

ANALGESIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF AERIAL PARTS OF *MALVASTRUM COROMANDELIANUM*BHUPESH CS^{1*}, ROHIT B², KALYANI D²¹Department of Pharmacology, Institute of Pharmaceutical Research, GLA University, Mathura, Uttar Pradesh, India. ²Department of Pharmacology, Acharya and B. M. Reddy College of Pharmacy, Bengaluru, Karnataka, India. Email: bhupesh115@gmail.com

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ABSTRACT

Objective: The aim of this study is to explore the antinociceptive property of hydroalcoholic extract of *Malvastrum coromandelianum*. Pain is an unpleasant sensory and emotional experience associated with actual and potential tissue damage. Various types of pain are seen in humans, somatic, visceral, and neuropathic pain. *M. coromandelianum* belongs to Malvaceae family. Traditionally, it is used as an emollient, anti-inflammatory, analgesic, and antidiysenteric.

Methods: The antinociceptive effects of hydroalcoholic extract of *M. coromandelianum* (HEMC) were evaluated by tail-flick, hot-plate, and acetic acid induced writhing method. Rats were divided into four groups. The first group which served as control was administered with aqueous 1% tragacanth suspension. The second group received standard drug, morphine (5 mg/kg) orally as a suspension. Third and fourth group received HEMC (200 and 400 mg/kg) and served as test drug treatment group, 30 minutes after treatment the reaction time and number of writhes was noted.

Results: HEMC produced significant antinociceptive effects against thermally induced pain. In tail-flick method, the peak effect of HEMC 400 mg/kg was shown at 60 minutes which is nearly equal to the peak effect of morphine 5 mg/kg. In hot-plate method, the HEMC showed significant analgesic effect up to 3 hrs after the treatment, whereas morphine showed significant effect up to 6 hrs.

Conclusion: On the basis of finding, it may conclude that the HEMC shows its central analgesic action probably through inhibition of central pain receptors, whereas peripheral analgesic effect may be mediated by prostaglandin inhibition.

Keywords: Pain, Analgesia, Hydroalcoholic extract of *Malvastrum coromandelianum*, Morphine.

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INTRODUCTION

Pain is a warning signal that helps to protect the body from tissue damage [1]. Damaging stimuli activate and sensitize primary afferent nerve cells whose activity is to project the spinal cord to the brain, causing pain sensation [2]. Various pains are acute, chronic, somatic, visceral, cutaneous neuropathic pain, and phantom limb pain. Acute pain is defined as short-term but extreme pain that comes on quickly but last only for a brief period of time. It is a symptom that may be dealt with unhesitatingly and effectively with drugs, by injection if necessary, at the same time as the causative disease is addressed [3]. Chronic pain was originally defined as pain that has lasted 6 months or longer. It is now defined as pain that outlasts the precipitating tissue injury [4]. Somatic pain originates from ligaments, tendons, bones, blood vessels, and even nerves themselves. It is detected with somatic nociceptors. Visceral pain is poorly localized, diffuse and often in the midline, with the exception of joints and the mesentery [5]. Cutaneous pain is caused by injury to the skin or superficial tissues. Cutaneous nociceptors terminate just below the skin and due to the high concentration of nerve endings, produce a well-defined, localized pain of short duration [6]. Neuropathic pain or "neuralgia" can occur as a result of injury or disease to the nerve tissue itself. This can disrupt the ability of the sensory nerves to transmit correct information to the thalamus and hence the brain interprets painful stimuli even though there is no obvious or known physiologic cause for the pain [7]. Phantom limb pain is the sensation of pain from a limb that has been lost or from which a person no longer receives physical signals. It is an experience almost universally reported by amputees and quadriplegics [8].

Malvastrum coromandelianum is commonly known as false mallow, broom weed or clock plant [9]. It is a weed of waste places and plantations. It is found in waste ground, roadsides, in abandoned city,

etc., [10]. The plant is considered to be emollient; leaves are applied to inflamed sores and wounds [11]. The essential oil from the leaves shows fungi toxicity against the damping-off fungi, *Pythium aphanidermatum* Fitzp (92.31%), *Pythium debaryanum* Hesse (82.22%), and *Rhizoctonia solani* Kuhn (72.22%) [12]. The flowers are given as a pectoral and diaphoretic. It is used as antipyretic. It has smooth muscle relaxant property [13]. It is used as an anti-inflammatory, pectoral, diaphoretic, and antidiysenteric agent [14].

