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RESEARCH ARTICLE

Analgesic and anti-inflammatory activity of crude leaf and bark extract of *Lantana Camara*

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ABSTRACT

The purpose of present study was to investigate the analgesic and anti-inflammatory activity of *Lantana camara (Verbenaceae*) leaf and bark extract. The methanol extract of *Lantana camara* (MELC) was screened for possible analgesic activity by acetic acid, hot plate and anti-inflammatory activity by carrageenan and histamine induced paw edema. The result showed that, MELC (100 mg/kg and 200 mg/kg) leaf and bark possessed significant (P <0.01 and P <0.05) anti-inflammatory response and 200 mg/kg dose of MELC leaf and bark showed more significant (P <0.01) analgesic activity as compared to the 100 mg/kg dose. Preliminary phytochemical screening result shows the presence of several phytochemicals which may responsible for its anti-inflammatory and analgesic actions. The results indicate the MELC showed great potential for analgesic and anti-inflammatory activity and may be useful for the medical purpose.

Keywords: *Lantana camara*; anti-inflammatory activity; analgesic activity.

1. Introduction:

From the thousands of the years, many countries are using the plants as a source of traditional medicine. Various Indian systems (Ayurveda, Unani, and Siddha) were using medicinal plants and their extracts for the treatment of various disorders [1]. The chemical diversity of plants has made them one of the main sources for the isolation of the active compounds. Inflammation is generally treated by opioids and non-steroidal anti-inflammatory drugs (NSAIDs). Severe side effects are produced by the both the class like renal damage, GI disturbances, and respiratory depression. Traditionally used plant show different uses [2, 3].

Though the different type of analgesic and anti-inflammatory agents are available in the market, to overcome their side effects, searching for the new effective agent from plant source is still progressing [4].

Lantana camara comprises about 150 varieties over 50 countries. It is evergreen shrub commonly called as wild sage and lantana weed. Different species of lantana have been used from many years in the treatment and medical condition such as for the ulcers, cuts, tumor, eczema [5-8].

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Various plant parts reported for its pharmacological properties like anti-lymphocytic and immunosuppressive, hepatoprotective, antimotility, anti-filarial, *in-vivo* cytotoxic and antimicrobial activity [9-12].

On the basis of above findings, the present work was performed to investigate the analgesic and anti-inflammatory potential of *L. camara* leaves methanol extract in different animal models.

2. Materials and methods

Collection and identification of plant material:

The leaf and bark of *Lantana camara* were procured from MPKV, Rahuri, Maharashtra, India. The identity of plant specimen is confirmed by botanist Dr. J. Jayanthi (HOD, Scientist, Govt. of India, BSI, Western, Regional Centre, Pune). A voucher with number SOSLAC2 was deposited to BSI, Pune.

Extraction of plant material:

The leaves and bark of plant was collected and washed thoroughly in distilled water and bark cut into small pieces. The leaves and bark was shade dried at room temperature. Dried parts were consistently ground using the mechanical grinder to make delicate powder. The soxhlet apparatus used for the extraction process, 100g powdered of leaves and bark was separately extracted in methanol. The filtrate was concentrated by using rotary evaporator for methanol elimination and both the extract were preserved for further use [13].

Drugs and chemicals

The reference standard drugs pentazocine and indomethacin was commercially purchased. The Carrageenan and acetic acid were obtained from Himedia Lab, Mumbai, India.

Preliminary Phytochemical screening

The phytochemical analysis of the extracts was carried out as per the standard procedure [14-16].

Experimental animals:

The animals were procured from NTC, Pune, India and kept under the standard conditions like temperature, humidity and light/dark cycle, and provide them standard diet and allowed to drink water *ad libitum*. For evaluation of analgesic and anti-inflammatory activity wistar albino rat (180-200g) and Swiss albino mice (25-30g) were used. The experiments were carried out as per the standards of institutional animal ethical committee guidelines for the care of laboratory animals of MES College of Pharmacy, Sonai, India. The protocol was approved by IAEC no. MESCOP-1211/PO/Re/S08/CPCSEA.

Acute toxicity

As per the Organization for Economic Cooperation and Development guidelines the acute oral toxicity study on wistar albino rat was performed [17].

