European Review for Medical and Pharmacological Sciences 2013; 17: 1748-1751

# Naloxone blocks the beneficial effects of aqueous extract of *Murraya koenigii* (L.) Spreng leaves in models of pain

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**Abstract. – AIM: This study investigated the antinociceptive effects of aqueous extract of *Murraya koenigii* (AEMK) leaves (200, 400 and 800 mg/kg, orally) on animal models of acute and persistent pain and its modulation by naloxone.**

**MATERIALS AND METHODS: Antinociceptive effects were assessed using tail-flick, hot plate and formalin tests in mice. To differentiate between central and peripheral antinociceptive effect of AEMK, naloxone (2 mg/kg) was administered along with the 800 mg/kg dose of extract. Morphine was used as a standard drug.**

**RESULTS: AEMK and morphine significantly increased the tail-flick latency (tfl) and paw licking/jumping latency in tail-flick and hot plate tests, respectively, in comparison to control. Also, in both the tests AEMK and morphine significantly increased the $AUC_{0\text{-}120\ min}$. In formalin test, AEMK (400 mg/kg and 800 mg/kg) and morphine significantly reduced licking time in both early and late phases in comparison to control.**

**CONCLUSIONS: Thus, in all three pain models AEMK showed antinociceptive effect, which was blocked by naloxone suggesting the involvement of opioidergic central mechanism.**

*Key Words:*
*Murraya koenigii*, Tail-flick test, Hot plate test, Formalin test, Morphine, Antinociception.

## Introduction

Phytochemical studies on leaves of *Murraya koenigii (L) Spreng* have revealed the presence of carbazole alkaloids, volatile oil, gycozoline, xanthotoxine and sesquiterpione[1]. Some investigations have shown the beneficial effects of ethanolic and methanolic extracts of *Murraya koenigii* leaves in neuropathic pain and inflammatory pain, respectively, in rats[2,3]. However, its underlying mechanism (central or peripheral) has not been well explored in above mentioned investigations. This study, therefore, investigated the antinociceptive effects of aqueous extract of *Murraya koenigii* (AEMK) on well established animal models of acute and persistent pain (tail-flick, hot plate and formalin test) in mice. In addition, naloxone was used to determine the role of opioid system in its antinociceptive effect and to differentiate between central and peripheral antinociceptive effect of AEMK.

## Materials and Methods

Swiss albino male mice (25-30 g) were procured from the Central Animal House, University College of Medical Sciences, Delhi, maintained at 25 ± 2°C, humidity 65 ± 5 under a 12 h light-dark cycle and given *ad libitum* access to food and water. The study was approved by institutional animal Ethics Committee and care of the animals was as per guidelines of committee for the purpose of control and supervision of experiments on animals, India.

Leaves of *Murraya koenigii* were purchased from Tapovan Ayurved Sadan, Delhi. The sample of leaves was authenticated by a Botanist at University of Delhi, and the voucher specimen (AC. no. 13550) kept in the Herbarium of the University. Fresh leaves were air-dried at room temperature and milled into a fine powder in an electric blender. A total of 500 g of the ground powder was soaked in 1.5 L of distilled water at room temperature for 48 hrs with intermittent stirring. The extract was filtered with Whatman filter paper and filterate was evaporated under reduced pressure and vacuum dried followed by collection as powdered form (yield = 11.6% w/w). The powder was diluted by distilled water and administered as 200, 400 and 800 mg/kg, by oral route.


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The preliminary phytochemical screening for alkaloids, glycosides, triterpenoids, tannins and flavonoids was carried out[4].

Morphine sulphate (ampoules) and formalin were obtained from hospital (UCMS and GTBH) supplies. Naloxone was purchased from Sigma-Aldrich (Sigma-Aldrich Chemical Co., St Louis, MO, USA).

