analgesic activity of Momordica cochinchinensis and Momordica balsamina fruit extracts

Mohan R. Agrawal¹*, Anilkumar N. Aher², Subodh C. Pal², Deelip V. Derle²

¹Department of Pharmacognosy, Sitabai Thite College of Pharmacy, Pune, Maharashtra, India, ²Department of Pharmacognosy, MVP’s College of Pharmacy, Nashik, Maharashtra, India

Abstract

Introduction: In the present study, fruit extracts of Momordica cochinchinensis (Cucurbitaceae) and Momordica balsamina (Cucurbitaceae) were investigated for analgesic activity by Eddy’s hot plate and Tail immersion method.

Materials and Methods: The extracts were prepared successively using powdered material with petroleum ether, ethanol, and water, and concentrated under vacuum and were evaluated for analgesic activity at three dose level (100, 200, and 400 mg/kg).

Results and Discussion: In Eddy’s hot plate method, oral administration of petroleum ether extracts of both the plants at the dose of 200 mg/kg ($P < 0.01$) and 400 mg/kg ($P < 0.001$) significantly reduced the thermal stimulation. Analgesic activity of petroleum ether extracts of both plants at the dose of 400 mg/kg after 90 min was comparable to standard drug pentazocine (10 mg/kg). In tail immersion method, petroleum ether extract at the dose of 100 mg/kg ($P < 0.05$), 200 mg/kg, and 400 mg/kg ($P < 0.01$) and alcoholic extract at the dose of 200 mg/kg and 400 mg/kg ($P < 0.05$) of both plant material has shown significant analgesic activity and was comparable to standard drug pentazocine (10 mg/kg) after 90 min. Conclusion: It is concluded that petroleum ether extracts of both plant material have central analgesic effects.

Key words: Analgesic activity, Eddy’s hot plate, Momordica balsamina, Momordica cochinchinensis, phytosterols, tail immersion

INTRODUCTION

Momordica is a genus of about 60 species of annual or perennial climbers herbaceous or rarely small shrubs belonging to the family Cucurbitaceae, natives of tropical and subtropical Africa, Asia and Australia.¹,²

Momordica cochinchinensis (Gac) is a Southeast Asian fruit found throughout the region from Southern China to Northeastern Australia, mostly Vietnam and throughout India. It grows on dioecious vines and is usually collected from fence climbers or wild plants. The vines can be commonly seen growing on lattices at the entrances to rural homes or in gardens. It bears fruits annually and is found in local markets. The fruit becomes a dark orange color on ripening, and is typically round or oblong, maturing to a size of about 13 cm in length and 10 cm in diameter. The exterior skin is covered in small spines, while dark red interior consists of clusters of fleshy pulp and seeds.³ Gac fruit, M. cochinchinensis Spreng, is one of the special fruits containing extraordinarily high levels of carotenoids, especially β-carotene (>16 mg/100 g), and lycopene (>50 mg/100 g), mainly in the red aril.⁴ Conventionally, Gac has been used as both food and medicine and promotes healthy vision by relief of dry eyes. It also possesses antioxidant, antimicrobial, and antidiabetic properties. The seeds are considered to be good for cough and pains in the chest.⁵-⁷

Momordica balsamina Family: Cucurbitaceae is climber with bright green leaves bears striking orange to red spindle-shaped ripe fruit. Shrub is fairly common and widespread in Malaya, Australia, West Asia, Africa, America, and India (Sind, Gujarat, and Deccan). Conventionally used as a purgative agent, purification of blood and dissipate

Address for correspondence:
Mohan R. Agrawal, Department of Pharmacognosy, Sitabai Thite College of Pharmacy, Pune, Maharashtra, India. Tel.: +91-2138-222680/9860947093.
E-mail: agrawalmohan19@gmail.com

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melancholia, stimulant, and gross humors. In Africa, as a medicinal plant for curing various diseases such as diabetes and malaria.[5,8]

**MATERIALS AND METHODS**

**Plant Material**

The herbarium of the *M. cochinchinensis* and *M. balsamina* was authenticated by Botanical Survey of India, Pune and Veer Narmad South Gujarat University, Surat, respectively, and voucher specimens were deposited in the library.

**Preparation of Extracts**

Fruits of *M. cochinchinensis* and *M. balsamina* were extracted successively by Soxhlet extractor with petroleum ether and macerated with ethanol and water. All the extracts were stored in tightly closed glass bottles in refrigerator at 2–8°C.

