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## Research



### Evaluation of Analgesic Activity of Ethanolic Leaf Extract of *Madhuca Longifolia*

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	<b>Abstract</b>
Published on: 27 Aug 2025	<p>In this study, investigate the analgesic activity of the ethanolic leaf extract of <i>Madhuca longifolia</i> animal models using Eddy's hot plate method. In which to explore the analgesic activity of ethanolic leaf extract of <i>Madhuca longifolia</i> using Eddy's hot plate method. To compare the effect of ethanolic leaf extract of <i>Madhuca longifolia</i> with the commonly used analgesic drug pentazocin. This study was conducted by using Eddy's hot plate method. A total of 12 Adult swis albino mice were grouped into four groups of 4 animals in each group. These groups were Group I: Control (normal saline), Group II: Standard (Pentazoicin, 3mg per kg) Group III: Ethanolic root extract of Leaf extract of <i>Madhuca longifolia</i>(200mg per kg) &amp; Group IV: Ethanolic root extract of Leaf extract of <i>Madhuca longifolia</i>(400mg per kg). The reaction time was recorded at 0, 30, 60 &amp; 90 minutes after injecting normal saline, standard drug, and extract. Result: The increase in mean reaction time of the extract was statistically significant (<math>p &lt; 0.0001</math>) at 30, 60, 90 and 120 min compared to that of the control. There was less increase in the reaction time of 400 mg of <i>Leaf extract of Madhuca longifolia</i>in treated group compared to that of the pentazocin-treated group at 30 min &amp; 60 min points. Based on above rseult discussion ,the present study was conclude that the analgesic activity of 400 mg of <i>Leaf extract of Madhuca longifolia</i>in was significantly more than that of the control group. Peak analgesic activity occurred at 60 and 90 minutes.</p>
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<b>Keywords:</b> Analgesic activity, <i>Madhuca longifolia</i> , Eddy's hot plate method	

## INRODUCTION

Pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage," according to the International Association for the Study of Pain (IASP).<sup>1</sup> Although it could be more precisely defined, pain is nearly always linked to medical conditions. It is frequently triggered by unpleasant stimuli, which may originate internally or externally.<sup>2</sup> Pain interferes with day-to-day activities and the body's overall ability to function. The pain is the main reason why most patients see a doctor. It is the primary symptom of a lot of illnesses.<sup>3</sup> Analgesics, both central and peripheral, reduces pain without impairing consciousness. Opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are the two categories of analgesics. While NSAIDs are used to treat pain related to inflammation and tissue damage, opioids are recommended for deep-seated visceral pain. Three NSAIDs and opioids use remain the mainstay for the treatment of pain. However, both groups of drugs are well known for their common & severe side effects. The studies conclude that opioids lead to tolerance, physical dependence, and addiction, whereas NSAIDs lead to gastrointestinal bleeding & ulcers.<sup>4</sup>

For that, looking for an alternative compound with minimal or no side effects for pain management is necessary. Medicinal plants could be the better option to overcome these side effects.

Conventional drug therapy for diseases can occasionally have severe adverse effects. All over the world, there is a trend to explore traditional medicines. Siddha is an old medicine branch famous amongst the Tamil-speaking people. There are various sources of the drugs. These can be broadly classified into plants, animals, microorganisms & minerals. There is a need to identify the accurate source of the drug, which can give good results with minimal side effects. The availability of plant sources is more as compared to the other sources. The newly discovered active compound should have higher efficacy, safety, and fewer side effects.

Numerous chemical compounds found in medicinal plants may have a variety of therapeutic applications. Around the world, medicinal plants have been used to treat a wide range of illnesses. Nevertheless, medications are made using the active ingredients found in medicinal plants. The main benefit of herbal medicine is that it is safer than synthetic medicine because it is less expensive and has fewer side effects, which developing nations like India would accept. Medicinal plant parts have been used for centuries to treat a variety of illnesses, particularly in India.<sup>5</sup> The Sapotaceae family, which includes *Madhuca longifolia* leaf extract, is known by several names in various languages, including Sapota in Hindi, American bully in English, Simaiyiluppai in Tamil, and Sapotasima in Telugu.