METHODS**Plant material**

Crude drug, i.e., aerial parts of *M. coromandelianum* was collected from the forest of Ghulewadi range and dried under shade. The crude drug was authenticated from Botanical Survey of India by PSN Rao, Joint Director of BSI, Pune. Then, the crude drug was dried and minced with a grinder into a powder and passed through sieve number 40 for the preparation of the extract.

Preparation of the extract

The powder was packed into a soxhlet apparatus and extracted with petroleum ether (60-80°C) for 18 hrs. The same marc was successively extracted with chloroform and afterward with hydro-alcohol (30:70) for 18 hrs. The extracts were dried at 75°C in water bath for 5 hrs when a solid mass was obtained in the case of hydroalcoholic extract, respectively, and were stored in airtight containers in the refrigerator at below 10°C. The extracts thus obtained were subjected to phytochemical analysis for the various phytoconstituents.

Phytochemical estimations of the extracts

The preliminary phytochemical screening was carried out on the petroleum ether, chloroform, and ethanolic extract of aerial parts of *M. coromandelianum* for qualitative identification. Tests for common

phytochemicals were carried out by standard methods described in practical pharmacognosy books [15,16].

Experimental animals

Albino rats (Wistar) weighing 150-200 g and mice 20-25 g of either sex were used in this study. The animals were acclimatized for 1 week under laboratory conditions. They were housed in polypropylene cages and maintained at 30°C±2°C under 12 hrs dark/light cycle. They were fed with standard diet and water *ad libitum*. The ethical clearance for handling the animals was obtained from the Institutional Animal Ethics Committee before the beginning of the project work.

EXPERIMENTAL METHODS

Tail-flick method

Fasted rats of either sex were divided into four groups (n=6). Each rat served as its own control. Thus, before treatment, its reaction time was determined thrice at 0, 20 and 40 minutes intervals. The mean of these three determinations constituted the "initial reaction time" - i.e., reaction time before treatment of the rat. The first group which served as control was administered with aqueous 1% tragacanth suspension. The second group received standard drug, morphine (5 mg/kg) orally as a suspension. The hydroalcoholic extract of *M. coromandelianum* (HEMC) was administered orally at 200 mg/kg to the third group and 400 mg/kg to the fourth group as suspension. Thirty minutes after treatment the reaction time was again evaluated at 30, 60, 90, 120, 150 and 180 minutes [17].

Hot-plate method

Fasted rats were divided into four groups (n=6). Each rat served as its own control. Thus, before treatment, its reaction time was determined thrice at 0, 20 and 40 minutes intervals. The mean of these three determinations constituted the "initial reaction time." The first group which served as control was administered with aqueous 1% tragacanth suspension. The second group received standard drug, morphine (5 mg/kg intraperitoneal [IP]) as suspension. The HEMC was administered by IP route at 200 mg/kg to the third group and 400 mg/kg to the fourth group as suspension. Thirty minutes after treatment the reaction time was again evaluated at 0.5, 1, 2, 3, 6 and 24 hrs [18].

Acetic acid-induced writhing model

In this model, animals were pretreated with drugs 30 minutes before induction of writhing. The Group I animals received vehicle and Group II animals received the reference standard indomethacin (10 mg/kg p.o.). Analgesic activity of HEMC at doses 200 and 400 mg/kg p.o. (Groups III and IV) was accessed by counting the number of writhes induced by 0.7% acetic acid. The numbers of writhes per animal were counted for 20 minutes. Percent reduction in writhing syndrome was calculated and compared with the standard drug [19].

$$\text{Inhibition(\%)} = \frac{\text{Number of Writhes [Control]} - \text{Number of Writhes [Treatment]}}{\text{Number of Writhes [Control]}} \times 100$$

RESULTS

The preliminary phytochemical analysis like alkaloids, glycosides, carbohydrates, steroids, flavanoids, sponin, tannin, protein and amino acids was done with different extract of *M. Coromandelianum* according to standard procedure. The results of the preliminary phytochemical analysis of all the extracts were shown in Table 1.

