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Research Article

In vitro anti-inflammatory activity of hydroalcoholic extract of *Asparagus racemosus* roots

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ABSTRACT

Asparagus racemosus commonly known as Shatavari belonging to family Liliaceae. In India, it is found in Himalaya's up to an altitude of 1300-1400m and all tropical part. Its various uses are galactogogue, tonic, diuretic, rheumatism, inflammation, nervine disorder etc. The aim of study is to evaluate anti-inflammatory activity of hydroalcoholic extract of *Asparagus racemosus* (ARHE) roots by using human red blood cell membrane stabilisation (HRBC) method and protein denaturation method. The inhibition of hypotonicity induced HRBC membrane lysis and denaturation of protein was taken as a measure of the anti inflammatory activity. The percentage of membrane stabilisation and protein denaturation for hydroalcoholic extracts and Diclofenac sodium were done at different concentrations. The maximum membrane stabilization and denaturation of protein of *A. racemosus* extracts was found to be 76.63 % at a dose of 1600 µg/ml and 545.91 at a dose of 2000 µg/ml respectively.

Keywords: Anti-inflammatory; *A. racemosus;* Liliaceae; HRBC; Protein denaturation

INTRODUCTION

Inflammation is the protective mechanism of the local microcirculation to tissue injury which caused by physical trauma, noxious stimuli by chemical agents, heat, antigen-antibody reaction and microbial effect. (Mahesh G et al., 2011) It is a body defence reaction in order to eliminate or limit the spread of injurious agents. (Paschapur MS et al., 2009) It is known to be involved in the inflammatory reactions such as release of histamine, bradykinin, prostaglandins, fluid extravasations, cell migration, tissue breakdown and repair which are aimed at host defence and usually activated in most disease condition. (Kumar V et al., 2012) The mechanism of inflammation injury is attributed, in part, to release of Reactive Oxygen species from activated neutrophil and macrophages. This over production leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. (Lavanya R et al., 2010) Nature has provided a complete store-house of remedies to cure all aliments of mankind. (Ravi V et al., 2009) Many herbal-based remedies are believed to have a range of biomedical efficacies including treatment of inflammation, hyperlipemia, arteriosclerosis, osteoporosis and bone resorption etc. (Talhouk RS et al., 2008) Plants with analgesic and anti-inflammatory activities have become more interesting because some of them are part of the arsenal of modern medicine and many people are aware of problems associated with the over-prescription and misuse of usual drugs. (Marzouk B et al., 2011) Many plants belonging to family Liliaceae with wide range of pharmacological and biological activity. *Asparagus racemosus* is one of the plant of that family. The present study is to investigate about anti-inflammatory property of *A. racemosus* by in-vitro method.

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MATERIALS AND METHODS

Plant material

Roots of *A. racemosus* (Family: Liliaceae) were procured in the month of May 2012 from Lucknow, India. After procurement, the roots were dried in shade and ground mechanically into a coarse powder and kept into an air-tight container for use in the study.

Preparations of extract

The powder plant material was extracted with hydroalcohol (30% Ethanol and Water 70%). The extract (ARHE) was filtered and evaporated to dryness to yield the dry extract. The dry extract was kept in a vacuum desiccator until use.

Evaluation of anti-inflammatory activity by HRBC method

HRBC method used for the estimation of anti-inflammatory activity in-vitro. (Mohamed Saleem TK et al., 2011) Blood was collected from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution. This blood solution was centrifuged at 3000

rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the estimation of anti-inflammatory property. Different concentrations of extract, reference sample and control were separately mixed with 1mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of HRBC suspension. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged at 3000 rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

Percentage protection= 100 (OD sample/ OD control) x 100

Table 1: HRBC method of ARHE

Sl. No.	Concentration (µg/mL)	% protection
1.	Control	-
2.	100	49.15
3.	200	57.21
4.	400	61.16
5.	800	66.55
6.	1600	76.63

Table 2: HRBC method of Diclofenac Sodium

Sl. No.	Concentration (µg/mL)	% protection
1.	Control	-
2.	100	57.25
3.	200	60.61
4.	400	67.06
5.	800	70.51
6.	1600	80.35

