Journal of Applied Pharmaceutical Science Vol. 7 (02), pp. 156-161, February, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.70221
ISSN 2231-3354 

Exploration of Analgesic and Antiinflammatory potential of *Lagerstroemia speciosa*

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ARTICLE INFO

Article history:
Received on: 09/11/2016
Accepted on: 03/12/2016
Available online: 27/02/2017

Key words:
Lagerstroemia speciosa,
Analgesic;
Antiinflammatory;
Antinociceptive, Banaba.

ABSTRACT

This study was designed to assess the analgesic and antiinflammatory actions of an aqueous ethanolic extract of *Lagerstroemia speciosa* L. (Family: Lythraceae). The analgesic investigations were carried out against two types of noxious stimuli, chemical (formalin-induced pain and acetic acid-induced writhing) and thermal (hotplate and tail immersion tests). The effects of standard drugs, aspirin and naloxone pretreatments were also studied. For antiinflammatory activities, the carrageenan-induced oedema of the hind paw of rats was used and the paw volume measured plethysmometrically 3h after injection. This was compared to a standard drug aspirin (300 mg/kg). The results were subjected to statistical analysis. The crude plant extract significantly increased the reaction time of hotplate and immersion tests. It decreased the writhings of acetic acid-induced abdominal contractions and lickings of formalin-induced pain. Aspirin had no effect on hotplate and tail immersion tests but showed an effect on writhing test. These results showed that the plant had both central and peripheral acting effects and this was confirmed by its effect on both phases of formalin-induced pain. The extract also significantly decreased the rat paw oedema volume at 200 mg/kg and above. In conclusion, *Lagerstroemia speciosa* has central and peripheral analgesic properties as well as antiinflammatory activities.

INTRODUCTION

Lagerstroemia speciosa L. is commonly known as banaba, and it has been traditionally used for the treatment of various disorders as diabetes, obesity and kidney malfunction (Klein *et al*., 2007; Stohs *et al.*, 2012). *Lagerstroemia speciosa* is a deciduous tropical flowering tree from family Lythraceae that grows in India, Bangladesh, Malaysia, Thailand, Philippines, Indonesia, and Japan. Today, it also grows in some parts of California and Florida in USA too. Most studies have focused on corosolic acid, which is isolated with an organic solvent from the leaves of the plant, and corosolic acid is used to standardize banaba extracts (Ulbricht *et al.*, 2007; Park and Lee, 2011).

Earlier phytochemical investigations have revealed that *Lagerstroemia speciosa* possessed triterpenes and sterols (Hou *et al.*, 2009; Ragasa *et al.*, 2005), phenolic compounds, including ellagic and gallic acid (Nutan *et al.*, 2013), ellagitannins and several flavonoids (Song *et al.*, 2013, Bai *et al.*, 2008). *In vivo* and *in vitro* studies indicated that banaba extract shows anti-α-glucosidase activity (Hou *et al.*, 2009), anti-HIV-1 protease and reverse transcriptase activities (Nutan *et al.*, 2013), anti-human rhinovirus activity (Song *et al.*, 2013), hypoglycemic activity (Bai *et al.*, 2008, Liu *et al.*, 2001) and free radical scavenging and anti-inflammatory effects (Priya *et al.*, 2008).

A survey of the literature showed that no detail studies on their potential antinociceptive activity have been undertaken. The present investigation reports pharmacological evaluation for antinociceptive and anti-inflammatory activities in experimental animals, in order to validate their popular use, for these pharmacological properties.

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MATERIAL AND METHODS

Plant Collection and Identification

The leaves of *Lagerstroemia speciosa* were freshly collected from the road side of Lucknow, Uttar Pradesh. The leaves were identified and authenticated taxonomically by Dr. A.K.S. Rawat, scientist, Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute (NBRI), Lucknow, India. The herbarium, (NBRI / CIF /256 / 2011), was preserved at department for future reference.

