Original Research Article

A study to evaluate the analgesic activity of *Origanum vulgare* in mice using tail flick method

Siddhi Raveendran¹, Vikram Rajadnya²*, Revati Kothari¹, A. V. Tilak¹, Sayan Das¹, Rahul Bhalsinge³

INTRODUCTION

Pain is an enteroceptive sensation which warns of the danger of bodily harm and alerts to trauma and injury. Analgesia commonly known as painkillers is used by number of patients who seek treatment of pain which is the most important therapeutic objective of any analgesic. However, half of the patients using analgesics seem to be dissatisfied with available pharmacological options. In addition, many of these drugs cause serious side effects. The adverse effect profile associated with opiates (like pentazocine) consist of physical dependency, tolerance and addiction while that of non-steroidal anti-inflammatory drugs (like paracetamol) consist mostly of gastrointestinal disorders.

It is known that herbal plants with medicinal properties have been used since ancient times which show potent analgesic activity with minimal adverse effects. *Origanum vulgare*, a traditional medicine, is known to have various properties like diuretic, stimulant, antimicrobial, anti-inflammatory, antioxidant and anticancer. Several studies have reported that *Origanum vulgare*...
vulgare has potent analgesic activity, so this study was conducted to evaluate analgesic efficacy of *Origanum vulgare* compared to pentazocine using tail flick method in mice.

**METHODS**

The study was commenced after Institutional animal ethics committee approval was granted and is conducted in accordance with Committee for the purpose of control and supervision of experiments on animals guidelines.\(^5\)

**Study animals**

Experimentally naive mice were selected for the study. Swiss albino mice, experimentally naive of either sex, weighing 18-22 gm were selected for study. The mice were procured from animal house of Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pune-18.

**Study population**

The study was conducted on 24 Swiss albino mice; 8 mice in each group.

**Study period**

The study was conducted from January 2018 to June 2018.

**Animal feed**

**Food**

Mice were fed with commercially available ‘Nutrimix Std-1020’ manufactured by Baramati Agro Ltd, acquired from Nuvivet Life Sciences, Pune. The nutrition provided by the pellet feed was contained energy 3620 kcal/kg, crude protein 22.15%, crude fibre 62.48%, ash 5.11% and sand silica 1.15%.

**Water**

Drinking tap water supplied by Pimpri Chinchwad Municipal Corporation was provided to the mice through the feeding bottles with stainless steel nozzle in each cage. Food and water were replenished once daily in the morning.

**Animal housing**

Mice were housed in groups of four in standard big polypropylene cages under standard condition of temperature (25±5°C) and relative humidity (55±10%) and 12/12 hour light or dark cycle. Apart from daily replenishment of food and water, they were left undisturbed.

**Study drugs**

*Origanum vulgare* (leaf extract; Jesus Heals Ayurveda Centre, Pune) was used as test drug.

Pentazocine (from Pharmacy, Dr. D.Y. Patil Medical College, Pimpri, Pune) was used as standard analgesic drug used in study. Pentazocine solution was freshly prepared in distilled water and given in a dose of 1.4 mg/kg i.p.

**Study design**

Mice were divided into three groups consisting 8 in each group.

**Table 1: Groups showing drugs and dose.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drugs and dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled water (1 ml, i.p.)</td>
</tr>
<tr>
<td>II</td>
<td><em>Origanum vulgare</em> (84 mg/kg, i.p.)</td>
</tr>
<tr>
<td>III</td>
<td>Pentazocine (1.4 mg/kg, i.p.)</td>
</tr>
</tbody>
</table>

**Methods for evaluation of analgesic activity**

**Screening**

Prior to employing different methods to study the analgesic activity, a preliminary screening was done. Those mice showing reaction time of less than 15 seconds were included in the study.\(^6\) Analgesic activity of aqueous extract of *Origanum vulgare* was studied with help of tail flick method: widely used to evaluate opioid analgesics.

