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EVALUATION OF ANTI-INFLAMMATORY PROPERTIES OF ETHANOLIC LEAF, FLOWER AND SEED EXTRACTS OF *LAGERSTROEMIA SPECIOSA* (L.) PERS (LYTHRACEAE) AGAINST CARRAGEENAN-INDUCED ACUTE INFLAMMATION IN ALBINO RATS

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ABSTRACT

Lagerstroemia speciosa medicinal properties suggest that it may have anti-inflammatory effects on acute inflammation. The present study was performed to investigate the anti-inflammatory effect of *L. speciosa* ethanolic leaf, flower, and seed extract (LELE, LEFE, and LESE) on carrageenan-induced acute inflammation in rats. This plant is commonly called Pride of India refers to the various parts of this plant, including the leaves, flowers, and seeds, which are used as folk medicine. It has been demonstrated that the phytochemical and anti-inflammatory activities are effective in the treatment of inflammatory conditions in traditional medicine. The qualitative phytochemical investigation of *L. speciosa* leaf, flower, and seed revealed that it contained steroids, terpenoids, glycosides, polyphenolic compounds, amino acids, saponins, alkaloids, flavonoids, reducing sugars, tannins, and many other active metabolites. Studies have shown active anti-inflammatory ingredients in *L. speciosa* ethanolic extracts of leaf (LELE), flower (LEFE), and seed (LESE). The ethanolic extracts of *L. speciosa* (LELE, LEFE and LESE) were investigated for anti-inflammatory activity in carrageenan-induced paw edema in albino rats and compared to control and standard drug, Diclofenac injection. The experimental plant extracts were given orally at a concentration of 250 and 500 mg/kg b.w. before carrageenan injection. In the paw edema evaluation, the paw volume was measured in the early (from 0 min-3 h) and late (4-6 h) phases of edema formation. In acute inflammation paw models, the ethanol extracts significantly reduced paw edema. The result obtained is indicative that the ethanolic extract of leaf and seed with high dose of 500 mg/kg b.w. produced 42% of inhibition and is low as compared to the standard drug. Low dose of ethanolic flower extract of *L. speciosa* inhibitory is equal to the low dose of seed extract; it is 1% lower than the standard and 2% lower than the control.

Keywords: *Lagerstroemia speciosa*, Ethanolic leaf, flower and seed extracts, Qualitative phytochemical analysis, Anti-inflammatory, Paw edema.

1. INTRODUCTION

Medicinal plants in India play a key role in the treatment of a number of diseases, and they are the only source of folk medicine for the majority of people. Currently, 25% of drugs are derived from plants. The advantage of herbal medicine is that it minimises side effects and is relatively low-cost compared to synthetic medicines. The Western Ghats are one of the rich biodiversity regions of India, especially Coimbatore in Tamil Nadu. *Lagerstroemia speciosa* (L.) Pers. (Family: Lythraceae) is commonly known as Queen's Flowers and Queen Crape Myrtle in English, Poomaruthu in Tamil, Manimaruthu in Malayalam. The active components of banaba leaf extract are arjunolic acid [1], ellagic acid, corosolic acid, and tannic acid [2]. A wide variety of phytochemical compounds, such as secondary metabolites, are synthesised by plants. The secondary metabolites of medicinal plants have very strong anti-inflammatory properties and act as an efficient source of natural antioxidants [3]. Inflammation is a protective response to injury. The early stage of acute inflammation from minutes to hours starts with vasodilation, oedema, and chemotaxis. At the cellular level, changes occur in the endothelial cells and the movement of phagocytes and leukocytes into the area of infection. Inflammation occurs due to leakage of serum protein and fluids into tissues. By decreasing membrane permeability and subsequently preventing the drainage of proteins and fluids, membrane

stability can be ensured and an anti-inflammatory response can be manifested [1]. Arjunolic acid has been shown to influence cyclooxygenase-catalysed arachidonic acid metabolism. This phytochemical might have contributed to the plant's anti-inflammatory activity [4, 5]. On the other hand, quercetin and isoquercitrin were found to be effective eosinophilic inflammation suppressors. These phytocomponents have the potential to treat allergies [6]. Again, Gallic acid was reported to exhibit anti-inflammatory activity in an animal model [7]. The African continent is richly endowed with diverse medicinal plants with anti-inflammatory properties that have been shown to be effective in the treatment of inflammatory conditions in traditional medicine. Inflammation is part of the body's immune response. There can be four primary indicators of inflammation: pain, redness, heat or warmness and swelling. Plants have the ability to synthesize a wide verity of phytochemical compounds as secondary metabolites which shows anti-inflammatory activity.

