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Research Article

Analgesic and anti-inflammatory properties of the aqueous extract leaves of *Murraya koenigii linn* in small animal models

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ABSTRACT

Background: The present study was carried out to evaluate the analgesic activity of aqueous extract leaves of *Murraya koenigii* linn in Albino rats using tail flick method, Eddy's hot plate methods and anti-inflammatory activity in Carrageenan induced paw edema in rats.
Methods: The analgesic activity was evaluated using Eddy's hot plate induced hyperalgesia and tail flick method, which served as thermal induced pain, where the animal were placed on the hot plate and the reaction time to (lick the paw/ jump out) from the hot plate was observed, 0, 30, 60, 90 mins. Murraya 300 mg, 600 mg/kg/body weight (BW) and ibuprofen (5 mg/kg BW) was administered per oral. The anti-inflammatory activity was measured by Carrageenan induced paw edema volumes at 0, 1, 2, 3 and 4 hrs using mercury plethysmometer, which served as chemical induced pain models.
Results: The mean reaction time in Murraya at a dose of 600 mg/kg at 0 min 5.45±0.72, at 30 mins 6.52±1.03, at 60 mins 7.6±0.81, at 90 mins 8.8±0.63 respectively. The mean reaction time increased significantly with Murraya at dose of 600 mg/kg when compared with control.
In the ibuprofen group, the mean reaction time at 0 hr was 0.28±0.04, at 1 hr 0.34±0.05, at 2 hrs 0.46±0.03, at 3 hrs 0.61±0.05, at 4 hrs 0.76±0.05. The mean reaction time Murraya in group 600 mg/kg at 0 hr 0.27±0.04, at 1 hrs 0.39±0.03, at 2 hrs 0.48±0.06, at 3 hrs 0.68±0.05, at 6 hrs 0.80±0.03, respectively.
Conclusions: The results indicate that the aqueous extract of Murraya (leaf) extract revealed significant analgesic and anti-inflammatory in thermal and chemical induced pain models.

Keywords: *Murraya koenigii* linn, Anti-inflammatory, Analgesic, Tail flick method, Hot plate, Ibuprofen, Carrageenan

INTRODUCTION

The international association of study of pain definition states "pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage."[1] Pain is a major symptom in many medical conditions and significantly interferes with a person's quality of life and general functioning. Analgesics are drugs that selectively relieve pain by acting on the central nervous system or peripheral pain mechanism without altering the consciousness. Non-steroidal anti-inflammatory drugs and opioid analgesics are the most commonly used drugs for symptomatic relief of pain.

Most of the drugs used at present for analgesic effect are synthetic in nature and prolonged use of which causes severe side-effects and have toxic effects.[2] In this context, there arises new scope for evaluation of herbs for treatment of pain. Plants still remain a large untapped source of structurally novel compounds that might serve as a lead for the development of novel drugs.

Murraya koenigii (MK) commonly known as "curry tree" belongs to the family Rutaceae. It has been extensively used in the tradiontional system of Ayurveda.[3]

The literature survey revealed that very few studies have been done about analgesic activity of MK. There is a need

for better analgesics, which can effectively relieve pain without causing adverse effects. Furthermore, this plant is easily available and has insignificant side-effects.

Aim and objectives

1. To evaluate the analgesic effects of MK leaves extract in chemical and thermal induced pain in rats
2. To compare the analgesic activity of MK with that of standard analgesic drug ibuprofen.

MK is a tropical tree native to India and extensively used in the traditional medicinal system Ayurveda.

