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Research Article

EVALUATION OF ANALGESIC ACTIVITY OF *MURRAYA KOENIGII* AND *CORIANDRUM SATIVUM* LEAVES EXTRACT IN ANIMAL MODEL

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ABSTRACT

Objective: The objective of the study was to investigate the analgesic activity of hydroalcoholic extract of *Murraya koenigii* and *Coriandrum sativum* leaves and compared it with standard drug in an animal model.

Methods: Hydroalcoholic extracts of *M. koenigii* and *C. sativum* leaves were obtained using Soxhlet apparatus. The central analgesic property was screened by hot plate method in mice and tail flick method in rats. The pain reaction time (PRT) was measured at 30, 60, and 120 min. The peripheral analgesic activity was evaluated by acetic acid induced writhing in mice.

Results: In hot plate method *M. koenigii* leaves extract at both doses and tramadol showed significant increase in PRT at 30, 60, and 120 min compared with control group. *C. sativum* leaves extract showed significant increase in PRT only at 60 and 120 min compared to control group. In tail flick method *M. koenigii* leaves extract at both doses, higher dose of *C. sativum* leaves extract and tramadol showed significant increase in PRT at 30, 60, and 120 min compared with control group. Higher dose of *M. koenigii l*eaves extract (200 mg/kg) was comparable with standard drug tramadol in both the methods. *M. koenigii l*eaves extract at both dose showed significant reduction in the number of writhing but *C. sativum l*eaves extract failed to show any significant reduction in the number of writhing compared with control. Higher dose of *M. koenigii* leaves extract was comparable with standard drug tramadol.

Conclusion: *M. koenigii* leaves extract showed both peripheral and central analgesic effect while *C. sativum* leaves extract showed only peripheral analgesic effect.

Keywords: Analgesic, Acetic acid, Tail flick, Hot plate, *Murraya koenigii, Coriandrum sativum.*

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INTRODUCTION

Pain is protective warning signal, primarily protective in nature, but causes discomfort and suffering. It is the most important symptom that brings the patient to physician. Inflammation and pain are common non-specific manifestations of many diseases. Although nonsteroidal anti-inflammatory drugs and opiates have been used classically in these conditions, some adverse reactions occur with these drugs such as gastrointestinal disturbances, renal damage, respiratory depression, and possible dependence [1,2].

It is, therefore, essential to find out novel effective analgesic agents with minimum side effects. Medicinal plants have been repeatedly considered as one of the main sources of medicines for the treatment of several health problems in humans. In India, 65% of the population in rural areas uses medicinal plants to help meet their primary health-care needs [3].

Despite such intensive research on various herbal medicines, many species of plants are still left unexplored. *Murraya koenigii* and *Coriandrum sativum* one such highly valued plant used for seasoning in Indian cooking.

M. koenigii, commonly known as *"Curry patta"* has wide array of properties that include antidiabetic, hypocholesterolemia, antidiarrheal, antimicrobial, anti-inflammatory, and antiulcer activities [4,5]. *C. sativum* is commonly known as "*Dhania"* possesses antidiabetic, hypolipidemic, and antibacterial activity [6-8].

From the perusal of literature it appears that, of all the biological effects studied on *M. koenigii* and *C. sativum*, the analgesic activities have been less investigated. Therefore, the present study was planned to evaluate the analgesic activity of *M. koenigii* and *C. sativum* leaves extract in experimental models.

METHODS

Collection and identification of plant materials

M. koenigii (curry leaf) and *C. sativum* (*Dhania*) were collected from the local market and authenticated by botanist. The leaves were then carefully cleaned, shade-dried, powdered, and stored in an airtight container for use.

Preparation of extract

The powder of both plants was extracted according to the procedure of Mahanta and Mukherjee using Soxhlet apparatus [9]. A total of 40–45 g of dried powder of each plant was packed in thimble and extracted using 70% ethanol and 30% distilled water (i.e., hydroalcoholic extract) at 5–55°C from morning 8 am to 4 pm for 6 days (total 48 h). The extracts were concentrated in a ventilated oven at 45°C for 24 h. It was dissolved in 0.5% carboxymethyl cellulose (CMC) before administering it to the experimental animals. The extracts were freshly prepared each time before using it in experiment.

Drugs and chemicals

Tablets of diclofenac sodium and tramadol of Novartis, Mumbai, were procured from local medical store. All other solvents and chemicals

of analytical grade used were obtained from SD fine – chemical, Mumbai.

Ethical clearance

Ethical clearance was taken from Institutional Animal Ethics Committee of the institute before commencement of the study.

