



## ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC EFFECTS OF *MOLLUGO PENTAPHYLLA* L.

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### ABSTRACT

The present study was aimed at evaluation of the anti-inflammatory, analgesic and antipyretic activities of petroleum ether and ethanolic extract of aerial parts of *Mollugo pentaphylla* Linn. In experimental standard models. The results showed that the ethanolic extract significantly reduce the edema induced by carrageenan within 1 to 3 hrs. post dosing and Cotton-pellet induced granuloma models than the petroleum ether extract at all the dose levels used. In analgesic activity by tail immersion and acetic acid induced writhing models, ethanolic extract significantly reduce the painful stimulus than petroleum ether extract. This confirms central and peripheral effects of the drugs. It also possess antipyretic activity, ethanolic extract significantly reduces fever at higher doses within 3 hrs. than petroleum ether extract on Brewer's yeast induced pyrexia model in rats.

**Keywords:** mollugo pentaphylla, anti-inflammatory, analgesic, antipyretic.

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### INTRODUCTION

The problem of uncontrolled pain led early humans to seek remedies from any materials that they could lay their hands on. In recent times, focus on plant research has increased and non-steroidal antiinflammatory drugs (NSAID) constitute one of the most widely used classes of drugs<sup>1</sup>. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects. Herbal medicines are in line with nature, with less hazardous reactions. *Mollugo pentaphylla* Linn. (family- Aizoaceae) is commonly known as carpet weed (English), Pita-gohun (Oriya). It is an erect slender, much branched annual herb, up to 30 cm. high, commonly found in dry as well as moist areas. Leaves are falsely whorled or opposite, linear-lanceolate to obovate. Flowers are white, greenish, orange or pink, in terminal compound cymes. Capsules are globose with many dark reddish brown seeds. Roots are creaper and adventitious<sup>2</sup>. The plant contains carotene, traces of vitamin C, saponin and potassium nitrate. It is also having numerous applications in traditional medicine as stomachic, aperient, antiseptic, emmenagogue and is also used in poultices for sore legs. An infusion of the plant is given to women to promote the menstrual discharge. Leaves are bitter and antiperiodic; they are warmed after smearing with oil and applied to the ear to relieve earache<sup>3</sup>. It has been reported that the plant possesses antimicrobial<sup>4</sup>, whooping cough<sup>5</sup>, hepatitis<sup>6</sup>, anticancer<sup>7</sup>, spermicidal<sup>8</sup>, antibacterial<sup>9</sup> and antifungal activity<sup>10</sup>. Therefore, this present study was under taken to evaluate anti-inflammatory, analgesic and antipyretic activity potential of petroleum ether and ethanolic extract of leaves of the plant on various animal models.

### EXPREMENTAL

#### Plant material

The *Mollugo pentaphylla* plant was collected from the rural belt of Rayagada (Orissa) during the month of January and was authenticated by the Taxonomist of Botanical Survey of India, Howrah. The collected plant materials were washed under running tap to remove adhered dirt, then shed dried. Then the aerial part was ground in to coarse powder.

### Preparation of extract

The powdered plant material was extracted successively with petroleum ether (60-80°C) and then with 80% ethanol using soxhlet apparatus. The solvent was removed under reduced pressure to obtain dry extract, which gave a greenish-black coloured sticky residue. The extracts were stored in a desiccator for further use.

### Animals

Animals were kept in polypropylene cages and feed on standard laboratory diet and water *ad libitum*, exposing them to alternate cycle of 12h dark and light, temperature 25±2°C and relative humidity 55±10%. Animals were allowed to acclimatized for 7 days to the laboratory conditions before the conduct of experiment.

### Acute toxicity study

The acute toxicity of extracts were evaluated in female mice. The animals were fasted prior to the acute toxicity study. Different groups containing 6 no. of mice in each were orally administered with all extracts of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 gm/kg p.o respectively. The control group received only propylene glycol (vehicle). Drug treated and control groups were placed in propylene cage with free access of food and water. Mortality and general behavior of animals were observed continuously for initial 4h, and intermittently for 6h and then again at 24h and 48h after dosing. The parameters observed and recorded were sedation, hyperactivity, grooming, and loss of rightening reflex, respiratory rate and convulsion.<sup>11</sup>