Preparation of extract

The matured leaves were collected, washed with distilled water to remove dirt and soil, and shade dried up-to 20-25 days. Routine pharmacognostic studies including organoleptic tests, macroscopic and microscopic observations were carried out to confirm the identity of the materials. The dried materials were powdered by grinder and passed through a 10-mesh sieve. The coarsely powdered leaves were defatted by immersing the powder into petroleum ether up-to 12 hrs by regular shaking. Extraction was done by hot continuous soxhlet apparatus using 50% alcohol at 60^0C for six hours. After extraction the excess solvent was removed by using a rotary evaporator (Buchi, USA) and then freeze-dried (Freezone® 4.5, Labconco, USA) at high vacuum (133×10^{-3} mBar) and at temperature -40 ± 2^0C (Rao *et al.*, 2003a). A net yield of 12.8 gm per 100gm was obtained. The collected extract was stored in air-tight glass container for further use in the experiments. For the pharmacological tests the extract was suspended in double distilled water containing carboxy methyl cellulose (1%, w/v, CMC).

The optimum conditions for experiments were decided on the basis of initial pilot experiments performed on three rats per treatment. *Lagerstroemia speciosa* leaves extracts (LSE) were administered at up to 1 g/kg to individual rats in group. There was no mortality due to the treatment. Hence, for further studies; 400 mg/kg (p.o.) of maximum dose was employed (Amresh *et al.*, 2008).

Animals

Male albino Wistar rats (200-220 g) and albino mice (18-25 g) were kept in the departmental animal house of National Botanical Research Institute, Lucknow at 27 ± 2^0C and relative humidity 42%-54%, light and dark cycles of 10 and 14 h, respectively, for one week before and during the experiments. Animals were provided with standard rodent pellet diet and the food was with drawn 18–24 h before the experiment thought, water was allowed *ad libitum*.

All the studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No.222/2000/CPCSEA). The standard orogastric cannula was used for oral drug administration.

Drug and chemicals

All chemicals used were of analytical grade. Acetic acid (0.6%), carrageenan, aspirin, indomethacin, (Sigma Chemicals, St. Louis, MO, USA), morphine sulphate, naloxone hydrochloride dihydrate and Formaldehyde were obtained from local firms (India) and were of highest grade purity. Water represents the double distilled water.

Pharmacological Activity

Antinociceptive Activity

Formalin-induced pain

Pain was induced by injecting 0.05 ml of 2.5% formalin (40% formaldehyde) in distilled water in the subplantar of the right hind paw. Rats (six per group) were given extract (LSE) (100, 200, 400, mg/kg, p.o.), Indomethacine (10 mg/kg) and 1% CMC (p.o.) 30 min prior to injecting formalin. These rats were individually placed in a transparent Plexiglass cage (25 cm × 15 cm × 15 cm) observation chamber. The procedure described by Zeashan et al. (2009) was used but with slight modifications. The amount of time spent licking the injected paw was indicative of pain. The number of lickings from 0 to 5 min (first phase) and 15–30 min (second phase) were counted after injection of formalin. These phases represented neurogenic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987).

Tail immersion

Tail immersion involved immersing extreme 3 cm of the rat's tail in a water bath containing water at a temperature of 55 ± 0.5^0C Aydin et al. (1999). Within a few minutes, the rat reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. Each rat served as its own control and two readings were obtained for the control at 0- and 10-min interval. The average of the two values was the initial reaction time (T_b). The test groups were given extract (LSE) (100, 200, 400 mg/kg, p.o.), aspirin (300 mg/kg), morphine (5 mg/kg, s.c.), naloxone+extract (1 mg/kg, i.p.+400 mg/kg) and 1% CMC (p.o.). The reaction time (T_a) for the test groups was taken at intervals 0.5, 1, 2, 4 and 6 h after a latency period of 30 min following the administration of the extract and drugs (Verma *et al.*, 2010). The cut-off time, i.e. time of no response was put at 120 s. The reaction time was measured and calculated as

$$\text{Percentage analgesic activity} = \frac{T_a - T_b}{T_b} \times 100\%$$

Hotplate

Mice were screened by placing them on a hot plate maintained at 55 ± 1^0C and the reaction time was recorded in seconds for fore paw licking or jumping. Only mice which reacted with in 15 s and which did not show large variation when tested on four separate occasions, each 15 min apart, were taken for the test (Amresh *et al.*, 2007c). Each rat (six per group) acted as its own control. Prior to treatment, the reaction time of each mouse (licking of the forepaws or jumping response) was done at 0- and 10-min interval. The average of the two readings was obtained as

