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Original Article

Evaluation for Membrane Stabilizing Activity & Protein Denaturation activity of Leaves of *Wrightia tinctoria*

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ABSTRACT

Wrightia Tinctoria is known to possess potent anti-inflammatory activity. In the present study, the methanolic extract of the leaves of *Wrightia Tinctoria* significantly inhibited the hemolysis of erythrocytes and heat-induced protein denaturation in vitro models. Thus it can be postulated that anti-inflammatory activity of *Wrightia Tinctoria* could be because its membrane stabilizing action and inhibition of protein Denaturation.

Keywords- *Wrightia Tinctoria,* anti-inflammatory.

1. INTRODUCTION

Wrightia tinctoria (roxb) R.Br is a small deciduous tree belong to family Apocynaceae is commonly known as Dudhi (Sweet indrajao) found in central India, Burma, and Timor. It is extensively used in Indian system of medicine. Fresh leaves are pungent and gives relief from tooth ache.[1-2] A decoction of leaves used as astomachic and in treatment of abdominal pain.[1-2] Ethyl acetate, acetone and methanol extracts of *Wrightia tinctoria* bark shows antinociceptive activity in pinice and wound healing activity.[3] The reported constituents are alkaloids, triterpenoids & wright have demonstrated anti-inflammatory activity.[4,5,6]

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In our previous studies have reported anti-inflammatory activity of methanolic extract of leaves of *Wrightia tinctoria* in Carrageenan-induced paw edema test. [7] Therefore, the aim is to investigate the possible anti-inflammatory mechanism *Wrightia tinctoria* present study of was carried using in-vitro models.

2. MATERIALS AND METHODS

Plant Material

The leaves *of Wrightia tinctoria* collected from Jalgaon state Maharashtra in August 2007. The plant was identified and authenticated by T. Chakraborty Joint Director, Botanical Survey of India, Western circle, Pune.

Preparation of Extract:

The World Health Organization procedure of extraction was adapted for the study. A total of 100 gram of the powder *of Wrightia tincoria* leaves subjected to Soxhlet extraction in 500 ml of distilled water for 72 hours. The extract obtained was concentrated in a water bath until constant dark sticky residue obtained which further dried by a vacuum dryer and maintained in desiccators until a constant weight obtained.

In all experiments, three doses (50, 100 & 200 µg/ml) of the extracts were tested for in-vitro models for evaluation of membrane stabilizing and protein denaturation.

Drugs and chemicals- Diclofenac sodium was procured from Indoco Remedies, Mumbai. All the drugs and chemicals used were of analytical grade obtained commercially — double distilled water used throughout the study.

Evaluation of anti-inflammatory effects by membrane stabilizing property[8-9]

Alsever solution 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 collected from the median cubital vein of the first author [NVP 43 years old male]. The collected blood mixed with an equal volume of sterilized alsever solution. The blood centrifuged at 1000-2000 rpm, packed cells were washed with isosaline and a suspension in 10% (V/V)

Isosaline was made. Various concentrations of the *Wrightia tinctoria* extract prepared in a mixture of 1ml Phosphate buffer, 2ml Hyposaline, 0.5ml of HRBC suspension. Diclofenac sodium was used as reference drug. Instead of hyposaline, 2ml of distilled water was used

in control. The assay mixtures incubated at 37ºC for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution estimated using UV spectrophotometer (Shimadzu, UV 1800) at 560nm. The percentage haemolysis 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 calculated using this equation,

Percentage inhibition of Haemolysis = 100 X [OD1-OD2 /OD1]

Where OD1= Optical Density of the hypotonic buffered saline solution alone and OD2 = Optical Density of the test sample (*Wrightia tinctoria* extract and diclofenac) in the hypotonic medium.

Evaluation of in-vitro anti-inflammatory activity by Protein denaturation method [8-9]

The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffer saline (PBS, pH 6.4) and 4 mL of various concentrations of *Wrightia tinctoria* extract, so that final concentration became 50, 100 and 200 µg/mL. A similar volume of double-distilled water served as control. Then the mixtures was incubated at (370c ±2) in an incubator for 15 min and followed by heating at 700 c for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using the vehicle as blank. Diclofenac sodium was used as reference drug and treated similarly for absorbance and viscosity. The percentage inhibition of protein denaturation 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)* calculated according to the following 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 a hypotonic solution, and OD3=control sample heated or in a hypotonic solution.

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

3. RESULTS

Membrane stabilizing activity

In the study of membrane stabilization activity, the *Wrightia tinctoria* extract at a concentration range of 50-200 µg/ml protect the erythrocyte membrane significantly against lysis induced by heat as well as hypotonic solution. With a concentration of 200 µg/ml, the *Wrightia tinctoria* extract showed the highest % inhibition in Heat-induced and hypotonic solution induced Haemolysis when compared with blank. The details are summarized in Table 1.

Inhibition of protein denaturation

The inhibitory effect of different concentrations of *Wrightia tinctoria* extract on protein denaturation is shown in Table 2. *Wrightia tinctoria* extract (50-200 µg/ml) showed significant inhibition of denaturation of egg albumin in a concentration-dependent manner. *Wrightia tinctoria* extract at a concentration of 200 µg/ml showed significant inhibition of protein denaturation when compared with control and standard.

4. DISCUSSION

In the current study, in-vitro results confirmed the reported anti-inflammatory activity of *Wrightia tinctoria*. The present investigation suggests the membrane stabilizing the activity of *Wrightia tinctoria* may be playing a significant role in its anti-inflammatory action.

A possible explanation for stabilizing the activity of *Wrightia tinctoria* may be an increase in the surface area to volume ratio of the cells; which could be possibly brought about by an expansion of membrane or shrinkage of the cell, interaction with membrane proteins. [8-9]

Table 1: Effect of *Wrightia tinctoria* on heat-induced and hypotonic solution-induced hemolysis of erythrocyte membrane

Treatment	Concentration (µg/ml)	% Inhibition of hemolysis	
		Heat-induced	Hypotonic solution-induced
Control	-	-	-
Wrightia Tinctoria	50*	16.33 ± 0.277	54.43 ± 0.722
	100*	36.22 ± 0.677	63.12 ± 0.506
	200*	46.82 ± 0.023	66.05 ± 0.049
Diclofenac sodium	20*	24.21 ± 1.72	76.23 ± 1.35

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

Table 2: Effect of *Wrightia tinctoria* extract of on protein denaturation

Treatment	Concentration (µg/ml)	% Inhibition of protein denaturation
Control	-	-
Wrightia Tinctoria	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.

Denaturation of proteins is a well-documented cause of inflammation and rheumatoid arthritis. [8-9] Several anti-inflammatory drugs have shown dose-dependent ability to inhibit thermally-induced protein denaturation.[8-9] Knowledge of *Wrightia tinctoria* 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, are present in the extract of *Wrightia tinctoria* . [1-7,10] The anti-inflammatory activity of *Wrightia tinctoria* extract in the present study may be due to the presence of therapeutically active flavonoids and steroids. [1-7,10]

The data of our studies suggest that *Wrightia tinctoria* showed significant anti-inflammatory activity in both the in-vitro methods. Furthermore, studies involving the purification of the chemical constituents of *Wrightia tinctoria* may result in the development of a potent anti-inflammatory agent.

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