### Evaluation of antiinflammatory activity

#### Carrageenan induced hind paw edema model

Male Albino rats (120-150gm) were selected for the study. All experimental animals were divided into 6 groups of 6 animals in each and were given the following treatment orally. Group I (control) received 1ml vehicle (propylene glycol) only. Group II received (12.5mg/kg) of Diclofenac sodium standard drug suspended in propylene glycol. Group III and IV received (200 and 400 mg/kg) of petroleum ether extract respectively suspended in propylene glycol. Group V and VI received (200 and 400 mg/kg) of ethanolic extract respectively suspended in propylene glycol. After 1h of drug treatment, the animals were administered with subcutaneous injection (0.05ml) of 2% w/v Carrageenan solution into sub plantar tissue of right hind paw. The contra lateral hind paws were injected with 0.1ml of saline as control. The paw volume was measured plethysmographically at 0, 1, 2 and 3h after injection of odematogenic agent. The difference between initial and subsequent reading gave the actual edema volume.<sup>12</sup>

#### Cotton-pellet granuloma model

This model was employed to study the sub-chronic inflammation. Two sterilized cotton pellets weighing 10 ± 1 mg were implanted subcutaneously by incision on either side of the back of the albino rats under ether anesthesia. Drugs were administered daily orally for 7 consecutive days. Diclofenac (100mg/kg p.o.) was used as standard. After 7 days the animals were sacrificed by cervical dislocation and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60°C for 24hr, weighed and compared with control. Animals were divided into 6 groups of 6 animals in each and were given the following treatment orally. Group I (control) received 1ml vehicle (propylene glycol) only. Group II received (100 mg/kg) of Diclofenac sodium standard drug suspended in propylene glycol. Group III and IV received (200 and 400 mg/kg) of petroleum ether extract respectively suspended in propylene glycol. Group V and VI received (200 and 400 mg/kg) of ethanolic extract respectively suspended in propylene glycol.<sup>13</sup>

### Evaluation of analgesic activity

#### Tail immersion method

Swiss Albino mice of either sex (20-25gm) were divided into 6 groups of six animals each and placed into individual cages leaving the tail hanging out freely. The animals were allowed to adopt in the cage for 30min before the experiment. Group I received sodium lauryl sulphate (SLS) 0.5% orally (3ml/kg) to the control group. Group II received standard drug Pentazocin (30mg/kg) is suspended in 0.5% (w/v) SLS orally. Group III and IV received (200 and 400 mg/kg) of petroleum ether extract as

suspended in 0.5% (w/v) SLS orally. Group V and VI received (200 and 400mg/kg) of ethanolic extract suspended in 0.5 % (w/v) SLS orally. The lower 5cm portion of the tail was immersed in a beaker of freshly filled hot water maintained at  $55.0\pm 1.0^{\circ}\text{C}$ . The time taken to withdraw the tail was noted as reaction time. A cut off time of 10sec was maintained to prevent tissue damage. The reaction time was measured at 0, 15, 30, 45 and 60 min. respectively. The mean reaction time was found out for each group and compared with the value of standard drug.<sup>14</sup>

#### **Acetic acid induced writhing method**

Peripheral analgesic activity was evaluated by using acetic acid induced writhing test in mice (20-25 gm) were divided into six groups of six animals each. Group I received 2% tween 80 orally (3ml/kg) which served as control. Group II served as positive control and received Aspirin (50mg/kg). Group III and IV received (200 and 400 mg/kg) of petroleum ether extract and group V and VI received (200 and 400 mg/kg) of ethanolic extract respectively. All the test doses were administered orally 1hr prior to acetic acid injection (0.1 ml of 6% v/v i.p). Ten minutes after acetic acid injection, the mice were placed in a transparent box and number of writhing movements was counted for a period of 20 min. writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching with a jerk at the hind limb. Significant reduction in the number of writhes by the test treated group as compared to control is considered as positive analgesic response.<sup>15</sup>

#### **Evaluation of antipyretic activity**

The anti-pyretic activity was evaluated by using Brewer's yeast induced pyrexia method in Albino rats. Fever was induced by injecting 2.0ml/kg of 20% aqueous suspension of Brewer's yeast in normal saline and 18hr after yeast injection the test drugs were administered. Rectal temperature was recorded by clinical thermometer at 0, 1, 2 and 3hr after drug administration. The animals were divided into 6 groups of 6 animals in each and were given the following treatment orally. Group I (control) received 1ml vehicle (2% gum acacia) only. Group II received Paracetamol (200mg/kg) as standard drug suspended in 2% gum acacia. Group III and IV received (200 and 400 mg/kg) of petroleum ether extract suspended in 2% gum acacia. Group V and VI received (200 and 400 mg/kg) of ethanolic extract suspended in 2% gum acacia. Before the experiment, the rats were maintained in separate cages with food *ad libitum* for 7 days and the animals with approximately constant rectal temperature ( $37.5$  to  $38.5^{\circ}\text{C}$ ) were selected for the study. The mean rectal temperature was found out for each group and compared with the value of standard drug.<sup>16</sup>