Anti-inflammatory activity

Carragennan induced paw edema

The overnight fasted rats administered both the extracts, after 1h the carragennan (1 % w/v) suspension administered in the right hind paw by sub-planter injection to induce the inflammation. The six groups were prepared, each group consists six animals. The first group received 0.9 % normal saline in 3% Tween 80 (2ml/kg), and served as negative control, the second group received indomethacin (10 mg/kg), and served as a positive control, the third and fourth group received 100 mg/kg and 200 mg/kg leaves extract, the fifth and sixth group received 100 mg/kg and 200 mg/kg bark extract. After carragennan injection the animals were observed for change in paw volume at 0,1,3,4 and 5 h, the volume is measured by plethysmographically. Drugs were freshly prepared just before oral administration [18, 19].

Histamine induced paw edema

The 0.1% freshly prepared solution of histamine was used to induce the paw edema to the right hind paw of rats by sub-plantar administration. The change in paw volume was recorded at 0 and 1 h after histamine injection. Animals were divided into various groups to receive the leaves and bark extract (100 and 200 mg/kg) with 0.9 % normal saline in 3% Tween 80 (2ml/kg) as a negative control and indomethacin were administered at 10 mg/kg dose and serve as positive control. Prior to eliciting the paw edema the drugs and extracts were administered [20, 21].

Analgesic activity:

Acetic acid induced writhing:

The six groups of swiss albino mice were prepared with six animals in each group. The first group received normal saline 3% Tween 80 (2ml/kg), and serve as a normal control, the acetylsalicylic acid (100 mg/kg) received by second group, group third and fourth received 100 and 200mg/kg leaves extract, where as fifth and sixth group received 100 and 200mg/kg bark extract. One hour after administration of extract the acetic acid 0.6 % v/v (10ml/kg, i.p.) was injected to the positive and negative control groups. After acetic acid administration, for 20 min the number of writhes was counted [22, 23]. The extracts and acetylsalicylic acid suspended in tween 80 prior to administration and given orally.

Eddy's hot-plate method:

The six groups of swiss albino mice were prepared with six animals in each group. First group received normal saline in 3% tween 80 (2ml/kg) and served as normal control, second group received pentazocine (5 mg/kg), third and fourth group received 100 and 200 mg/kg leaves extract, fifth and sixth group received 100 and 200 mg/kg of bark extract. The individual animal place on the hot plate maintained at temperature of $(55+1)^0$C, 15 sec cut-off period considered to avoid the damage to the animal and response recorded for untreated mice such as paw licking or jumping whichever appear first considered at 0 min. [24, 25], the response time again observed after treatment at 30, 60,120 and 240 min, and changes in the reaction time were noted.

Statistical analysis

The one-way analysis of variance (ANOVA) used for the evaluation, and values were exhibits as Mean+SEM, followed by Dunnett's test for multiple comparisons using prism graphpad version 5.0, values of $P < 0.05$ were considered as statistically significant.

3. Result

Phytochemical Screening

The phytochemical analysis of the extract by qualitative study showed the presence triterpenoid and flavonoid and the absence of volatile oils, tannin, alkaloid and saponin.

Acute toxicity studies

Lantana extract at the dose of 2000 mg/kg does not exhibited any signs of toxicity up to 14 days and no animals died upon oral administration [26]. Therefore, the biological activity was carried out using 100 mg/kg and 200 mg/kg dose levels [27].

