ORIGINAL ARTICLE

Protective effect of *Lawsonia inermis* Linn. on chronic inflammation in rats

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Abstract

Background: Medicinal plants are being widely used, either as single drug or in combination in health-care delivery system. *Lawsonia inermis* Linn. is commonly known as henna, which is recognized in traditional system of medicine. **Aim:** In the present study, the anti-inflammatory and antioxidant activity was studied using an ethanolic extract of *L. inermis*. **Materials and Methods:** The bioactive compounds from *L. inermis* were identified by gas chromatography-mass spectrometry. The anti-inflammatory and anti-oxidants assays were followed by the standard methods. **Results:** The mice bioassay study was carried out by dose of 50, 100, 150, and 200 mg/kg bw, respectively. The ethanolic extracts showed the presence of Methyl salicylate (8.846), propanoic acid (9.608), ethyl (dimethyl) silyl ester 2, 1, 3-benzothiadiazole (12.258), diethyl phthalate (14.222), ethanol, 2-bromo (16.985), dibutyl phthalate (17.729), phytol (19.671), and disooctyl phthalate (23.272) all the compounds are response for anti-inflammatory activity. The extracts (100 and 200 mg/kg) showed the maximum inhibition of 39.49% and 55.98% at the end of 3 h with Carrageenan-induced in rat paw edema. A significant increase in the activities of superoxide dismutase, catalase, glutathione peroxidase, Vit-C, and Vit-E was observed in the tissue of tested groups and compared with standard and control groups. **Conclusion:** Through this study as a conclusion, the *L. inermis* possesses anti-inflammatory and antioxidant potential which may be used for therapeutic purposes mainly in the prevention of oxidative damage that occurs during inflammation.

Key words: Anti-inflammatory, antioxidants, carrageenan, Lowsonia inermis

INTRODUCTION

Inflammation is a universal host defense process involving a complex network of cell-cell, cell-mediator, and tissue interactions. It occurs in response to a variety of stimuli, namely physical, chemical, traumatic, antigen challenge, and infectious agents. Synthetic drugs both steroidal and nonsteroidal are being used for acute and chronic inflammation cause a number of side effects. Furthermore, they are not able to cure inflammation completely. Hence, the traditional medicine practitioners and scientists are turning toward medicinal plants for curing these ailments.[1] Herbal drugs can, therefore, be considered as a better alternative to synthetic anti-inflammatory drugs.

Medicinal plants are widely used in the management of diseases all over the world. Historically, the use of medicinal plants is as old as mankind and medicine. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceutical, coupled with their reduced cost, is encouraging both the consuming public and national health-care institutions to consider plant medicines as an alternative to synthetic drugs. Nowadays, herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness and no side effect in clinical experience. Large numbers of plants belonging to different families have been studied for their therapeutic properties. Previous studies have proved that the chemical constituents such as flavonoids, alkaloids, tannins, and terpenoids are promising agents in the treatment of inflammation.[2-5] However, *Lawsonia inermi*s belonging to family Lythraceae with having several medicinal properties.[3] In the present study we focused on the anti-inflammatory and antioxidant properties of ethanolic extracts of *Lawsonia inermi*s on carrageenan-induced paw edema i**n** albino rat*s*.

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MATERIALS AND METHODS

Collection and Authentication of Plant

Fresh, healthy *L. inermis* was collected from their natural habitat of Herbal Garden, AMET University campus Chennai, and authenticated by professionals in Department of Botany, St. Joseph's College, and Tiruchirappalli. According to Mukerjee,[4] the herbarium number of the plant is RVL001.