the initial reaction time (T_b). The reaction time (T_a) following the administration of the extract (LSE) (100, 200, 400, mg/kg, p.o.), aspirin (300 mg/kg), morphine (5 mg/kg, s.c.), naloxone + extract (1 mg/kg, i.p. + 400 mg/kg) and 1% CMC (p.o.), was measured at 0.5, 1, 2, 4, and 6 h after a latency period of 30 mins. The following calculation was made:

$$\text{Percentage analgesic activity} = \frac{T_a - T_b}{T_b} \times 100\%$$

Acetic acid writhing reflex

Mice (six per group) were injected intraperitoneally with 0.6% acetic acid at a dose of 10 ml/kg. The extract LSE (100, 200, 400 mg/kg, p.o.), morphine (5 mg/kg, s.c.), naloxone+extract (1 mg/kg, i.p. + 400 mg/kg, p.o.) and 1% CMC (p.o.) were administered 30 min prior to treatment with acetic acid (Amresh et al. 2007b). The writhing induced by the acid, consisting of abdominal constrictions and hind limbs stretching, were counted for 30 min after a latency period of 5 min. The percentage analgesic activity was calculated as follows:

$$\text{Percentage analgesic activity} = \frac{N - N_I}{N} \times 100$$

Where, N is the average number of stretching of control per group. N_I is the average number of stretching of test per group.

Antiinflammatory activity

Carrageenan-induced hind paw oedema

The acute hind paw oedema was produced by injecting 0.1 ml of carrageenan (prepared as 1% suspension in 1% CMC) locally into the plantar aponeurosis of the right hind paw of rats (Winter *et al.*, 1962). LSE (100, 200 and 400 mg/kg, p.o.) was administered to three different groups while the other two groups served as negative and positive controls and received vehicle (1 ml/kg, p.o.) and standard drug, acetylsalicylic acid (Aspirin, 300 mg/kg, p.o.), respectively. LSE and Aspirin were administered 1 h prior to the injection of carrageenan.

The rat pedal volume up to the ankle joint was measured using plethysmometer (Ugo Basile, 7140 Comerio-varese, Italy) at 0 h (just before) and 3 h after the injection of carrageenan. Increase in the paw oedema volume was considered as the difference between 0 and 3 h (Amresh *et al.*, 2007a).

Percent inhibition of oedema volume between treated and control groups was calculated as follows:

$$\text{Percent inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_c and V_t represent the mean increase in paw volume in control and treated groups, respectively.

Statistical analysis

The results were analyzed using one-way analysis of variance followed by Dunnett's test using Graph Pad Prism 5.0 (Graph-Pad Software Inc., San Diego, California, USA). The data are expressed as mean ± SEM. The value of $P<0.05$ was considered statistically significant.

RESULT

Antinociceptive Activity

Formalin-induced pain

The extract had analgesic effects on both first (0–5 min) and second phases (15–30 min) of formalin test as shown on Table 1. These phases corresponded to neurogenic and inflammatory pains, respectively. Its neurogenic-induced pain blockade occurred only at 400 mg/kg (35.23%, $P < 0.001$) whereas 100 and 200 mg/kg, the extract significantly blocked pain emanating from inflammation significantly in a dose dependent manner in second phase. The extract was found to inhibit the neurogenic-induced pain (35.23%, $P < 0.001$) better than the pain resulting from inflammation (27.71%, $P < 0.01$). Indomethacin was significantly active (35.63%, $P < 0.01$) only in the second phase.

Tail immersion

After a latency period of 0.5 h following oral administration of the *Lagerstroemia speciosa* extract there was a significant reduction of painful sensation due to tail immersion in warm water at a dose of 200 mg/kg (11.11%, $P <0.05$), and it was dose and time dependent, see Table 2. The inhibitory effects of the extract became pronounced between 1 and 3 h post-dosing and reached a maximum of 38.09% ($P < 0.001$) with the dose of 400 mg/kg. The analgesic activity of the extract was blocked by naloxone. Aspirin had no effect on this test while the antinociceptive property of the extract at 400 mg/kg was not as effective as that of morphine.

Table 1: Effect of *Lagerstroemia speciosa* extract on formalin induced pain.