Animals

Experimental animals used in this study were Wistar albino rats weighing 140±10 g and Swiss albino mice weighing 25±5 g of either sex. All animals were procured from King's Institute, Guindy, Chennai, and housed in central animal house of institute. The animals were housed in polypropylene cages, with dry paddy husk bedding and covered with stainless steel mesh lid. The environment of the room was maintained on a 12-h light/dark cycle at a constant room temperature of 26±2°C and relative humidity of 45–55%. The rats and mice had free access to standard rat and mice chow diet, respectively, and water *ad libitum*. The rats and mice were acclimatized to the surroundings for 1 week before the experiment. The animals were cared as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India.

Animal experimental design

Central analgesic activity

Hot plate method

Swiss albino mice weighing 25±5 g of either sex were used and divided into six groups of six animals in each group. Group 1 received 1% CMC orally as vehicle and was considered as control group. Group 2 and Group 3 received *M. koenigii* leaves extract at the dose of 100 mg/kg and 200 mg/kg body weight orally, respectively. Group 4 and group 5 received *C. sativum* leaves extract at the dose of 100 mg/kg and 200 mg/kg body weight orally, respectively. Group 6 received tramadol at a dose of 13 mg/kg orally and served as the standard control [10]. All the groups received same volume of preparations. The temperature of the hot plate was maintained at 55±1°C, mice were placed on the hot plate and the pain reaction time (PRT), or latency period determined with a stopwatch was recorded, which represented the time taken for the mice to react to the pain stimulus (paw licking or jumping) [11]. Cutoff time in the absence of response was 15 s to prevent the animals being burnt. Observations were made after administration of respective drugs at an interval of 30, 60, and 120 min.

Tail flick method

Wistar albino rats of either sex were used and divided into six groups of six animals in each group. Group 1 received 1% CMC orally as vehicle and was considered as control group. Group 2 and Group 3 received *M. koenigii* leaves extract at the dose of 100 mg/kg and 200 mg/kg body weight orally, respectively. Group 4 and group 5 received *C. sativum* leaves extract at the dose of 100 mg/kg and 200 mg/kg body weight orally, respectively. Group 6 received tramadol 9 mg/kg and served as the standard control [10]. Rats were placed into restrainer leaving tail exposed outside restrainer. A light beam was focused (exerting radiant heat) to the proximal third of the tail. PRT, i.e., tail flick was measured and recorded [12]. A cutoff time of 20s was imposed as a protection against tissue damage. Before doing actual experiment albino Wistar rats were screened for sensitivity test. Any animal that failed to withdraw its tail within 5 s was rejected from the study. Observations were made after administration of respective drugs at an interval of 30, 60, and 120 min.

Peripheral analgesic activity

Acetic acid induced writhing test

The peripheral antinociceptive activity of *M. koenigii* and *C. sativum* leaves extracts were assessed using acetic acid induced writhing test (abdominal constriction test) in mice [13]. Swiss albino mice weighing 25±5 g of either sex were used and divided into six groups of six animals in each group. Acetic acid solution (0.6%) in a dose of 10 ml/kg was injected intraperitoneal (i.p) to all the animals, 30 min after the administration of the test drug. Group 1 received 1% CMC as vehicle orally and was considered as control. Group 2 and Group 3 received *M. koenigii* leaves extract at the dose of 100 mg/kg and 200 mg/kg body weight orally, respectively. Group 4 and Group 5 received *C. sativum* leaves extract at the dose of 100 mg/kg and 200 mg/kg body weight orally, respectively. Group 6 received diclofenac sodium 10 mg/kg body weight orally and served as the standard control [14]. The numbers of contraction of the abdominal muscles together with stretching of hind limbs (writhes) were noted for 30 min, and percent inhibition of writhing was also calculated.

Statistical analysis

The results were presented as mean±standard deviation. Statistical analysis was performed using one-way analysis of variance followed by *post hoc* test Bonferroni. $p<0.05$ was considered as statistically significant.

RESULTS

Effect of *M. koenigii* and *C. sativum* leaves extract on PRT by hot plate method

The result showed that there was no significant difference in the PRT during the pre-drug testing time (O minutes) in all groups. The group which received *M. koenigii* leaves extract at 100 mg/kg bw and 200 mg/kg bw showed a significant increase in PRT compared to control group at 30, 60, and 120 min. The group which received *C. sativum* leaves extract at 100 mg/kg bw and 200 mg/kg bw showed a significant increase in PRT at 60 and 120 min but failed to show increase in PRT at 30 min compared to control group. The group which received a higher dose (200 mg/kg bw) of both plant extracts was found better than the lower dose (100 mg/kg bw). Standard drug tramadol was found better than both doses of *C. sativum* leaves extract, and lower dose of *M. koenigii* leaves extract at 30, 60, and 120 min but there was no significant difference between a higher dose of *M. koenigii* leaves extract and tramadol at 60 and 120 min (Table 1).