Preparation of Extraction

The coarse powder plant material was extracted with ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure to get the crude extract. Standard methods were used for preliminary phytochemical screening of the extract, which was performed to know the phytoconstituents in the extract.[5]

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of the sample was performed using a Shimadzu GCMS-QP2010 GC-MS interfaced with a Turbo Mass quadrupole mass spectrometer, fitted with an Rtx-5 fused silica capillary column (30 mm × 0.25 mm, with 1 cm film thickness). The oven temperature was programmed from 100°C to 320°C at 100°C/min and hold for 10 min. Helium was used as carrier gas at flow 1.0 mL/min. The injector temperature was 250°C, injection size 1 µL neat, with split ratio 1:10. The interface and MS ion source were maintained at 320°C and 200°C, respectively, and the mass spectra were taken at 70eV with a mass scan range of 40–700 amu (atomic mass unit).

Identification of Compounds

Interpretation of mass spectrum of GC-MS was conducted using the mass spectral database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Animals

Wistar rats 7–8-week-old, weighing 150–200 g were used for the present study. To maintain the animal house under the standard condition of temperature (24 ± 2°C) and relative humidity (30–70) with a 12:12 light:dark cycle. The animals were fed with standard pellet diet and water. The animal handling was performed according to good laboratory practice. Ethical clearance was obtained from the Institutional Animal Ethical Committee (CPCSEA/265/2015) and conducted according to Indian national science academy guidelines for the use and care of experiments.

Acute toxicity Study

Animals were randomly allotted in 5 groups (each group contains six mice). The ethanol extract was administered orally at doses of 50,100, 200, and 300 mg/kg of body weight. The control group received only the normal saline (10 mL/kg b.w). The animals were observed during the first 2 h for toxic signs, and then mortality was recorded for each group at 24, 48, and 72 h after dose administration.

Evaluation of Anti-inflammatory Activity

Anti-Inflammatory activity was tested on an extract of *L. inermis* against carrageenan-induced paw edema in rats. The reductions of paw edema of rats are compared with the standard drug, i.e., indomethacin.

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

*Increase in paw volume in 3 h.

Animal Grouping

Animals were divided into five groups (6 animals in each)

- Group 1: Normal control (normal saline)
- Group 2: Negative control (carrageenan, 1%)
- Group 3: Positive control (indomethacin, 5 mg/kg)
- Group 4: Ethanolic extract of *L. inermis* (100 mg/kg)
- Group 5: Ethanolic extract of *L. inermis* (200 mg/kg)

Preparation of Tissue Homogenate

The tissue samples were homogenized in a solution containing 5% trichloroacetic acid and 5mM EDTA at 4°C and centrifuged for 10 min at 15,000 g in 4°C.[6]

Estimation of Antioxidants

Enzymatic antioxidants

The estimation of enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) was measured by the following methods. The activity of SOD was assayed by the method of Kakkar *et al*.,[7] CAT was estimated by the method of Sinha,[8] and GPx measured by the method described by Rotruck *et al*.[9]

Non-enzymatic antioxidants

The estimation of non-enzymatic antioxidants Vitamins E, Vitamin C, and GSH was estimated. Vitamins C by the method of Omaye *et al*.,[10] Vitamin E was estimated by the method of Baker *et al*.,[11] and GSH was measured by the method of Ellmann *et al*.[12]

RESULTS

Photochemical Analysis

The preliminary phytochemical screening results of *L. inermis* showed [Table 1] various bioactive secondary metabolites constituents such as alkaloids (0.42%), flavonoids (0.62), saponins (4.5%), tannins (1.0%), terpenoids (0.20%), carbohydrates (0.16), and protein (2.5).

GC-MS

The GC-MS analysis, a total of 8 compounds identified from the *L. inermis* extract [Figure 1] such as methyl salicylate (8.846), propanoic acid (9.608), ethyl (dimethyl) silyl ester 2, 1, 3-benzothiadiazole (12.258), diethyl phthalate (14.222), ethanol, 2-bromo (16.985), dibutyl phthalate (17.729), phytol (19.671), and disooctyl phthalate (23.272).

