having the synonym *Poranopsis paniculata* belongs to the family *Convolvulaceae* is an ever green creeper most abundantly available across India and well as different areas of Andhra Pradesh³. Even though the plant is having medicinal uses both in ayurveda and folklore, its phytopharmacological nature was unrevealed. The plant posses its significant use in the ayurveda for Febrile excitement (Traditional Medicines for Larynx, Lungs and Heart), Blowing with diastole (Traditional Medicines for Larynx, Lungs and Heart)⁴. In Rajasthan, tribal people found its use in jaundice⁵. The leaves are made into paste and applied to the fractured bone in tribal areas of Chhattisgarh, India⁶. After thorough literature review it was came to know that *Porana paniculata* plant is one of the most important medicinal plants which were less explored. So far, there is no scientific data available on the analgesic and anti inflammatory activities, in the present study an attempt was made to screen the analgesic and anti inflammatory activities of whole plant of *Porana paniculata* by using standard methods.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

For the present study, *Porana paniculata* whole plant was collected from forest area

near to the Madanapalli of Chittoor district of Andhra Pradesh and the plant was botanically identified and authenticated by Dr. K. Madhava Chetty, Assistant professor, Department of Botany, S.V. University, Tirupati, A.P., India and a voucher specimen (RIPER/ASK/001) was preserved in division of pharmacognosy, RIPER, Anantapur for further reference.

EXTRACTION

For the present study, 1000 gm of the powdered *Porana paniculata* was extracted by cold maceration method with ethanol: water (3:2) mixture as solvent. The maceration was continued for 72 hours with occasionally agitation after which, the contents were filtered and concentrated by rota evaporator. A resinous greenish extract was obtained which was calculated from the yield, designated with HAPP and stored in desiccator till further study⁷.

PHYTOCHEMICAL SCREENING

Hydroalcoholic extract of *Porana paniculata* whole plant was subjected to standard battery of preliminary phytochemical screening^{7,8}.

ANIMALS

Adult Swiss albino mice for acute toxicity studies (20–30 g) and Wistar rats (150-200 g), males or females for pharmacological screening at about 6–8 weeks of age were used in the study. The animals were maintained with free access to food and water and kept at 25 ± 2 °C under a controlled 12 h light/dark cycle. Twelve hours before each experiment animals received only water, in order to avoid food interference with substances absorption. The care and maintenance of the animals were carried out as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi. The research protocols were approved by the Institutional Animal Ethical Committee (IAEC) of Raghavendra Institute of Pharmaceutical Education and Research, Krishnam Reddy Palli cross, Chiyyedu, Anantapur-515721, Andhra Pradesh, India

Gross behavioural and toxicity studies of hydro alcoholic extract of *P. paniculata*

The hydro alcoholic extract of whole plant of *P. paniculata* was screened for the gross behavioural and toxicity studies in selected Swiss albino mice. Groups of mice comprising six animals each were treated with 100, 200, 400, 800, 1000, 2000 and 3000 mg/kg of the extract suspended in 0.5% w/v sodium carboxy methyl cellulose were administered orally, via a gastric catheter. The animals were then observed continuously for first four hours for any behavioural changes and for mortality if any at the end of 72 h⁹. However, no mortality was observed in the animals. Hence HAPP was selected to screen for its anti inflammatory and analgesic activities in selected models at dose level of 250 mg/kg and 500 mg/kg body weight.