Effect of *M. koenigii* and *C. Sativum* leaves extract on PRT by tail flick method

The result showed that there was no significant difference in the PRT during the pre-drug testing time (O minutes) in all groups. The group which received *M. koenigii* leaves extract at 100 mg/kg bw and 200 mg/kg bw showed a significant increase in PRT compared to control group at 30, 60, and 120 min. The group which received *C. sativum* leaves extract at 100 mg/kg bw showed a significant increase in PRT at 60 and 120 min but failed to show significant increase in PRT at 30 min while higher dose 200 mg/kg bw showed significant increase in PRT at 30, 60, and 120 min compared to control group. The group which received higher dose (200 mg/kg bw) of both plant extracts was found better than the lower dose (100 mg/kg bw). Standard drug tramadol was found better than both doses of *C. sativum* leaves extract, and lower dose of *M.koenigii* leaves extract at 30, 60, and 120 min but there was no significant difference between a higher dose of *M. koenigii* leaves extract and tramadol at 60 and 120 min (Table 2).

Effect of *M. koenigii* and *C. Sativum* leaves extract on acetic acid-induced writhing

The groups which received *M. koenigii* leaves at a dose 100 and 200 mg/kg bw exhibited a significant reduction in writhing with approximately 30.98% and 60.56% of inhibition, respectively, when compared with control group. The groups received *C. sativum* leaves at a dose 100 and 200 mg/kg failed to exhibit a significant reduction in writhing with approximately 11.01% and 16.90% of inhibition, respectively, when compared with control group. Standard drug diclofenac showed a significant reduction in writhing with approximately 67.60% when compared with control group. There was no significant difference between higher dose of *M. koenigii* leaves extract and tramadol in reduction in writhing (Table 3).

DISCUSSION

This study was conducted to investigate analgesic activities of leaves extract of *M. koenigii* and *C. sativum,* highly valued plants used for

Table 1: Effect of oral administration of leaves extract of *M. koenigii* and *C. sativum* on pain reaction time by hot plate method in mice (n=6)

Groups (n=6)	Reaction time in seconds at various time intervals			
	0 min	30 min	60 min	120 min
Group 1 - Control vehicle	1.5±0.5	1.7±0.5	2.3±0.5	2.3±0.5
Group 2 - MK 100	1.5±0.5	2.3±0.5*	4±0.6*	6.5±0.5*
Group 3 - MK 200	1.7±0.5	2.7±0.5*	6.7±0.5*,#	8.5±0.8*,#
Group 4 - CS 100	1.3±0.5	1.7±0.5	4.5±0.5*	5.5±0.5*
Group 5 - CS 200	1.2±0.4	2±0.6	5.3±0.5*	6.2±0.8*
Group 6 - Tramadol (13 mg/kg)	1.7±0.5	5.2±0.4*	7.2±0.4*	9.2±0.4*

Data are expressed as mean±SD. n=6 in each group, *p<0.05 was considered as statistical significant when compared to control group, #not significant when compared to standard tramadol treated group. MK: *Murraya konigii* leaves extract, CS: *Coriandrum sativum* leaves extract, 100: 100 mg/kg bw, 200: 200 mg/kg bw, SD: Standard deviation

Table 2: Effect of oral administration of leaves extract of *M. koenigii* and *C. sativum* in pain reaction time by tail flick method in rats (n=6)

Groups (n=6)	Reaction time in seconds at various time intervals			
	0 min	30 min	60 min	120 min
Group 1 - Control vehicle	0.8±0.1	0.9±0.1	1±0.2	1.1±0.2
Group 2 - MK 100	0.8±0.2	1.4±0.5*	1.7±0.4*	2.5±0.4*
Group 3 - MK 200	0.8±0.1	3±0.6*	4.9±0.5*,#	6.2±0.8*,#
Group 4 - CS 100	0.7±0.1	1.3±0.5	2.2±0.4*	2.4±0.5*
Group 5 - CS 200	0.9±0.1	1.6±0.5*	3.8±1.2*	5.3±0.5*
Group 6 - Tramadol (9 mg/kg)	0.9±0.1	4.9±0.5*	5.4±0.4*	6.9±0.4*

Data are expressed as mean±SD. n=6 in each group, *p<0.05 was considered as statistical significant when compared to control group, #not significant when compared to standard tramadol treated group. MK: *Murraya konigii* leaves extract, CS: *Coriandrum sativum* leaves extract, 100: 100 mg/kg bw, 200: 200 mg/kg bw, SD: Standard deviation

Table 3: Effect of oral administration of hydroalcoholic leaves extract of *M. koenigii* and *C. sativum* in acetic acid-induced writhing test in mice

