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Evaluation of analgesic and anti-inflammatory activity of alcoholic extract of leaves of *Malachra Capitata* in male Wistar rats

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ABSTRACT

Aim: The present study was carried out to evaluate analgesic and anti-inflammatory activity of alcoholic extract of leaves of *Malachra Capitata* in male Wistar rats.

Method: The collected plant material (Leaves) *Malachra capitata* was evaluated for its analgesic activity and anti-inflammatory activities. Analgesic activities was carried out by Acetic acid induced writhings and Tail immersion methods, were as anti-inflammatory activity was evaluated by Carageenan induced paw edema and cotton pellet granuloma method.

Result: The results revealed that in the analgesic activity the plant extract shown significant dose dependant inhibition of Writhing's and significant increase in time taken for removal of tail from hot water in treated animals were noted. In anti-inflammatory activity there was 41.38% reduction of edema volume and the extract shown dose dependent decrease in both dry and wet cotton pellets weight.

Conclusion: The present study revealed that *Malachra capitata* found to exhibit significant analgesic and anti-inflammatory properties.

Key words: Analgesic activity, Anti-inflammatory activity, *Malachra capitata*, Carageenan.

INTRODUCTION

Pain and Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. However, inflammation is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen [1].

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process [1, 2].

Drugs which are in use presently for the management of inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars [3, 4]. On the contrary many medicines of plant origin had been used since long time without any adverse effects.

In spite of tremendous development in the field of synthetic drugs during recent era, they were found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents.

Plants have been used in traditional medicine for several thousand years. The knowledge of medicinal plants has been accumulated in the course of many centuries' based on different medicinal systems such as Ayurveda, Unani and Siddha. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world [5, 6].

Plants and their extracts have immense potential for the management and treatment of inflammation. The phyto-medicines for inflammation are not only cheap and affordable but are also purportedly safe as hypersensitive reactions are rarely encountered with the use of these agents. However, there is a need for scientific validation, standardization and safety evaluation of plants of the traditional medicine before these could be recommended for inflammation. Keeping this fact in view, the present study was undertaken to investigate the analgesic and anti-inflammatory effects of the plant *Malachra capitata* (L.) [5].

Malachra capitata (L.) is an herb belongs to family: Malvaceae. Description: Mostly erect, coarse, annual or perennial herb 1-2 m tall, throughout densely whitish- or yellowish-tomentose with stellate hairs and usually also moderately to copiously hispid with simple or stellate hairs to 2 mm long; roots long-petioled; stipules lanceolate, 5-15 mm long; blades orbicular to ovate, 2-10 cm long, palmately sinuate to 3-, 5-, or 7-lobed, lobes mostly obtuse, crenate to serrate, the base obtuse or truncate; flowers in axillary, pedunculate, bracteate heads, bracts 1-2 cm long, stipitate and subtended by paired, filiform bracteoles, conduplicate, sub orbicular to ovate, obtuse or acute, entire or once or twice dentate, obtuse to cordate at base, prominently veined and whitish basocentrally; involucre bracts wanting; calyx tubular-campanulate, 4-8 mm long, 5-lobed to below middle,

lobes ovate-lanceolate, white with brownish or reddish nerves; petals yellow, obovate, 10-15 mm long, slightly exceeding staminal column; mericarps 3-3.5 mm long, muticous, reddish veined, puberulent; seed obovoid-cuneate, about 2.5 mm long, black, whitish-pubescent about hilum. The root of the *Malachra capitata* (L.) is a traditional remedy for the many diseased conditions such as pain, hepatic cirrhosis, inflammation, diarrhea, convulsion, dementia, pyrexia, ulcer, healing of wounds [7, 8, 9].

However, there is no ethno medicinal information and scientific findings for the above said traditional claim for pain and inflammation. Therefore, to justify the traditional claims the present study was undertaken to find out if alcoholic extract of *Malachra capitata* (L.) leaves demonstrates the analgesic and anti-inflammatory activity in rats. Hence, the present study was designed to verify the claims of the native practitioners.

MATERIAL AND METHODS

Collection of Plant Material

Malachra capitata Leaves was collected from Tirumala hills, Tirumala, Chittoor DT, A.P, India. The plant was identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi, A.P, and India.

Preparation of Extract:

The collected plant material (Leaves) *Malachra capitata* was washed thoroughly in water, and air dried for two weeks at 35-40°C temperature. Extraction was done by using Soxhlet apparatus with 70% methanol (alcoholic) as solvent. The extracts were concentrated under reduced pressure dried and stored at 4°C temp in air tight containers for further studies.

Phytochemical Screening:

The phytochemical examination of aqueous extract of leaves of *Malachra capitata* (L.) was performed by the standard methods [10].

