

Anti-inflammatory and analgesic activity of aqueous extracts of dried leaves of *Murraya koenigii* Linn.

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ABSTRACT

Background: *Murraya koenigii*, commonly known as karipatta or curry leaf, is analgesic and can be used effectively against inflammation and itching. The various pharmacological activities such as vasodilation, antimicrobial, antidiabetic, antiulcer, analgesic, phagocytic, and antioxidant activities of this plant have also been reported. **Aims and Objectives:** To evaluate the anti-inflammatory and analgesic activity of aqueous extract of dried leaves of *M. koenigii* Linn. in male Wistar rats. **Materials and Methods:** Adult male Wistar rats (100–150 g body weight) were used in this study. Aqueous extract of *M. koenigii* Linn. was used to evaluate acute anti-inflammatory and analgesic activity by plethysmometer and hot plate method by oral administration at doses of 100, 300, and 500 mg/kg body weight in healthy albino rats. **Result:** In acute studies, the aqueous extract showed anti-inflammatory activity by significant reduction in the paw edema volume, in a dose-dependent manner when compared with the control and standard drug. Aqueous extract of *M. koenigii* Linn. significantly and dose-dependently reduced the number of acetic acid-induced writhing and significantly increased the latency of paw licking in hot plate method. Statistical analysis was carried out by one-way ANOVA, followed by Turkey's test. **Conclusion:** Aqueous extract of *M. koenigii* Linn. possesses both anti-inflammatory and analgesic activity in a dose-dependent manner.

KEY WORDS: *Murraya koenigii*; Anti-inflammatory; Analgesic; Carrageenan; Paw Edema


INTRODUCTION

Plants have been the major source of drugs in Indian system of medicine and other ancient systems in the world. Rig-Veda (2500–1800 BC) offers the earliest depiction of healing properties of medicinal plants, while Charka Samhita and Sushruta Samhita unfold elaborately on several medicinal herbs.^[1–4] At present, the application of plant-derived natural products in the synthesis of drugs becomes a drift. Hence, there is a requirement to update data on the properties, uses, effectiveness, and safety of medicinal plant products.^[5,6]

Most of the drugs used at present for anti-inflammatory and analgesic effects are synthetic in nature and prolonged use of which causes severe side effects and exhibit toxic effects.^[7] In this regard, a novel possibility of evaluating herbs in pain therapy arises. Plants still remain a vast unexploited resource of structurally novel molecules that can aid in the development of novel drugs.

Murraya koenigii, commonly known as karipatta or curry leaf, is a native plant of India, Sri Lanka, and other south Asian countries.^[8] The plant is greatly appreciated as its leaves are a vital component in an Indian cuisine to promote hunger and digestion. The leaves are used in treatment of dysentery and against vomiting. They can be applied externally to bruises and infection. The leaves are analgesic and can be used effectively against inflammation and itching. The various pharmacological activities such as vasodilation, antimicrobial, antidiabetic, antiulcer, analgesic, phagocytic, and antioxidant activities of this plant have also been reported.^[9–11]

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

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koenigine were recognized for highest antioxidant activity.^[12] Aqueous extract of leaves produced a significant dose-dependent decrease in serum urea and creatinine level and a marked increase in the levels of plasma antioxidant capacity in diabetic rats, compared with the control (nondiabetic) subjects. Histological studies of the kidneys of these animals showed comparable tissue regeneration by the aqueous extract.^[13] There have also been several recent studies that have anticipated *M. koenigii* to possess anti-inflammatory activity mainly owing to the presence of its carbazole alkaloids.^[14] In this study, we further corroborated the anti-inflammatory and analgesic activity of the aqueous extract of the leaves of *M. koenigii*.

MATERIALS AND METHODS

Plant Material

Fresh leaves of plant *M. koenigii* were collected in the summer season (April 2015) locally from Jodhpur market. The taxonomic identification of the plant was confirmed and processed for further investigations. Collected leaves were washed thoroughly under running water for 2–3 times. Washed leaves were dried under the shade for 30 days. The dried leaves were powdered and stored in a sterile bottle at room temperature.

