



Analgesic and Anti-inflammatory Potential in *Misopates orontium*

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Abstract: The *Misopates orontium* (L.) Raf has been reported for its medicinal importance in the literature. This plant has been studied for its antioxidant and hepatoprotective studies. In the current study the *in vitro* analgesic and anti-inflammatory potentials of ethanol extracts of an ethnomedicinal plant *Misopates orontium* (L.) Raf was evaluated in a dose-dependent manner. The analgesic activity was determined *via* hot plate, tail flicking/immersion methods, and paw licking in mice, whereas, the anti-inflammatory potential was investigated by using carrageenan-induced hind paw edema in albino rats. The doses of 100, 200, and 300 mg/kg concentrations were used. The extract of 300 mg/kg displayed significant results within the range of 22.4 ± 0.79 and 7.2 ± 0.83 at 1.5 h for hot plate and tail immersion methods, respectively, in comparison with the standard drug aspirin. In the paw licking method the most effective dose was 300 mg/kg at which the licking and biting were reduced within 30 minutes, *i.e.* 3 ± 0.58 at 10 min. In the anti-inflammatory activity, the expansion in paw volume was measured after carrageenan injection. Results indicated that after carrageenan injection the paw volume increased but after a few intervals of time, it again reduced, *i.e.* 6.17 ± 0.15 at 2 h as compared to standard drug diclofenac sodium. This study reveals the anti-inflammatory and analgesic effectiveness of this plant, as it is also used locally to reduce pain and inflammations. Further studies on the isolation of active metabolites from the ethanol extract of *M. orontium* may lead to the discovery of new and novel molecules which can be used as drug candidates.

Keywords: *Misopates orontium* (L.) Raf., anti-inflammatory, diclofenac sodium, analgesic, aspirin.

1. INTRODUCTION

Analgesia means suppression of ache or pain and analgesics are those substances that reduce pain sensitivity. Paracetamol, aspirin, and morphine are among commonly used analgesics. On the other hand, inflammation is a reaction of the human body towards external stimuli or foreign particles. It is a defensive mechanism that eliminates or limits the spread of injurious agents. Inflammation is characterized *via* pain, swelling, and redness which might be due to edema or granuloma formation and leukocyte infiltration [1-2]. It can be chronic and

acute based on its distinct features and is almost associated with every health risk. Currently, the drugs used for the treatment of inflammation are either NSAIDs (Non-steroidal anti-inflammatory drugs) or narcotics that have toxic effects and over usage of these drugs could lead to severe lethal effects [3]. NSAIDs usually destroy the gastric mucosa by inhibiting the COX (Cyclooxygenase) enzyme thus leading to gastrointestinal damage [4].

Many agents have been introduced to cure inflammation and analgesia but long-term use of these curative agents often directs to bone marrow

suppression, gastric intolerance, and salt and water retention. On the other hand, natural products derived from herbal and other medicinal plants are less toxic, easily available, and have good absorption capability, therefore, they are being used since ancient times [5-6]. Almost 80% population depends entirely on plants for their health [7-8]. The plant kingdom has provided a great source of biologically active molecules that can be used as new drug candidates. Therefore, it is of dire necessity to explore new medicinal plants to develop effective, cheaper, and safe anti-inflammatory and analgesic drugs [9].

Misopates orontium (L.) Raf. family Scrophulariaceae is a herb that belongs to southwestern and central Europe. Its Latin name is: *Antirrhinum orontium* L. In Greek, *anti* means against and *rhis* means snout which means appressed closed palate of the flowers [10]. Plant of this family was typically autotrophic, while some hemiparasitic or parasitic plants were also found. Their habitat includes wet and swampy places, near and within the water bodies like ponds, rivers, and ditches [4]. Ethnobotanically, this plant is used for the treatment of scurvy, tumors, liver disorder, and inflammations and as a pain killer [11-13]. Keeping in mind the medicinal importance of this plant, the current investigation was focused to evaluate the analgesic and anti-inflammatory effects of ethanolic extract of *M. orontium*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The plant material *M. orontium* was collected from the local area of District Bhimber Azad Jammu and Kashmir in March 2016. The plant was authenticated from herbarium in the Department of Botany; Mirpur University of Science and Technology having voucher number of specified sample MUST.BOT.5353.

2.2 Preparation of Ethanolic Extract

The whole plant material of *M. orontium* 250 g was grounded using pestle and mortar and soaked in 1500 mL ethanol for seven days. The ethanolic extract was evaporated by using a rotary evaporator to get crude extract and the dark green thick extract was obtained. The extraction procedure was

repeated three times. This crude extract dissolved with distilled water 200 μ L was used during oral dosage to each mice and rat.

2.3 Animals

Swiss mice of 25-30 g and Wistar rats of 132-151 g were kept at the animal house of College of Pharmacy, the University of Punjab at 25 °C. The animals were fed with the standard diet and water under suitable environmental conditions.