**Procedure**

Mice weighing 18-22 g were used and placed into small cages, leaving the tail exposed. The tail of the mice was held gently. A light beam was focused (exerting radiant heat) to the proximal third of the tail. The mice tried to pull the tail away and rotated its head, a reaction known as escape reaction. The test drug and standard drug were administered intraperitoneally.

The same procedure was repeated and the reaction time was noted after 30, 60, 90 and 120 mins.

A lengthening of the reaction time was interpreted as an analgesic action of the test drug. At each time interval those mice that showed higher reaction time than the time before drug administration were regarded as positive.\(^5,7\)

**Statistical analysis**

The data was compiled and analyzed with the help of statistical package, Primer of biostatistics, version 7.0. Results were expressed in mean and SD were analysed using one-way repeated measures analysis of variance.
(ANOVA) followed by Tukey’s test for multiple comparisons and p value <0.05 was considered to be statistically significant.

RESULTS

Figure 1 shows the results of analgesic activity of *Origanum vulgare* and pentazocine as compared with control. *Origanum vulgare* in a dose of 84 mg/kg showed statistically significant prolongation in the reaction time after 60 min of administration.

However, on comparison, we see that in tail flick method, pentazocine showed statistically significant increase in the reaction time after 30 min of administration as compared to the control group.

Table 2: Results of tail flick method.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time in min (mean±SD )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>4.37±1.68</td>
</tr>
<tr>
<td><em>Origanum vulgare</em></td>
<td>4.37±1.68</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>4.37±1.68</td>
</tr>
</tbody>
</table>

*: Comparison with control group (p<0.05). #: Comparison with *Origanum vulgare* (p<0.05).

Figure 2 shows the comparison of reaction time in pentazocine and *Origanum vulgare* treated groups. *Origanum vulgare* shows significant effect at dose 84 mg/kg i.p. compared to control but when the analgesic effect of *Origanum vulgare* was compared with pentazocine, it exhibited significant analgesic effect as compared to *Origanum vulgare*.

DISCUSSION

Pentazocine is a potent opiate analgesic which is used to relieve pain and apprehension caused due to it. However, it is associated with various adverse effects so there is a need to find a novel drug having similar analgesic activity with minimal adverse effects.

The present study was carried out by using tail flick method to evaluate the analgesic activity of aqueous extract of *Origanum vulgare* compared to that of pentazocine.

The results of the present study suggest that *Origanum vulgare* exhibits significant effect at dose 84 mg/kg i.p. compared to the control but when the analgesic effect of *Origanum vulgare* was compared with pentazocine, it exhibited significant analgesic effect as compared to *Origanum vulgare*.

Thus, the findings of the present study are similar to the findings of study done by Arzi et al. The latter evaluated the hydroalcoholic extract of *Origanum vulgare* in rats by formalin test where maximum analgesic effect was seen at a dose of 600 mg/kg. This analgesic effect of *Origanum vulgare* was less than analgesic effect exhibited by aspirin (300 mg/kg) and morphine (2.5 mg/kg).

Another study done by Khaki et al evaluated antinociceptive activities in aqueous extract of *Origanum vulgare* using tail flick test.

It is reported that the analgesic effect of *Origanum vulgare* can be attributed to the compound named carvacrol. Carvacrol has an inhibitory effect on prostaglandins. Inhibition of prostaglandins cause antinociception.

In the present study, *Origanum vulgare* showed statistically significant increase in reaction time after 30 mins of administration as compared to control group.
However, pentazocine showed statistically significant increase in reaction time after 30 mins compared to control and *Origanum vulgare*.

Isolation, purification and characterization of active compounds of the extract were not a part of this study. Further such studies are needed as the active compound may be better in efficiency as compared to standard analgesic drug.

Also, the combination of standard drug with *Origanum vulgare* has not been studied. It might be possible that its extract potentiates analgesic activity of standard drugs which may result in lowering of dosages and helps in minimizing the undesirable effects of such drugs.

Further, toxicity studies and clinical trials are needed to establish it as a drug in mild to moderate painful conditions.

**ACKNOWLEDGEMENTS**

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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