Preparation of Plant Extract

We have collected aqueous extract of *M. koenigii* through Soxhlet apparatus by hot continuous extraction method. The use of commercially available Soxhlet apparatus is a convenient way to prepare crude plant extract. The dried and powdered drug was packed. Soxhlet apparatus is an automatic, continuous method that does not require further manipulation. This method is not time-consuming, as, for a standard-sized sample (50 g), extraction time is 48 h. The yield of the aqueous extract was 9.52%. The extract was stored in refrigerator until further studies.

Drugs

Lambda carrageenan (Sigma Chemical, Co.), ibuprofen, diclofenac sodium, aspirin (Cipla), acetic acid (ASES Chemical Works, Jodhpur), and sodium chloride (ASES Chemical Works)

Procurement of Animals

Male Wistar rats weighing (100–150 g) were obtained. They were housed in ventilated cages and fed with a normal pellet diet and water *ad libitum*. All experiments were in agreement with ethical guidelines for investigations of experimental plant in conscious animal. Research protocol was approved by the Animal Ethics Committee.

Carrageenan-Induced Edema in Rats

Five Groups of six animals were used. Paw swelling was induced by subplantar injection of 0.1 mL 1% sterile lambda carrageenan in saline into the right hind paw. The aqueous extracts of *M. koenigii* at dose of 100, 300, and 500 mg/kg were administered orally 30 min before carrageenan injection.

Ibuprofen (50 mg/kg) was used as reference drug. Control group received the vehicle only (10 mL/kg). The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer (Medicaid system) at time 0 min, 30min, and 1, 2, and 3 h after carrageenan injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined, and the percent inhibition of edema was calculated in comparison with the control animals.

Procedure

Thirty minutes after drug or test compound (extracts) administration, 0.1 mL of 1% carrageenan in distilled water was injected into the subplantar region of right hind paws of all groups. A mark was put on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw edema volume was measured with the help of plethysmometer at 0 h (immediately after injecting carrageenan). The same procedure was repeated at 30 min and 1, 2, and 3 h. The difference between 1 h and subsequent hours reading was taken as actual edema volume. The percentage inhibition of paw edema in the various treated groups was then calculated by using the formula:

$$\text{Percentage inhibition} = (1 - V_t/V_c) \times 100$$

where V_t = the edema volume in the drug treated group;

V_c = the edema volume in the control group.

The five groups are as follows:

Group I: served as control and treated with carrageenan;

Group II: standard group, ibuprofen 50 mg/kg;

Group III: aqueous extract of *M. koenigii* at dose of 100 mg/kg;

Group IV: aqueous extract of *M. koenigii* at dose of 300 mg/kg;

Group V: aqueous extract of *M. koenigii* at dose of 500 mg/kg.

Antinociceptive Activity after Acute Administration

- *Acetic acid-induced writhing method:* Antinociceptive activity of AEMK was assessed by counting the number of writhes induced by 0.6% acetic acid (10 mL/kg, i.p.) in the following 20 min. Aspirin (100 mg/kg, p.o.) was used as a reference standard. Percentage protection against writhing was taken as an index of analgesia.

% inhibition = [(Number of writhing in control group - Number of writhing in treated) × 100] / (Number of writhing in control group).

- *Hot plate test:* The hot plate was used to measure response latencies according to the method described by Eddy and Leimbach (1953). The rats were placed on a Techno hot plate maintained at 56°C, and the time between placement of the rat on the platform and shaking or licking of the paws or jumping was recorded as the hot plate latency. Rats with baseline latencies higher than 10 s were eliminated from the study. Twenty-four hours later, animals were treated with the aqueous extract of *M. koenigii* (at dose of 100, 300, and 500 mg/kg p.o.) or with diclofenac sodium (10 mg/kg p.o.)

60 min, before the test. Control animals received the same volume of saline solution (10 mL/kg).