2.4 Analgesic Activity

Analgesic activity of ethanolic extract of *M. orontium* was examined by using three different assays (i) Tail flicking assay, (ii) Formalin assay and (iii) Hot plate assay

2.4.1 Hot Plate Assay

A hot plate assay was carried out using the method of Lanhers *et al.* [7].

2.4.2 Tail Flicking Assay

Tail flicking assay was used to determine the analgesic activity of the crude extract of *Misopates orontium* according to the selected assay of Gupta *et al.* [8].

2.4.3 Formalin Assay

Into the right hind paw of mice, 15 μ L of 1% formalin was injected hypodermically. After injecting formalin, the licking and biting responses were measured, the first phase comprises of 10 min, while the second phase was within 20-30 min. Oral administration of the extract (100, 200, and 300 mg/kg), and aspirin (300 mg/kg) was done 30 min before formalin injection. The Control group received saline water 15 mL/kg orally.

2.5 Antiinflammatory activity

Antiinflammatory effect was investigated by following assay.

2.5.1 Carrageenan-induced Paw Edema Assay

This assay examined the antiinflammatory effect of crude extract of *M. orontium* according to the

reported method of Ishola *et al.*, and Kakoti *et al.* [14-15].

Plant extracts of different doses and standard drugs were given orally. Rats were distributed into five groups and labeled as:

Group 1 = Negative control (Standard saline 15 mL/kg/day orally)

Group 2 = Positive control (Diclofenac sodium 300 mg/kg/day orally).

Group 3 = Ethanolic extract of *M. orontium* L. 100 mg/kg/day.

Group 4 = Ethanolic extract of *M. orontium* L. 200 mg/kg/day.

Group 5 = Ethanolic extract of *M. orontium* L. 300 mg/kg/day.

$$\% \text{ Inhibition} = \frac{\text{Edema of control} - \text{Edema of treated}}{\text{Edema of control}} \times 100$$

2.6 Statistical analysis

The data was obtained from experiment analysis by graph pad prism. The results were obtained in terms of mean \pm SEM. ANOVA (analysis of variance) and Bonferroni post-test were used to analyze the data statistically [11].

3. RESULTS AND DISCUSSION

3.1 Appraisal of analgesic activity

To investigate the analgesic effect in a crude extract of the whole plant of *M. orontium* doses of different concentrations were used *i.e.* 100, 200, and 300 mg/kg at different parameters which are hot plate method, tail flicking method, and formalin test. For the test and control specimen, five groups of mice were used. The ethanolic extract of 300 mg/kg showed significant results and reduced latency of pain which was close to standard drug aspirin at 300 mg/kg. The Control group did not exhibit good results. A comparison of all the results are shown in Table-1, -2, -3, and -4. These results were compared with the standard and control groups.

3.2 Investigation of anti-inflammatory activity

To examine the anti-inflammatory activity in a crude extract of the whole plant of *Misopates orontium* dose of different concentrations were used

i.e. 100, 200, and 300 mg/kg, a formalin-induced inflammation activity was carried out as explained by [16] 300 mg/kg ethanolic extract significantly reduced inflammation which was close to standard drug diclofenac sodium at 300 mg/kg. The Control group did not show good results. The results of tested groups were compared with that of controlled groups (Table-4).

In the current study, the analgesic and anti-inflammatory effects of *M. orontium* were investigated. The results indicated that the ethanolic extract of *M. orontium* exhibited potent antiinflammatory and analgesic activity at 300 mg/kg in animal models.

The crude extracts of the whole plant of *M. orontium* (L.) Raf. with different concentrations were evaluated for analgesic activity against thermally and chemically induced pain to explore their central and peripheral pain inhibition potential. Hot plate method and tail flicking test are the simplest and common methods which are used as thermal nociception models for evaluating analgesic efficacy of drugs or compounds.

In the hot-plate method three doses of 100, 200, and 300 mg/kg concentrations were used. Maximum heat tolerance recorded by crude extract of *M. orontium* was 22.4 ± 0.79 at 1.5 h, 26.6 ± 0.52 at 2 h, and 26.6 ± 0.51 at 2.5 h at the dose of 300 mg/kg that was close to the latency of pain in comparison with the standard drug aspirin at 300 mg/kg (Table-1).

In the tail immersion method, three doses of 100, 200, and 300 mg/kg were also used as compared to the standard drug aspirin at 300 mg/kg. Results indicated that at 300 mg/kg of ethanolic extract the latency of pain become low *i.e.* 7.2 ± 0.83 at 1.5 h, 8.6 ± 0.30 at 2 h, and 8.8 ± 0.35 at 2.5 h and has comparable activity with the standard aspirin at 300 mg/kg (Table-2).