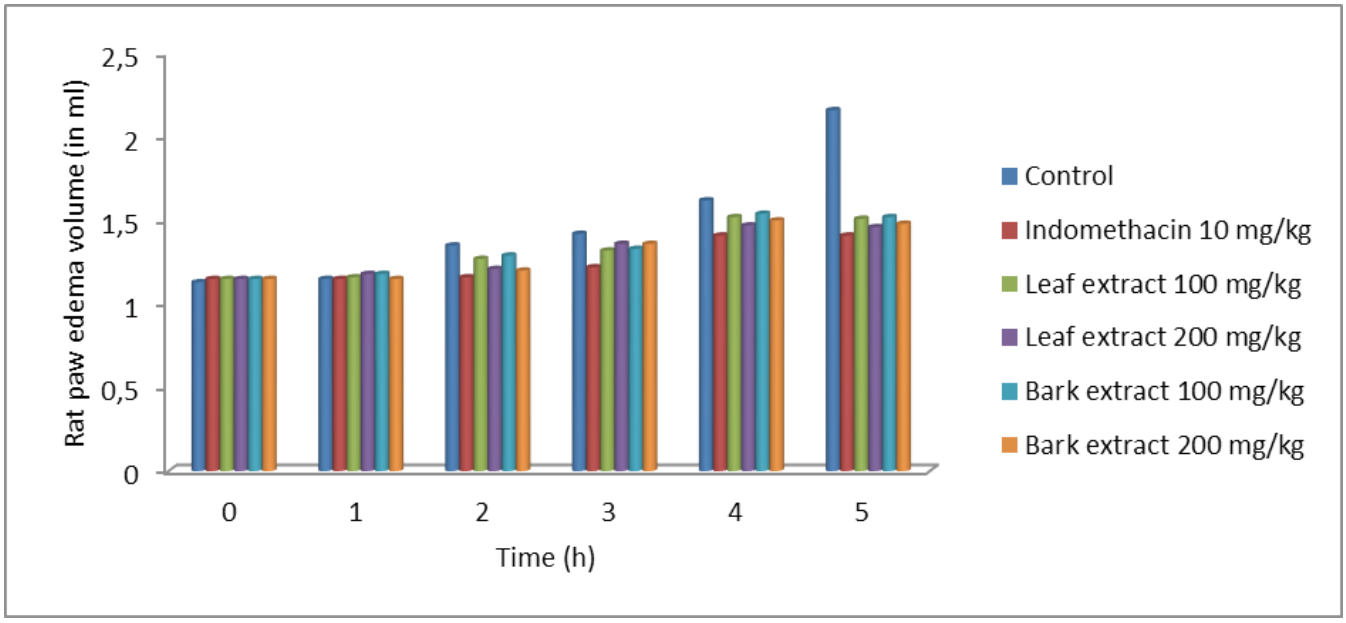
Carrageenan induced paw edema

The bark and leaves extract treated animals showed significant (P <0.01 and P <0.05) reduction in paw volume at 100 and 200mg/kg dose when compared with standard group from 2 h onwards (Figure 1 and Table 1). Both the extracts showed anti-inflammatory action up to 5h of both the doses. The extract treatment reduced inflammation in a dose-dependent manner.

Table 1. MELC effect on carrageenan induced paw edema.

Treatment	Dose (mg/kg)	Paw edema volume (mm)					
		0 h	1 h	2 h	3 h	4h	5h
Control		1.13 + 0.12	1.15 + 0.02	1.35 + 0.01	1.42 + 0.02	1.62 + 0.01	2.16 + 0.04
Indomethacin	10	1.15 + 0.02	1.15 + 0.22	1.16 + 0.03**	1.22 + 0.01**	1.41 + 0.01**	1.41 + 0.01**
Leaf Extract	100	1.15 + 0.02	1.16 + 0.21	1.27 + 0.00**	1.32 + 0.01**	1.52 + 0.02**	1.51 + 0.01**
Leaf Extract	200	1.15 + 0.03	1.18 + 0.01	1.21 + 0.01**	1.36 + 0.02**	1.47 + 0.02**	1.46 + 1.23**
Bark Extract	100	1.15 + 0.02	1.18 + 0.01	1.29 + 0.02*	1.33 + 0.01**	1.54 + 0.01**	1.52 + 0.02**
Bark Extract	200	1.15 + 0.03	1.15 + 0.02	1.20 + 0.01**	1.36 + 0.02**	1.50 + 0.02**	1.48 + 0.01**

Mean + SEM (n=6). **P <0.01, *P<0.05 compared with control group.



Mean + SEM (n=6). **P <0.01, *P<0.05 compared with control group.

Figure 1. MELC effect on carrageenan induced paw edema.