Statistical Analysis

The results are expressed as mean \pm SD ($n = 6$). Statistical significance was determined by ANOVA and subsequent Turkey's test. P values less than 0.05 were considered as indicative of significance.

RESULTS

Carrageenan-Induced Edema in Rats

The anti-inflammatory effects of the aqueous extracts of *M. koenigii* on carrageenan-induced edema in rat's hind paws are presented in Table 1. There was a gradual increase in edema paw volume of rats in the control group. However, in the test groups, the extract showed a significant reduction in the edema paw volume. As indicated in the oral administration of aqueous extracts of *M. koenigii* at doses of 100, 300, and 500 mg/kg p.o. 30 min before carrageenan, a dose-related inhibition of hind paw edema between 1 and 3 h was exhibited. The inhibitory effect was highest with 500 mg/kg. Significant effects were demonstrated by the extract. Ibuprofen as reference drug (50 mg/kg orally) produced a significant inhibitory effect comparable with the extract. Extract and ibuprofen exhibited 52% and 66% inhibition of edema formation, respectively, at 3 h after carrageenan administration.

Acetic Acid-Induced Writhing Method

Aqueous extract of *M. koenigii* leaves (AEMK) significantly ($P < 0.05$) and dose-dependently reduced the number of acetic acid-induced writhing, when compared with vehicle-treated group, indicating significant peripheral antinociceptive activity. The percentage inhibition on single administration of test substance was found to be increased up to 53.52 with AEMK of 300 mg/kg, p.o., and 61.03 with AEMK of 500 mg/kg, p.o. Aspirin, used as reference standard, produced maximum inhibition (73%; Table 2).

Hot plate Test

Diclofenac sodium at a dose of 10 mg/kg and AEMK produced significantly increased the pain latency, when compared with the control group. AEMK at doses of 100, 300, and 500 mg/kg, p.o., produced significantly percentage increase in pain (42.40 ± 2.70 ,

Table 2: Effect of *M. koenigii* on acetic acid-induced writhing in rats

Treatment	Number of writhes	%Inhibition
Control	42.6 \pm 0.81	—
Asprin (100)	11.5 \pm 1.55*	73
AEMK (100)	27.2 \pm 0.86*	36.15
AEMK (300)	19.8 \pm 1.15*	53.52
AEMK (500)	16.6 \pm 0.93*	61.03

The observations ($n = 6$) are mean \pm SEM.

* $P < 0.05$, compared with control (one-way ANOVA, followed by Turkey's test).

AEMK, aqueous extract of *M. koenigii* leaves.

55.34 \pm 1.14, and 77.16 \pm 0.47) 2 h after drug administration, as shown in Table 3.

DISCUSSION

This study provides evidence that the AEMK acts as an anti-inflammatory agent in rats in acute inflammation model. Carrageenan-induced inflammation is the majorly applied experimental model for determining the anti-inflammatory effectiveness of compounds or natural products.^[15] In addition, the experimental model displays a good degree of reproducibility.^[16] The paw edema induced by carrageenan is a biphasic event. The release of histamine, serotonin, and kinins forms the first phase, while the release of prostaglandins (PGEs), protease, and lysosome results in the second phase of edema. This phase is responsive to majority of clinically efficient anti-inflammatory drugs.^[17] The results of this study indicate the role of AEMK against carrageenan-induced acute inflammation to be significant. The AEMK at a dose of 100 and 300 mg/kg suppressed only the second phase of carrageenan-induced inflammation but, at a dose of 500 mg/kg, significantly suppressed both first and second phases of carrageenan-induced inflammation. The standard (ibuprofen 50 mg/kg) significantly suppresses the biphasic response of carrageenan-induced inflammation. So, the anti-inflammatory effect of AEMK at a dose of 500 mg/kg may be owing to its suppression action on PGE, protease, or lysosome synthesis or activity.