Screening of analgesic activity by Hot plate method in rats

For hot plate method, Swiss albino rats weighing between 100-150g were divided into four group comprising six animals each. First group received normal saline and served as normal control, second group received pentazocin 30mg/kg and served as standard, third and fourth group received 250 and 500mg/kg HAPP and served as test one and two. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. The test and standard drugs were given orally. The paws of rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The hot plate, which is commercially available, consists of an electrically heated surface. The temperature is controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals from each group were placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch at the time interval of 0, 60, 120, 180 and 240 minutes.^{10, 11}

Screening of analgesic activity by tail immersion method in rats

For tail immersion method, Swiss albino rats weighing between 100-150g were divided into four group comprising six animals each First

group received normal saline and served as normal control, second group received pentazocin 30mg/kg and served as standard, third and fourth group received 250 and 500mg/kg HAPP and served as test one and two. Prior to analgesic experiments, the animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55⁰ C – 55.5⁰ C . The animal immersing the tail from hot water with in 5 second was selected for the study. After administration of the drugs, the reaction time was measured at 0, 60, 120, 180 and 240 minutes.^{10, 11}

Screening of analgesic activity by Acetic acid-induced writhing

In this method, Male albino mice, weighing 18–25 g, were randomly divided into four groups, six animals each. First group received normal saline and served as normal control, second group received pentazocin 30mg/kg and served as standard, third and fourth group received 250 and 500mg/kg HAPP and served as test one and two. In this method, acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. Test samples and vehicle were administered orally 60 minutes prior to intraperitoneal administration of 0.75% v/v acetic acid solution (0.1mL/10g) but Pentazocin (30 mg/kg) was administered 15 minutes prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups was observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it¹². This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared with the standard group received pentazocin 30mg/kg.^{10, 11}

Screening of anti inflammatory activity by carrageenan induced model in rats

For carrageenan induced inflammatory model, Swiss albino rats weighing between 100-150g were divided into four group comprising six

animals each. First group received normal saline and served as normal control, second group received ibuprofen 10 mg/kg and served as standard, third and fourth group received 250 and 500mg/kg HAPP and served as test one and two. The acute hind paw oedema in animals was produced by Carrageenan, where 0.1 ml of carrageenan (prepared as 1% w/v suspension in saline) locally injected into sub plantar region of the left hind paw of rats. Animals from all the groups were administered with the treatment orally 1 hour prior of carrageenan injection. The rat paw volume up to the ankle joint was measured at 0 (30 min before carrageenan injection), 60, 120, 180 and 240 minutes after the injection of carrageenan using digital plethysmomete^{11, 13}. Percent inhibition of paw volume between treated and control groups were calculated as follows:

$$\text{Percentage inhibition} = \frac{V_0 - V_t}{V_0} \times 100$$

Where, V_0 = volume of the paw of control, V_t = volume of the paw of test at 240 minutes

Histamine induced paw oedema

The chemical mediator viz. histamine, 5-HT, bradykinin and PEG₁ are involved in the genesis of acute inflammation which is reported to be released from the mast cell degradation during the first hour of carrageenan induced artificial paw oedema. In the present study, histamine was used directly as the edematogenic agent and anti-inflammatory activity of the extract studied. The procedure carried out was same as that of carrageenan model with a minor difference in the use of the edematogenic agent and grouping of animals for screening was shown in table 5. Histamine (0.1% w/v in normal saline) was injected into the paw of each rat at a dose of 0.1ml to induce oedema. The paw volume was measured at 0, 1, 2, 3 and 4 hour respectively. The anti-inflammatory effect was expressed as percent inhibition of oedema^{11, 13}. Percent inhibition of paw volume between treated and control groups were calculated as follows:

$$\text{Percentage inhibition} = \frac{V_0 - V_t}{V_0} \times 100$$

Where, V_0 = volume of the paw of control and V_t = volume of the paw of test

STATISTICAL ANALYSIS

All the results are expressed as the mean \pm S.E.M. The data were analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by Bonferroni's test using computerized Graph Pad Prism, version 4.5 software (Graph Pad Software Inc) and statistical significance was set accordingly.

RESULTS

Extraction

Whole plant powder of *Porana paniculata* was subjected to maceration using ethanol and water mixture as solvent at ration of 3:2 because pure organic solvents were found to be least soluble for phenolic compound. A thick green viscous matter about 28.9 gm was obtained from 1000 gm of plant material and the percentage was found to be 2.89% w/w.