**Animals**

Male Wistar rats (200–250 g) and Swiss albino mice (25–40 g) were used and procured from National Toxicological Centre, Pune. The animals were maintained in colony cages at 25 ± 2°C, relative humidity 50–55% maintained under 12 h light and dark cycle (6–10 h light and 18–6 h dark). The animals were fed with standard animal feed, and water was applied *ad libitum*. All animals were acclimatized to the laboratory conditions before experimentation.

**Acute Toxicity Studies**

Acute toxicity studies were carried out using the acute toxic class method as per the OECD guidelines 425.[9] Acute toxicity for various plant extracts was carried out using groups of three Swiss Albino mice by administrating a dose of 2000 mg/kg, in 1% carboxymethylcellulose (CMC) p.o., while the control group received only the vehicle. The groups were observed for mortality and behavioral changes during 48 h.

**Preliminary Phytochemical Analysis of Extracts**

All the extracts were tested for the presence of various chemical constituents.[10,11]

**Analgesic Studies**

**Eddy’s hot plate method**

Hot plate method developed by Woolfe and McDonalds[12,13] was followed. Wistar rats (*n* = 6) were placed in Eddy’s hot plate kept at a temperature of 55 ± 0.5°C. A cutoff time of 30 s was fixed to avoid damage to the paw. The reaction time of response was recorded using a stopwatch. Control animals were treated with vehicle (0.3% CMC, oral) and test groups were pretreated with a single dose of 100, 200, and 400 mg/kg p.o. of petroleum ether, ethanol, and water extract of fruits of *M. cochinchinensis* and *M. balsamina*. Pentazocine (10 mg/kg) was used as a positive control. The latency was recorded before and after 30, 60, 90, 120, and 180 min following oral administration of each extract. Percentage analgesia was calculated using the following formula.

\[
\text{% Analgesia} = \frac{\text{Latency (test)} - \text{Latency (control)}}{\text{Latency (test)}} \times 100
\]

**Tail immersion method**

Wistar rats were administered orally with vehicle (0.3% CMC, oral) (3 ml/kg), Pentazocine (10 mg/kg) as a standard and test groups with petroleum ether, ethanolic, and aqueous extracts of fruits of *M. cochinchinensis* and *M. balsamina* at the dose 100, 200, and 400 mg/kg. The distal part of the tails of the animals was immersed in hot water maintained at 55.0 ± 1.0°C. The time taken to withdraw the tail was noted as reaction time. A cutoff time of 10 s was maintained at 55°C to prevent tissue damage. The reaction time was measured before and 30, 60, 90, and 120 min after treatment, respectively. Percentage of elongation was calculated using the following formula.[14]

\[
\text{Elongation} (%) = \frac{\text{Latency (test)} - \text{Latency (control)}}{\text{Latency (test)}} \times 100
\]

**Statistical Analysis**

All experimental data were expressed as the mean ± standard error of the mean. Statistical analysis was carried out using one-way ANOVA followed by Dunnett’s *t*-test and two-way ANOVA followed by Bonferroni Posttests. The values of *P* < 0.05 were considered as statistical significant.

**RESULTS**

**Preliminary Phytochemical Screening**

The results of preliminary phytochemical screening of various extracts of fruits of *M. chinchinensis* and *M. balsamina* revealed the presence of glycosides, flavonoids, steroids, phenolic compounds, carotenoids, and carbohydrates [Table 1].

**Acute Toxicity**

All extracts of fruits of *M. cochinchinensis* and *M. balsamina* were evaluated for acute toxicity in mice by oral administration. No behavioral changes were observed after 4 h. It was found that all the extracts were safe at the highest
A dose of 2000 mg/kg and no mortality was shown even after 14 days of extract administration. Moreover, no mortality was observed during the toxicity study.

**Analgesic Studies**

**Eddy’s hot plate method**

Analgesic effects of petroleum ether, alcohol, and water extracts of fruits of *M. cochinchinensis* and *M. balsamina* evaluated by Eddy’s hot plate method are shown in Figures 1 and 2, respectively. It revealed that oral administration of petroleum ether extracts of both the plants at the dose of 200 mg/kg ($P < 0.01$) and 400 mg/kg ($P < 0.001$) significantly reduced the thermal stimulation. Analgesic activity of petroleum ether extracts at the dose of 400 mg/kg of *M. cochinchinensis* (44.07%) and *M. balsamina* (46.02%) after 90 min was comparable to standard drug Pentazocine (10 mg/kg) (57.66%) [Table 2]. Alcoholic and water extracts at all doses were nonsignificant. Analgesic activity of petroleum ether extracts at the dose of 400 mg/kg of *M. cochinchinensis* (40.57%) and *M. balsamina* (43.82%) after 90 min was comparable to standard drug Pentazocine (10 mg/kg) (44.83%) [Table 3].

