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Research Article

EVALUATION OF MEMBRANE STABILIZING ACTIVITY & PROTEIN DENATURATION ACTIVITY OF LEEA MACROPHYLLA EXTRACTS

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Leea macrophylla herb is known to possess potent anti-inflammatory activity. In the present study the ethanolic extract of Leea macrophylla significantly inhibited the haemolysis of erythrocytes and heat induced protein denaturation in vitro models. Thus it can be postulated that the anti-inflammatory activity of Leea macrophylla could be due to its membrane stabilizing action and inhibition of protein denaturation.

INTRODUCTION

Leea macrophylla (Roxb.) (family: Leeaceae) is a herb or herbaceous shrub with a very big size leaf like an elephant-ear. Ethnobotanical survey of this plant shows some important therapeutic uses in cancer, dysentery, body-ache, and sexual disability (R.S. Singh and A.N. Singh, 1981; K.D. Jadhao and M.P. Wadekar, 2010;). It has some other traditional uses for tonsillitis, tetanus, nephrolithiasis, rheumatism, arthritis, snake bites, sore, pain, and blood effusion (R.S. Singh and A.N. Singh, 1981; K.D. Jadhao and M.P. Wadekar, 2010;). Pharmacologically the plant has been reported to possess antiurolithiatic (A.N. Nizami *et al.* 2012;) and anti-inflammatory activities (S.Dewanjee *et al* 2013;). Therefore, with the aim to investigate the possible anti-inflammatory mechanism *Leea macrophylla* present study of was carried using in-vitro models.

MATERIALS AND METHODS

Plant material: *Leea macrophylla* was obtained from different places in Karad western Maharashtra. The plant was identified and authenticated by Department of Botany, Yashwantrao Chavan College of Science, Karad.

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Preparation of the extract; The dried whole plant was minced and extracted with 70% ethanol-water in the proportion of 70:30, being stirred and macerated at room temperature (22-28°C) for 15 days. The ethanol was evaporated and the extract (yield 8%) was concentrated to the desired level and stored in a refrigerator. In all experiments three doses (50, 100 & 200 µg/ml) of the extracts was tested in vitro models for evaluation of membrane stabilizing and protein denaturation activity.

Drugs and chemicals: Diclofenac sodium was procured from Indoco Remedies, Mumbai. All the drugs and chemicals were of analytical grade obtained commercially. Double distilled water was used throughout the study. Evaluation of anti-inflammatory effects by membrane stabilizing property, **(**Shinde *et al.* 1999;M. Gambhire,*et al.* 2009;): Alsever solution was prepared by dissolving 2% dextrose,0.8% Sodium citrate, 0.05% citric acid and 0.42% sodium chloride in distilled water, which was later sterilized. Blood was collected from median cubital vein of the second author [ARC 30 years old male]. The collected blood was mixed with equal volume of sterilized alsever solution. The bloodwas centrifuged at 1000-2000 rpm and packed cells were washed with isosaline and a suspension in 10% (V/V) Isosaline was made. Various concentrations of the *Leea macrophylla* extract was prepared in a mixture of 1ml Phosphate buffer, 2ml Hyposaline and 0.5ml HRBC suspension. Diclofenac sodium was used as the reference drug. Instead of hyposaline, 2ml of distilled water was used

in control. The assay mixtures were incubated at 37ºC for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated using UV spectrophotometer (Shimadzu, UV 1800) at 560nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC [Human Red Blood Cells] membrane stabilization or protection was calculated using this equation, Percentage inhibition of Haemolysis = 100 X [OD1-OD2 /OD1] Where, OD1= Optical Density of hypotonic buffered saline solution alone and OD2 = Optical Density of test sample (*L. macrophylla* extracts and diclofenac) in hypotonic medium.

Evaluation of in vitro anti-inflammatory activity by Protein denaturation method (Shinde *et al*. 1999; Gambhire *et al*. 2009;). The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 4 mL of varying concentrations of *Leea macrophylla* extract so that final concentrations become 50, 100 and 200µg/mL. Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37^0c ±2) in a incubator for 15 min and then heated at 700 c for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank. Diclofenac sodium was used as reference drug and treated similarly for determination of absorbance and viscosity. The percentage inhibition of protein denaturation was calculated by using the following formula, % inhibition = 100 x (Vt / Vc - 1) Where, Vt = absorbance of test sample, Vc = absorbance of control.