Preliminary phytochemical analysis

Preliminary phytochemical analysis of whole plant of *Porana paniculata* was carried out and it showed the presence of alkaloids, carbohydrates, saponins, tannins and flavonoids.

Pharmacological activity Analgesic activity Hot plate method & Tail immersion method

Animals treated with the standard drug showed a significant ($p < 0.05$) increase in mean reaction time that was found to be increased highly at time interval of 120, 180 and 240 minutes of study where as the plant extract showed a slight increase in the mean reaction time at its lower dose level that is 250 mg/kg which was found to be significant ($p < 0.05$) at the terminating time of study where as it was significant ($p < 0.05$) at its higher test dose 500 mg/kg (figure 01) at the time interval of 180 and 240 minutes of study when compared to control group. The results were tabulated in table number 01 & table number 02.

Acetic acid induced writhing method

In case of writhing model, 500mg/kg test dose number of writhes were reduced and found to be statistically significant and percentage inhibition of writhing test was found to be 50.90 which are nearly comparable with standard drug 62.73. the results were tabulated in table number 03.

Anti inflammatory activity**Carrageenan induced model**

In this model, plant extract 250 mg/kg exhibited significant ($p<0.05$) mean paw volume at 180 and 240 minutes of study with a percentage inhibition value of 22.22 and 25.86 respectively where as significant ($p<0.05$) mean paw volume at 180 and 240 minutes of study with a percentage inhibition value of 27.08 and 43.10 respectively in its 500 mg/kg dose (figure 4) when compared to the control group. The results were tabulated in table number 04.

Histamine induced model

In this model, plant extract 250 mg/kg exhibited significant ($p<0.05$) mean paw volume at 120, 180 and 240 minutes of study with a percentage inhibition value of 21.05, 07.99 and 13.41 respectively where as significant ($p<0.001$) mean paw volume at 120, 180 and 240 minutes of study with a percentage inhibition value of 78.66, 52.90 and 54.87 respectively in its 500 mg/kg dose when compared to the control group. The results were tabulated in table number 5.

Table 1
Analgesic activity of *Porana paniculata* whole plant by hot plate method

Group (treatment)	Reaction time in Sec				
	30 min	60 min	120 min	180 min	240 min
Control	2.50± 1.23	2.64± 1.28	3.29± 1.30	3.20± 1.29	3.19± 1.29
Pentazocine (30 mg/kg)	2.67± 1.26	4.63± 2.71	5.95± 2.85	6.30±2.58*	6.58± 2.94*
HAPP (250 mg/kg)	2.48± 1.22	3.12± 1.68	4.32± 1.84	4.67± 1.67	5.01± 1.72*
HAPP (500 mg/kg)	2.63± 1.24	3.76± 1.95	5.32± 2.22	5.45±2.23*	5.77± 2.32*

All values were expresses as Mean± SEM, one way ANOVA followed by Bonferroni's test, * $p<0.05$ when compared to control group; HAPP- Hydroalcoholic extract of *Porana paniculata* whole plant

Table 2
Analgesic activity of *Porana paniculata* whole plant by tail immersion method

Group (treatment)	Reaction time in Sec				
	30 min	60 min	120 min	180 min	240 min
Control	2.28± 0.854	2.33± 1.079	2.46± 1.172	2.51± 1.113	2.47± 1.094
Pentazocine (30 mg/kg)	2.30± 0.995	3.45± 1.581	4.57± 1.95	5.14± 2.06**	6.42± 2.46**
HAPP (250 mg/kg)	2.36±0.885	3.24± 1.329	3.44± 1.492	3.92± 1.68*	4.47± 2.04*
HAPP (500 mg/kg)	2.56±1.049	3.91± 1.604	3.98± 1.618	5.10± 2.05**	5.33± 2.1**

All values were expresses as Mean± SEM, one way ANOVA followed by Bonferroni's test, * $p<0.05$ and ** $p<0.01$ when compared to control group; HAPP- Hydroalcoholic extract of *Porana paniculata* whole plant