Treatment	Dose (mg/kg)	Number of Licks (s)			
		0-5 min		15-30 min	
		Score of pain	Percentage inhibition	Score of pain	Percentage inhibition
Control	0	65.75 ± 6.56	0	136.42 ± 5.94	0
Extract	100	62.27 ± 5.92	5.29	132.78 ± 7.21	2.67
Extract	200	56.61 ± 5.32	13.90	112.38 ± 5.63	17.62*
Extract	400	42.58 ± 4.69	35.23***	98.62 ± 4.38	27.71**
Indomethacin	10	56.36 ± 6.63	14.28	87.82 ± 4.12	35.63***

Values are expressed as mean ± S.E.M. for six rats (n=6), *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Table 2: Effect of *Lagerstroemia speciosa* extract on pain using tail immersion test.

Treatment	Dose (mg/kg)	Latency period (hr)						
		0	0.5	1	2	3	4	6
Control	0	2.51±0.11	2.49±0.20	2.63±0.09	2.53±0.32	2.62±0.12	2.42±0.17	2.48±0.12
Extract	100	3.09±0.23	2.81±0.27	2.94±0.08	2.32±0.37	2.27±0.91	2.13±0.37	2.24±0.23
Extract	200	2.31±0.91	2.57±0.23*	2.91±0.17*	3.19±0.97**	2.71±0.16	2.27±0.17	2.21±0.17
Extract	400	2.24±0.25	2.61±0.23*	3.21±0.16**	3.84±0.15***	3.32±0.21**	2.48±0.17	2.18±0.06
Aspirin	300	3.04±0.31	3.14±0.12	3.09±0.17	2.91±0.34	3.06±0.11	3.27±0.63	3.14±0.37
Morphine	5	3.92±0.38	9.29±1.59***	9.69±0.92***	8.18±1.28***	6.93±0.97***	5.87±1.52***	4.48±0.94*
Naloxone + Extract	1+400	2.49±0.29	2.51±0.31	2.54.0.21	2.57±0.18	2.53±0.18	2.47±0.36	2.46±0.31

Values are expressed as mean ± S.E.M. for six rats (n=6) and units are in seconds. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001.

Table 3: Effect of *Lagerstroemia speciosa* extract on pain induced by hotplate.

Treatment	Dose (mg/kg)	Latency period (hr)						
		0	0.5	1	2	3	4	6
Control	0	4.68±0.27	4.31±0.12	4.12±0.14	3.93±0.16	3.96±0.13	3.74±0.19	4.12±0.15
Extract	100	4.31±0.39	4.70±0.71	4.52±0.58	5.68±0.68**	5.32±0.51*	4.83±0.42	4.46±0.36
Extract	200	3.75±0.31	5.31±0.58**	5.83±0.46**	6.72±0.79***	5.26±0.89*	3.84±0.52	3.63±0.73
Extract	400	3.69±0.21	5.01±0.51**	6.82±0.63***	8.63±0.52***	5.73±0.32***	4.21±0.17*	3.71±0.38
Aspirin	300	4.71±0.12	4.83±0.35	4.81±0.27	4.86±0.31	4.79±0.11	4.63±0.16	4.12±0.37
Morphine	5	4.41±0.11	6.24±0.72***	7.41±0.63***	8.31±0.67***	6.73±0.71***	5.21±0.41*	4.39±0.09
Naloxone + Extract	1+400	4.75±0.31	4.83±0.24	4.69±0.23	4.83±0.27	4.96±0.37	5.03±0.29	4.47±0.58

Values are expressed as mean ± S.E.M. for six mice (n=6) and units are in seconds $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001.