Against the first phase of formalin-induced pain in mice, the study exposed that the ethanolic extract of *M. orontium* showed an analgesic effect (Table-3). It was also observed that the dose level of 300 mg/kg exhibited a better analgesic effect as compared to the other dose levels and from the control. These dose levels, however, were as

Table-1: Analgesic effect of ethanolic extract of *M. orontium* by hotplate method in mice

Response time in sec. at various intervals (h)	Groups				
	Normal saline	Aspirin (300 mg)	<i>M. orontium</i> extract (100 mg)	<i>M. orontium</i> extract (200 mg)	<i>M. orontium</i> extract (300 mg)
0	8.2 ± 0.48	8.2 ± 0.48	8.4 ± 0.52	9.4 ± 0.67	9.4 ± 0.67
0.5	8.6 ± 0.89	17.4 ± 0.84	10.2 ± 0.30	13.4 ± 0.55	17.4 ± 0.84
1.0	9.6 ± 0.14	20.2 ± 0.17	9.8 ± 0.84	16.4 ± 0.14	20.6 ± 0.52
1.5	8.6 ± 0.52	25.6 ± 0.85*	11.6 ± 0.34	17.8 ± 0.28	22.4 ± 0.79*
2.0	8.6 ± 0.14	31.2 ± 0.95**	12 ± 0.6	19.2 ± 0.10	26.6 ± 0.52*
2.5	8.8 ± 0.48	31.8 ± 0.77*	10.8 ± 0.11	19 ± 0.20	26.6 ± 0.51**
3.0	9.4 ± 0.34	29.8 ± 0.92	10 ± 0.21	20 ± 0.22	25 ± 0.71
3.5	9 ± 0.22	26 ± 0.66	11.6 ± 0.23	19 ± 0.71	24 ± 0.71
4.0	8.4 ± 0.34	25.4 ± 0.97	8 ± 0.58	16.2 ± 0.45	20.4 ± 0.89
4.5	9 ± 0.71	20.8 ± 0.11	9.2 ± 0.30	12.8 ± 0.48	16.2 ± 0.92

Results were expressed as Mean ± SEM (standard error mean), n=5. P < 0.05*, 0.01** and 0.001*** were considered statistically significant, very significant and highly significant respectively.

Table-2: Analgesic effect of crude extract of *M. orontium* by tail immersion method in mice.

Response time in sec. at various intervals (h)	Groups				
	Normal saline	Aspirin (300 mg)	<i>M. orontium</i> extract (100 mg)	<i>M. orontium</i> extract (200 mg)	<i>M. orontium</i> extract (300 mg)
0	2.2 ± 0.44	2.4 ± 0.54	2.2 ± 0.54	2.2 ± 0.44	2.2 ± 0.44
0.5	2 ± 0.30	3 ± 0.71	2.1 ± 0.54	2.8 ± 0.44	2.8 ± 0.44
1	2.6 ± 0.54	6.4 ± 0.13	2.3 ± 0.43	4.6 ± 0.53	5.6 ± 0.54
1.5	2.4 ± 0.54	8.2 ± 0.30*	2.4 ± 0.54	6.2 ± 0.83	7.2 ± 0.83*
2	2.8 ± 0.43	10.6 ± 0.88**	2.5 ± 0.83	7.2 ± 0.83	8.6 ± 0.30*
2.5	2.4 ± 0.54	11.2 ± 0.64***	2.6 ± 0.45	7.2 ± 0.85	8.8 ± 0.35**
3	2.4 ± 0.54	10.8 ± 0.84	2.3 ± 0.71	6.8 ± 0.84	8.2 ± 0.35
3.5	2.2 ± 0.45	9.6 ± 0.45	2.4 ± 0.55	6.4 ± 0.89	5.2 ± 0.89
4	2.4 ± 0.55	8.4 ± 0.30	2.3 ± 0.71	4.4 ± 0.52	3.4 ± 0.52
4.5	2.2 ± 0.46	6.4 ± 0.65	2.2 ± 0.44	2.8 ± 0.44	3 ± 0.71

Results were expressed as Mean ± SEM (standard error mean), n=5. P < 0.05*, 0.01** and 0.001*** were considered statistically significant, very significant and highly significant respectively.

effective as aspirin (standard drug).

Analgesic activity of *M. orontium* has been evaluated by formalin-induced paw licking and biting assay. At dose level of 300 mg / kg of ethanolic extract these results were obtained 8 ± 0.93 at 0 h, 3 ± 0.58 at 10 min, 2 ± 0.51** at 20 min and 1 ± 0.71*** at 30 min. The results indicated that the maximum analgesic activity was observed at 300 mg/kg dose level might be due to one or more groups of the secondary metabolites detected in the extracts.

Results of the anti-inflammatory activity of crude extract of plant *M. orontium* were significant as compared to the control group. The anti-inflammatory activity of the extract was studied by using the carrageenan-induced hind paw edema

model which involves the production of PGs and reactive oxygen species (ROS).