The leaves extract showed a significant reduction in carrageenan-induced paw edema and analgesic activity evidenced by increase in the reaction time by Eddy's hot plate method.[4]

Feeding the leaves to rats produced hypoglycemia by increasing the hepatic glycogenesis as evident by increased activity of glycogen synthetase. A decrease in glycogenolysis and gluconeogenesis is reported and was evident form decreased activity of glycogen phosphorylase and gluconeogenic enzymes.[5] A significant reduction, in fasting blood sugar and postprandial blood sugar, was observed by feeding (12 g) leaves powder to non-insulin dependent diabetes mellitus patients.[6,7] The dose dependent nitric oxide scavenging property of aqueous extract suggests that it might be a novel and potent therapeutic agent for free radical scavenging. Mahaimbine and koenigine were identified for maximum antioxidant activity. Koenigine also showed a high degree of free radical-scavenging activity.[8] Murrayanine, girinimbine and mahanimbine isolated from the stem bark showed anti-fungal activity against human pathogenic fungi.[9] Essential oil and aqueous extract of leaf were found active against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Streptococcus* species. Crude extract and chloroform soluble fraction and petroleum ether soluble fraction showed a promising antibacterial activity against all the tested bacteria. The crude extract of MK roots showed strong antibacterial activity.[10]

METHODS

The animals used for the study were Albino rats (150-250 g) of either sex. Animals are housed at Central Animal House of Narayana Medical College in standard polypropylene cages and maintained under standard conditions (room temperature 24-27°C and humidity 60-65%) with 12 hrs light and dark cycle. The food will be given in the form of dry pellets and water will be available *ad libitum*. Food and water will be withdrawn 6 hrs prior to the start of experiment. Each group consists four animals of either sex. The animal experiments are conducted after approval by the Institutional Animal Ethics Committee. Animal will not be sacrificed. (Protocol/Proposal Number –28/2013/NMC Dated: 20.12.2013).

- For anti-inflammatory activity, Carrageenan induced paw edema in rats was performed
- For analgesic activity, Eddy's hot plate method and Tail flick method in rats was performed.

Inclusion criteria

1. Healthy Albino rats of either sex weighing 150-250 g.

Exclusion criteria

1. Pregnant rats or those who have delivered
2. Diseased rats.

Plants materials/preparation of the extract

Fresh leaves of the plant (MK) were obtained locally. The fresh leaves were oven dried at 45°C, ground into powder and 20 g will be soaked in 300 ml of distilled water overnight at room temperature. The filtrate obtained was evaporated in a hot-air oven at 45°C. The extract was weighed (2 g) and reconstituted in appropriate volume of distilled water before administration to the rats.

Drugs

The standard drug: Ibuprofen will be dissolved in water and administered in a dose (orally) of 5 mg/kg.

Test drug: - MK linn extract will be dissolved in water and administered in an orally dose of 300 and 600 mg/kg.

Grouping

There are four groups each group consist of n=6 rodents total n=24 rodents.

Group A - Administered with 0.5 ml of normal saline.
Group B - Administered with 5 mg/kg body weight (BW) of ibuprofen.
Group C - Administered with the extract of MK linn 300 mg/kg BW.
Group D - Administered with the extract of MK linn 600 mg/kg BW group treated with test drug.

Statistical analysis

The results are expressed as mean±standard deviation (SD). Statistical analysis is performed using one-way analysis of variance, followed by Scheffe's *post-hoc* test. $p<0.05$ will be considered as statistically significant.

Table 1: Eddy's hot plate in rats (n=6).

Group	Dose (orally)	Mean reaction time in minutes			
		0	30	60	90
I	Control N saline - 5 ml/kg	6.16±0.90	6.26±0.81	6.42±0.83	6.33±0.80
II	Ibuprofen - 5 mg/kg	6.25±0.65	9.45±0.58**	11.56±0.73***	12.40±0.73***
III	MK - 300 mg/kg	6.42±0.89	6.85±0.83	7.64±0.98*	8.60±0.78*
IV	MK - 600 mg/kg	6.30±0.71	7.56±0.92*	8.70±0.68*	9.54±0.78**

ANOVA followed by Tukey's multiple comparison tests was used for analysis of data between the four groups.*p<0.05, **p<0.01, ***p<0.001, when compared with control, MK: *Murraya koenigii*

Table 2: Effects of MK on tail flick method in rats (n=6).