### Tail-flick and Hot Plate Test

Tail flick latency (tfl) was measured using an analgesiometer (Ugo Basile, model 37360, Varese, Italy)[5]. In hot plate test, mice were placed in a plexiglass cylinder with a heated surface (temperature –52 ± 0.5°C) individually and the latency time of the animal to lick its hindpaw (or jump) was measured[6]. In our studies, the maximum time of heat exposure to avoid tissue damage was 15 s and 20 s in tail-flick and hot plate test[7], respectively.

Normal saline, morphine (5 mg/kg, i.p.) or AEMK (200, 400 or 800 mg/kg) were given 30 min prior to the tests. Two separate groups of mice were pre-treated with naloxone (2 mg/kg, i.p.) 10 min prior to the administration of 800 mg/kg AEMK and one each was subjected to the two tests. The tfl and licking latency were recorded before and 30, 60, 90 and 120 min after the administration of each solution. Prolongation of the tfl and latency time was taken as an indicator of antinociceptive activity.

### Formalin Test

In formalin test, the mice were injected with 20 µl of 1% formalin [formaldehyde solution 37% (w/w) diluted in distilled water] into the subplantar space of the right hind paw. The test was carried out in a transparent plastic chamber (30 × 30 × 30) cm with a mirror placed at the base of the chamber to allow clear observation. After formalin injection, mouse starts licking and biting the formalin injected paw. This behavior is observed in two phases; first phase (0-5 min) and second phase (20-30 min). The total time (seconds) spent in licking and biting the injected paw in both phases was measured[8]. Normal saline, morphine (5 mg/kg) or AEMK (200, 400 or 800 mg/kg) were given 45 min before formalin injection. One separate group was pre-treated with naloxone (2 mg/kg, i.p.) 10 min before administration of 800 mg/kg AEMK and subjected to the formalin test.

### Statistical Analysis

The data are presented as mean ± SD for visualization only. Data from tail-flick test and hot plate test were analyzed using one way ANOVA after converting each value into percentage of maximum possible effect (% MPE) followed by Tukey's test. % MPE was calculated as: 100 × (post drug value – baseline value)/(cut off value – baseline value). The trapezoidal rule was used to calculate area under the % MPE versus time curves (AUCs) for each animal. AUC data and formalin test data were analyzed using one way ANOVA followed by Scheffe's test. $p < 0.05$ was considered statistically significant.

## Results

The preliminary phytochemical screening revealed the presence of alkaloids, glycosides, triterpenoids, tannins and flavonoids.

### Tail-Flick and Hot Plate Test

In the tail-flick test, administration of 800 mg/kg AEMK and morphine significantly lengthened tfl at 30, 60 and 90 min in comparison to control [$F(6, 35) = 12.2$, 42.9 and 13.3; $p < 0.01$ for each] (Table I). 200 and 400 mg/kg AEMK significantly increased tfl only at 60 min ($p < 0.01$ for both). In hot plate test, all 3 doses (200, 400 and 800 mg/kg) of AEMK and morphine significantly increased the licking/jumping latency in comparison to control group at 30 min, 60 min and 90 min [$F(6, 35) = 17.4$, 258.5 and 54.5; $p < 0.01$ for each comparison]. Naloxone treatment, prior to 800 mg/kg dose of AEMK and morphine 5 mg/kg, completely blocked their antinociceptive action in both tail-flick and hot plate tests. $AUC_{0\text{-}120\ min}$ showed a dose-response relationship regarding antinociceptive effect of AEMK both in the tail-flick test [$F(6, 35) = 25.4$, $p < 0.01$] and the hot plate test [$F(6, 35) = 165.2$, $p < 0.01$].

### Formalin Test

AEMK produced a dose-dependent antinociceptive activity in both the early and late phases in the formalin test (Figure 1). In both early and late phases, 400 and 800 mg/kg doses of AEMK significantly reduced the paw-licking time in comparison to control [$F(6, 35) = 11.4$ and 26.6 ($p < 0.05$ for each comparison)].