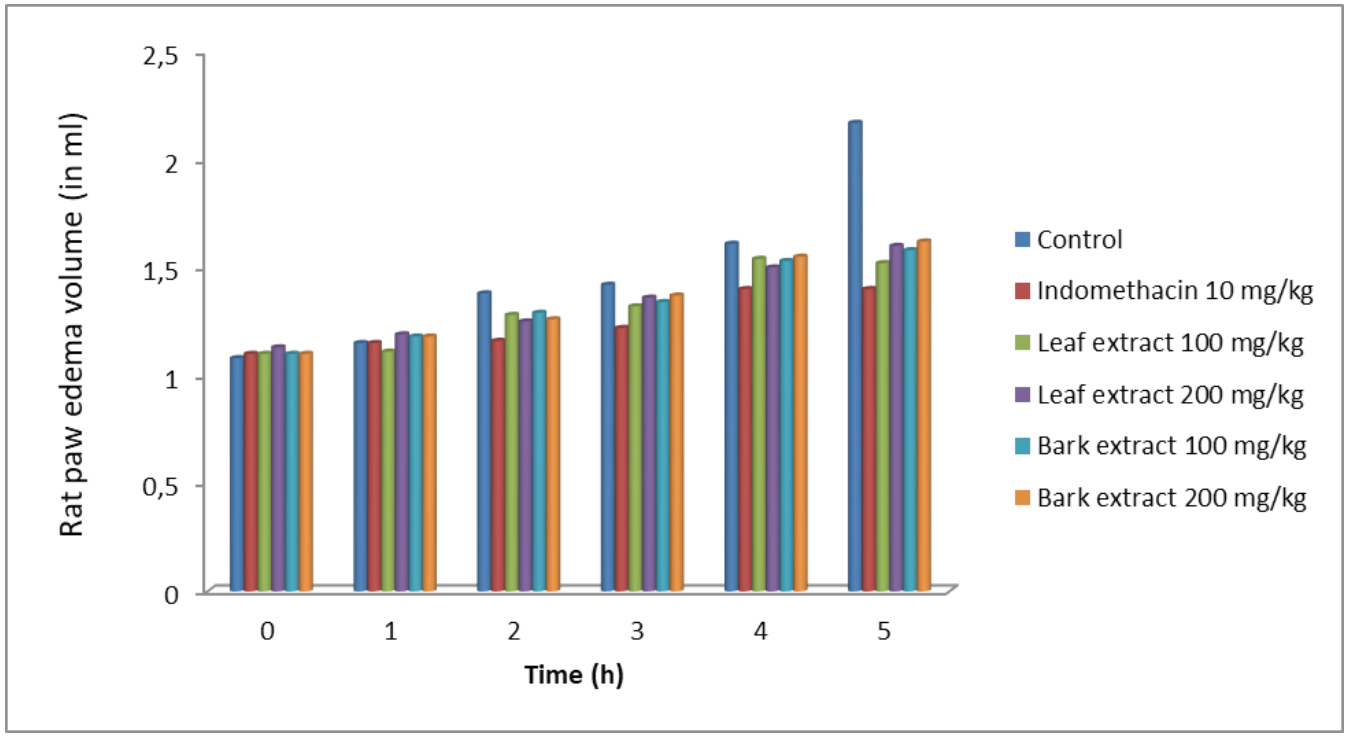
Histamine induced edema

The indomethacin, leaves and bark extract at both the doses showed significant(P <0.01 and P <0.05) reduction in histamine induced paw edema from 2h onwards as compared to the control group (Figure 2 and Table 2). Both the extract showed anti-inflammatory action up to 5h of both the doses. The extract shows dose-dependent anti-inflammatory actions.

Table 2. MELC effect on histamine induced paw edema.

Treatment	Dose (mg/kg)	Paw edema volume (mm)					
		0 h	1 h	2 h	3 h	4h	5h
Control		1.08 + 0.01	1.15 + 0.02	1.38 + 0.01	1.42 + 0.01	1.61 + 0.01	2.17 + 0.01
Indomethacin	10	1.10 + 0.02	1.15 + 0.02	1.16 + 0.03**	1.22 + 0.02**	1.40 + 0.00**	1.40 + 0.00**
Leaf Extract	100	1.10 + 0.03	1.11 + 0.03	1.28 + 0.02**	1.32 + 0.01**	1.54 + 0.01**	1.52 + 0.00**
Leaf Extract	200	1.13 + 0.03	1.19 + 0.00	1.25 + 0.01**	1.36 + 0.02**	1.50 + 0.02**	1.60 + 0.01**
Bark Extract	100	1.10 + 0.01	1.18 + 0.01	1.29 + 0.00**	1.34 + 0.03**	1.53 + 0.00**	1.58 + 0.02**
Bark Extract	200	1.10 + 0.02	1.18 + 0.02	1.26 + 0.01**	1.37 + 0.00**	1.55 + 0.01*	1.62 + 0.00**

Mean + SEM (n=6). **P <0.01, *P<0.05 compared with control group.



Mean + SEM (n=6). **P <0.01, *P<0.05 compared with control group.

Figure 2. MELC effect on histamine induced paw edema.