Group	Dose (orally)	Mean reaction time in minutes			
		0	30	60	90
I	Control N saline - 5 ml/kg	5.47±0.89	5.28±0.63	4.9±1	5.14±0.75
II	Ibuprofen - 5 mg/kg	5.43±0.8	7.12±0.75**	8.33±0.54***	10.0±0.65***
III	MK - 300 mg/kg	5.12±0.51	5.7±0.54	6.25±0.63*	6.89±0.54*
IV	MK - 600 mg/kg	5.45±0.72	6.52±1.03*	7.60±0.81**	8.80±0.63**

ANOVA followed by Tukey's multiple comparison tests was used for analysis of data between the four groups.*p<0.05, **p<0.01, ***p<0.001, when compared with control, MK: *Murraya koenigii*

Table 3: Effect of MK on carrageenan induced rat paw edema in rats (n=6).

Group	Dose (orally)	Mean increase in paw volume in ml at hours				
		0 hr	1 hrs	2 hrs	3 hrs	4 hrs
I	Control N saline – 5 ml/kg	0.27±0.04	0.50±0.05	0.67±0.03	0.88±0.03	1.04±0.06
II	Ibuprofen - 5 mg/kg	0.28±0.04	0.34±0.05**	0.46±0.03**	0.61±0.05**	0.76±0.05**
III	MK - 300 mg/kg	0.28±0.03	0.46±0.04	0.58±0.05*	0.77±0.04	0.92±0.06
IV	MK - 600 mg/kg	0.27±0.04	0.39±0.03*	0.48±0.06**	0.68±0.05*	0.80±0.03*

ANOVA followed by Tukeys multiple comparison tests was used for analysis of data between the four groups. *p<0.05, **p<0.01 ***p<0.001, when compared with control. MK: *Murraya koenigii*

Table 4: Percentage inhibition MK on carrageenan induced rat paw edema (n=6).

Group	Dose (orally)	% inhibition of paw volumes at different time points			
		1 hr	2 hrs	3 hrs	6 hrs
I	Control N saline – 5 ml/kg	-	-	-	-
II	Ibuprofen - 5 mg/kg	32	31.34	30.68	26.9
III	MK - 300 mg/kg	8	19.4	12.5	11.53
IV	MK - 600 mg/kg	22	28.35	22.72	18.26

MK: *Murraya koenigii*

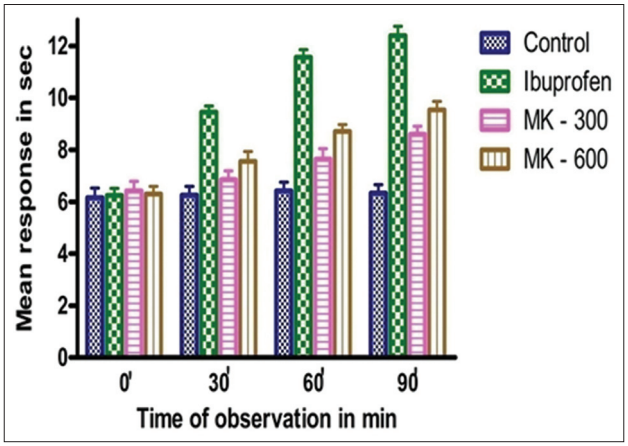

Figure 1: Eddy's hot plate method.

RESULTS

The mean reaction time in control group at 0 min was 6.16±0.90, at 30 mins 6.26±0.81, 60 mins 6.42±0.83, at 90 mins 6.33±0.80. For ibuprofen group the mean reaction time at 0 min 6.25±0.65, at 30 mins 9.45±0.58, at 60 mins 11.56±0.73, at 90 mins 12.40±0.86 respectively. The mean reaction time of MK in dose of 300 mg/kg at 0 min 6.42±0.89, at 30 mins 6.85±0.83, at 60 mins 7.64±0.98, at 90 mins 8.60±0.76, and in 600 mg/kg at 0 min 6.30±0.71, 30 mins 7.56±0.92, at 60 mins 8.70±0.68, at 90 mins 9.54±0.78 respectively. The mean reaction time increased significantly with MK at a dose of 600 mg/kg when compared to control.