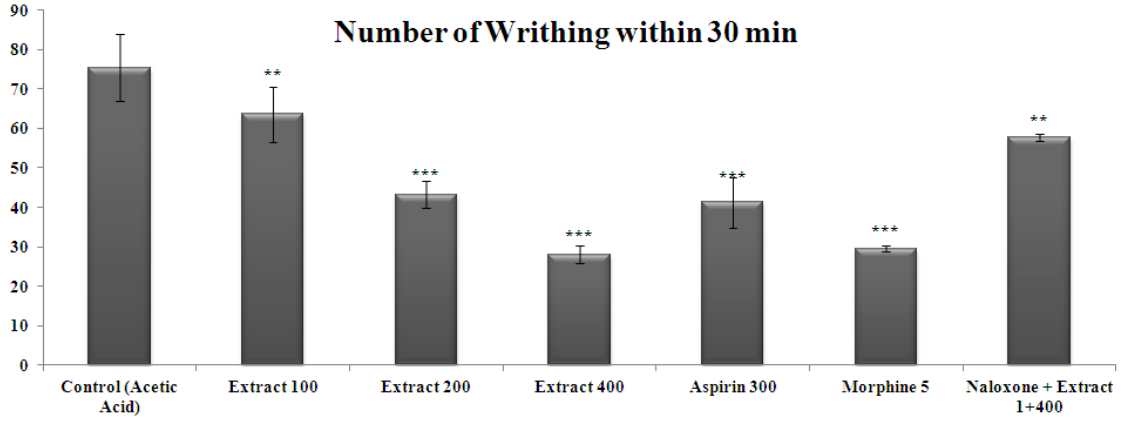

Fig 1: Effect of *Lagerstroemia speciosa* extract on writhing induced by acetic acid within 30 minutes.
Values are expressed as mean ± S.E.M., for six mice (n=6), Doses are expressed in mg/kg $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001.

Hotplate

The results of the hotplate test are summarized in Table 3 revealed that the reaction time for the mice were significantly increased from the dose of 100 mg/kg (32.38%, P < 0.01) and above. This augmentation in reaction time reached 133.87% (P < 0.001) with 400 mg/kg. Pretreatment with naloxone (1 mg/kg) drastically reduced the analgesic potentials of the extract. In the presence of naloxone, the effect of the extract was inhibited. Aspirin at 300 mg/kg did not offer any protection against the heat-induced pain. Morphine sulphate at 5 mg/kg significantly showed its maximum protective effect of 88.43% (P < 0.001) after 2 h compared to 133.87% (P < 0.001) for 400 mg/kg of the extract.

Acetic acid writhing reflex

Writhings and stretchings induced by 0.6% acetic acid at a dose of 10 ml/kg was reduced by *Lagerstroemia speciosa* significantly. The significant protective effect was dose dependent with 15.64% (P < 0.01) reduction observed for 100 mg/kg to 62.78% (P < 0.001) seen for 400 mg/kg dose. Aspirin (300 mg/kg) had only 45.28% (P < 0.001) inhibition and morphine (a centrally acting analgesic) had 60.89% (P < 0.001) inhibition. Pretreatment with naloxone blocked the protective effect of the *Lagerstroemia speciosa* extract (Fig 1.).

Antiinflammatory activity

Carrageenan-induced hind paw oedema

The mean increase in paw oedema volume was about 0.89±0.11 ml in the vehicle-treated control rats. LSE (200 and 400 mg/kg, p.o.) significantly (P < 0.01) reduced the mean paw oedema volume at 3 h after carrageenan injection. LSE (100, 200 and 400 mg/kg, p.o.) exhibited antiinflammatory activity in a dose-dependent manner with the percentage inhibition of paw oedema 31.46, 53.93 and 62.92, respectively as compared to control group. However, the standard drug, aspirin (300 mg/kg, p.o.) showed highly significant (P < 0.001) anti-inflammatory activity with the percent inhibition of 76.40 (Fig 2).