PHARMACOLOGICAL ACTIVITY

Experimental animals

Healthy adult male rats weighing 150-200 gm's were obtained from Raghavendra enterprises (Bangalore). The animals were housed in stainless steel cages at a controlled room temperature of 24°C, under a 12 h light and 12 h dark cycle. After one week of acclimatization, the animals were used for experimentation. The experimental protocol was approved by the Institutional Animal Ethical Committee. (JKKMMR/FCP/IAEC/2013/003)

Acute toxicity studies

The acute toxicity of Methanolic Leaf extract of *Malachra*

capitata (L.) leaves was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, the doses of 200mg/kg and 400mg/kg were selected for further study [11].

Assessment of Analgesic activity:

Acetic acid induced Writhing Method

The method used in this test has been described by Koster R *et al*, 1959 [12]. The rats were divided into 5 groups of 6 animals each. The first group was given 10 ml/kg of normal saline i.p and served as control, the second group received Diclofenac sodium (50 mg/kg), p.o and served as standard and the groups 3 and 4 was treated with alcoholic leaves extract of *Malachra capitata* (200 & 400 mg/kg), p.o, and serves as treatment group. The total number of writhings following by intraperitoneal administration of acetic acid solution (1%, 10ml/kg) was recorded over a period of 10 min, starting from 5 min after acetic acid injection. The rats were treated with the alcoholic leaves extract of *Malachra Capitata* (200 and 400 mg/kg), or vehicle (tween 80 (5%)), 30 min before administration of acetic acid. The number of writhings and stretching was recorded and permitted to express the percentage of protection. The percentage inhibition of writhings was calculated using the expression.

$$\text{Inhibition (\%)} = \frac{\text{mean number of writhings (Control)} - \text{mean no of writhings (test)} \times 100}{\text{Mean number of writhings (control)}}$$

Tail immersion method

Healthy rats were selected and tested according to tail immersion method. The tip of the tail was placed in hot water which was maintained at $55 \pm 0.5^\circ\text{C}$ and tail withdrawal from water was taken as reaction time. 200mg/kg and 400mg/kg MEMC was given p.o and the reaction time readings were measured at 30min, 60min, 120min, 180min, and 240min. A cut off period of 25sec was observed to avoid damage to the tail.

Assessment of Anti-inflammatory activity:

Carageenan induced Paw edema:

Carageenan induced paw edema is one of the anti inflammatory activity to check the anti inflammatory response of the test drug under investigation. In this method the animals are divided into four groups of 6 animals each. Group I serves as control which is treated with normal saline, II serves as Standard which is treated with standard drug, Group III & IV serves as Test-1 & Test-2 which are treated with plant extract of 200 & 400 mg/kg body weight respectively. In this method initially Carageenan is administered to all groups of animals through intraplantar route and then the effect of test drugs for its anti inflammatory activity is compared with control and standard respectively and it is done as a 14 days study.

Cotton pellet Granuloma Method:

This study was carried out as described by Victor BO *et al*, 2003 [13]. A sterilized cotton pellet weighing 10mg was implanted subcutaneously into the groin region of rats after which four groups were treated (once daily) with 200mg/kg and 400mg/kg as low and high doses of extract for seven consecutive days. Animals in control and reference groups received normal saline and Dexamethasone Sodium Phosphate injection (0.5 mg/kg) respectively. The animals were sacrificed on the 8th day. Thereafter, the pellets surrounded by granuloma tissue were dissected out carefully and the weight of wet cotton pellets were noted and there after the cotton pellets were dried in oven at 60°C for 24 hrs to obtain a dry cotton pellet weight, the mean weight of granuloma tissue formed around each pellet was obtained and the percentage inhibition was determined.

$$\text{Percentage inhibition (\%)} = \frac{\text{Control-Treated} \times 100}{\text{Control}}$$

Statistical analysis

All the data was expressed as Mean $\pm$ S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad.). Statistical significance was taken as $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical investigation:

The results of preliminary phytochemical investigation of methanolic extract of *Malachra capitata* (L) leaves (MEMC) is as shown in **Table No 1**. It mainly shows the presence of carbohydrates, phenols, flavonoids, glycosides, terpenes, alkaloids, tannins, and saponins.

Table No 1: Showing the Qualitative Phytochemical analysis of *Malachra capitata*.

S.NO	Tests	Extract
1	Alkaloids	+
2	Carbohydrates	+
3	Proteins	—
4	Glycosides	+
5	Flavanoids	+
6	Tannins	+
7	Saponins	+
8	Fixed oils	—
9	Steroids	—
10	Phenols	+
11	Terpenes	+

Acute toxicity study

Acute toxicity study in which the animals treated with the MEMC at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioural changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Effect of the crude extract on Analgesic activity:***In acetic acid induced writhing test method***

The effect of methanolic leaf extract of *Malachra capitata* studied at the doses of 200mg/kg & 400mg/kg is as shown in the **Table No 2**. The results revealed that the methanolic extract of *Malachra capitata* shows dose dependant inhibition of Writhing's in treated animals.

In tail immersion Method:

The effect of methanolic leaf extract of *Malachra capitata* on tail immersion method is as shown in **Table No 3**. The results revealed that the MEMC showed dose dependent effect on tail immersion method, here the dose of MEMC at 400 mg/kg shown significant increase in time taken for removal of tail from hot water.