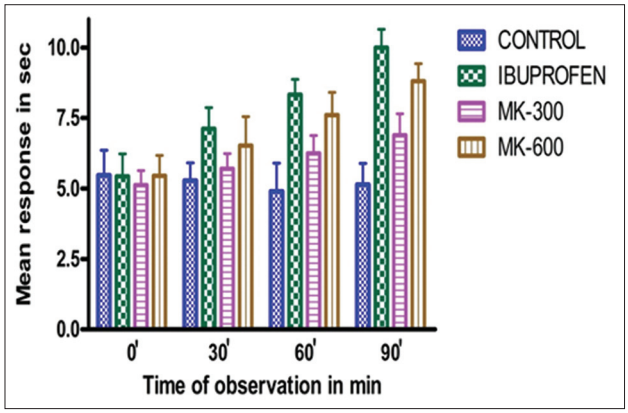

Figure 2: Tail flick method.

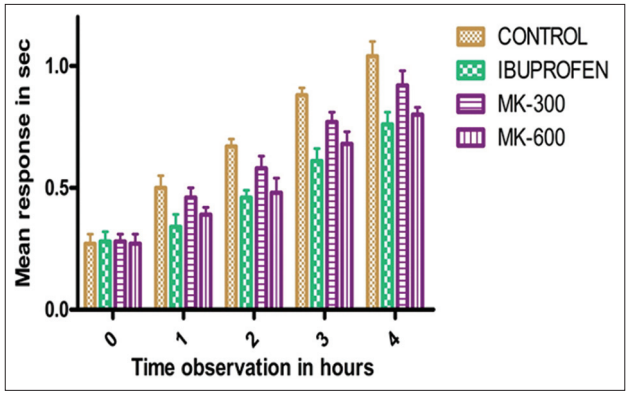

Figure 3: Anti-inflammatory activity.

MK in the dose of 600 mg/kg shows statistically significant analgesic activity.

The mean reaction time in control group at 0 min 5.47 ±0.89, at 30 mins 5.28±0.63, at 60 mins 4.9±1 and at 90 mins 5.14±0.75. In ibuprofen group the mean reaction time at 0 min 5.43±0.8, at 30 mins 7.12±0.75, at 60 mins 8.33±0.54, at 90 mins 10.0±0.65. The mean reaction time MK at a dose of 300 mg/kg at 0 min 5.12±0.51, at 30 mins 5.7±0.54, at 60 mins 6.25±0.63, at 90 mins 6.89±0.54 and in group of 600 mg/kg at 0 mins 5.45±0.72, at 30 mins 6.52±1.03, at 60 mins 7.6±0.81, at 90 mins 8.8±0.63 respectively. The mean reaction time increased significantly with MK at a dose of 600 mg/kg when compared to control.

The mean reaction time in control group at 0 hr 0.27±0.04, at 1 hr 0.50±0.05, at 2 hrs 0.67±0.03, at 3 hrs 0.88±0.03 at 4 hrs 1.04±0.06. In ibuprofen group the mean reaction time at 0 hr was 0.28±0.04, at 1 hrs 0.34±0.05, at 2 hrs 0.46±0.03, at 3 hrs 0.61±0.05, at 4 hrs 0.76±0.05. The mean reaction time MK at a dose of 300 mg/kg at 0 hr 0.28±0.03, at 1 hr 0.46±0.04, at 2 hrs 0.58±0.05, at 3 hrs 0.77±0.04, at 4 hrs 0.92±0.06 and in group 600 mg/kg at 0 hr 0.27±0.04, at 1 hr 0.39±0.03, at 2 hrs 0.48±0.06, at 3 hrs 0.68±0.05, at 6 hrs 0.80±0.03 respectively. The mean reaction time increased significantly with MK at a dose of 600 mg/kg when compared to control.

DISCUSSION

The medicinal plants or their bioactive compounds have been utilized by developing countries for primary and traditional healthcare system since very long period of time. In several ancient systems of medicine including Ayurveda, Siddha and Unani, MK, a medicinally important herb from mainly Asian origin has vast number of therapeutic applications such as in bronchial disorders, piles, vomiting, skin diseases etc. The medicinal utilities have been described especially for leaf, stem, bark and oil. MK contains a number of chemical constituents that interact in a complex way to elicit their pharmacodynamic response. A number of active constituents responsible for the medicinal properties have been isolated and characterized. This plant has been reported to have anti-oxidative, cytotoxic, antimicrobial, antibacterial, anti-ulcer, positive inotropic and cholesterol reducing activities.[11] The present study was aimed at evaluating the analgesic and anti-inflammatory activity of MK linn. properties in comparison with standard drugs using animal models.

