EVALUATION OF ANALGESIC EFFECTS OF LEAF EXTRACT OF MORINGA OLEIFERA IN SWISS ALBINO MICE

Dr. Tshema V.1*, Dr. S. Anusha2 and Dr. R. Mani3

1Final Year Post Graduate, Department of Pharmacology, Coimbatore Medical College, Coimbatore.
2Assistant Professor - Department of Pharmacology, Coimbatore Medical College, Coimbatore.
3Professor & Head, Department of Pharmacology, Coimbatore Medical College, Coimbatore.

ABSTRACT

Background: Pain is one of the common reasons to seek medical care. Even though NSAIDS and opioids are effective in pain management, they have many adverse effects. Herbal medication may provide an alternative therapy. Moringa oleifera (Drumstick plant) is known since antiquity and its medicinal properties are used traditionally in India.

Aim: To evaluate the analgesic effects of Ethanolic leaf extract of Moringa oleifera (EMO) in Swiss Albino Mice.

Materials and methods: This study was approved by Institutional Animal Ethical Committee (IAEC). 24 Swiss albino mice of either sex, 6 weeks old, 25 to 30 grams each were divided into 4 groups with 6 mice in each group. Group I (control) received normal saline 2ml/kg orally. Group II (standard) received morphine 1mg/Kg intra-peritoneally. Both Group III and Group IV were test groups, that received EMO Leaf Extract orally for 7 days, at the dosage of 200mg/Kg (0.1 ml) and 400mg/Kg (0.2ml) respectively. The analgesic effect was evaluated using Eddy’s Hot plate. Statistical analysis was done using ANOVA and Probability (P) value.

Result: The reaction time of groups was observed using Eddy’s Hot Plate. Groups II (morphine), III (EMO-200 mg/kg) & IV (EMO-400 mg/kg) showed 10.4 seconds, 7.35sec & 8.45 sec respectively at 60 minutes. Among the test groups, Group IV (400 mg/kg of EMO) showed the maximum reaction time (significant with p-value <0.05).
**Conclusion:** These results conclude that Moringa oleifera leaf extract has analgesic effect at higher concentration of extract. This plant extract has medicinal and nutritional properties. This study has demonstrated the central analgesic activity using Eddy’s hot plate. This study justifies the use of Moringa oleifera in chronic pain disorders.

**KEYWORDS:** Analgesic, Drumstick, Eddy’s Hot Plate, Mice, Moringa oleifera leaf extract.

**INTRODUCTION**

Pain is one of the most common reasons for patients to seek medical advice. According to International Association for the study of pain (IASP) “Pain is defined as, an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.”[1] The increase in the incidence of diseases with chronic pain such as Osteoarthritis, pain due to spine related problems and muscle sprains, requires prolonged administration of analgesics.

NSAIDS and opioids are commonly prescribed analgesics. Even though NSAIDS and opioids are effective, they have many adverse effects. NSAIDS have adverse effects like gastritis and nephrotoxicity. Opioids have abuse potential and dependence on prolonged use.[2] Herbal medication may provide an alternative therapy. The Moringa oleifera leaf extract contains antinociceptive and anti-inflammatory activities.[3] Traditional medicine has already been declared by WHO as promotive, preventive, curative and rehabilitative and can serve as an alternative in this regard.[4]

Moringa oleifera is a native plant of south Asia, now grown worldwide. It is known as Drumstick or Horseradish tree in English and Mungna in Hindi. Moringa oleifera (Drumstick plant) is known since antiquity and its medicinal properties are used traditionally in India. Drumstick trees are commonly found in South India. It is a medium sized, fast growing tree grown in hot arid regions and thrives well even in drought. Different parts of Moringa contains a profile of important minerals, protein, vitamins, beta-carotene, amino acids and various phenolics.[5] The active components such as flavonoids, anthocyanin, cinnamates, vanillin, saponins, tannins and terpenoids could be responsible for various pharmacological effects such as antipyretic and anti-inflammatory activity.[6] Many folklore claims exist about this plant. The history about the use of this plant has been documented in texts of Ayurveda, Romans and Egyptians. African bushmen had used the drumstick leaves as poultice for headaches, abdominal pain and joint pain. (Homa et al).[23] The active
components in the leaves of Moringa oleifera are flavonoids, saponins, tannins and terpenoids that could be responsible for the analgesic effects.\textsuperscript{[6]} Hence this study is done to evaluate the analgesic property of Moringa oleifera in mice.

