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RESEARCH ARTICLE

ANTI-INFLAMMATORY ACTIVITY OF *Lawsonia ulba* Linn., IN WISTAR ALBINO RATS

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Anti-inflammatory activity of *Lawsonia ulba linn.*, on carrageenan induced paw edema in wistar male rats Aqueous, ethanolic and methanolic extracts of *Lawsonia ulba linn.*, were investigated for anti-inflammatory activity in carrageenan induced paw edema in wistar male rats, and compared to a positive control drug, Voveran. These extracts were given (ip) in a concentration of 20 and 50 mg/kg b.w. before carrageenan injection. Methanolic extracts of *Lawsonia ulba linn.*, with a concentration of 20 mg/kg b.w. and ethanolic extract with a concentration of 50 mg/kg b.w. showed maximum (90.9%) inhibition on carrageenan induced rat paw edema. The effect was significantly ($P< 0.05$) higher than that of the standard drug Voveran (72.72%). Methanol extract with a concentration of 50 mg/kg b.w. produced 81.81% inhibition, which was also high as compared to the standard drug. Ethanolic extract with a dose of 20 mg/kg b.w and the two doses of aqueous extract produce less percentage of inhibition as compared to the standard drug voveran.

Key words: *Lawsonia ulba linn.*, anti-inflammation, carrageenan

INTRODUCTION

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Thus, the present investigation was carried out to evaluate the anti-inflammatory potential of *Lawsonia ulba* Linn., in experimental animal models. *Lawsonia ulba* Linn., belonging to the family of Lythraceae is an evergreen plant. It has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties. Most of the plant parts are used in traditional system of medicine in India. From its leaves a red-orange dye agent is extracted. This agent has an affinity for bonding with proteins, and thus is used to dye human body parts (skin, hair, fingernails), as well as leather, silk and wool. *Lawsonia ulba* Linn., also acts as an anti-fungal and a preservative for leather and cloth (Bosoglu *et al.*, 1998). *Lawsonia ulba* Linn., leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments as rheumatoid arthritis, headache, ulcers, diarrheoa, leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and colouring agent (Chetty, 2008; Singh,1982). *Lawsonia ulba* Linn., is a medicinal plant, whose parts were pharmacologically proved to possess hypoglycemic, antibacterial, anti-HIV activity and anti-diarrhea effects (Indira, and Mohan, 1993; Aguwa, 1987). *Lawsonia ulba* Linn., leaves showed significant anti-inflammatory effect with some active principles (Gupta, 1986). Hence, the present study has been made to investigate the phytochemical.

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On the basis of these common uses of this plant in traditional folk medicine and its above reported activities in the literature, we have evaluated the anti-inflammatory effect of various extracts of *Lawsonia ulba* Linn.

MATERIALS AND METHODS

Collection of plant materials

The fully mature *Lawsonia ulba* Linn., were collected in January 2006 from Tiruchirappalli district, Tamil nadu, India. The plant was identified and authenticated Dr. Anusha Baskar, Principal, Dhanalakshmi Sreenivan College for women, Perambalur, Tamil Nadu, India confirmed with the voucher specimen kept in the Rapinat Herbarium, St.Joseph's College, Tiruchirappalli, TamilNadu, India.

Aqueous extract

500 g arial parts of *Lawsonia ulba* Linn., were collected, washed thoroughly and dried in shade. It was then crushed and taken in a round-bottomed flask. 500 ml distilled water was added to cover the material, refluxed in a water bath for 1 h at 90 - 95ºC. The supernatant was removed and the extraction repeated once again. The supernatant obtained were combined and filtered through a Whatman No. 1 filter paper. The filtrate was concentrated at low temperature by lyophilization. The residue was designated as aqueous extract.

Methanol extract

Dried arial parts of *Lawsonia ulba* Linn.,were reduced to a fine powder with a mechanical grinder. The powder plant material

(200 g) was soaked in 3 l of 70% methanol and allowed to stand for 3 days for extraction. The extract was concentrated to dryness using a rotary evaporator attached to a vacuum pump and stored at a temperature of -4ºC until use.

Table 1: The analysis of phytochemicals in the aqueous extract, methanol extract and ethanol extract of *Lawsonia Ulba* Linn.

Secondary Metabolites	Aqueous extract	Methanol extract	Ethanol extract
Alkaloids	+	+	+
Amino acids	+	+	+
Anthraquinones	-	-	-
Flavonoids	+	+	+
Glycosides	+	+	+
Phytosterol	+	+	+
Saponins	-	-	+
Steroids	+	+	+
Tannins	+	+	+
Triterpenoids	+	+	+

+=presence; - = absence

Table 2. Anti-inflammatory activity of the aqueous, ethanolic and methanolic extract of *Lawsonia ulba* Linn.