Anti-inflammatory Activity of L. inermis

In the present study, the anti-inflammatory activity of ethanol extract of *L. inermis* against carrageenan-induced paw edema shows that the extracts have a significant effect on inflammation and markedly reduced the swelling. The percentage reduction in the paw volume in the group of animals treated with *L. inermis* extracts 100 mg was 39.49% and for the 200 mg/kg was 55.98% at 3 h. It shows that the plant extract has significant ($P < 0.01$; $P < 0.001$) anti-inflammatory effect and the results were compared with indomethacin 10 mg/kg and showed percentage paw volume reduction of 58.13% [Table 1].

Antioxidant Activity of *L. inermis*

Enzymatic Antioxidants L. inermis

Table 2 shows that the activities of SOD, CAT, and GPx were significantly decreased in the tissue of inflammation control rats due to the inadequacy of the antioxidant defenses in combating reactive oxygen species (ROS) mediated damage. The decreased levels of enzymatic antioxidants status were seen in erythrocyte lysate, and inflammatory was observed in carrageenan alone treated rats when compared with control group. Ethanolic extracts of *L. inermis* at a dose of 200 mg/kg b.w significantly normalized the enzymatic antioxidant such SOD, CAT GPx in carrageenan-treated animals.

Table 1: Phytoconstituents Investigation of *L. inermis*

Phytoconstituents	% of phytoconstituents in *L. inermis*
Alkaloids	0.42
Flavonoids	0.62
Saponins	4.5
Tannins	1.0
Terpenoids	0.20
Carbohydrates	0.16
Protein	2.5

L. inermis: Lowsonia inermis

Non-enzymatic Antioxidants *L. inermis*

Table 3 showed that decreased levels of non-enzymatic antioxidant Vitamin C and E were in inflammation rats, when compared to that of control rats. The non enzymatic antioxidant decreased level was observed after treatment significantly increased in tissue of inflammatory rats by treating with crude extract of *L. inermis* when compare with stranded drug. GSH has a multifaceted role in the anti-oxidant defense. It is a direct scavenger of free radicals as well as a cosubstrate for peroxide detoxification by GPx [Table 4].

DISCUSSION

The plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population.[13] There is a growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities.[14]

In the present study, the quantitative phytochemical investigations of ethanolic extract of *L. inermis* show the presence of tannins, carbohydrates, glycosides, phenols, alkaloids, terpenoids, and flavonoids. The GC-MS analysis, a total of 8 compounds identified from the *L. inermis* extract [Figure 1] such as methyl salicylate (8.846), propanoic acid (9.608), ethyl (dimethyl) silyl ester 2, 1, 3-benzothiadiazole (12.258), diethyl phthalate (14.222), ethanol, 2-bromo (16.985), Dibutyl phthalate (17.729), phytol (19.671), and

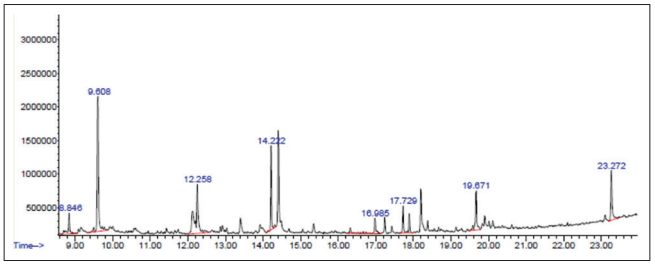

Figure 1: Gas chromatography-mass spectrometry spectra of *Lowsonia inermis*

Table 2: Anti-inflammatory activity of *L. inermis*

Treatment (mg/ml)	Mean increase in paw volume				% of paw volume after 3 h
	0 h	1 h	2 h	3 h	
Control	39.63±2.16	85.11±4.15	103±2.33	123.31±9.33	-
L.inermis extract (100 mg/kg)	22.11±2.18*	39.73±4.05*	63.35±4.18*	74.77±3.58*	39.49%
L.inermis extract (200 mg/kg)	31.37±1.98*	71.37±2.67*	71.16±2.18*	54.41±1.69*	55.98%
Indomethacin (10 mg/kg)	25.71±1.69**	28.43±1.94*	49.11±1.69*	51.75±2.15**	58.13%

