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Original Research Article

Antinociceptive effect of methanolic extract of *Murraya koenigii* leaves in swiss albino mice

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ABSTRACT

Background: The objective of the study was to evaluate anti-nociceptive effect of methanolic extract of *Murraya koenigii* leaves on thermal and mechanical pain in swiss albino mice.

Methods: Thirty adult male swiss albino mice weighing 25-30 grams were selected and allocated in to five groups. Each group consists of six animals. The control group received vehicle (10 ml/kg), standard group received morphine (10 mg/kg) and test groups received dried methanolic extract of *Murraya koenigii* leaves (100 mg/kg, 200 mg/kg, 400 mg/kg per oral respectively) 1 hour before placing the animal over the hot plate at temperature of 55°C . A cut off period of 10 sec was observed to avoid damage of the paw. The response in the form of withdrawal of paws or licking of the paws. The delay in the reaction time denotes analgesic activity. The latency was recorded before and after 15, 30, 60, 120 minutes administration of drug. After washout period of 1 month the same group of animals were utilized to evaluate the analgesic effect by tail clip method for better comparison.

Results: All the doses of *Murraya koenigii* leaves significantly delayed reaction time in hot plate method and tail clip method. The results were comparable to that produced by standard drug morphine.

Conclusions: *Murraya koenigii* leaves has analgesic activity which was comparable to morphine.

Keywords: Analgesia, Hot plate method, *Murraya koenigii*, Tail clip method

INTRODUCTION

Algesia is derived from Greek word Algesis means sensitive to pain.¹ It is an ill-defined sensation, usually evoked by an external or internal noxious stimulus. Clinically pain can be superficial pain, deep pain.² Analgesics are drugs which relieve pain without loss of consciousness. Analgesics are classified into opioid and non-opioid drugs which carry potential toxic effects like gastrointestinal bleeding, sedation and CNS depression. Many medicines of plant origin had been used with less adverse effects.

Murraya koenigii L. (curry tree), belonging to family Rutaceae, is a tropical to sub-tropical tree native to India.^{3,4} It is known as karuveppilei (Tamil Nadu and Kerala), karepaku (Andhra); karibeva (Karnataka). Traditionally, the plant is used as tonic, stomachic, and carminative. Antioxidant, anti-tumour, antimicrobial, anti-inflammatory, antioxidant, anti-tumor activities of this plant may be due to presence of carbazole alkaloids.^{5,6}

Aims and objectives of the study was to evaluate the antinociceptive activity of methanolic extract of *Murraya*

koenigii leaves (MEMK) using hot plate method and tail clip method in mice.

METHODS

Plant material

The leaves of *Murraya koenigii* were collected from the local garden, identified and authenticated by Assistant Professor of Botany, American College, Madurai.

The study was undertaken after obtaining approval of Institutional Animal Ethics Committee (Ref. No: Roc No 12677/ E1 / 5 / 2012).

Preparation of leaves extract

The leaves were dried under shade and the dried powder was loaded into Soxhlet extractor and subjected to extraction for about 24hrs with methanol. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50°C. This crude extract was used for antinociceptive activity analysis.

Selection of animals

The study was undertaken after obtaining approval of Institutional Animal Ethics Committee. A total of 30 swiss albino mice (20-25 g) of either sex, bred locally in the central animal house were selected for the study. All

animals were allowed free access to water ad libitum. Animals were kept under fasting for overnight and weighed before the experiment.

Study design

The mice were randomly allocated into five groups of six each.

Table 1: Study design.

Groups	Study	Treatment
1	Control	2% gum acacia
2	Standard	Morphine 10 mg/kg
3	MEMK-1	100 mg/kg
4	MEMK-2	200 mg/kg
5	MEMK-3	400 mg/kg

MEMK- methanolic Extract of *Murraya koenigii* leaves.

Eddy's hot-plate method

Mice were placed on hotplate kept at 55±1°C and the duration between placement of the mouse on the platform and licking of the paws or jumping was recorded as the reaction time.^{7,8} Mice with baseline latencies more than 10 sec were eliminated from the study. The animals were treated with MEMK at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg, morphine (10 mg/kg) as the standard drug. Control animals received 2% gum acacia. Reaction time were noted before (0) and 30, 60, 90 and 120 min after administration of the extract and standard drug.

Table 2: Effect of MEMK on thermally induced analgesia (Eddy's hot plate) in mice.