**MATERIALS AND METHODS**

**Plant material**

Leaves of drumstick were purchased from Local market. It was identified by a botanist of Tamil Nadu Agriculture University, Coimbatore.

**Preparation of Ethanolic extract of Moringa oleifera**

Leaves of M. oleifera were collected from local market in Coimbatore, dried in the shade and made into powder. Using the soxhlation method, the powder was extracted utilizing 90\% ethanol for 18 hours, the extract was filtered with Whitman filter paper No. 1 and concentrated to yield a semi solid mass of 46 g (yield 9.2\% w/w). Extract was refrigerated at 4°C and used later.

**Animals Used**

The study was started after getting the approval of the Institutional Animal Ethical Committee (IAEC). 24 Swiss albino mice of either sex weighing 25 - 30 grams, were procured from Central Animal House, Coimbatore Medical College, Coimbatore. The animals were housed in standard polypropylene cages and maintained under controlled conditions with 12-hour light and 12-hour dark cycle. All the mice were provided with commercially available rodent chow diet and tap water ad libitum. The animals were acclimatized to laboratory conditions for 7 days before the experiment.

**Evaluation of Pharmacological Activities**

**Analgesic Activity**

Analgesic action was evaluated using Eddy’s hot plate test for the central analgesic activity in albino mice.

**Eddy’s hot plate method:** This test was used to determine the antinociceptive activity by evoking thermal induced noxious stimulus. Swiss Albino mice were placed on a pre-heated plate set at 55 ± 1°C. The time taken from placement to reactivity towards pain, which is observed by animal licking its paw or jumping was taken as the reaction time or latency time.
Latency time was determined before and after drug administration. The cut-off period was taken as 15 seconds.

**Procedure**
The experiments were carried out by dividing the animals into 4 groups, each containing six animals.

Group I: Control group - normal saline (2 ml/kg body weight, oral)
Group II: Standard - Morphine (1 mg/kg) injected intraperitoneally
Group III: Test - Ethanolic extract of Moringa oleifera leaves (EMO) (200 mg/kg dose \(^6\) given orally for 7 days)
Group IV: Test – EMO (400 mg/kg doses \(^6\) given orally for 7 days)

The test drugs (EMO) are given to the test groups (III & IV) for a period of 7 days by oral route. On the 7\(^{th}\) day, baseline latency time was noted for the 4 groups. The drugs were administered by oral route to control and test group, intraperitoneally to standard group. Nociceptive stimuli were induced by placing the mice over Eddy’s Hot Plate. The latency time was recorded at 30, 60, 90, 120 min after administration of control, standard and test drug.

**RESULTS**
The reaction time of Groups II (morphine), III (EMO-200 mg/kg) & IV (EMO-400 mg/kg) were 10.4 seconds, 7.35 sec & 8.45 sec at 60 minutes respectively were significant when compared with saline (significant with p-value <0.05).

Among the test groups, Group IV (400 mg/kg of EMO) showed the maximum reaction time at 60 minutes.

The one-way Anova for standard and test groups at 60 minutes showed P value <0.05, which is significant.

**Table 1: Mean Reaction Time.**

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>0 min (Pre Drug)</th>
<th>30 min Mean±SD</th>
<th>60 min Mean±SD</th>
<th>90 min Mean±SD</th>
<th>120 min Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I SALINE 2mL/kg p.o</td>
<td>3.3±01</td>
<td>3.20±0.01</td>
<td>3.28±0.01</td>
<td>3.08±0.01</td>
<td>3.02±0.01</td>
</tr>
<tr>
<td>Group II Morphine(1 mg/kg ip)</td>
<td>3.37±0.02</td>
<td>9.37±0.05</td>
<td>10.4±0.01</td>
<td>8.99±0.56</td>
<td>7.85±0.47</td>
</tr>
<tr>
<td>Group III</td>
<td>Group IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>----------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMO (200mg/kg p.o.)</td>
<td>EMO (400mg/kg p.o.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.27±0.01</td>
<td>3.26±0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.7±0.09</td>
<td>6.19±0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.32±0.06</td>
<td>8.45±0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.27±0.02</td>
<td>7.38±0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.91±0.19</td>
<td>6.33±0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p.o – per oral, ip – intraperitoneal, SD – Standard Deviation. The one way Anova for standard and test groups at 60 minutes showed P value <0.05, which is significant.