Each value is standard error of mean±5 individual observations *P<0.05; **P<0.01 compared paw edema induced control versus drug-treated rats. *L. inermis: Lowsonia inermis*

Table 3: Enzymatic antioxidants *L. inermis*

Groups	Enzymatic antioxidants		
	GPX (U/gmHb)	SOD (U/gmHb)	CAT (U/gmHb)
Control	15.79±1.10	8.02±0.89	5.90±0.44
Carrageenan + *L.inermis* (100 mg/kg)	10.54±1.37*	5.02±0.50*	4.01±0.59*
Carrageenan + *L.inermis* (200 mg/kg)	13.09±1.20**	6.45±0.28**	5.09±0.47**
Indomethacin (10 mg/kg)	15.02±1.25**	6.77±0.81**	5.89±0.21**

Each value is standard error of mean±5 individual observations *P<0.04; **P<0.01 compared paw edema induced control versus drug-treated rats. *L. inermis: Lowsonia inermis,* GPx: Glutathione peroxidase, SOD: Superoxide dismutase, CAT: Catalase

Table 4: Non-enzymatic antioxidants *L. inermis*

Groups	Non-enzymatic antioxidants		
	GSH (mg/dl)	Vitamin E (mg/dl)	Vitamin C (mg/dl)
Control	27.36±1.81	1.41±0.15	1.64±0.11
Carrageenan + *L.inermis* (100 mg/kg bw)	21.76±1.66*	1.16±0.14*	1.31±0.10*
Carrageen + *L.inermis* extract (200 mg/kg)	23.79±1.80**	1.21±0.10**	1.54±0.10**
Indomethacin (5 mg/kg)	22.15±1.23**	1.26±0.18**	1.14±0.28**

Each value is standard error of mean±5 individual observations *P<0.04; **P<0.01 compared paw edema induced control versus drug-treated rats. *L. inermis: Lowsonia inermis,* GSH: Glutathione

disooctyl phthalate (23.272). All these bioactive compounds are response for anti-inflammatory properties.

The inflammation is a biological complex of vascular tissues in harmful stimulated by pathogens and irritants[15] and has been major health problems in the world.[16] The anti-inflammatory effects can be elicited by a variety of chemical agents and that there is little correlation between their pharmacological activity and chemical structure.[17] Carrageenan-induced hind paw edema is the standard experimental model of inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility.[18] Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin, and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances which peek at 3 h.[19] It has been reported that the second phase of edema is sensitive to drugs such as hydrocortisone, phenylbutazone, and indomethacin. The indomethacin is a cyclooxygenase inhibitor, the ethanol extract has an activity which is comparable to indomethacin and can be said to inhibit the cyclooxygenase enzyme, but lipoxygenase inhibitors also possess significant anti-inflammatory activity against carrageenan-induced paw edema, so inhibition of carrageenan-induced paw edema by the crude extract. In the present study, the acute toxicity showed that *L. inermis* was not a cause of death at a single dose of even 200 mg/kgbw. A transient hypoactivity, loss of appetite, and piloerection were observed at a dose of 2000 mg/kg bw and recovered within 12 h, while no sign or symptom of toxicity was observed in a group of 50, 100, 150, and 200 mg/kg bw treated. Hence, 1/5 (200 mg/kg bw) and 1/10 (100 mg/kg bw) were considered as the appropriate dose range for further anti-inflammatory studies.

In the present study, the anti-inflammatory activity of ethanol extract of *L. inermis* against carrageenan-induced paw edema shows that the extracts have a significant effect on inflammation and markedly reduced the swelling. The percentage reduction in the paw volume in the group of animals treated with *L. inermis* extracts 100 mg was 39.49% and for the 200 mg/kg was 55.98% at 3 h. It shows that

the plant extract has significant ($P < 0.01$; $P < 0.001$) anti-inflammatory effect and the results were compared with indomethacin 10 mg/kg and showed percentage paw volume reduction of 58.13% [Table 1].