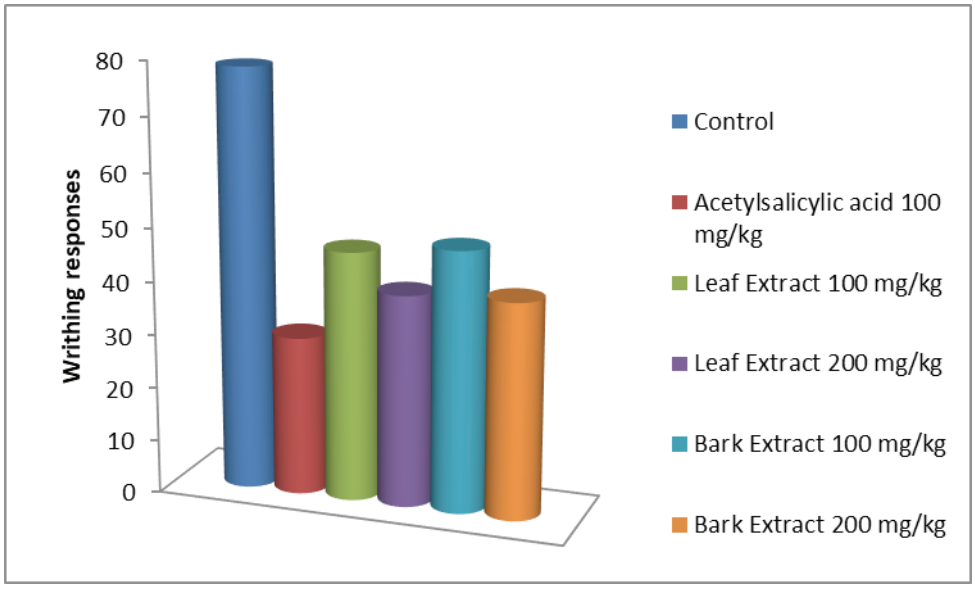
Acetic acid induced writhing

The acetic acid induced writhing responses in mice. Pretreated MELC and acetylsalicylic acid significantly (P < 0.01) inhibited writhing responses as compared to the control group (Figure 3 and Table 3). Also, the 200 mg/kg leaf and bark extract showed significant (P < 0.01) inhibition of writhing responses than 100 mg/kg dose.

Table 3. Effect of MELC on acetic acid writhing response.

Treatment	Dose (mg/kg)	No. of writhing/ 20 min
Control		78.5 + 0.22
Acetyl salicylic acid	100	29.66 + 0.42**
Leaf Extract	100	46.5 + 0.42**
Leaf Extract	200	39.5 + 0.42**
Bark Extract	100	48.5 + 0.22**
Bark Extract	200	40.16 + 0.40**

Mean + SEM (n=6). **P < 0.01 MELC leaf and bark extract 100, 200 mg/kg and acetylsalicylic acid 100 mg/kg compared with control group.



Mean + SEM (n=6). **P < 0.01 MELC leaf and bark extract 100, 200 mg/kg and acetylsalicylic acid 100 mg/kg compared with control group.

Figure 3. Effect of MELC on acetic acid writhing response.