Groups (n=6)	Average number of writhes	Percent inhibition
Group 1 - Control vehicle	11.8±1.2	0
Group 2 - MK 100	8.2±0.8*	30.98
Group 3 - MK 200	4.7±1*	60.56
Group 4 - CS 100	10.5±1.2	11.01
Group 5 - CS 200	9.8±0.6	16.90
Group 6 - Diclofenac sodium (10 mg/kg)	3.8±0.8*	67.60

Data are expressed as mean±SD. n=6 in each group, *p<0.05 was considered as statistical significant when compared to control group, #not significant when compared to standard Tramadol treated group. MK: *Murraya konigeii* leaves extract, CS: *Coriandrum sativum*, 100: 100 mg/kg bw, 200: 200 mg/kg bw, SD: Standard deviation

seasoning in Indian cooking by experimental animal models. We investigated analgesic effect of two plants simultaneously to reduce the number of animals as in separate studies there will be need of extra control group and standard group.

The centrally mediated analgesic activity was evaluated using hot plate and tail flick methods [15]. The time is taken to withdraw tail from the thermal source and paw licking or jumping recorded as PRT in tail flick and hot plate method, respectively. The opioid-like analgesics increase threshold time of such tail flicking and paw licking [16]. These methods have also advantage of differentiating central opioid-like analgesics from peripheral analgesics. The behavior of pain resulting from this method is based on reflex mediated at spinal and supraspinal level. Tail flick is a spinal reflex [17], and hot plate is mostly supraspinally integrated response. Tramadol is considered as a potent opioid analgesic that activates opioid receptors (μ, δ, and κ) through the action on these receptors is weaker than morphine [18].

M. koenigii leaves extract and standard drug tramadol produced a significant increase in PRT compared to control group in hot plate model at 30, 60, and 120 min. *C. sativum* leaves extract failed to show any significant increase in PRN at 30 min but showed significant increase in PRN at 60 and 120 min when compared to control group. Higher dose of both plants extracts was found better than the lower dose. *M. koenigii* leaves extract was found better than *C. sativum* leaves extract in increasing the reaction time. Higher dose of *M. koenigii* leaves extract was comparable with standard drug tramadol.

Similar types of results were seen in tail flick method except higher dose of *C. sativum* leaves extract produced significant increase in PRT at 30 min also. These findings from this study suggest that *M. koenigii* and *C. sativum* leaves extract has central antinociceptive activity which may be due to their activation of opioid receptors.

The writhing induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. i.p administration of acetic acid in rats irritates serous membranes and provokes a stereotyped behavior in mouse known as writhing.The number of writhes is counted for 30 min following acetic acid injection. The percentage reduction in the number of abdominal contractions (writhing) indicates the level of analgesia in the acetic acid writhing reflex model [19]. i.p injection of acetic acid causes pain by liberating endogenous substances such as prostaglandins (PGs), serotonin, histamine, bradykinins, and substance P, which stimulate nerve endings [20]. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response [21].

M. koenigii leaves extract at both doses showed significant dose-dependent reduction in the number of writhing when compared to *C. sativum* leaves extract which failed to show significant reduction in the number of writhing when compared with control group. Standard drug diclofenac showed a significant reduction in the number of writhing when compared with the control group but there was no significant difference between higher dose of *M. koenigii* leaves extract and standard drug. This suggest that *M. koenigii* leaves extract has both central and peripheral analgesic activity while *C. sativum* leaves extract exerts only central analgesic activity.

The chemical compounds responsible for the analgesic effect of the extracts were not identified in the present study, and future studies are

planned. Qualitative phytochemical screening showed the presence of flavonoids, tannins, and alkaloids in *M. koenigii* leaves extract which may be responsible for analgesic activity as all the three constituents are well known for their ability to inhibit pain perception [22-26]. However, qualitative phytochemical screening of *C. sativum* showed only presence of the flavonoids and polyphenols [27,28]. This may be the reason behind the lower analgesic activity of *C. sativum* than *M. koenigii*.

CONCLUSION

The present study demonstrates the analgesic activity of two common seasoning plants *M. koenigii* and *C. sativum*. *M. koenigii* leaves extract showed both peripheral and central analgesic effect while *C. sativum* leaves extract showed only peripheral analgesic effect. The analgesic activity may be due to its agonistic action on opioid receptors while the peripheral analgesic effect may be due to inhibition of PG generation. The analgesic activity of *M. koenigii* leaves extract may be due to the presence of alkaloids, flavonoids, and tannins and in *C. sativum* leaves extract due to the presence of flavonoids and polyphenolic compounds. Further elaborative work is necessary for the better understanding of the mechanism of their antinociceptive activity of both plants.

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