CALCULATION

The percentage inhibition of haemolysis in tests *(b)* and *(c)* was calculated according to the equation: % inhibition of haemolysis = 100 x [1- (OD2-OD1 / OD3-OD1)] Where, OD1= test sample unheated or in isotonic solution; OD2=test sample heated or in hypotonic solution; and OD3=control sample heated or in hypotonic solution.

Table I Effect of *Leea macrophylla* on heat-induced and hypotonic solution-induced haemolysis of erythrocyte membrane

Treatment	Concentration (µg/ml)	% Inhibition of haemolysis	
		Heat-induced	Hypotonic solution-induced
Control	-	-	-
Leea macrophylla	50*	18.33 ± 0.277	54.43 ± 0.722
	100*	38.22 ± 0.677	63.12 ± 0.506
	200*	49.68 ± 0.023	66.05 ± 0.049
Diclofenac sodium	20*	25.12 ± 1.72	76.23 ± 1.35

Values are mean ± S.D., *n*=6; *P< 0.01 vs. control, Student's t-test.

Table II Effect of *Leea macrophylla* extract of on protein denaturation

Treatment	Concentration (µg/ml)	% Inhibition of protein denaturation
Control	-	-
Leea macrophylla	50*	21.22 ± 0.633
	100*	34.77 ± 0.722
	200*	46.55 ± 0.317
Diclofenac sodium	10*	82.83 ± 1.602

Values are mean ± S.D., *n*=6; *P< 0.01 vs. control, Student's t-test.

Statistical analysis Data were statistically analyzed by Student's *t*-test and P< 0.001 vs. control were considered to be significant.

RESULTS

Membrane stabilizing activity

In the study of membrane stabilization activity the *Leea macrophylla* extract at concentration range of 50-200 µg/ml protect significantly the erythrocyte membrane against lysis induced by heat as well as hypotonic solution. At a concentration of 200 µg/ml, the *Leea macrophylla* extract showed highest % inhibition in Heat induced and hypotonic solution induced Haemolysis when compared with blank. The details are summarized in Table I.

Inhibition of protein denaturation

The inhibitory effect of different concentrations of *Leea macrophylla* extract on protein denaturation is shown in Table II. *Leea macrophylla* extract (50-200 µg/ml) showed significant inhibition of denaturation of egg albumin in concentration dependent manner. *Leea macrophylla* extract at concentration of 200 µg/ml showed significant inhibition of protein denaturation when compared with control and standard.

DISCUSSION

In the current study in-vitro results confirm the reported anti-inflammatory activity of *Leea macrophylla*. The present investigation suggests that the membrane stabilizing activity of *Leea macrophylla* may be playing a significant role in its anti-inflammatory activity. A possible explanation for the stabilizing activity of *Leea macrophylla* could be an increase in the surface area to volume ratio of the cells which could be brought about by an expansion of membrane or shrinkage of the cell, and an interaction with membrane proteins. Denaturation of proteins is a well documented cause of inflammation and rheumatoid arthritis. (Shinde *et al*. 1999; M. Gambhire, *et al*. 2009;). Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. (Shinde *et al*. 1999; M. Gambhire *et al*. 2009;). Ability of *Leea macrophylla* extract to bring down thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity. Several bioactive molecules, such as flavonoids, steroids and terpenoids, have been shown to be present in the extract of *Leea macrophylla*. R.S. Singh and A.N. Singh, 1981; K.D. Jadhao, and M.P. Wadekar, 2010; A.N. Nizami *et al*. 2012; S. Dewanjee *et al*. 2013;). The anti-inflammatory activity of *Leea macrophylla* extract in the present study may be due to the presence of therapeutically active flavonoids and steroids. The data of our studies suggests that *Leea macrophylla* showed significant anti-inflammatory activity in both the in-vitro methods tested. Further studies involving the purification of the chemical constituents of *Leea macrophylla* may result in the development of a potent anti-inflammatory agent.

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