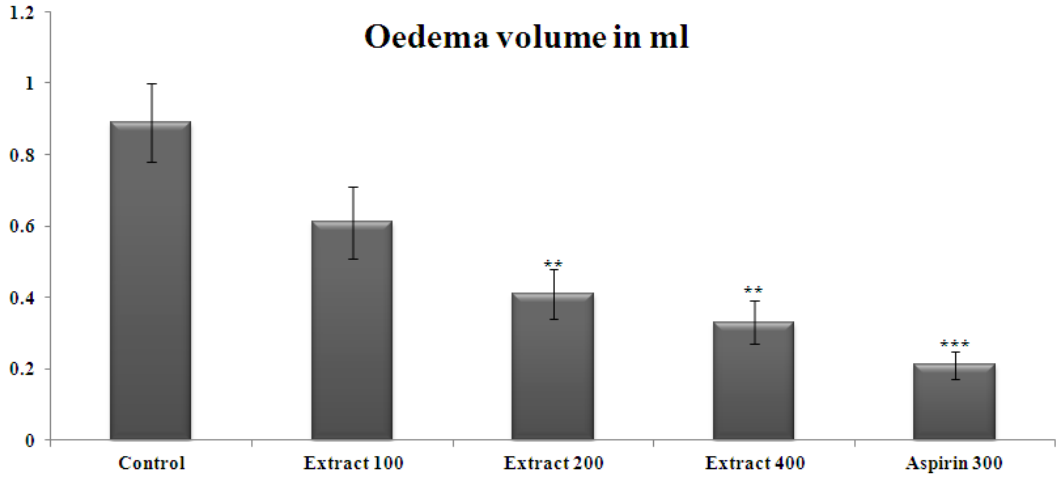

Fig 2: Percentage inhibition of *Lagerstroemia speciosa* extract on carrageenan induced oedema.
Values are expressed as mean ± S.E.M., for six rats (n=6), Doses are expressed in mg/kg. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

DISCUSSION

The study indicated that 50% aqueous ethanolic extract of *Lagerstroemia speciosa* leaves has both peripheral and central analgesic properties. Its peripheral analgesic activity was deduced from its inhibitory effects on chemically (acetic acid and formalin, inflammatory phase) induced nociceptive stimuli (Rao *et al.*, 2005). At 200 mg/kg (42.59%), the peripheral analgesic action of the extract on acetic acid induced pain was found to be comparable to 300 mg/kg (45.28%) of aspirin but less so with inflammatory phase of formalin test (27.71%) at the dose of 400 mg/kg. The centrally acting protective effects of the extract were corroborated by the first phase of formalin-induced pain, hotplate and immersion tests results (Rao *et al.*, 2003b). In these experiments, aspirin or indomethacin was inactive. The tail immersion test indicated that the pharmacological actions were mediated by mu (μ) opioid receptors rather than kappa (k) and delta (δ) receptors (Schmauss and Yaksh, 1984; Aydin *et al.*, 1999).

A comparison done on acetic acid-induced pain showed that morphine at 5 mg/kg had 60.89% ($P < 0.001$) protection similar to 400 mg/kg of the extract (62.78%, $P < 0.001$). Similar comparison between effects of morphine sulphate and *Lagerstroemia speciosa* on tail immersion test showed that at 5 mg/kg, morphine sulphate had very potent analgesic effect with significant percentages of inhibition ranging from 49.74% to 147.19% compared to the extract (16.51%–71.42%). The fact that the neurogenic (0–5 min) pain was significantly blocked by the extract meant that it also acted through opioid receptors which were more centrally located than peripheral. This showed that the extract was a weaker opioid receptor agonist (Amresh *et al.*, 2007b). Due to their central location, a higher therapeutic concentration (400 mg/kg) of the extract was therefore required for the analgesia as revealed by the first phase of formalin-induced pain test (Verma et al, 2012).

The carrageenan test was selected because of its sensitivity in detecting orally active antiinflammatory agents particularly in the acute phase of inflammation (Rao *et al.*, 2005). The antiinflammatory effects of *Lagerstroemia speciosa* extract on acute inflammatory process such as carrageenan -induced oedema in rat paw was dose dependent. At the dose 200 mg/kg, the extract showed at least 53.93% inhibitory activity throughout the measurement intervals and the efficacy of aspirin (300 mg/kg) was comparable to 400 mg/kg of the extract. Aspirin blocks the production of prostaglandins by inhibiting cyclooxygenase (prostaglandin H synthase), with greater selectivity towards the COX-1 isoform.

These data validated the traditional uses of this plant in the diseases like pain, headache as well as inflammatory diseases like gout, rheumatism, cystitis and nephritis. Although these inflammatory diseases are chronic in nature, this study has focused mainly on the acute inflammatory properties of the plant.

CONCLUSION

It can be concluded that the 50% ethanolic extract of *Lagerstroemia speciosa* leaves showed an antiinflammatory and antinociceptive activity (peripheral and centrally acting) by the various models used in the present study.

Financial support and sponsorship: Nil.

Conflict of Interests: There are no conflicts of interest.

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How to cite this article:

Gupta A, Agrawal VK, Rao CV. Exploration of Analgesic and Antiinflammatory potential of *Lagerstroemia speciosa.* J App Pharm Sci, 2017; 7 (02): 156-161.