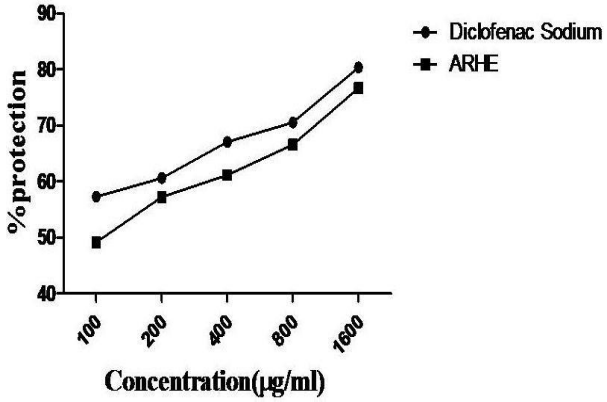

Figure 1: Effect of ARHE and Diclofenac sodium on HRBC method

Evaluation of anti-inflammatory activity by protein denaturation method

The reaction mixture (50 mL) consisted of 2 mL of egg albumin (from hen's fresh egg), 28 mL of phosphate buffered saline (PBS, pH 6.4) and 20 mL of varying concentrations of ARHE so that final concentrations become 10, 50, 100, 200, 400, 800, 1000, 2000 µg/mL. Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37±2) °C in a BOD incubator for 15 min and then heated at 70 °C for 5 min on water bath. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank and their viscosity was determined by using Ostwald viscometer. Diclofenac sodium at the final concentration of (10, 50, 100, 200, 400, 800, 1000, 2000 µg/mL) 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:

$$\% \text{ inhibition} = 100 \times (Vt / Vc - 1)$$

Where,

Vt = absorbance of test sample

Vc = absorbance of control sample

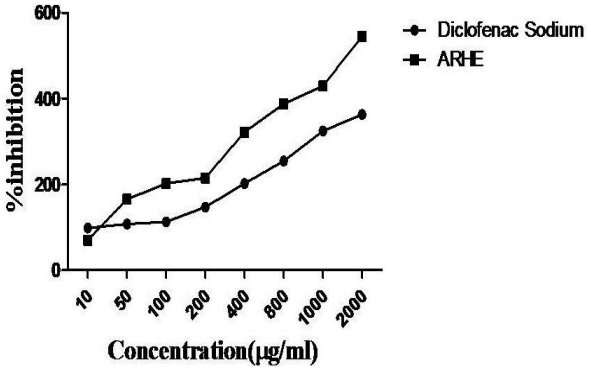

Figure 2: Effect of ARHE and Diclofenac sodium on Protein denaturation method

RESULTS AND DISCUSSION

Effect of ARHE on HRBC membrane stabilization

The results of the human red blood cell membrane stabilization test were shown in Table 1 and 2 and Fig:1. The ARHE showed a concentration dependent anti inflammatory activity, and the protection percent increased with increase in the concentration of the drug. All the results were compared with control and diclofenac (standard) which showed 80.35% protection (Table 2).

Effect of ARHE on protein denaturation method

In the present investigation, the anti-inflammatory effect of *A. racemosus* hydroalcoholic extract (ARHE) was evaluated against the denaturation of protein. The results are summarized in (Table: 3 and 4 and Fig:2). The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by (ARHE) throughout the concentration range from 10 to 2000 µg/ml. Diclofenac sodium (at the concentration range from 10 to 2000 µg/ml) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation, however, the

Table 3: Protein denaturation method of ARHE

S. No.	Concentration (µg/ml)	% inhibition	Viscosity (cps)
1.	Control	--	1.40
2.	10	70.40	0.71
3.	50	165.30	0.75
4.	100	202.04	0.77
5.	200	215.30	0.82
6.	400	322.44	1.03
7.	800	387.75	1.08
8.	1000	429.59	1.10
9.	2000	545.91	1.13

Table 4: Protein denaturation method of Diclofenac sodium

S.No.	Concentration (µg/ml)	%inhibition	Viscosity (cps)
1.	Control	-	1.40
2.	10	98.08	0.93
3.	50	107.66	0.94
4.	100	112.99	1.02
5.	200	147.07	1.03
6.	400	202.44	1.05
7.	800	254.63	1.08
8.	1000	324.92	1.09
9.	2000	363.25	1.10

effect of Diclofenac sodium was found to be less as compared with ARHE. In the present study, the relatively high viscosity of control dispersion substantiated this fact. The viscosities of the ARHE and Diclofenac sodium, of all concentrations were always less than that of control. Therefore, form the results of the present preliminary study it can be stated that *A. racemosus* possessed marked anti-inflammatory effect against the denaturation of protein.

DISCUSSION

The *A. racemosus* roots exhibited membrane stabilization effect by inhibiting hypo tonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. (Rahman H et al., 2012) Denaturation of tissue proteins is one of the well documented causes of inflammation. (Sakat SS et al., 2010) Production of auto-antigens in certain inflammatory diseases may be due to denaturation of proteins *in vivo*. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The mechanism of denaturation probably involves alteration electrostatic hydrogen, hydrophobic and disulphide bonding. The increments in absorbances of test sample with respect to control indicate stabilization of protein. It has been reported that the viscosities of protein solutions increase on denaturation. (Chandra S et al., 2012).

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