Effect of analgesic activity of hydroalcoholic extract of *M. Coromandelianum* by tail-flick method

HEMC produced dose-dependent analgesic activity significantly (p<0.01) against tail-flick method. The antinociceptive effects of the HEMC by the tail-flick test in rats are summarized in Table 2. Pre-treatment with the extract significantly increased reaction time in

comparison to control. At a dose of 400 mg/kg of HEMC exhibited analgesic effect to the same degree as morphine 5 mg/kg up to 150 minutes and at 180 minutes its effect was significant but lesser than the morphine 5 mg/kg in comparison with control. The peak effect of HEMC 400 mg/kg was shown at 60 minutes which is nearly equal to the peak effect of morphine 5 mg/kg.

Effect of analgesic activity of hydroalcoholic extract of *M. coromandelianum* by Hot-plate method

The antinociceptive effect of HEMC by the hot-plate method was produced dose-dependently on mice and was showed in Table 3. At the dose of 200 mg/kg, the effect was not significant after 30 minutes of its administration but its 400 mg/kg dose produced significant (p<0.01) effect. The HEMC showed peak effect at the dose of 200 mg/kg and 400 mg/kg, respectively at 60 minutes after treatment while morphine was also showed peak effect at 60 minutes in comparison with control. Comparatively, the effect of high dose of HEMC was lesser than the effect of morphine. The HEMC showed significant analgesic effect up to 3 hrs after the treatment whereas morphine showed significant effect up to 6 hrs.

Effect of analgesic activity of hydroalcoholic extract of *M. coromandelianum* by Acetic acid induced writhing effect

The HEMC produced significant (p<0.01) reduction in the number of writhing in mice in dose-dependent manner. At 200 mg/kg and 400 mg/kg oral dose, percent reduction of writhing was 92.14% and 95.17%, respectively, as compared to control group, whereas the standard drug indomethacin (10 mg/kg p.o.) showed a reduction of 86.45% as shown in Table 4.

DISCUSSION

In indigenous system of medicine, several plants and herb have been used for the treatment of pain. *M. coromandelianum* is an herb which is used for the treatment of pain in various parts of Maharashtra district. Therefore, this study was an attempt to investigate the analgesic activity of *M. coromandelianum*. Preliminary phytochemical analysis showed that the HEMC revealed the presence of flavonoid, glycosides, saponin, triterpenes, and phenolic compounds. It is well-known reported that flavonoid, triterpenoid saponins, and phenolic compounds possess analgesic and inflammatory activity [19]. From the study, it was observed that the in all the screening model of analgesic activity the HECM showed analgesic effect and *M. coromandelianum* has both peripheral and central analgesic properties. Its central analgesic

Table 1: Phytochemical analysis of different extracts of *M. coromandelianum*

Pet ether	Chloroform	Hydro alcoholic		
Carbohydrates	Molish's test	-ve	-ve	-ve
	Benedict's test	-ve	-ve	-ve
	Feeling's test	-ve	-ve	-ve
Alkaloids	Mayer's test	-ve	-ve	-ve
	Wagner's test	-ve	-ve	-ve
	Dragendroff's test	-ve	-ve	-ve
	Hager's test	-ve	-ve	-ve
Glycosides	Modified borntagger's test	-ve	+ve	+ve
	Legal's test	-ve	+ve	+ve
Saponins	Froth test	+ve	+ve	+ve
Phytosterols and triterpenoids	Lieberman burchard test	+ve	+ve	+ve
	Salkowski's test (steroid)	+ve	+ve	+ve
Fats and oil	Stain test	+ve	+ve	+ve
Phenolic and tannins	Ferric chloride test	-ve	-ve	+ve
	Lead acetate test	-ve	-ve	+ve
Test for flavonoids	Shinoda test	-ve	-ve	+ve
Proteins and amino acids	Milon's test	-ve	-ve	-ve
	Biuret test	-ve	-ve	-ve
	Ninhydrin test	-ve	-ve	-ve

Table 2: Effect of analgesic activity of hydroalcoholic extract of *M. Coromandelianum* by tail-flick method

Treatment	Increase in reaction time (sec)					
	Different time interval (minute)					
	30	60	90	120	150	180
Control	-1.090±0.51	-0.412±0.55	0.178±0.86	0.060±0.73	0.060±0.73	-0.78±0.48
Morphine (5mg/kg)	6.146±0.58**	7.379±0.34**	3.829±0.45**	3.476±0.18**	2.651±0.27**	1.064±0.24**
HEMC (200mg/kg)	4.282±0.82**	5.112±0.71**	3.312±0.70**	3.095±0.44**	1.828±0.49**	0.650±0.41*
HEMC (400mg/kg)	4.690±0.70**	5.745±0.68**	3.453±0.51**	3.288±0.79**	1.998±0.40**	0.710±0.28*