Acute inflammation has two main components: vascular changes and cellular events. The presence of infection or injury is perceived by resident cells, mainly macrophages, but also by other cell types, which secrete molecules (cytokines and other mediators) that induce and regulate the inflammatory response. Carrageenan (a polysaccharide) is a floristic agent extensively used to induce an acute, non-immune, and highly reproducible inflammation framework in laboratory animals[8]. Sulphated sugars present in carrageenan are responsible for the activation of inflammatory mediators and the production of vascular and cellular events of inflammation [9].

The aim of the present study is, therefore, to find out:

- Phytochemical analysis of ethanolic leaf, flower, and seed extract of *L. speciosa*.
- To study the anti-inflammatory effect of *L. speciosa* on leaf, flower, and seed ethanolic extract,
- To investigate the anti-inflammatory activity of *L. speciosa* on leaf, flower, and seed ethanolic extracts in carrageenan-induced paw edema in albino rats.
- To compare the anti-inflammatory activities of carrageenan and diclofenac among the three samples of ethanolic extracts.

2. MATERIAL AND METHODS

2.1. Collection and Authentication of plant samples

The leaves, flowers and seeds of *L. speciosa* were collected from PG Girls hostel, Government Arts College (Autonomous), Coimbatore District, Tamil Nadu, India. The *L. speciosa* were identified and authenticated at, Botanical Survey of India, Coimbatore-03 (No: BSI/SRC/5/23/2020/Tech/51) and the voucher specimens was kept in Department of Zoology, Government Arts College, Coimbatore-18.

2.2. Plant extracts preparation

The collected samples of *L. speciosa* were observed carefully for any kind of disease or infection; the clean samples from those were isolated for the experiment. The selected plant parts were to be cleaned of dust and any other particles stuck to them. The samples were then kept under the shade at room temperature (27± 2°C) for about 2 weeks until they were completely dry. The dried leaves were powdered with the help of a mixer grinder. Then, 100g of the powder was soaked in 1000ml of ethanol solvent and stored in an airtight bottle and kept for 4 days with periodic shaking. The extract was then filtered using Whatman No. 1 filter paper and kept in Petri dishes to dry at room temperature [10].

2.3. Phytochemical analysis

The Horborne [11] and Trease and Evans [12] methodology was used to perform the qualitative phytochemical analysis of selected parts of *L. speciosa* ethanolic extracts.

2.4. Acute oral toxicity studies

The leaves, flowers and seeds of ethanolic extracts were orally fed for the three groups of 6 animals and they were observed to check for behavioural changes, if any. The toxicological study was done for 7 days to find out the mortality, if any. It was found to be safe so the experiment was continued.

2.5. Experimental animals

All the experimental procedures used in these studies were approved by the Institutional Animal Ethics Committee of KMCH College of Pharmacy, Coimbatore (Approval No: KMCRET/ReRe/Ph.D/23/2021).The animals were kept in polypropylene cages with stainless steel top grills having facilities for holding pellet food and drinking water in feeder bottle with sipper tube. Each cage contained 6 animals. All the experimental animals had free access to potable water and standard pelleted laboratory animal diet *ad libitum*. Paddy husk was used as bedding material.

2.6. Drugs

Three samples of ethanol low and high dose extracts (250 and 500 mg/kg) was uniformly suspended in 1% carboxymethyl cellulose (CMC) and water which was fed orally for 5 days to the animals before the start of the experiment. 0.1 ml of 1% freshly prepared suspension of carrageenan used as a control and 0.1 ml of diclofenac sodium (10 mg/kg) as a standard drug. The animals were divided into 8 groups.

2.7. Experimental design

2.7.1. Assay of anti-inflammatory activity

Carrageenan-induced paw edema model described by Winter *et al*. [13] was used for evaluating potential anti-inflammatory activities by plant extract.

2.7.2. Carrageenan-induced Model

One day before the experiment, three basal readings of hind paw in each group were recorded. Control group received 0.1ml of carrageenan, Group 2 received a standard drug 0.1ml of diclofenac injection, Groups 3, 5 and 7 received low dose of the ethanolic extract, whereas Groups 4, 6, and 8 received high doses of the ethanolic extract. Localized inflammatory pain was induced in all groups of animals by sub planar injection of carrageenan. The volume of the paw was measured immediately after induction six times with period of 1 hr and once in 24 hr until the inflammation disappeared [13]. The paw volume was marked at 0, 1, 2, 3, 4, 5 and 6thhrafter carrageenan injection using Vernier calipers. The difference between initial and subsequent reading gave the actual edema volume. Then percentage of inhibition of edema was calculated for each group with respect to the control group using the formula given below:

Percentage of inhibition of paw edema=(1- Vt/Vc)× 100

Where, Vt is the inflammatory increase in paw volume in drug-treated rats, and Vc is the inflammatory increase in paw volume in control group of rats.