#### **Statistical Analysis**

All values were expressed as mean $\pm$ SEM. The results were analysed for statistical significance using one-way ANOVA followed by Dunnet's 't' test with \* $P < 0.05$  and \*\* $P < 0.01$  were considered as significant.<sup>17</sup>

## **RESULTS AND DISCUSSION**

The preliminary phytochemical screening of the dried ethanolic extract showed the presence of carbohydrate, glycosides, tannins, saponin, alkaloids, flavonoids, terpenoids and steroids.<sup>18,19</sup>

#### **Acute toxicity study**

Upto 4000 mg/kg of petroleum and ethanolic ether extract do not show any toxic effect. As per the ranking system European Economic Community (EEC) for acute oral toxicity, the LD<sub>50</sub> dose of 2000mg/kg and above is categorized unclassified (E C Directive 83/467/EEC, 1983).

#### **Carrageenan induced hind paw edema test**

The effect of ethanolic and petroleum ether extract on carrageenan induced hind paw edema test in rats shown in table1. The results obtained indicates that the ethanolic extract possess significant anti-inflammatory activity than petroleum ether extract in rats.

#### **Cotton-pellet granuloma test**

The effects of petroleum ether and ethanolic extract on cotton-pellet granuloma test in rats were reported in table 2. In granuloma induced sub-chronic inflammation the ethanolic extract in dose of 200,400 mg/kg had significant anti-inflammatory activity than petroleum ether extract ( $P < 0.01$ ). The percentage inhibition of granuloma after drug administration shows that 400 mg/kg of ethanolic extract had 40.33 % and Diclofenac sodium had 52.99 % when compared to control group.

### Tail immersion test

Table-3 shows the results of the tail immersion test. In this model, 30 min after drug administration, reaction time increased significantly, ( $P < 0.01$  and  $0.05$ ) for the test and standard when compared to the control. The test drugs produced a dose dependent increase in reaction time at various time intervals.

Table-1: Anti-inflammatory activity of petroleum ether and ethanolic extracts of *Mollugo pentaphylla* by Carrageenan induced hind paw edema test.

Group	Treatment	Dose	Paw Edema Volume (ml)			
		mg/kg	0h	1h	2h	3h
I	Control	--	0.30±0.006	0.45±0.008	0.56±0.014	0.72±0.023
II	Diclofenac sodium	30	0.19±0.007**	0.23±0.004**	0.26±0.005**	0.29±0.005**
III	Pet. Ether Extract	200	0.28±0.004*	0.44±0.007	0.50±0.005**	0.66±0.014**
IV		400	0.27±0.005**	0.40±0.005**	0.46±0.008**	0.56±0.006**
V	Ethanolic Extract	200	0.22±0.004**	0.27±0.004**	0.31±0.005**	0.27±0.006**
VI		400	0.20±0.004**	0.31±0.003**	0.25±0.004**	0.22±0.003**

Values are expressed as mean±S.E. (n=6). \* $P < 0.05$  and \*\* $P < 0.01$  compared with vehicle control (ANOVA followed by Dunnet's t-test).

Table-2: Anti-inflammatory activity of pet ether and ethanolic extracts of *Mollugo pentaphylla* by Cotton-pellet granuloma test.

Group	Treatment	Dose(mg/kg)	Weight of dry cotton pellet granuloma (mg)	% Inhibition
I	Control	---	34.88±0.32**	---
II	Diclofenac Sodium	100	15.16±0.31**	56.53
III	Pet. ether extract	200	31.44±0.29**	9.86
IV		400	29.84±0.30**	14.44
V	Ethanolic extract	200	23.43±0.34**	32.82
VI		400	18.73±0.41**	46.3

Values are expressed as mean±S.E. (n=6). \* $P < 0.05$  and \*\* $P < 0.01$  compared with vehicle control (ANOVA followed by Dunnet's t-test).