Table 3
Analgesic activity of *Porana paniculata* whole plant by acetic acid induced writhing method

Group	No. of writhes	% of Inhibition
Control	36.66±1.31	-----
Pentazocine (30 mg/kg)	13.66±0.47*	62.73
HAPP 250 mg/kg	21.33±0.23	41.81
HAPP 500 mg/kg	18.0±0.409*	50.09

All values were expresses as Mean± SEM, one way ANOVA followed by Bonferroni's test, * $p<0.05$ when compared to control group; HAPP- Hydroalcoholic extract of *Porana paniculata* whole plant

Table 4
Anti inflammatory activity of *Porana paniculata* whole plant by carrageenan induced method

Group (treatment)	Paw volume (ml)					Percentage Inhibition at 4 th Hr
	0 min	60 min	120 min	180 min	240 min	
Control	0.24±0.109	0.39±0.177	0.48±0.239	0.45±0.266	0.58±0.28	-----
Ibuprofen (10 mg/kg)	0.18±0.061 (25)	0.22±0.081 (43.58)	0.32±0.129 (33.33)	0.34±0.015* (24.44)	0.30±0.12* (48.27)	48.27
HAPP (250 mg/kg)	0.21±0.088 (12.5)	0.32±0.122 (17.94)	0.34±0.17 (29.16)	0.35±0.184* (22.22)	0.43±0.177 (25.86)	25.86
HAPP (500 mg/kg)	0.19±0.068 (20.83)	0.28±0.102 (28.20)	0.30±0.157 (37.5)	0.32±0.17* (27.08)	0.33±0.136* (43.10)	43.10

All values were expressed as Mean± SEM, value in parenthesis represents percentage inhibition, one way ANOVA followed by Bonferroni's test, *p<0.05 when compared to control group; HAPP- Hydroalcoholic extract of *Porana paniculata* whole plant

Table 5
Anti inflammatory activity of *Porana paniculata* whole plant by histamine induced method

Group (treatment)	Paw volume (ml)					Percentage Inhibition at 4 th Hr
	0 min	60 min	120 min	180 min	240 min	
Control	0.58±0.02	1.07±0.008	1.14±0.019	0.913±0.01	0.82±0.006	-----
Ibuprofen (10 mg/kg)	0.33±0.008 (43.01)	0.42±0.01 (60.74)	0.54±0.006*** (52.63)	0.503±0.006*** (44.90)	0.39±0.004*** (52.43)	52.43
HAPP (250 mg/kg)	0.47±0.005 (18.96)	0.83±0.006 (22.42)	0.90±0.003* (21.05)	0.84±0.006* (7.99)	0.71±0.005* (13.41)	13.41
HAPP (500 mg/kg)	0.29±0.004 (50.00)	0.36±0.008 (66.35)	0.45±0.014*** (78.66)	0.43±0.007*** (52.90)	0.37±0.008*** (54.87)	54.87

All values were expressed as Mean± SEM, value in parenthesis represents percentage inhibition, one way ANOVA followed by Bonferroni's test, *p<0.05 and ***p<0.001 when compared to control group; HAPP- Hydroalcoholic extract of *Porana paniculata* whole plant

Figure 1
Analgesic activity of *Porana paniculata* whole plant by hot plate method

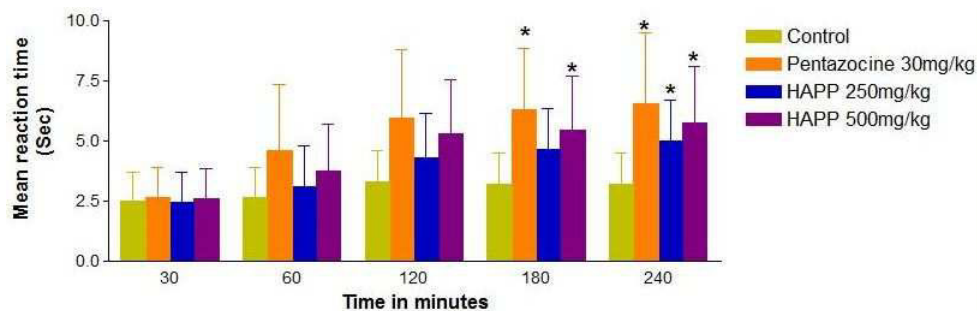


Figure 2
Analgesic activity of Porana paniculata whole plant by tail immersion method

