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Research Article

Evaluation of analgesic activity of aqueous extract of *Mangifera indica* leaves in albino rats

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ABSTRACT

Background: *Mangifera indica* (MI), also known as mango, aam, has been an important herb in the Ayurvedic and indigenous medical systems for over 4000 years. Mangoes belong to genus *Mangifera*, which consists of about 30 species of tropical fruiting trees in the flowering plant family Anacardiaceae. According to Ayurveda, varied medicinal properties are attributed to different parts of the mango tree. This study was undertaken to evaluate the analgesic activity of aqueous extract of MI leaves in albino rats.
Methods: Analgesic activity was evaluated by hot plate method and acetic acid induced writhing with four groups of six rats each. Group 1 was control, Group 2 received standard drug (tramadol and diclofenac for hot plate and writhing method, respectively), and Groups 3 and 4 received aqueous extract of MI leaves extract 200 mg/kg and 400 mg/kg.
Results: The reaction time (seconds, mean±standard error mean [SEM]) in hot plate method at 120 mins increased significantly ($p<0.001$) with MI 200 mg/kg and 400 mg/kg, 8.96±0.12 and 10.61±0.2, respectively. The acetic acid induced writhing test showed a significant ($p<0.001$) decrease in number of writhes (mean±SEM) in MI 200 mg/kg and 400 mg/kg 43.76±1.59 and 30.33±1.41 respectively in treated rats when compared with control 67.17±0.75.
Conclusion: Aqueous extract of MI extract has significant analgesic effect in rats.

Keywords: Analgesic, Diclofenac, *Mangifera indica*, Tramadol

INTRODUCTION

Pain has been defined by International Association for the Study of Pain as unpleasant sensory and emotional experience associated with actual or potential tissue damage.[1]

Clinically, the management of acute and chronic pain is effectively done by the use of non-steroidal anti-inflammatory drugs (NSAIDS), opioids, and other analgesics. However, the safety profile on their long-term use remains to be an area for concern as these drugs carries potential toxic effects. Morphine causes acute morphine poisoning, hypotension, dependence etc. whereas the NSAIDs are associated with gastric irritation, bleeding ulcers and perforation.[2,3] Hence, analgesic activity of drugs continues to be an area of great interest for research probably due to non-availability of safer and ideal analgesic drugs.

Mangifera indica (MI) (Anacardiaceae) is a tree, distributed in rural and semi urban parts of India. It is one of the most important tropical plants marketed in the world.[4] Phytochemical research from different parts of MI has demonstrated the presence of phenolic constituents, triterpenes, flavonoids, phytosterol, and polyphenols.[5-7]

There are traditional medicinal uses for the bark, roots and leaves of MI throughout the globe. MI is used medicinally to treat ailments such as asthma, cough, diarrhea, dysentery, leucorrhoea, jaundice, pains, and malaria.[8]

This species is purposed to possess numerous therapeutic uses including analgesic, anti-inflammatory,[9,10] anti-diabetic, antilipidaemic,[11] antioxidant[12] and immunomodulatory.[13]

The present study aims at assessing the effect of aqueous extract of MI leaves as analgesic agent, in comparison to the standard diclofenac and tramadol.

METHODS

Plant material

MI leaves available locally, were identified and used for the study.

Animals

Adult albino rats (200-250 g) of either sex were used in this study; animals were obtained from National Institute of Nutrition Hyderabad. The animals were stabilized for 1 week under standard conditions at temperature 25±1°C, 60±5% relative humidity and 12 hrs dark light cycles. They had been given free accesses to standard pellet diet and water *ad libitum*. Experiments were conducted according to the ethical norms approved by the Institutional Animal Ethics Committee guidelines of Navodaya Medical College, Raichur.

Drugs and chemicals

Diclofenac was obtained as free sample 2 g from Win Medicare Pvt. Ltd.

Tramadol - Micro Lab Limited, batch no: ULAH0049.

Acetic acid - 0.6%.

Preparation of extract

The shade dried leaves of MI were made in to a fine powder and passed through a sieve to make it a further fine powder, which was soaked in equal amount of water and stirred intermittently and was left over night. The macerated pulp was dried at reduced temperature. This dry mass served as an aqueous extract of leaves of MI for experimentation.[14]

Evaluation of analgesic effect was done by

I. Acetic acid induced writhing
II. Hot plate method.

Experimental protocol

Animals were divided into four groups of six animals each and treated as follows. All the drugs were given orally except, tramadol which was given intra peritoneal (IP).

Group 1: Control received vehicle only (distilled water).
Group 2: Standard received diclofenac 10 mg/kg orally for acetic acid induced writhing and tramadol 10 mg/kg IP for a hot plate method.
Group 3: Test rats received aqueous extract of leaves of MI 200 mg/kg.
Group 4: Test rats received aqueous extract of leaves of MI 400 mg/kg.

Acetic acid induced writhing

The writhing model represents a chemical stimulus of acute pain in the animal model. Contraction of abdomen, turning of trunk (twist) and extension of hind limbs (at least once) are considered as writhing reaction.[15] Writhing was induced in rats (n=6) by IP injection (10 ml/kg) of 0.6% acetic acid. The number of writhing was counted over a 20 mins period.[16] All the drugs were administered orally 30 mins before acetic acid injection.

Percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = W_c - W_t / W_c * 100$$

Where, W_c=Number of writhes in the control group, W_t=Number of writhes in test group.[17]

Hot plate method

Hot plate consists of an electrically heated surface, the temperature of which is maintained at 55-56°C. The rats were placed on hot plate 1 hr after oral administration of drugs and the reaction time between placing the animal on hot plate and licking of fore/hind limb (Paw response) or the jump response was recorded by a stop watch. The average normal reaction time being 5 sec.

A cut-off time 30 sec was followed to avoid any thermal injury to the paws. The latency was recorded before and after 20, 60, 90 and 120 mins following oral administration of vehicle or drugs.[18-20]

Statistical analysis

The results were expressed as mean±standard error mean in each group (n=6). Comparisons were made by one-way analysis of variance test. Inter group significance was analyzed by Dunnet's test. $p<0.05$ considered as significant.

RESULTS

Compared to control 67.17±0.7491 the mean number of writhes are decreased with MI that is 43.67±1.585 for 200 mg/kg and 30.33±1.406 for 400 mg/kg. Here, we can observe that there is dose-dependent decrease in the number of writhes. Hence, compared with control there is definite analgesic effect, but less compared to standard diclofenac 14.50±0.562 (Table 1).

Table 1: Acetic acid induced writhing.

Groups (n=6)	Number of writhes (mean±SEM)	% Inhibition of writhes
I. Control	67.17±0.7491	-
II. MI 200 mg/kg	43.67±1.585*	34.98%
III. MI 400 mg/kg	30.33±1.406*	54.84%
IV. Standard diclofenac	14.50±0.562*	78.41%

*p<0.001, MI: *Mangifera indica*, SEM: Standard error mean

Table 2: Hot plate method.

Groups (n=6)	Pre drug reaction time (in seconds)	Post drug reaction time in seconds (mean±SEM)			
		20 mins	60 mins	90 mins	120 mins
I. Control	4.822±0.121	4.612±0.182	4.693±0.1596	5.067±0.2253	4.620±0.2397
II. MI 200 mg/kg	5.102±0.2121	5.090±0.1026*	6.373±0.1586*	7.067±1.678*	8.962±0.1203*
III. MI 400 mg/kg	5.380±0.2062	5.640±0.1497*	6.672±0.2719*	8.453±0.2481*	10.61±0.2054*
IV. Standard tramadol 10 mg/kg	5.153±0.1864	6.015±0.1279*	8.707±0.1698*	10.61±0.4457*	11.90±0.3062*

*p<0.001, MI: *Mangifera indica*, SEM: Standard error mean

There is no much change in the pre drug reaction time, but post drug reaction time has shown increase in various time periods that is 20, 60, 90 and 120 mins. Here, we can observe that the increase in reaction time is maximum at 120 mins, and with MI there is dose-dependent increase in reaction time, that is 8.962±0.1203 for 200 mg/kg MI and 10.61±0.2054 for 400 mg/kg MI compared with control 4.620±0.2397 and standard tramadol 11.90±0.3062 (Table 2).

DISCUSSION

Acetic acid induced writhing method is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors.[21] The abdominal contraction is related to the sensitization of nociceptive receptors by prostaglandins.[22]

The method depends on the principle that an irritant directly administered in to the peritoneum cavity of the animal produces severe pain and irritation in the ventral part. The writhing is characterized by typical stretching behavior of body and rodent tries to touch its ventral part to the ground.[23] In our present study, acetic acid induced writhing model, the aqueous extract of MI reduced the writhes significantly in dose-dependent manner.

The hot plate method has been found to be suitable for the evaluation of centrally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance.[24] The analgesic effect of the extract may be either due to its action on visceral receptor sensitive to acetic acid, to the inhibition of the alogenic substances or the inhibition of transmission of painful message to the central level.[25,26]

The abdominal injection of acetic acid in rodents has been attributed to the release of arachidonic acid, which results in synthesis of prostaglandin via the cyclooxygenase enzyme.[27]

Therefore, it has been suggested that the inhibition of prostaglandin synthesis is remarkably efficient as an anti-nociceptive mechanism in visceral pain.[28]

In our study, the two doses of extract (200 mg/kg and 400 mg/kg) had increased the reaction time in dose-dependent manner; 400 mg/kg of MI extract had exhibited anti-nocioceptive effect to thermal stimulus at 120 mins, which is comparable to standard drug tramadol.

The analgesic effect exhibited by the aqueous extract of leaves of MI may be due to inhibition of prostaglandin synthesis.

CONCLUSION

Aqueous extract of MI leaves has shown significant analgesic activity, in both models of pain.

Hence, when it comes to management of chronic painful conditions, instead of increasing the dose, or changing to other analgesics, which definitely has some potential side-effects on long-term use, the natural alternatives like MI extract can be used safely with conventional analgesics for better pain management.

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