Effect of the crude extract on Anti inflammatory activity:***In Carageenan induced Paw edema:***

The MEMC showed decrease in paw edema. There was 41.38% reduction of edema volume in the rats treated with 200 mg/kg p.o. MEMC at 5 h. In standard drug treated group the paw edema reduced significant with time from 6.25% at 0 min to 93.10% at 5 h. the results are as shown in **Table No 4**.

In Cotton pellet granuloma method:

The results revealed that the extract at the dose of 400mg/kg had shown 55.3% inhibition in weight of wet cotton pellets and 64.06% inhibition in weight of dry cotton pellets, while the extract at the dose of 200mg/kg had shown 67% inhibition in weight of wet cotton pellets and 79.93% inhibition in weight of dry cotton pellets when compared to that of control group animals as shown in the following **Table No 5**. Therefore the decrease in granuloma weight indicates suppression of the proliferative phases, which was effectively inhibited by *Malachra capitata* in the present study.

Table No 2: Evaluation of analgesic activity of MEMC by acetic acid induced Writhings method.

S.No	Groups	Time(min)	No. of Writhings	%inhibition
1	CONTROL	5	12.5±0.5	0
2	DICLOFENAC(10mg/kg)	5	2.5±0.5***	0.83%
3	MEMC (200mg/kg)	5	5.5±0.5***	0.58%
4	MEMC (400mg/kg)	5	4.0±0.0***	0.66%

The data were expressed as Mean ± S.E.M (n=6). ; Tukey Kramer multiple comparison test: * p<0.05, **p<0.01, ***p<0.001.

Table No 3: Effect of methanolic leaf extract of *Malachra capitata* on tail immersion method.

S.No	Groups	Time				
		30 min	60 min	120 min	180 min	240 min
1	CONTROL	1±0.0	1±0.0	1±0.0	1±0.0	1±0.0
2	DICLOFENAC(10mg/kg)	4.5±0.5*	3.5±0.5*	3±0.0	2.5±0.5	1.5±0.5
3	TEST 1(MEMC 200mg/kg)	3.5±0.5*	3.0±0.0	2.5±0.5	1.5±0.5	1.0±0.0
4	TEST 2(MEMC 400mg/kg)	8.5±0.5***	6.5±0.5**	5.5±0.5	3.5±0.5**	1.5±0.5

One- way Analysis of Variance ANOVA: p value found to be 0.0001 is considered extremely significant. The data were expressed as Mean ± S.E.M (n=4); Tukey Kramer multiple comparison test * p<0.05, **p<0.01, ***p<0.001.

Table No 4: Effect of *Malachra capitata* on Carrageenan induced paw edema in Rats.

Treatment groups	Dose (mg/kg)	Inflammatory volume after carrageenan Injection					
		0hr	1hr	2hr	3hr	4hr	5hr
Control	Vehicle	0.16±0.01	0.18±0.01	0.19±0.04	0.22±0.01	0.24±0.01	0.22±0.01
Standard	10	0.15±0.01	0.15±0.01	0.13±0.01	0.11±0.01**	0.08±0.01**	0.02±0.01***
MEMC	200	0.16±0.01	0.15±0.01	0.14±0.01	0.13±0.01*	0.11±0.01*	0.06±0.01**
MEMC	400	0.17±0.01	0.15±0.01	0.14±0.01	0.12±0.01*	0.10±0.01**	0.04±0.01***

One- way Analysis of Variance ANOVA: p value found to be 0.0001 is considered extremely significant. The data were expressed as Mean ± S.E.M (n=4); Tukey Kramer multiple comparison test * p<0.05, **p<0.01, ***p<0.001

Table No 5: Effect of *Malachra capitata* on Cotton pellet granuloma model.

S.No	Treatment group	Mean of wet cotton pellets (mg)	Percentage inhibition	Mean of dry cotton pellets (mg)	Percentage inhibition
1.	Control	161.5±1.057	-	62.17±1.579	-
2.	Standard	81.65±1.057	50%	39.83±1.662**	64.06%
3.	MEMC (200mg/kg)	109.4±1.057	67%	47.21±0.8342*	79.93%
4.	MEMC (400mg/kg)	89.33±2.679	55.3%	39.83±1.740**	64.06%

One- way Analysis of Variance ANOVA: p value found to be 0.0001 is considered extremely significant. The data were expressed as Mean ± S.E.M (n=4); Tukey Kramer multiple comparison test * p<0.05, **p<0.01, ***p<0.001.

CONCLUSION

The present study revealed that *Malachra capitata* found to exhibit significant analgesic and anti-inflammatory properties. Further the phytochemical screening reveals the presence of Alkaloids, Carbohydrates, Glycosides, Flavonoids, Tannins, Saponins, Phenols and Terpenes. Therefore, it is assumed that these compounds may be responsible for the observed analgesic and anti-inflammatory activities.

Conflict of interest: Authors declare no Conflict of interest.

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