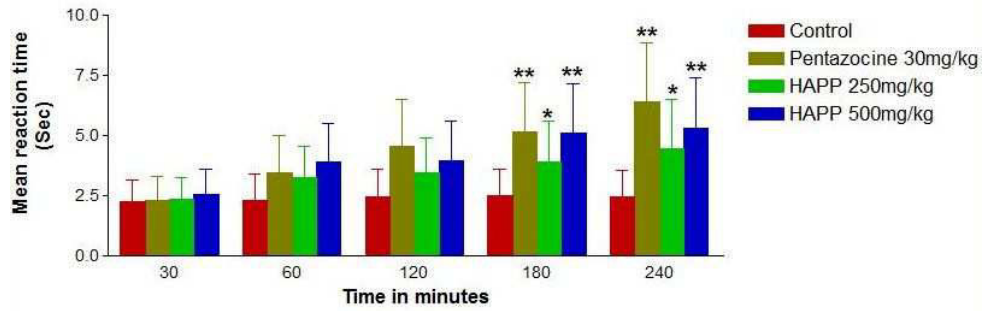


Figure 3
Analgesic activity of Porana paniculata whole plant by acetic acid Induced writhing method

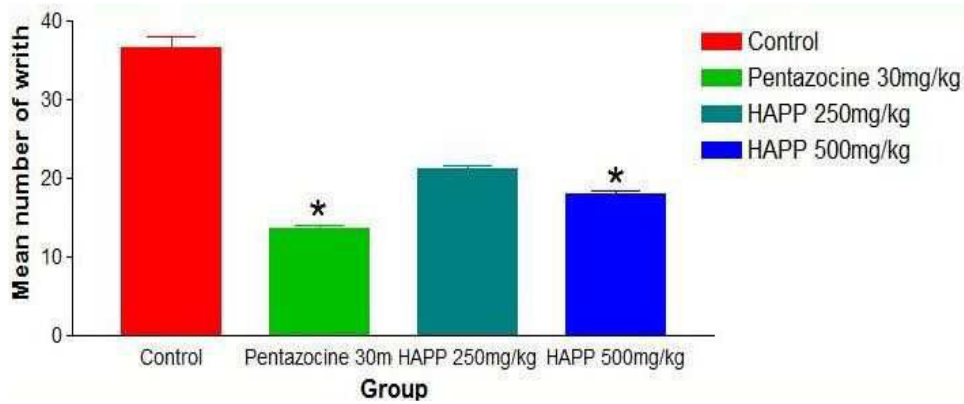


Figure 4
Anti inflammatory activity of Porana paniculata whole plant by carrageenan induced method