*Madhuca longifolia* is a large tree with a thick crown and rough, lumpy, shady grey bark. The elliptic-oblong, 7.5–12.5 cm long leaves have tiny, distinct secondary branching nerves and shine from both sides<sup>6</sup>. Flowers are long. The round fruits have light brown pulp and five to six large seeds<sup>7</sup>. The hard seeds have a single white border and are either brown or black. Alkaloids, flavonoids, saponins, tannins, steroids, glycosides, and phenolic compounds are among the phytochemicals found in the seeds. Since seeds also contain hydrocyanic acid, they should be removed before consuming the fruit<sup>8</sup>. *Madhuca longifolia* leaf extract has been shown to have anthelmintic, hypoglycemic<sup>9</sup>, diuretic, and antibacterial properties<sup>10</sup>. The antimicrobial and anticancer properties of bark extract have been evaluated<sup>11</sup>. The plant's leaves contain antioxidants, analgesics, and anti-inflammatory properties<sup>12</sup>. Hypoglycemic, and hypocholesterolemic activities.<sup>13</sup> The roots of the plant are found to have hypoglycemic activity.<sup>14</sup>

Although many pharmacological investigations have been carried out based on the constituents present in it, a lot more can still be explored and utilized in a therapeutic manner.

The anti-inflammatory activity of seeds of Leaf extract of *tridax procumbance* has already been demonstrated in experimental animals.<sup>15</sup> As pain always accompanies inflammation, we have set forward a study to discover the analgesic activity of the ethanolic leaf extract of *tridax procumbance* in animal models using Eddy's hot plate method.

## MATERIALS AND METHODS

### Material and Methods

This study was conducted after the approval of the Institutional Ethics Committee (IEC). The study followed the Helsinki Declaration. The leaf extract of *madhuca longifolia* were obtained from a forest near the experimental institute. These fresh seeds were thoroughly cleaned with water to remove dirt. Seeds were dried for one week in the shed. After complete drying, the seeds were powdered. The powder was packed in a sealed bottle and stored in a dry, cool, and dark place.

The extract is prepared with the help of Soxhlet's apparatus (hot extraction) and extracted with 95% ethanol at a temperature of 60°-80°C. Ethanolic extract was subjected to filtration. It was then dried under reduced pressure to obtain a solid mass that was free from the solvent. The dried extract was dissolved in distilled water.

The hot plate assay method was employed for the purpose of preferential assessment of possible centrally mediated analgesic effects of ethanolic leaf extract of *Madhuca longifolia*. The central analgesic drug pentazocine was used for positive control group. In this experiment, four groups (n=6) of Swiss albino mice (20-25 g) were placed on a hot plate maintained at room temperature for 15 min. Food was withdrawn on the preceding night of the experiment. Group I- Normal Control received CMC (0.5%), and Group II- standard treated with pentazocine (3 mg/kg i.p), whereas group III and IV- animals were treated orally with ethanolic leaf extract of *Madhuca longifolia*. (200 and 400 mg/kg respectively).

Group I : Normal control (CMC)

Group II : Standard (Pentazocine 3 mg/kg)

Group III : Test Drug I 200mg (Ethanolic leaf extract of *Madhuca longifolia*)

Group IV : Test Drug II 400mg (Ethanolic leaf extract of *Madhuca longifolia*)