In both early and late phases, maximum antinociceptive actvity was produced by morphine

**Table I.** Effect of aqueous extract of *Murraya koenigii* (AEMK) and morphine *i.p.* on tail-flick latency and licking/jumping latency in mice

| Groups | Tail flick latency (s) | | | | | | Paw licking latency (s) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min | AUC | 0 min | 30 min | 60 min | 90 min | 120 min | AUC |
| Control | 4.2 ± 1.2 | 4.5 ± 1.4 | 4.1 ± 1.6 | 4.0 ± 1.0 | 4.3 ± 1.0 | 0.1 | 10.8 ± 1.2 | 11.1 ± 1.4 | 11.1 ± 1.5 | 10.9 ± 1.4 | 10.8 ± 0.8 | 2.1 |
| AEMK 200 mg/kg | 4.8 ± 1.4 | 5.4 ± 2.8 | 8.1 ± 3.7[a] | 5.6 ± 3.0 | 4.6 ± 1.7 | 46.0[a] | 11.4 ± 1.9 | 15.7 ± 2.6[a] | 20.4 ± 1.4[a] | 15.5 ± 1.2[a] | 11.4 ± 1.9 | 81.4[a] |
| AEMK 400 mg/kg | 4.6 ± 1.2 | 6.2 ± 0.5 | 9.1 ± 0.7[a] | 6.4 ± 0.7 | 5.2 ± 0.8 | 68.5[a] | 11.4 ± 1.6 | 17.5 ± 1.7[a] | 26.3 ± 1.7[a] | 18.5 ± 1.9[a] | 11.5 ± 1.6 | 133.5[a] |
| AEMK 800 mg/kg | 4.7 ± 1.2 | 8.2 ± 0.8[a] | 13.1 ± 0.7[a] | 7.8 ± 0.8[a] | 5.0 ± 1.0 | 130.6[a] | 10.4 ± 1.1 | 19.3 ± 2.2[a] | 29.3 ± 1.6[a] | 19.6 ± 1.6[a] | 10.6 ± 1.2 | 166.9[a] |
| AEMK 800 mg/kg + naloxone 2 mg/kg | 4.6 ± 1.3 | 4.6 ± 1.4[b] | 5.2 ± 1.3[b] | 4.9 ± 1.3 [b] | 4.9 ± 1.2 | 9.6[b] | 10.7 ± 1.3 | 12.3 ± 1.0[b] | 12.8 ± 0.5[b] | 11.6 ± 0.6[b] | 10.1 ± 0.7 | 17.6[b] |
| Morphine 5 mg/kg | 4.7 ± 1.3 | 8.7 ± 1.2[a] | 14.6 ± 0.4[a] | 9.4 ± 1.2[a] | 5.3 ± 0.9 | 164.4[a] | 10.8 ± 1.5 | 21.0 ± 1.5[a] | 29.7 ± 0.5[a] | 23.9 ± 0.8[a] | 10.9 ± 1.1 | 193.6[a] |
| Morphine 5 mg/kg + naloxone 2 mg/kg | 4.1 ± 1.0 | 4.4 ± 0.9[b] | 4.5 ± 0.8[b] | 4.5 ± 0.7[b] | 4.3 ± 1.1 | 10.0[b] | 10.8 ± 1.2 | 12.4 ± 1.0[b] | 13.9 ± 0.8[b] | 11.1 ± 0.9[b] | 10.9 ± 0.8 | 20.9[b] |

Data are mean ± SD of 6 mice in each group. AUC: mean areas under the time-action curves ($AUC_{0\text{-}120\ min}$ s × min; normalized for time-interval of 30 min. Data analyzed by one way ANOVA followed by Tukey's test. [a]$p < 0.05$ significant in comparison to control. [b]$p < 0.05$ significantly different from group above.