**p<0.01: Very significant, *p<0.05: Significant, Number of animals n=6, values are expressed as mean±SEM, SEM: Standard error of mean, *M. coromandelianum*: *Malvastrum coromandelianum*, HEMC: Hydroalcoholic extract of *M. coromandelianum*

Table 3: Effect of analgesic activity of hydroalcoholic extract of *M. coromandelianum* by Hot-plate method

Treatment	Increase in reaction time (sec)					
	Different time interval (h)					
	0.5	1	2	3	6	24
Control	0.623±0.07	0.704±0.15	0.266±0.16	0.498±0.17	0.503±0.13	0.4842±0.19
Morphine (5 mg/kg)	5.493±0.73**	9.476±0.50**	6.976±0.64**	5.241±0.5**	2.491±0.45**	0.7242±0.19ns
HEMC (200 mg/kg)	2.415±0.58ns	3.177±0.49**	2.618±0.37**	1.808±0.24*	0.493±0.07ns	0.2700±0.06ns
HEMC (400 mg/kg)	5.281±1.19**	5.281±1.19**	5.281±1.19**	2.729±0.16**	1.083±0.20ns	0.6608±0.31ns

**p<0.01: Very significant, *p<0.05: Significant, Not significant: p>0.05, Number of animals n=6, values are expressed as mean±SEM, SEM: Standard error of mean, *M. coromandelianum*: *Malvastrum coromandelianum*, HEMC: Hydroalcoholic extract of *M. coromandelianum*

Table 4: Effect of analgesic activity of hydroalcoholic extract of *M. coromandelianum* by acetic acid induced writhing effect

Treatment	Number of writhing	Percentage reduction
Control	77±1.67	0.0
Indomethacin (10 mg/kg)	8.33±0.63*	86.45
HEMC (200 mg/kg)	4.83±0.47*	92.14
HEMC (400 mg/kg)	2.67±0.25*	95.17

*p<0.01: Very significant, Number of animals n=6, values are expressed as mean±SEM, SEM: Standard error of mean, *M. coromandelianum*: *Malvastrum coromandelianum*, HEMC: Hydroalcoholic extract of *M. coromandelianum*

activity was confirmed through tail-flick and hot-plate method and its peripheral analgesic property was confirmed by writhing test. Effect of HEMC on tail-flick response provides a confirmation of its central effect since the tail-flick is predominantly a spinal reflex and is considered to be selective for centrally acting analgesic compounds such as morphine, pethidine, while peripheral analgesic is known to be inactive on this kind of painful stimulus [20,21]. Nociceptive pathways activated in the tail-flick and hot-plate tests are not the same [22,23]. Therefore, in different antinociceptive tests the same opioid ligand can elicit different responses, even though the same receptors are involved. This is also supported by our results since the extent of analgesic activity exhibited by tail-flick method is greater than the hot-plate method. Its peripheral analgesic activity was confirmed from its inhibitory effects on chemical (acetic acid) induced nociceptive stimuli. The IP injection of acetic acid increase the mediators of pain especially prostaglandin E2 and prostaglandin 2 α which elicited writhings [24,25] (a syndrome characterized by a wave of abdominal musculature contraction followed by extension of the hind limbs). Moreover, acetic acid also increases the vascular cell permeability, mast cell degranulation, and smooth muscle contraction. Eosinophils and chemotaxis stimulate the nociceptor and produce pain response. Peripheral analgesic effect of plant's extract may be mediated by prostaglandin inhibition, whereas the central analgesic action of the extract probably mediated through inhibition of central pain receptors. Analgesic effect of extract exhibited after 30 minutes of its administration. Analgesic effect of extract last after 3 hrs and shows a maximum antinociceptive effect after 1 hrs its administration. Both the doses showed analgesic effect. The dose-dependent inhibition of writhings induced by acetic acid in this study

by *M. coromandelianum* suggests a peripherally mediated analgesic activity based on the association of the model with stimulation of peripheral receptors, especially the local peritoneal receptors at the surface of cells lining the peritoneal cavity. This suggests that chemical constituents by the virtue of them showed pharmacological action has a rapid onset of action and of the short duration of action.

CONCLUSION

The analgesic activity of *M. coromandelianum* was conducted, and a significant activity was found. Hence, it can be used to develop analgesic medications.

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