Two different analgesic testing methods were used in the current investigation with the objective to identifying possible peripheral and central effects of the AEMK. Using both hot plate

Table 1: Anti-inflammatory effect of AEMKL in carrageenan-induced paw edema

Group no.	Treatment	Dose	1 h	2 h	3 h	Average reading	%Inhibition
1	Carrageenan	0.1 mL 1% sol.	1.43 \pm 0.08	1.50 \pm 0.06	1.42 \pm 0.04	1.45	—
2	Ibuprofen	50 mg/kg	0.50 \pm 0.05	0.60 \pm 0.06	0.64 \pm 0.04	0.58	60
3	AEMK	100 mg/kg	0.79 \pm 0.12	0.92 \pm 0.15	0.96 \pm 0.11	0.89	39
4	AEMK	300 mg/kg	0.89 \pm 0.17	0.90 \pm 0.09	1.10 \pm 0.12	0.93	36
5	AEMK	500 mg/kg	0.75 \pm 0.09	0.72 \pm 0.1	0.66 \pm 0.12	0.71	52

Values are mean \pm SD ($n = 6$).

Table 3: Analgesic effect of aqueous extract of AEMKL in the hot plate test

Groups	0 min	30 min	60 min	120 min
Control	0.87 ± 0.03	0.97 ± 0.02	1.10 ± 0.02	0.97 ± 0.05
DS	1.0 ± 0.04	1.76 ± 0.02 (9.34 ± 4.81)	2.77 ± 0.02 (46.05 ± 1.22)	4.69 ± 0.16 (61.67 ± 0.81)
AEMK (100)	0.98 ± 0.04	1.58 ± 0.06 (8.49 ± 6.57)	1.88 ± 0.06 (37.29 ± 3.23)	3.33 ± 0.09 (42.40 ± 2.70)
AEMK (300)	1.07 ± 0.02	1.73 ± 0.02 (12.61 ± 4.32)	2.46 ± 0.06 (45.17 ± 1.39)	4.73 ± 0.12 (55.34 ± 1.14)
AEMK (500)	1.10 ± 0.03	2.83 ± 0.03 (14.15 ± 3.25)	4.89 ± 0.04 (65.75 ± 0.53)	5.39 ± 0.26 (77.16 ± 0.47)

Values are mean ± SD ($n = 6$); values in bracket indicate percentage inhibition in pain.

test and acetic acid-induced writhing response in rats, it was observed that the plant extracts possessed analgesic effects against both models. The observations also indicated that the extracts exhibit both central and peripheral effects. To evaluate for a possible central antinociceptive effect of the AEMK, the hot plate test was used,^[18] possibly acting on a descending inhibitory pain pathway.^[19] The paw-licking hot plate reaction is a more intricate supraspinally ordered activity.^[20] In general, the μ receptor has been considered as the receptor type related to pain relief and exhibited to be effective in controlling thermal pain.^[21] Activation of μ_2 opioid subtype results in spinal analgesia and generally via constipation unfavorable effect.^[22] Therefore, by considering several reports, the antinociceptive activity of AEMK is likely to be mediated centrally.

The AEMK also showed antinociceptive activity in the acetic acid test. Acetic acid produces the constriction reaction of abdomen, which forms a sensitive method for peripheral analgesic agents. Acetic acid promotes a raise in the levels of PGEs (PGE2 and PGF2 α) in peritoneal fluid, concerning in part with peritoneal receptors^[23] and inflammatory pain by inducing capillary permeability.^[24] AEMK exhibited significant reduction in number of writhing, suggesting involvement of PGEs. The presence of alkaloids, triterpenoids, and flavonoids in *M. koenigii* may be responsible for antinociceptive activity as all the three constituents have been reported to possess analgesic and anti-inflammatory activities.^[25,26]

CONCLUSION

In this study, aqueous extract of *M. koenigii* (500mg/kg, p. o.) significantly reduced edema induced by carrageenan in all the phases.. Aqueous extract of *M. koenigii* Linn. significantly and dose-dependently reduced the number of acetic acid-induced writhing and significantly increased the latency of paw licking in hot plate method.

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