Two different phases were observed while inducing the paw edema by carrageenan; the first phase (1 or 1.5 h) consisted of non-phagocytic edema followed by a second phase (2-5 h) which resulted in increased edema development that persisted up to 5 h. *M. orontium* showed an anti-inflammatory effect against formalin-induced edema in albino mice. The ethanolic extract of *M. orontium* significantly suppressed the inflammation when treated at different concentrations. In the first two stages, 100 and 200 mg/kg doses are used and in the third stage, a dose of 300 mg/kg was used which was more effective and reduced paw diameter as compared to the other treated groups [17,18]. The maximum result showed by ethanolic extract

Table-3: Analgesic effect of crude extract of *M. orontium* by formalin-induced pain method in mice.

Groups	Mean paw-licking and biting time(sec) ± SD			
	0 h	10 min	20 min	30 min
Normal saline	10 ± 0.71	5 ± 0.71	3 ± 0.51	2 ± 0.51
Aspirin (300 mg)	8 ± 0.30	2 ± 0.13*	1 ± 0.24**	0 ± 0.54***
<i>M. orontium</i> extract (100 mg)	9 ± 0.51	7 ± 0.51	5 ± 0.24	4 ± 0.20
<i>M. orontium</i> extract (200 mg)	9 ± 0.68	5 ± 0.71	3 ± 0.86	2 ± 0.71
<i>M. orontium</i> extract (300 mg)	8 ± 0.93	3 ± 0.58*	2 ± 0.51**	1 ± 0.71***

Results were expressed as Mean ± SEM (standard error mean), n=5. P < 0.05*, 0.01** and 0.001*** were considered statistically significant, very significant and highly significant respectively

Table-4: Anti-inflammatory evaluation of crude extract of *M. orontium* by carrageenan-induced paw edema in rats

Group	Paw size (mm) after carrageenan injection				
	0 h	1 h	2 h	3 h	4 h
Carrageenan control	5.19 ± 0.35	8.59 ± 0.40	9.01 ± 0.25	9.19 ± 0.20	8.82 ± 0.30
Diclofenac sodium (300 mg/kg)	5.19 ± 0.20	6.16 ± 0.20	5.87 ± 0.20*	5.38 ± 0.25**	5.12 ± 0.15***
<i>M. orontium</i> (100 mg/kg)	5.32 ± 0.35	8.38 ± 0.20	8.68 ± 0.15	8.69 ± 0.25	8.17 ± 0.20
<i>M. orontium</i> (200 mg/kg)	5.46 ± 0.38	7.36 ± 0.26	7.11 ± 0.15	6.65 ± 0.18	6.29 ± 0.20
<i>M. orontium</i> (300 mg/kg)	5.35 ± 0.35	6.61 ± 0.35	6.17 ± 0.15*	5.82 ± 0.15**	5.53 ± 0.20***

Results are expressed as Mean ± SEM (standard error mean), n=5. P < 0.05*, 0.01** and 0.001*** were considered statistically significant, very significant and highly significant respectively.

at 3 h *i.e.*, 5.82 ± 0.15**. The effects of diclofenac were compared with three different doses but the plant extract at dose 300 mg/kg showed to reduce inflammation.

4. CONCLUSIONS

The analgesic and anti-inflammatory activities of the whole plant of *Misopates orontium* were evaluated. Analgesic activity investigated by using a hot plate, tail flicking or immersion method and formalin-induced paw licking in mice. In hot-plate method ethanolic extract of *Misopates orontium* at dose 300 mg/kg showed significant results 22.4 ± 0.79 at 1.5 h, 26.6 ± 0.52 at 2 h and 26.6 ± 0.51 at 2.5 h that was close to the latency of pain as compared to standard drug aspirin 300 mg/kg. In tail immersion method results indicated that at 300 mg/kg the latency of pain become low *i.e.* 7.2 ± 0.83 at 1.5 h, 8.6 ± 0.30 at 2 h, and 8.8 ± 0.35 at 2.5 h. In the paw licking method, results showed that licking and biting were reduced at 300 mg/kg after some time *i.e.* 3 ± 0.58 at 10 min, 2 ± 0.51 at 20 min, and 1 ± 0.71 at 30

min. Ethanolic extract of *M. orontium* showed an anti-inflammatory effect against formalin-induced edema in albino mice. Plant extract at dose 300 mg/kg was more effective and reduced paw diameter as compared to the other treated group. The effects of diclofenac were compared with the three different doses but the plant extract at dose 300 mg/kg was most effective against inflammation. This study revealed that the ethanolic extract of *M. orontium* contains metabolites that might be responsible for the anti-inflammatory and analgesic activity. Further purification and isolation of pure compounds will be helpful in future studies.

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