**Tail immersion method**

Extracts were subjected to evaluation of analgesic effects by tail immersion method [Figures 3 and 4]. Petroleum ether extract at the dose of 100 mg/kg ($P < 0.05$), 200 mg/kg, and 400 mg/kg ($P < 0.01$) and alcoholic extract at the dose of 200 mg/kg and 400 mg/kg ($P < 0.05$) of fruits of *M. cochinchinensis* and *M. balsamina* have shown significant analgesic activity, while alcoholic extract at the dose of 100 mg/kg and all doses of water extract were non significant. Analgesic activity of petroleum ether extracts at the dose of 400 mg/kg of *M. cochinchinensis* (40.57%) and *M. balsamina* (43.82%) after 90 min was comparable to standard drug Pentazocine (10 mg/kg) (44.83%) [Table 3].

**DISCUSSION**

The universal role of the plants in the treatment of diseases is exemplified by their role in the all major system of medicine. Herbal medicines are the oldest form of health care known to mankind and are used by all cultures throughout history. Plants used in traditional medicine contain wide variety of chemical constituents that can be used to treat chronic and acute diseases. Preliminary phytochemical screening of petroleum ether, ethanolic and water extracts of fruits of *M. cochinchinensis* and *M. balsamina* were carried out and revealed the presence of glycosides, flavonoids, steroids, phenolic compounds, carotenoids, and carbohydrates.

Acute toxicity study showed that all the extracts of both the plants caused no mortality up to a dose of 2000 mg/kg, and no behavioral changes observed in any group. Based on these data three doses, that is, 100 mg/kg, 200 mg/kg, and 400 mg/kg of each extract were selected for further study. In the present study, analgesic activity of petroleum ether, ethanolic, and water extracts of fruits of *M. cochinchinensis* and *M. balsamina* was evaluated by Eddy’s hot plate method and tail immersion method. The results showed that petroleum ether extracts of both the plants have significant analgesic activity compared to standard drug Pentazocine. Ethanol and water extracts were found to be nonsignificant. The preliminary phytochemical screening revealed the presence of phytosterols, glycosides, carbohydrates, flavonoids, alkaloids, tannins, saponins, carotenoids, and phenolic compounds. The acute toxicity study showed that all the extracts of both the plants caused no mortality up to a dose of 2000 mg/kg, and no behavioral changes observed in any group.

The results of the current study indicate that *M. cochinchinensis* and *M. balsamina* have significant analgesic activity and can be considered as potential candidates for the treatment of pain-related disorders. Further studies are needed to evaluate the mechanisms of action and possible applications of these plants in the treatment of pain-related conditions.
and *M. balsamina* was evaluated by two models, that is, Eddy’s hot plate method and tail immersion method.

In Eddy’s hot plate method, there is marked central analgesic effect as evidenced by significant increase in reaction time. Results depicted that petroleum ether extracts of both plant materials have shown increase in latency period in a dose-dependent manner. Petroleum ether extracts at the dose of 400 mg/kg after 90 min significantly reduced the pain compared to Pentazocine; this central analgesic effect may be due to inhibition of prostaglandins synthesis and presence of phytosterols in these extracts. Alcoholic and aqueous extracts were found to be ineffective.

**Table 2:** Comparative effects of petroleum ether extracts on the latency of rats exposed to hot plate at 90 min

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean latency time</th>
<th>% Protection</th>
<th>Treatment</th>
<th>Mean latency time</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>5.042±0.095</td>
<td>--</td>
<td>Vehicle control</td>
<td>5.210±0.060</td>
<td>--</td>
</tr>
<tr>
<td>Pentazocine (10 mg/kg)</td>
<td>11.91±0.240</td>
<td>57.66</td>
<td>Pentazocine (10 mg/kg)</td>
<td>12.36±0.3216</td>
<td>58.57</td>
</tr>
<tr>
<td>PEMC 100 mg/kg</td>
<td>6.158±0.181</td>
<td>18.12</td>
<td>PEMB 100 mg/kg</td>
<td>6.463±0.1396</td>
<td>19.38</td>
</tr>
<tr>
<td>PEMC 200 mg/kg</td>
<td>7.925±0.201**</td>
<td>36.37</td>
<td>PEMB 200 mg/kg</td>
<td>8.762±0.068**</td>
<td>40.53</td>
</tr>
<tr>
<td>PEMC 400 mg/kg</td>
<td>9.015±0.155***</td>
<td>44.07</td>
<td>PEMB 400 mg/kg</td>
<td>9.652±0.116**</td>
<td>46.02</td>
</tr>
</tbody>
</table>