Table 2: Comparison of Reaction Time.

DISCUSSION
In the present study, the anti-nociceptive property of ethanolic extracts of Moringa oleifera was assessed by thermal (i.e., hot-plate test) model. The increase in the reaction time in response to thermal test denotes analgesic effect of the extract. Several mechanisms of anti-nociception could be present. The central analgesic effect is screened using Eddy’s Hot plate model.

All parts of the drumstick plant such as leaves, flowers, seeds, bark, roots contain important phytoconstituents such as terpenoids, tannis, alkaloids, reducing sugars and steroidal aglycones. The leaves are highly nutritious with good source of beta carotenes, amino acids (methionine, cysteine, tryptophan, lysine), Vitamin C, vitamin B1, vitamin B2, vitamin B3, iron, potassium, calcium, zinc and sodium. It is a Potential source of natural antioxidants. Different parts of the plant have valuable pharmacological action such as anti-diabetic, anti-inflammatory, anticancer, anti-microbial, and antipyretic. The other

In a study by Dr. Ravi Shankar, the analgesic effects of Moringa Oleifera Aqueous extracts was observed when it was compared with standard drug Lornoxicam in Wister Male albino Rats using Digital Analgesiometer by tail flick latency test.[20] In a study done by Ayon Bhattacharya et al, the reaction time in Eddy’s Hot plate method was 9.6 sec after 60 minutes of administration of ethanolic leaf extract of Moringa oleifera.[6] The reaction time in this study is 8.45 seconds (EMO 400mg/kg) at 60 minutes which is similar to the above study.

Multiple mechanisms are being postulated, for the production of analgesic effect of Ethanolic extract of Moringa oleifera leaves. In an interesting study conducted by Kanchan P Upadhye et al; The ethanolic extract of the leaves of Moringa Oleifera were tested for peripheral and central antinociceptive activities using three models: Acetic acid induced writhing, formalin induced paw licking and tail flick test using Analgesiometer respectively. It was observed that the extract possessed both peripheral and central antinociceptive activities. The involvement of opioid receptors and analgesic effect reversal by naloxone, an opioid receptor antagonist; indicates both the central and peripheral opioid receptors are involved in alleviating pain. This study also observed that Moringa Oleifera leaves have analgesic activity.[21] In a study done by Harith Jameel Mahdi showed that ethanolic extract of Moringa oleifera possessed both peripheral and central antinociceptive action in arthritis in rats.[22] 

It could be postulated that EMO leaf extract could act through release of endogenous peptides such as endorphins or enkephalins from periaqueductal gray matter. The impulses from the dorsal horn of spinal cord are inhibited by these peptides.[6] Leaves of Moringa oleifera contain active components such as flavonoids, saponins, tannins and terpenoids that could be responsible for the analgesic effects.[6] 

Our study showed similar results, observed by prolongation of latency time, denoting the analgesic effect of Ethanolic extract of leaves of Moringa oleifera.

CONCLUSION
The present study discusses the medicinal use & pharmacological activity of this plant.
The EMO leaf extract showed a significant analgesic effect at 200, and 400 mg/kg. The exact mechanism of action of the extract needs to be explored in detail. These results conclude that Moringa oleifera leaf extract has analgesic effect at higher dosage.

**Future Prospects**

The mechanism of EMO leaves could be due to its action on the central receptors or promoted release of endogenous peptides, which needs further evaluation in future studies. The adverse effects on chronic usage of Moringa oleifera should be evaluated.

**ACKNOWLEDGEMENT**

I sincerely thank my Professor Dr.R. Mani M.D.Pharmacology and Dr.S.Anusya M.D.Pharmacology, Assistant Professor for their guidance and support throughout the experiment.

**REFERENCES**

8. Al-Malki AL, El Rabey HA. The Antidiabetic Effect of Low Doses of Moringa oleifera Lam. Seeds on Streptozotocin Induced Diabetes and Diabetic Nephropathy in Male Rats