Table-3: Analgesic activity of pet ether and ethanolic extracts of *Mollugo pentaphylla* by tail immersion model.

Group	Treatment	Dose	Average tail withdrawing time (sec)				
		mg/kg	0 min.	15 min.	30 min.	45 min.	60 min.
I	Control	--	2.16±0.013	2.20±0.014	2.29±0.019	2.22±0.018	2.23±0.018
II	pentazocin	30	2.18±0.012	2.39±0.027**	3.18±0.016**	3.42±0.034**	3.89±0.258**
III	pet.ether extract	200	2.17±0.014	2.25±0.018	2.20±0.015	2.38±0.024**	2.45±0.026**
IV		400	2.16±0.011	2.28±0.011	2.34±0.017*	2.38±0.036**	2.50±0.026**
V	Ethanolic extract	200	2.17±0.015	2.41±0.023	2.99±0.084**	3.20±0.050**	3.42±0.028**
VI		400	2.18±0.018	2.40±0.008	3.24±0.025**	3.49±0.021**	3.99±0.084**

Values are expressed as mean±S.E. (n=6). \* $P < 0.05$  and \*\* $P < 0.01$  compared with vehicle control (ANOVA followed by Dunnet's t-test).

### Acetic acid induced writhing test

Dose dependent antinociceptive effect was noted with the extracts at the tested dose levels shown in table 4. Maximum percentage of inhibition of writhing response exhibited by ethanolic extract at 400 mg/kg.

Table-4: Analgesic activity of pet ether and ethanolic extracts of *Mollugo pentaphylla* by Acetic acid induced writhing test.

Group	Treatment	Dose(mg/kg)	Number of writhings/20 min	% inhibition.
I	control	---	40.83±1.13	---
II	Aspirin	50	21.33±0.49**	47.75
III	Pet. ether extract	200	34.66±0.80**	15.11
IV		400	31.50±0.56**	22.85
V	Ethanolic extract	200	27.00±0.57**	33.87
VI		400	21.50±0.42**	47.34

Values are expressed as mean±S.E. (n=6). \*P<0.05 and \*\*P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test)

### Antipyretic activity test

The results of anti pyretic activity of petroleum ether and ethanolic extracts were shown in table 5. It was observed that ethanolic extract at 400 mg/kg showed maximum antipyretic activity than petroleum ether extract.

Table-5: Antipyretic activity of petroleum ether and ethanolic extracts of *Mollugo pentaphylla* by Brewer's yeast induced pyrexia

Group	Treatment	Dose	Initial Temp. °C	Rectal Temperature °C in mean± SEM			
				0 Hour	1 Hour	2 Hour	3 Hour
I	Control	10ml/kg	37.44±0.07	39.00±0.03	39.12±0.04	39.32±0.03	39.16±0.03
II	Paracetamol	30mg/kg	37.35±0.01	39.053±0.02	38.83±0.01**	38.37±0.03**	38.09±0.01**
III	Pet. ether extract	200mg/kg	37.44±0.02	39.02±0.01	39.19±0.01	39.27±0.01	39.02±0.01**
IV		400mg/kg	37.43±0.02	39.01±0.01	39.12±0.01	39.16±0.01**	38.99±0.01**
V	Ethanolic extract	200mg/kg	37.45±0.03	39.01±0.02	38.96±0.01**	38.73±0.03**	38.30±0.01**
VI		400mg/kg	37.44±0.01	39.02±0.01	38.81±0.01**	38.61±0.01**	38.28±0.02**

Values are expressed as mean±S.E. (n=6). \*P<0.05 and \*\*P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test).

### CONCLUSION

From these results it was concluded that the ethanolic extract of *Mollugo pentaphylla* possess significantly both peripheral and central analgesic activity along with marked anti-inflammatory and antipyretic activity in rats than that of petroleum ether extract and also the present study provoke the traditional use of *Mollugo pentaphylla* for the purpose of various ailments like anti-inflammatory, analgesic and antipyretic.

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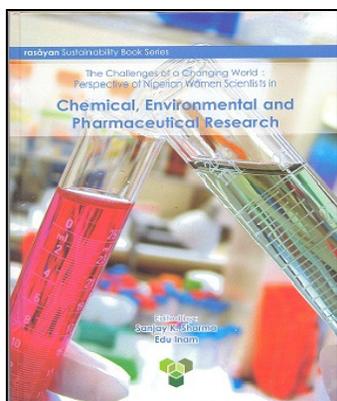
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