Exploration of Analgesic and Antiinflammatory potential of Lagerstroemia speciosa

Amresh Gupta¹*, Vipin K. Agrawal², Chandana Venkateswara Rao³

¹Department of Pharmacognosy, Faculty of Pharmacy, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, U.P., India.
²Department of Pharmaceutics, Invertis Institute of Pharmacy, Invertis University, NH 24 Lucknow Bareilly Highway, Bareilly, UP, India.
³Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Council of Scientific and Industrial Research, Lucknow, India.

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.

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.

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 banana extract shows anti-a-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.
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 Ethnomedecumanalogy 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°C 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°C (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°C 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.), Indomethacin (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°C 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 (T₀). 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 (Tₕ) 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

Percentage analgesic activity =\(\frac{T₀ - Tₕ}{T₀}\) × 100%

Hotplate
Mice were screened by placing them on a hot plate maintained at 55 ±1°C 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 \((T_c)\) 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:

\[
\text{Percentage analgesic activity} = \frac{N - N_t}{N} \times 100\%
\]

Where, \(N\) is the average number of stretching of control per group. \(N_t\) 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)** | **0-5 min**      | **15-30 min**    | **Score of pain** | **Percentage inhibition** | **Score of pain** | **Percentage inhibition** |
| Control          | 0                | 65.75 ± 6.56     | 136.42 ± 5.94    | 0                | 0.001               | 136.42 ± 5.94     | 2.67                        |
| Extract          | 100              | 62.27 ± 5.92     | 132.78 ± 7.21    | 5.29             | 0.001               | 132.78 ± 7.21     | 2.67                        |
| Extract          | 200              | 56.61 ± 5.32     | 112.38 ± 5.63    | 13.90            | 0.001               | 112.38 ± 5.63     | 17.62**                     |
| Extract          | 400              | 42.58 ± 4.69     | 98.62 ± 4.38     | 35.23**          | 0.001               | 98.62 ± 4.38      | 27.71**                     |
| Indomethacin     | 10               | 56.36 ± 6.63     | 87.82 ± 4.12     | 14.28            | 0.001               | 87.82 ± 4.12      | 35.63**                     |

Values are expressed as mean ± S.E.M. for six rats (n=6), \(P<0.05\), \(P<0.01\), \(P<0.001\).
with 15.64% 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**

*Carageenan-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).

**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 88.43% (P < 0.001) after 2 h.
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 isofrom.

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.

REFERENCES

Amresh G, Singh, PN, Rao ChV. Antinociceptive and antiarthritic activity of 

Aydin S, Demir T, Ozturk Y, Baser KHC. Analgesic activity of 

Bai N, He K, Roller M, Zheng B, Chen X, Shao Z, Peng T, 
Zheng Q. Active compounds from *Lagerstroemia speciosa*. insulin-like 
glucose uptake-stimulatory/inhibitory and adipocyte differentiation- 
inhibitory activities in 3T3-L1 cells. J Agric Food Chem, 2008; 56(24): 
11668–11674.

Hou W, Li Y, Zhang Q, Wei X, Peng A, Chen L, Wei Y. 
Triterpene acids isolated from *Lagerstroemia speciosa* leaves as 

Hunskaar S, Hole K. The formalin test in mice: dissociation 
103–114.

Hussain Z, Amresh G, Singh S, Rao ChV. Antinociceptive 
activity of *Amaranthus spinosus* in experimental animals. J Ethnopharm, 
2009; 122(3): 492–496.

Klein G, Kim J, Himmeldirk K, Cao Y, Chen X. Antiobesity 
and anti-obesity activity of *Lagerstroemia speciosa*. Evid Based 

Liu F, Kim JK, Li YS, Liu XQ, Li J, Chen XZ. An extract of 
*Lagerstroemia speciosa* L. has insulin-like glucose uptake-stimulatory and 
adipocyte differentiation-inhibitory activities in 3T3-L1 cells. J Nutr, 
2001; 131(9): 2242–2247.

Nutan M, Goel T, Das T, Malik S, Suri S, Rawat AKS, 
Srivastava SK, Tuli R, Malhotra S, Gupta SK. Ellagic acid & gallic acid 
from *Lagerstroemia speciosa* L. inhibit HIV-infection through inhibition 
of HIV-1 protease & reversetranscriptase activity. India J Med Res, 

Park C, Lee JS. Banaba: the natural remedy as anti diabetic 

Priya TT, Sabu MC, Jolly CI. Free radical scavenging and anti 
inflammatory properties of *Lagerstroemia speciosa* (L.). 

Ragasa CY, Ngo HT, Rideout JA. Terpenoids and sterols from 

Rao ChV, Amresh G, Kartik R, Irfan A, Rawat AKS, 
Pushpangadan P. Protective effect of *Aegle marmelos* fruit in 
gastrointestinal dysfunction in rats. Pharmaceutical Biology, 2003a; 41(8): 
358-563.

Rao ChV, Kartik R, Ojha SK, Amresh G, Rao GMM. 
Antiinflammatory and antinociceptive activity of stem juice powder of 
*Tinospora cordifolia* Miers. in experimental animals. Hamdard Medicus, 

Rao ChV, Ojha SK, Amresh G, Mehrotra S, Pushpangadan P. 
Analgesic, anti-inflammatory and antiulcerogenic activity of the unripe 
fruits of *Aegle marmelos*. Acta Pharmaceutica Turcica, 2003b; 45(2): 85-
91.

Schmauss C, Yaksh TL. In vivo studies on spinal receptor 
systems mediating antinociceptive. II. Pharmacological profiles suggesting 
a differential association of mu, delta and kappa receptor with visceral 
chemical and cutaneous thermal stimuli in the rat. J Pharmacol Exp Ther, 
1984; 228(1):1-12.

Song JH, Park KS, Kwon DH, Choi HJ. Anti-human rhinovirus 
2 activity and mode of action of quercetin-7-glucoside from 

Stohs SJ, Miller H, Kaats GR. A review if the efficacy and 
safety of banaba (*Lagerstroemia speciosa* L.) and corosolic acid. 

Ulbricht C, Dam C, Milkin T, Seamon E, Weissner Woods J. 
Banaba (*Lagerstroemia speciosa* L.); an evidence-based systematic review 
by the natural standard research collaboration. Journal of Herbal 

Verma N, Amresh G, Sahu PK, Mishra N, Rao ChV, Singh AP. 
Anti-inflammatory and antinociceptive activity of hydroethanolic extracts 
of *Woodfordia fruticosa* Kurz flowers. Der Pharmaceutica Sinica, 2012; 

Verma N, Singh AP, Amresh G, Sahu PK, Rao ChV. Anti 
inflammatory and anti-nociceptive activity of *Rhododendron arboresum*. 

Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema 
in hind paws of the rats as an assay for anti-inflammatory drugs. In: 
Proceedings of the Society for Experimental Biology and Medicine, 1962; 

---

**How to cite this article:**

Gupta A, Agrawal VK, Rao CV. Exploration of Analgesic and 
Antiinflammatory potential of *Lagerstroemia speciosa*. J App 