Each value is the mean±SEM of six determinations. *P<0.05, **P<0.01, ***P<0.001, Two-way ANOVA followed by Bonferroni posttests, when compared to control group. SEM: Standard error of the mean

**Table 3:** Comparative effect of petroleum ether extracts of *Momordica cochinchinensis* and *Momordica balsamina* on tail withdrawal reflex of rats induced by tail immersion method after 90 min of drug treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean reaction time</th>
<th>% Protection</th>
<th>Treatment</th>
<th>Mean reaction time</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>5.960±0.228</td>
<td>--</td>
<td>Vehicle control</td>
<td>5.848±0.160</td>
<td>--</td>
</tr>
<tr>
<td>Pentazocine (10 mg/kg)</td>
<td>10.31±0.150*</td>
<td>42.19</td>
<td>Pentazocine (10 mg/kg)</td>
<td>10.60±0.102*</td>
<td>44.83</td>
</tr>
<tr>
<td>PEMC 100 mg/kg</td>
<td>8.082±0.145*</td>
<td>26.25</td>
<td>PEMB 100 mg/kg</td>
<td>8.410±0.199**</td>
<td>30.46</td>
</tr>
<tr>
<td>PEMC 200 mg/kg</td>
<td>9.197±0.118**</td>
<td>35.19</td>
<td>PEMB 200 mg/kg</td>
<td>9.410±0.150**</td>
<td>37.85</td>
</tr>
<tr>
<td>PEMC 400 mg/kg</td>
<td>10.03±0.131**</td>
<td>40.57</td>
<td>PEMB 400 mg/kg</td>
<td>10.41±0.185***</td>
<td>43.82</td>
</tr>
</tbody>
</table>

Each value is the mean±SEM of six determinations. *P<0.05, **P<0.01, ***P<0.001, Two-way ANOVA followed by Bonferroni posttests, when compared to control group. SEM: Standard error of the mean

**Figure 2:** Analgesic activity of *Momordica balsamina* by Eddy’s hot plate method. Values are the mean±standard error of the mean from 6 animals in each group. Two-way ANOVA followed by Bonferroni posttest. # < 0.001, *P < 0.05, **P < 0.01, and ***P < 0.001 when compared to control group were considered as significant

**Figure 3:** Analgesic activity of *Momordica cochinchinensis* by tail immersion method. Values are the mean±standard error of the mean from 6 animals in each group. Two-way ANOVA followed by Bonferroni posttests. # < 0.001, *P < 0.05, **P < 0.01, and ***P < 0.001 when compared to control group were considered as significant

Tail immersion method is the second most commonly used test to assess analgesics. Substance P is released in excessive quantities due to the stimulation of nonmyelinated C fibers of rats’ tail after the application of thermal heat serving as noxious stimuli. Narcotic analgesics like Pentazocine are potential agonist of µ, κ, and δ receptors. These receptors are specific for endogenous narcotics such as endorphins and encephalin. After binding to these receptors, narcotic analgesics antagonize the action of substance P in the central nervous system (CNS) by producing post-synaptic inhibitory action on interneuron, which processes the nociceptive information
to be transmitted to the CNS.\textsuperscript{[14]} Petroleum ether and alcoholic extracts of both plants were effective in increasing reaction time in dose-dependent manner. Petroleum ether extracts at the dose of 400 mg/kg after 90 min significantly reduced the pain compared to Pentazocine. As our petroleum ether extracts of \textit{M. cochinchinensis} and \textit{M. balsamina} showed significant analgesic activity in the thermal heat method, it can be assumed that the extracts could act by a central anti-nociceptive mode like that of Pentazocine.

**CONCLUSION**

It was concluded from the present study that petroleum ether extracts of fruits of \textit{M. cochinchinensis} and \textit{M. balsamina} have significant central analgesic activity that may attribute to more amount of phytosterols present in these plants. However, further work has to be done to isolate the compounds responsible for these activities.

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**REFERENCES**


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