Our studies is supported by Gupta, et al., (2010)[4] employed two different analgesic testing methods with the objective of identifying possible peripheral and central effects of the methanolic extract of MK. Using, both hot plate test and formalin induced paw licking response in mice, it was observed that the plant extracts possessed analgesic effects against both models. The observations also indicated that the extracts have both central and peripheral effects. They concluded their study by stating that, analgesic effect of Murraya may be linked to processes involved in the prevention of sensitization of nociceptors, down regulation of the sensitized nociceptors and/or blockade of the nociceptors at peripheral and/or central levels.

The findings of the study conducted by Prasad et al., (2011)[12] were in conformity with our study, i.e. methanol extracts of leaves (400 mg/kg) of MK as potent anti-inflammatory agent in carrageenan induced inflammation in albino rats. Parmar et al., (2010)[13] showed that the mast cell stabilization and antihistaminic effects of MK were suggested to be the probable mechanisms for its anti-inflammatory action. Darvekar et al., (2006)[14] showed the ethanolic extract (250 mg/kg) of MK possessed significant anti-inflammatory effects as compared with petroleum ether and chloroform extracts in acute carrageen-induced paw edema method and yeast-induced hyperpyrexia method, respectively. Mathur et al., (2010)[15] showed significant reduction of carrageenan-induced paw edema with aqueous extracts. Petroleum ether and hexane extracts showed no reduction in paw edema. Mathur et al., (2011)[16] stated that 9, 12-octadecadienoic acid, a compound isolated from the methanolic extracts of MK leaves was reported to induce 85% reduction in paw edema at a dose of 150 µg/mL in reference to the standard anti-inflammatory drug aspirin which showed 68.62% reduction. Gupta et al., (2011)[4] demonstrated significant ($p<0.001$) anti-inflammatory activity by reduction in carrageenan-

induced paw edema and analgesic activity evidenced by increase in the reaction time by Eddy's hot plate method. The current study demonstrates significant anti-inflammatory and analgesic activity of MK in all three pain models, supporting their traditional use for the treatment. However the magnitude of anti-inflammatory and analgesic activities of MK was less when compared to ibuprofen.

CONCLUSIONS

The anti-inflammatory activities of MK in rats were demonstrated by measuring the volume of reduction in the paw edema after carrageenan injection into the paw of rats. The anti-inflammatory activity was observed at 1, 2, 3 and 4 hrs. There was maximum inhibition of the paw edema at end of 2 hrs.

The analgesic activity of MK was demonstrated by measurement of reaction time of rat to escape out/paw licking from the Eddy's hot plate and measurement of reaction time in tail flick method. The analgesic activity was observed at 30, 60 and 90 mins. The maximum analgesic effect was obtained at 60 and 90 mins, for both hot plate method and tail flick method.

The results were assessed and the mean, SD and percentage inhibition was noted. The statistical analysis was done by Tukey's test. The statistical significance was analyzed with the control group and standard group and p value were noted.

The current study demonstrates significant anti-inflammatory and analgesic of MK in thermal and chemical induced procedures/models, but the enormity of anti-inflammatory and analgesic activities of MK was statistically less significant when compared to ibuprofen.

MK linn is a polyherbal compound and there are no major side effects observed till now. Keeping in view the tremendous pharmacological activities and wealth of literature available, MK may be utilized to alleviate pain as evident from the pre-clinical data. We conclude that MK linn a polyherbal preparation has a potential to be used as an add on therapy in analgesic and anti-inflammatory. Although crude extract from various parts of curry neem have numerous medical applications, modern drugs can be developed after extensive investigation of its bioactivity, mechanism of action, pharmaco-therapeutics, toxicity and after proper standardization and clinical trials. The available literature and wide spread availability of MK in India thus makes it an attractive candidate for further pre-clinical and clinical research.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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