Extracts	Doses (mg/kg, ip)	Change in paw edema mean (mm)	% Edema inhibition relative to control at 3rd hour
Control (normal Saline, 0.9%)	0.3	1.1 ± 0.05	–
Aqueous extract	20	0.9 ± 0.03*	18.2
	50	0.7 ± 0.27*	36.3
Methanolic extract	20	0.1 ± 0.004*	90.9
	50	0.2 ± 0.007*	81.81
Ethanolic extract	20	0.4 ± 0.02*	63.6
	50	0.1± 0.003*	90.9
Voveran	20	0.3 ± 0.01*	72.72

Values are mean + S.E.M

Ethanol extract

Dried ariel parts of *Lawsonia ulba* Linn., were reduced to a fine powder with a mechanical grinder. The powder plant material (200 g) was soaked in 3 l of 80% ethanol and stand for 3 days. The extract was concentrated to dryness and stored at a temperature of – 4ºC until use.

Preliminary Phytochemicals screening

One gram of the aqueous extract, methanol extract and ethanol extracts of *Lawsonia ulba* Linn., were dissolved in 100 ml of its own mother solvents to obtain a stock of concentration 1% (v/v). The extracts thus obtained were subjected to preliminary phytochemical screening the methodology of Harborne (1998) and Kokate (2001).

Animal used for Anti-inflammatory activity

Wistar albino rats (150-180g) were used as experimental models and five rats were taken for each group. The rats were used after an acclimatization period of 7 days to the laboratory environment. They were provided with food and water *ad libitum*. The work was carried out in CPCSEA approved (Reg. No: 265/ CPCSEA) Animal House of Periyar College of Pharmaceutical Sciences, Tiruchirapalli, during the year 2005-2006.

Anti-inflammatory activity

Male Wistar rats (120 - 170 g) kept at the laboratory Animal home of the Faculty of Biochemistry, University of Kerala, India were used. The animals were maintained under standard environmental conditions and had free access to standard diet and water. Antiinflammatory activity was measured using carrageenan induced rat paw oedema assay (Winter *et al*., 1962; Adeyemi *et al*., 2002). Groups of 6 rats were given a dose of the extract (plant extracts were dissolved in sterile distilled water and administered intra peritoneally at different dose levels). After 1h, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured at hourly interval for 3 h (Bamgbose and Noamesi, 1981). Anti-inflammatory activity was measured as the percentage reduction in oedema level when drug was present, relative to control (Duffy *et al*., 2001) as shown in Table 1.

Statistical analysis

All data were expressed as mean ± SEM and one-way ANOVA was applied to determine the significance of the difference between the control groups and rat treated with the test compounds.

RESULTS AND DISCUSSION

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The successive extracts of *Lawsonia ulba* Linn., have revealed the presence of alkaloids, flavonoids, glycosides, lignins, phenols, saponins, sterols,and tannins are shown in Table 1. Carrageenan-induced rat paw oedema is used widely as a working model of inflammation in the search for new anti-inflammatory drug. The anti inflammatory activity of the aqueous, methanolic and ethanolic extract of *Lawsonia ulba* Linn., was evaluated by carrageenan-induced rat paw oedema method (Winter *et al*., 1962; Adeyemi *et al*., 2002) and the result is shown in Table 2. The extracts were tested at two different dose levels. The results showed that the methanolic extract with a dose of 20 mg/kg b.w and ethanolic extract with a concentration of 50 mg/kg b.w. showed 90.9% of inhibition on carrageenan induced rat paw edema at third hour. This result indicated that methanolic extract with a dose of 20 mg/kg b.w and ethanolic extract with a concentration of 50 mg/kg b.w. showed a maximum anti-inflammatory activity as compared to the reference drug Voveran, which showed only 72.72% inhibition. Methanolic extract with a dose 50 mg/kg b.wt produced 81.81% of inhibition and is also high as compared to the reference drug. Ethanolic extract with a dose of 50 mg/kg b.w produced 63.6% of inhibition and is low ascompared to the reference drug. Aqueous extract with two different doses 20 mg/kg b.w and 50 mg/kg showed only 18.2% and 36.3% inhibition respectively. It was lower as compared to the reference drug. The development of odema in the paw of the rat after the injection of carrageenan is due to release of histamine, serotonin and prostaglandin like substances (Vinegar *et al*., 1969). Significantly high anti-

inflammatory activity of methanolic extract 20 mg/kg b.wt) and ethanolic extract (50 mg/kg b.w) of *Lawsonia ulba* Linn., may be due to inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin. The present result indicates the efficacy of methanolic extract (20 mg/kg b.wt) and ethanolic extract (50 mg/kg b.wt) of *Lawsonia ulba* Linn., as an efficient therapeutic agent in acute anti-inflammatory conditions.

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