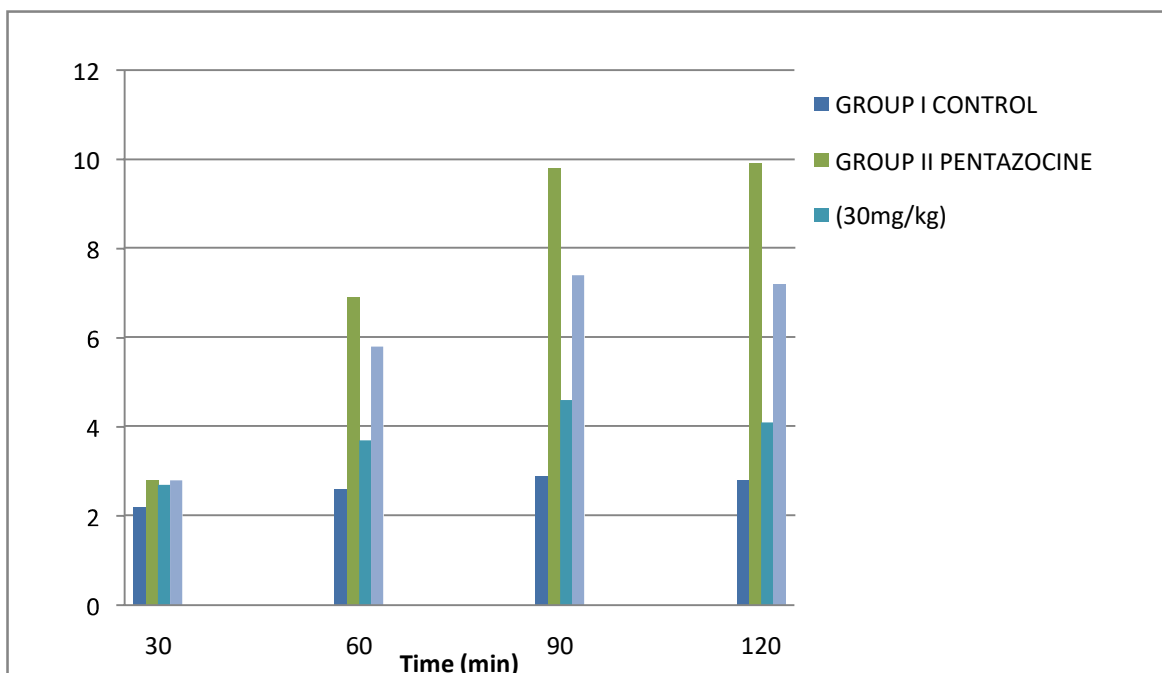
Each animal was then individually placed gently on Eddy's hot plate at 55°C. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate were determined at 30, 60, 90 and 120 min after administration of the drugs or vehicle.

Data were entered on a Microsoft Excel spreadsheet and statistically analyzed statically using the SPSS-20 software. Unpaired student t-tests were used to compare reaction times. A p-value of 0.05 or less than 0.05 is indicated as statistically significant.

### Observations and Results

The mean reaction time of all three groups, i.e., control, standard & test groups, at all time points is illustrated in Table 1. Compared to animals treated with saline, the mean reaction time of the aspirin and extract-treated group was increased, as shown in Figure 1.

GROUP	Paw licking or jumping in seconds			
	30min	60min	90min	120min
Group-I Control	2.2±0.22	2.6±0.12	2.9±0.21	2.8±0.10
Group-II Pentazocine (3mg/kg)	2.8±0.18	6.9±0.62**	9.8±0.64**	9.9±0.34**
Group-III (200mg/kg)	2.7±0.20	3.7±0.15*	4.6±0.21**	4.1±0.41**
Group-IV (400mg/kg)	2.8±0.14	5.8±0.37**	7.4±0.39**	7.2±0.44**



### **Analgesic effect of ethanolic leaf extract of *Madhuca longifolia* on hot plate test in Swiss albino mice**

When the analgesic activity of aspirin and extract was compared, it was found that there was less increase in the reaction time of the group treated with extract group compared to that of the pentazocin treated group. The reaction time of the extract-treated group at 30 min was found to increase significantly compared with the pentazocin treated group. Whereas the mean reaction time of extract treated group at 60 min & 90 min was not found to be significantly increased in comparison with the standard.