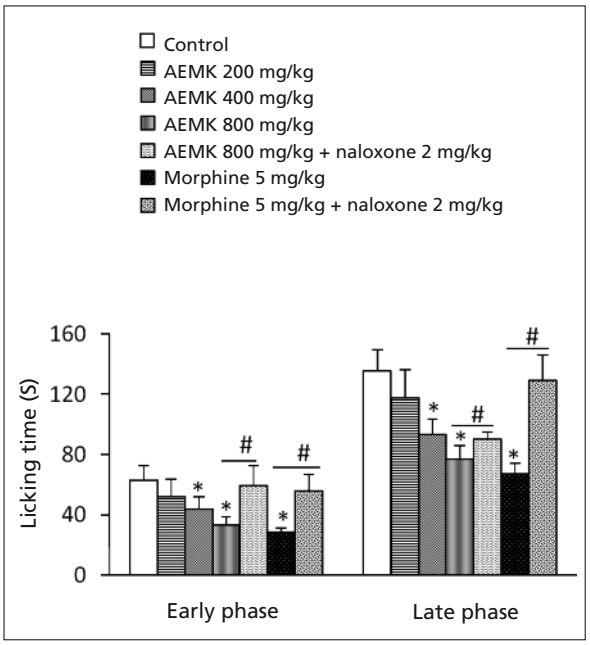


**Figure 1.** Effect of *Murraya koenigii* (200, 400, 800 mg/kg) in formalin test in mice (n = 6/ group). Results are shown as mean ± SD *$p < 0.05$ significant in comparison to control, [#]$p < 0.05$ significantly different from the compared group.

(5 mg/kg) which was blocked by prior naloxone treatment. In addition, the antinociceptive effect produced by the 800 mg/kg dose of AEMK following pre-treatment with 2 mg/kg naloxone was blocked in early phase, whereas in late phase it was partially blocked.

## Discussion

In this study, we examined the antinociceptive effect of AEMK (200, 400 and 800 mg/kg, p.o.) using thermal and chemical methods of nociception, i.e. tail-flick, hot plate and formalin tests in mice. In addition, to differentiate between central and peripheral antinociceptive effect of AEMK, naloxone was administered with AEMK (800 mg/kg). Naloxone is an opioid receptor antagonist, which blocks the antinociceptive effect of centrally acting analgesics/opioids. The tail-flick and hot-plate tests are sensitive in assessing the effectiveness of centrally acting analgesics[6]. The hot-plate test is a pain model in which opioid or central analgesics exert their analgesic effects via supraspinal and spinal receptors. The present study suggests that AEMK produces a central antinociceptive effect in a dose-dependent manner

which is maximized after 60 min, as evidenced by the prolonged tfls and paw-licking/jumping latencies. In addition, antinociceptive activity of AEMK started at 30 min and persisted up to 90 min period. $AUC_{0\text{-}120min}$ showed that this effect is close to that of morphine administered at a dose of 5 mg/kg. Further, naloxone administration blocked the antinociceptive activity of the AEMK in tail-flick and hot plate tests, suggesting the involvement of central opioid receptors. In formalin test, nociceptive behavior of the animal is observed in early and late phases reflecting neurogenic pain and inflammatory pain, respectively[9]. Centrally acting drugs inhibit both phases, while peripherally acting drugs only inhibit the second phase. 400 and 800 mg/kg doses are effective in both early and late phases, indicating central mechanism of its effect. Naloxone significantly inhibited the antinociceptive activity in early phase and partially in the late phase, implying that apart from exerting antinociceptive effect via opioid receptors, there could be involvement of mechanism other than opioid receptor stimulation.

## Conclusions

AEMK showed antinociceptive effect in tail-flick, hot plate and formalin tests in mice, which was blocked by naloxone, suggesting the involvement of opioidergic central mechanism. Further studies are required to evaluate the role of *Murraya koenigii* in clinical pain.

**Conflict of Interest**

None.

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