As evidenced by earlier studies induction of inflammation in experimental animal models is a huge task. Most studies revealed that so many factors play a role in the lack of uniformity in the induction of inflammation. The treatment effect of acute model preventive have very less effects of drugs are delayed, the chronic model is better adapted for studies on healing or resolution of inflammation. The results of the study supported the traditional use of this plant in some inflammation and painful conditions which confirm the presence of active chemical compounds related to these activities. Phytochemicals such as flavonoids, terpenoids, steroids, and phenolic compounds expressed their anti-inflammatory activity at least in part by modulation of pro-inflammatory gene expressions such as cyclooxygenase-2, inducible nitric oxide synthase, and several pivotal cytokines. These are considered to be reasonable candidates for new anti-inflammatory drugs.[20] The results of the present study showed that carrageenan injection induced the paw edema volume and observed edema volume was higher at 3 h.[21] Sharma *et al*. observed that different doses of *Banksia serrata* and indomethacin pretreated rats showed inhibition of carrageenan-induced paw edema in all observed time intervals. In high concentration of *B. serrata* extract administered rats showed decreased the level of inflammation when compared with stranded drug administrated rats.

Free radicals have long been implicated as mediators of tissue damage in inflammation patients, which are released in large amounts into the surrounding tissue. To neutralize this charge, free radicals try to withdraw an electron from or donate an electron to, a neighboring molecule. Other antioxidants work against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage. For example, certain enzymes in the body, such as SOD, CAT, GPx, and GSH work with other chemical to transfer free radical into harmless molecules.[22]

Oxidative stress is a condition of a reduction in antioxidative enzymes such as SOD, CAT, GPx, and GST.[23] The antioxidant enzymes SOD and CAT play an important role in reducing cellular stress. SOD scavenges the superoxide radical by converting it to hydrogen peroxide and molecular oxygen, while CAT brings about the reduction of hydrogen peroxides and protects higher tissues from the highly reactive hydroxyl radicals.[24] Table 2 shows that the activities of SOD, CAT, and GPx were significantly decreased in tissue of inflammation control rats due to the inadequacy of the antioxidant defenses in combating ROS mediated damage. The decreased levels of enzymatic antioxidants status were seen in erythrocyte lysate, and inflammatory was observed in carrageenan alone treated rats when compared with control group. Ethanolic extracts of *L. inermis* at a dose of 200 mg/kg b.w significantly normalized the enzymatic antioxidant such SOD, CAT, and GPx in carrageenan-treated animals.

The decreased activities of CAT and SOD may be a response to increased production of H_2O_2 and O_2 by the autoxidation. These enzymes play an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides generated from inadvertent exposure to carrageen.[25] Treatment with extract of *L. inermis* increased the activity of these enzymes and may help to control free radicals when compared to inflammation rats. The effect produced by plant extract was comparable with that of standard drug indomethacin

Vitamin C plays a central role in the antioxidant protective system, protecting all lipids undergoing oxidation and diminishing the number of apoptotic cells[26,27] and it also regenerates the oxidized Vitamin E.[28] Vitamin E, on the other hand, acts as a non-enzymatic antioxidant and reduces chain reactions of lipid peroxidation.[29] Table 3 showed that decreased levels of non-enzymatic antioxidant Vitamin C and E were in inflammation rats when compared to that of control rats. The non enzymatic antioxidant decreased level was observed after treatment significantly increased in tissue of inflammatory rats by treating with crude extract of *L. inermis* when compare with stranded drug. GSH has a multifaceted role in the antioxidant defense. It is a direct scavenger of free radicals as well as a cosubstrate for peroxide detoxification by GPx.

CONCLUSION

The results of the present study justified and supported scientifically to the ethnopharmacological use of the plant as an anti-inflammatory agent to treat pain and inflammation. Further attempts will be taken to isolate and define the active analgesic and anti-inflammatory fraction and its components.

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