## **DISCUSSIONS**

Recently developed allopathic drugs still exhibit mild to severe side effects. Various plants are still unique because of having fewer adverse effects. Therefore, the analgesic effect of plant products has been studied systematically.

As pain is the most common manifestation of most of the diseases. Many synthetic NSAIDs have been used as the primary analgesic, with common adverse side effects. Therefore, It is beneficial to investigate an alternative analgesic therapy.

Analgesics relieve pain selectively without affecting it by acting on the central or peripheral nervous system. Centrally-acting analgesics lower the pain threshold and alter the body's typical physiological reaction to pain. Conversely, peripherally acting analgesics prevent impulse generation at the chemoreceptor site for pain.

Eddy's hot plate method is the animal model employed for screening analgesic activity in this study. In this experimental model for analgesia, an increase in reaction time is an important measure or parameter of central and peripheral analgesic activity.

As mentioned earlier, many animal experiments have been conducted and proven many activities of Leaf extract of *Madhuca longifolia*, according to Ayesha Khan et al. The whole plant extract of Leaf extract of *Madhuca longifolia* Linn possesses analgesic activity. Therefore, in this study, particularly seeds of the Leaf extract of *Madhuca longifolia* plant were screened for analgesic effects by using Eddy's hot plate.

Leaf extract of *Madhuca longifolia* extract showed a moderate to significant analgesic effect, which was proved by the significant increase in the reaction time, as depicted in Table 1 and Figure 1. Compared to the control, the extract's reaction time was similar at 0 min and slowly increased from 30, 60, 90 120 min. The increase in mean reaction time of the extract was statistically significant (\*  $p < 0.0001$ ) at 30, 60, 90 120 min compared with that of the control. From this, it can be stated that the crude extract of Leaf extract of *Madhuca longifolia* roots an effective analgesic agent.

When the analgesic activity of aspirin and Leaf extract of *Madhuca longifolia* extract were compared, it was observed that there was less increase in the reaction time of the group treated with extract compared to that of the pentazocin treated group at 30 min & 60 min points of time. The Significant difference (#  $p < 0.001$ ) in the analgesic activity of aspirin and the extract was found at 30 min. The analgesic effect of the extract-treated group at 90 min was found to be the same, but the reaction time for aspirin at 90 min was decreased compared to the earlier values, as shown in Table. From this, we can state that the analgesic activity of aspirin has decreased earlier than that of the extract of seeds.

The observed analgesic effect of Leaf extract of *Madhuca longifolia* is attributed to the crude extract. Any crude extract contains many phytochemicals such as steroids, alkaloids, flavonoids, phenolic compounds, etc. Furthermore, the analgesic activity of seed extract may be attributed to the specific phytochemicals in the extract. A single constituent or more than one constituent may be responsible for the analgesic effect. Preliminary phytochemical screening of crude extract is needed. There is a need for additional studies to identify the actual phytochemicals in the crude extract of Leaf extract of *Madhuca longifolia*, which are responsible for analgesic activity.

## **CONCLUSION**

The extract under study had a significant analgesic effect compared with the analgesic activity of control in an established analgesic screening method. The analgesic effect was peaking at 60 minutes and 90 minutes. Treating 400mg/kg of ethanolic extract of Leaf extract of *Madhuca longifolia* increases the reaction time on Eddy's hot plate. Hence, the present study concluded a significant analgesic effect of Leaf extract of *Madhuca longifolia* extract. Hence forth, the preparation of Leaf extract of *Madhuca longifolia* could be used as one of the drugs in multi-drug therapy for pain relief. Though, more research is needed in separating and describing the active compound or compounds

responsible for the analgesic effect. Further work is also needed for the determination of the exact mechanism of action.

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