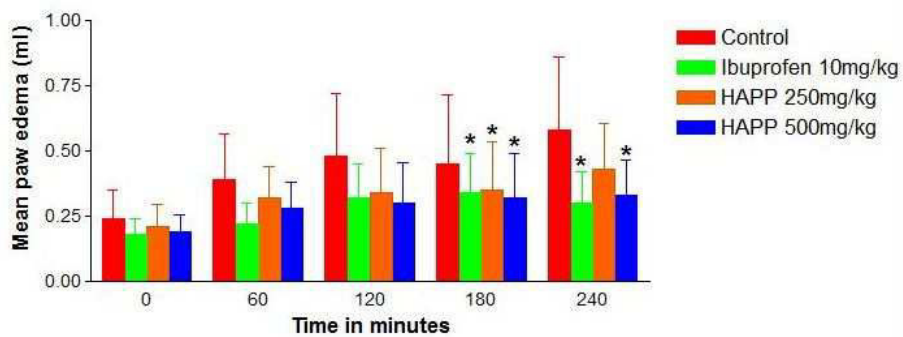
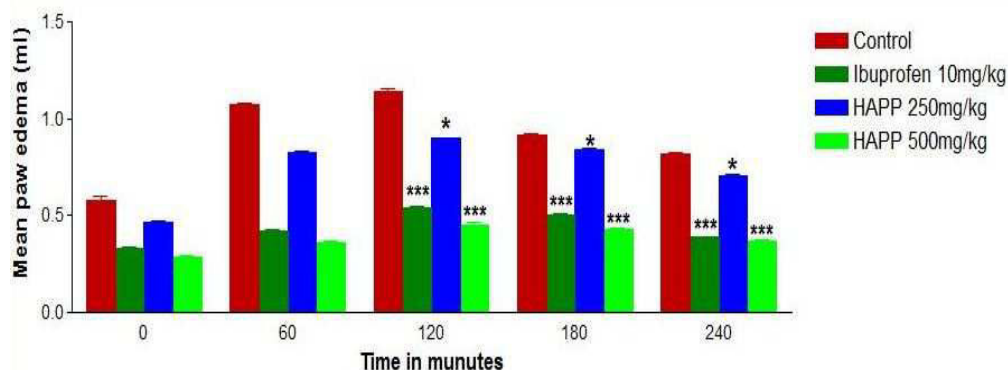


Figure 5
Anti inflammatory activity of *Porana paniculata*
whole plant by Histamine induced method



All values were expressed as Mean \pm SEM, One way ANOVA followed by Bonferroni's test, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to control group; HAPP- Hydroalcoholic extract of *Porana paniculata* whole plant

DISCUSSION

Plants are serving as a good source of food and medicine for human beings since from the beginning. They are forever will be offering a wide range of therapeutic agents for treatment of various ailments in humans as well as the animals. Currently research associated with the structured pharmacognostic study, extraction, characterization and pharmacological screening of therapeutically important phytochemicals gaining much interest. India, since ancient time serving as a rich source of various plant based therapeutic agents. There is an urgent need for research on the medicinally important plants which are available in and around us. The therapy of pain and inflammation with plant based medicines is not new to the human beings^{5, 14}. The treatment of pain and inflammation with the synthetic compounds is always leaving adverse effects like toxicity to human beings and the environment. So it is always appreciable to use the natural drugs. *Porana paniculata* is one such plant which found its significant use in the treatment of different ailments of the human beings both in traditional and folklore medicine. In analgesic activity, first the plant extract was evaluated by a hot plate method, in which the plant extract significantly increased the latency time at its both the tested levels. Later, in tail immersion method, heat source was the hot water through which the plant analgesic activity was comparable with that of the standard drug

employed^{15, 16}. Anti inflammatory action of the plant material was evaluated by carrageenan and histamine induced inflammatory model. In carrageenan model, the percentage inhibition of the plant extract was found to be significant at its higher test dose and in histamine induced model it was significant at both of the tested doses¹⁷. It is generally known fact that the pharmacological action of the plant is due to the presence of secondary metabolites. A plenty number of plants were evaluated for their analgesic and anti inflammatory activities, in which the action of plant material on pain and inflammation was attributed to the secondary metabolites present in that. Similarly, from our findings it can be claimed that the analgesic and anti-inflammatory activity of *Porana paniculata* whole plant is due to the secondary metabolites of the plant like alkaloids and flavonoids^{16, 17}.

CONCLUSION

From the above study, it can be concluded that whole plant of *Porana paniculata* possesses a significant analgesic and anti inflammatory activities in all the tested models stated earlier. However, comprehensive phytochemical and pharmacological studies are essential to reveal the exact mechanism by which the plant extract is exerting these pharmacological actions.

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