Treatment groups	Dose (mg/kg)	Basal reaction time (sec) mean±SEM	Reaction time in seconds at various time interval			
			30 mts	60 mts	90 mts	120 mts
Control	-	3.00±0.63	2.50±0.54	2.63±0.40	3.13±0.51	3.03±0.40
Morphine	10	3.17±0.75	3.83±0.75**	4.67±0.51**	6.00±0.63**	8.17±0.75**
MEMK	100	2.67±0.51	3.17±0.75	3.00±0.63	3.67±0.52	4.33±0.52*
MEMK	200	2.67±0.51	3.40±0.63*	4.33±0.51*	5.33±0.54*	6.27±0.78*
MEMK	400	3.00±0.89	3.33±0.51*	4.67±0.51*	5.47±0.62*	7.67±0.81*

Values are mean±SEM (n=6), * indicates when p<0.05, ** indicates when p<0.001 compared with control group.

Table 3: Effect of MEMK on pressure induced analgesia (tail clip method) in mice.

Treatment groups	Dose (mg/kg)	Basal reaction time	Reaction time in seconds at various time interval			
			30 mts	60 mts	90 mts	120 mts
Control	-	5.11±0.35	4.83±0.98	5.00±0.89	5.33±0.85	5.15±0.29
Morphine	10	5.19±0.25	7.93±0.75**	8.81±0.54**	9.87±0.75**	10.34±0.18**
MEMK	100	5.02±0.89	5.30±0.54	5.48±0.63	6.51±0.53*	6.62±0.73*
MEMK	200	5.10±0.15	5.73±0.51*	5.87±0.51*	6.92±0.86*	7.24±0.53*
MEMK	400	5.03±0.09	5.67±0.48*	6.83±0.75*	7.20±0.082*	8.63±0.37*

Values are mean±SEM (n=6), * indicates when p<0.05, ** indicates when p<0.001 compared with control group.

Haffner's tail clip method

After washout period of 30 days the same group of animals were subjected to tail clip method to test

analgesia.^{7,9,10} A metal artery clip was applied to the root of the mouse's tail to induce pain. The tail clip was applied 30, 60, 90, 120 mins after oral administration of MEMK (100 mg/kg, 200 mg/kg, 400 mg/kg), Morphine

(10 mg/kg). Whereas vehicle treated group served as control.

Statistical analysis

The results were expressed as the mean±S.E.M. Data was analyzed by oneway ANOVA, followed by student's test, p-value of <0.05 was considered as statistically significant

RESULTS

The present study evaluated the actions of MEMK on different animal models of pain such as hot plate method and Haffner's tail clip methods.

Hot-plate method showed that morphine has significant pain reduction at 30 min, 60 min, 90 min, 120 min, in comparison test drug MEMK shows significant pain reduction at doses 200 mg/kg, 400 mg/kg at 30 min, 60 min, 90 min, 120 min (p<0.05), it raised the pain threshold compared with control, at lower doses 100 mg/kg significant pain reduction noted only at 120 min (Table 2).

Tail clip method showed that morphine has significant pain reduction at 30 min, 60 min, 90 min, 120 min, in comparison test drug MEMK shows significant pain reduction at doses 200 mg/kg, 400 mg/kg at 30 min, 60 min, 90 min, 120 min (p<0.05), it raised the pain threshold compared with control, at lower doses 100 mg/kg significant pain reduction noted only at 90 min and 120 min (Table 3).

Moreover, in this study that oral administration of MEMK did not produce any allergic reactions, abnormal behavior, and mortality of the animals within 72h of observation period, revealing its nontoxic profile.

DISCUSSION

Analgesics can act on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptors site of pain, while centrally acting analgesics not only raise the threshold for pain, but also alter the physiological response to pain and suppress the patient's anxiety and apprehension. According to the studies done by Nushrat et al, MEMK shows excellent analgesic property, so in this study also used MEMK.¹¹ The hot plate and tail-clip method were used for evaluation of the central pain at the supraspinal and spinal levels respectively, possibly acting on a descending inhibitory pain pathway .

This study results were comparable to the similar studies conducted with the same plant *Murraya koenigii* like Gupta et al reported that *Murraya koenigii* leaves having analgesic activity.¹² Patil et al found that *Murraya koenigii* had analgesic and anti-inflammatory properties and Salwe et al confirmed *Murraya koenigii* leaves

extract having anti nociceptive property in experimental models.^{13,14}

The tail-clip response is believed to be a spinally mediated reflex and the paw-licking response in hot plate is more complex supraspinally organized. In the hot plate model, nociceptive reaction toward thermal stimuli in mice is a well-validated model for detection of opiate analgesics as well as several types of analgesics drugs from spinal origin. The tail clip model is also used to evaluate analgesic agents acting through central nervous system. MEMK was found to be effective in both the models.

CONCLUSION

It can be interpreted that MEMK possesses promising analgesic property in dose dependant manner which is probably mediated via central inhibitory mechanism. Further studies on isolation and fractionation of the active components from the leaf of *Murraya koenigii* and its mechanism of action to ascertain its analgesic activity will throw light on mode of action.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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