2.8. Statistical Analysis

Values are expressed as the mean±S.D. Statistical significance (p) by one way ANOVA followed by Dunnett's test. ns- not significant, **P< 0.05 calculated by comparing treated group with control group.

3. RESULTS AND DISCUSSION

Carrageenan-induced rat paw edema is used widely as a working model of inflammation in the research for a new anti-inflammatory drug. The result obtained is indicative that the ethanolic extract of leaf and seed with high dose of 500 mg/kg b.w. produced 42% of inhibition and is low as compared to the standard drug. Low dose of ethanolic flower extract of *L. speciosa* inhibitory is equal to the low dose of seed extract; it is 1% lower than the standard and 2% lower than the control. The development of edema in the paw of the rat after the injection of carrageenan is due to the release of histamine, serotonin and prostaglandin like substances [14]. The anti-inflammatory activity of the ethanolic leaf, flower, and seed extracts of *L. speciosa* was evaluated by carrageenan-induced rat paw edema method [13, 15] and the result is shown in Table 1. There were 8 groups of 6 animals (48 animals in total) used and observed every hour for 6 hours. The first group was injected with carrageenan, second with a combination of carrageenan and Diclofenac, third with carrageenan and Low dose of Leaf ethanolic extract (L.D LELE), fourth with carrageenan and High dose of Leaf ethanolic extract (H.D LELE), fifth with carrageenan and Low dose of Flower ethanolic extract (L.D LEFE), sixth with carrageenan and High dose of Flower ethanolic extract (H.D LEFE), seventh with carrageenan and Low dose of Seed ethanolic extract (L.D LESE), and the final group with carrageenan and High dose of Seed ethanolic extract (H.D LESE). Significantly high anti-inflammatory activity of ethanolic extract of leaf and seed (500 mg/kg b.w) of *L. speciosa* may be due to inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin. The result is compared with the control and the diclofenac group. Ethanolic extract of leaf and seed with high dose of 500 mg/kg b.w. produced 42% of inhibition and is low as compared to the standard drug. The ethanolic leaf extract with low dose and high dose of flower extract is compared to the control group. The inhibition rate is found to be closer to the control group. The mean volume of each group taken during each hour is show in Table 1.

In the control group there was a progressive increase in the mean paw volume, whereas in the standard and test groups there was a progressive decrease in the mean paw volume from the 1st to the 4th hour. The percentage inhibition was calculated for each group for every hour, it was found that the standard drug had 61% inhibition and was found to be the highest among all groups and H.D LELE, H.D LEFE being the lowest with 52% inhibition in Table 2. The mean volume of the control group shows 9.38±0.351 and the Standard group shows 8.7±0.193in the 4th hr. The paw inhibition in LELE and LESE is significantly higher and there is no cytotoxicity compared

to the standard group in the 4th hr. Group III and Group VI shows moderate paw inhibition in Table 2.

The different stages of inflammation are inhibited by the anti-inflammatory drugs. Chemical tests were carried out to identify the phytoconstituents present in the different extracts. [16]. The anti-inflammatory effect of *L. speciosa* can also minimize inflammation because of the presence of lagerstroemin, corosolic acid, and elagitannin. Polyphenolic phytochemicals may stimulate gene expressions and exhibits antioxidant and anti-inflammatory properties. The *L. speciosa* flower has furfural which contains the most potent anti-inflammatory compound - furandicarboxylic. The Dodecanoic acid is present in *L. speciosa* seed which helps in reducing the inflammation. Phenolic compounds are secondary plant metabolites and are essential in defence mechanisms of plants against pathogenic and free radicals [17]. These compounds are used in humans to modulate lipid peroxidation because of their antioxidant and anti-inflammatory activity [18]. Tannins are used in medicine primarily because of their astringent properties. Polyphenols particularly tannins, flavonoids are well known natural antioxidants [19]. Ethanol extract only answered positively for glycosides. Glycosides were found to show higher activity than quercetin [20]. Earlier studies suggest that the inflammatory tissue damage is due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites [21]. The migration of leucocytes is inhibited by the tannins' anti-inflammatory property [22]. Tannins may produce effects in a non-specific manner by their astringent properties on cell membranes thus affecting cell functions.