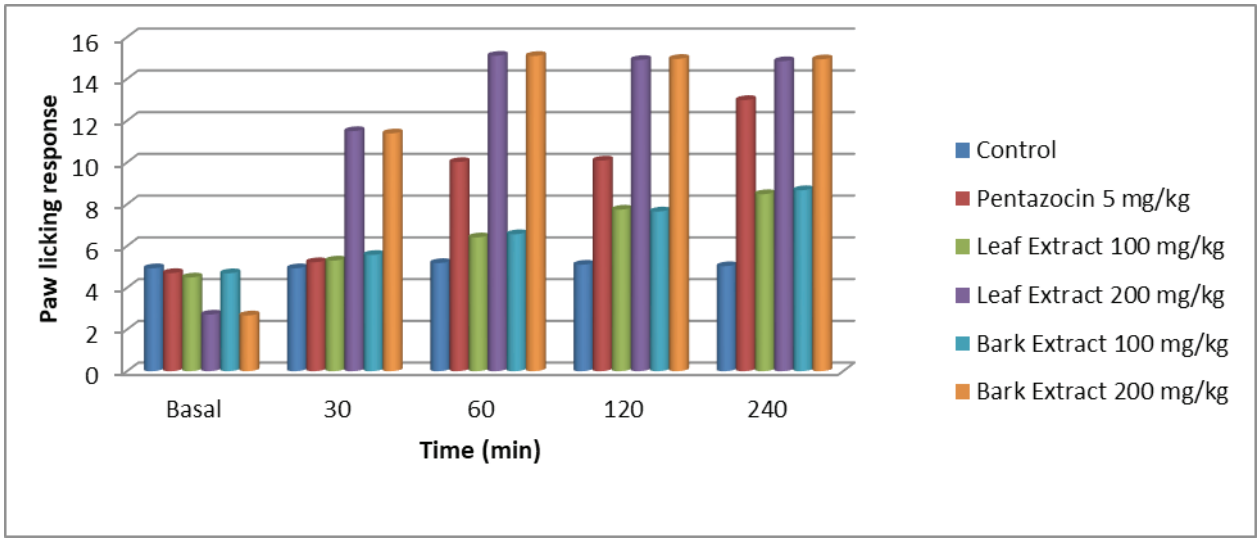
Eddy's hot-plate mediated pain reaction

The pentazocine and MELC (leaf and bark) showed significant (P <0.05 and P <0.01) increase in reaction time compared with control group at 100 and 200mg/kg dose. (Figure 4 and Table 4.) The MELC 200 mg/kg produced higher analgesic efficacy (P < 0.01) than the MELC 100 mg/kg dose.

Table 4. Effect of MELC on Eddy's hot-plate induced pain in mice.

Treatment	Dose (mg/kg)	Paw Licking response (Sec)				
		0 h	30 min	60 min	120 min	240 min
Control		4.93 + 0.03	4.93 + 0.03	5.18 + 0.15	5.1 + 0.03	5.03 + 0.05
Pentazocin	5	4.7 + 0.13	5.22 + 0.02	10.02 + 0.05**	10.09 + 0.06**	12.99 + 0.05**
Leaf Extract	100	4.49 + 0.11*	5.3 + 0.05	6.41 + 0.08**	7.74 + 0.11**	8.48 + 0.19**
Leaf Extract	200	2.71 + 0.12**	11.5 + 0.13**	15.11 + 0.04**	14.91 + 0.02**	14.85 + 0.03**
Bark Extract	100	4.69 + 0.10	5.57 + 0.11**	6.56 + 0.13**	7.65 + 0.11**	8.67 + 0.15**
Bark Extract	200	2.67 + 0.10**	11.39 + 0.18**	15.1 + 0.05**	14.96 + 0.03**	14.94 + 0.02**

Mean + SEM (n=6), **P < 0.01, *P < 0.05 MELC (leaf and bark) 100 and 200 mg/kg and pentazocine 5 mg/kg compared with control group.



Mean + SEM (n=6), $^{**}P < 0.01$, $^{*}P < 0.05$ MELC (leaf and bark) 100 and 200 mg/kg and pentazocine 5 mg/kg compared with control group.

Figure 4. Effect of MELC on Eddy's hot-plate induced pain in mice.

4. Dıscussıon

The various experiments are using carrageenan and histamine induced inflammation model for evaluation of anti-inflammatory activity. Due to the antigenic nature, reproducible and absence of apparent systemic effects carragennan model is commonly used. In the first phase of inflammatory response histamine, kinins and serotonin like inflammatory mediators released. The prostaglandins released in the second phase. Anti-inflammatory actions involved the inhibition of cyclooxygenase, which is responsible for formation of prostanoids, including thromboxane and prostaglandins. Carrageenan and histamine-induced edema are responsible for release of inflammatory mediators. The extract shows inhibition of paw edema induced by histamine and carragennan [28].

At 3, 4 and 5 h the MELC treated animals showed significant anti-inflammatory activity in carrageenan and histamine induced inflammatory model as compared to control group animals. We have conducted analgesic effects of both the extracts of *Lantana camara* for evaluation of peripheral and central anti-inflammatory action. Acetic acid showed induction of visceral pain, abdominal constrictions in mice due to the production of prostaglandin in the peritoneal fluid [29].

From the above findings, the analgesic action of both the extracts may be due to inhibition of the formation of inflammatory mediators or prevent transmission of inflammatory signals. In hot-plate model the pain perception take place by activation of central nociceptive action mediated at supraspinal level [30].

The opioid receptors mediated actions are responsible for the increase in reaction time after administration of MELC. The central and peripheral analgesic action of both the extract are dose dependant in both the models. The phenolic, flavonoid, and tannin compounds of MELC may responsible for its anti-inflammatory and analgesic action. Although the main chemical constituents of the plant that are the responsible for the analgesic and anti-inflammatory actions still remain speculative.

From above findings the present study represent that extract of *L. camara* leaf and bark possess anti-inflammatory and analgesic action. These findings support the folkloric use of *L. camara*.

Conflict of interest statement:

We declare that we have no conflict of interest.

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