Table 1: Anti-inflammatory activity of ethanolic extracts of *L. speciosa* in carrageenan-induced paw edema

Group	Mean paw volume before carrageenan injection	Paw Volume after induction withcarrageenan Increase in paw volume (ml) aftercarrageenan injection (mean ± SD)					
	0 min	1h	2h	3h	4h	5h	6h
Control	3.69 ± 0.429	6.275± 0.228	7.29± 0.123	8.573± 0.174	9.38± 0.351	8.8± 0.357	8.613± 0.265
Standard	3.788 ± 0.218	6.248± 0.184^{ns}	7.935± 0.228	8.66± 0.225**	8.7± 0.193^{ns}	7.33± 0.168*	6.095± 0.406***
Carrageenan + L.D LELE	3.61 ± 0.174	6.305± 0.051^{ns}	7.85± 0.127*	8.428± 0.556^{ns}	8.673± 0.342^{ns}	7.323± 0.472*	7.32± 0.284*
Carrageenan + H.D LELE	3.56 ± 0.191	6.795± 0.440^{ns}	7.508± 0.148^{ns}	8.13± 0.290^{ns}	8.893± 0.126^{ns}	7.338± 0.217*	6.71± 0.160***
Carrageenan + L.D LEFE	3.85 ± 0.0722	6.553± 0.167^{ns}	7.86± 0.344**	8.595± 0.206**	9.025± 0.346^{ns}	7.258± 0.416*	7.595± 0.409^{ns}
Carrageenan + H.D LEFE	3.863 ± 0.175	7.365± 0.117*	7.643± 0.287^{ns}	8.668± 0.288^{ns}	9.12± 0.357^{ns}	7.643± 0.208*	7.075± 0.179**
Carrageenan + L.D LESE	3.928± 0.111	6.715± 0.427^{ns}	7.503± 0.280^{ns}	8.723± 0.462^{ns}	8.4± 0.159^{ns}	7.5± 0.136^{ns}	7.363± 0.278*
Carrageenan+ H.D LESE	3.813 ± 0.112	7.195± 0.220*	7.235± 0.183^{ns}	8.813± 0.192**	9.483± 0.546^{ns}	6.858± 0.397**	6.843± 0.133**

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by Dunnett's. ns- Not significant, $^{*}P< 0.001$, $^{**}P< 0.05$, $^{***}P < 0.01$, *calculated by comparing treated group with control group.*

Table 2: Inhibition of edema in ethanolic extracts of *L. speciosa*in carrageenan-induced paw edema

Group	Percent inhibition of edema (%)					
	1h	2h	3h	4h	5h	6h
Control	59%	51%	43%	39%	42%	43%
Standard	61%	48%	44%	44%	52%	62%
Carrageenan + L.D LELE	57%	46%	43%	42%	49%	49%
Carrageenan + H.D LELE	52%	47%	44%	40%	48%	53%
Carrageenan + L.D LEFE	59%	49%	45%	43%	53%	51%
Carrageenan + H.D LEFE	52%	50%	44%	42%	50%	55%
Carrageenan + L.D LESE	58%	52%	45%	47%	52%	53%
Carrageenan+ H.D LESE	53%	52%	43%	40%	56%	56%

The present study exhibited the anti-inflammatory activity of the ethanol extracts of *L. speciosa*. The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzyme, which is responsible for the conversion of arachidonic acid to prostaglandin (PGH) [23].

The non-steroidal drugs act either by inhibiting the lysosomal enzymes or by stabilizing lysosomal

membranes. Since HRBC (Human red blood cell) membranes are similar to lysosomal membrane components, the prevention of hypotonicity-induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity of drugs [24].

The drugs used to reduce inflammation are NSAIDs (Non-steroidal anti-inflammatory drugs). These drugs block COX-1 (Cyclooxygenase-1) and COX-2 (Cyclooxygenase-2) enzyme activity. COX (Cyclooxygenase) enzymes assist with prostaglandin production. Prostaglandins influence blood flow, digestion and wakefulness [25]. Therefore, blocking prostaglandin production can result in a wide range of health problems. NSAIDs and other anti-inflammatory drugs simply mask symptoms-they do not correct the underlying cause or address the long-term ramifications of an overactive inflammatory response. These drugs also have numerous side effects. NSAIDs can cause abdominal cramping, gas, constipation, diarrhoea, dizziness, fatigue, headaches, nausea, heartburn, ringing in the ears and many other ailments. It is estimated that up to 60% of individuals taking NSAIDs will experience side effects. The NSAIDs (naproxen) was shown to contribute to a 50% higher risk of heart attack and stroke with long term use. The standard drug has the above-mentioned side effects; the research carried out reveals that *L. speciosa* can be a promising alternative that has the anti-inflammatory property with no side effects.

4. CONCLUSION

Inflammation is an immediate response by the body to any form of infection or injury, which induces the plasma proteins that leads to swelling in the injured area. Due to the migration of neutrophils, leukocytes and other migratory cells from the blood vessels, water gets accumulated in the infected area. Corosolic acid and ellagitannin present in the leaves of *L. speciosa* uptake the glucose in the cells and heals the inflammation. The flower of *L. speciosa* contains phytochemical component flavonoids in the form of quercetin and the seed contains dodecanoic acid which has anti-inflammatory properties. We concluded that the ethanolic extract of leaf, seed and flower of *L. speciosa* plant is a natural anti- inflammatory drug.

Conflict of interest

None declared

Source of funding

None declared

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