

Research Article

## *In vitro* Anti-inflammatory Activity of *Aerva lanata*

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### ABSTRACT

**Objective:** To evaluate the invitro anti-inflammatory activity of *Avera lanata* by HRBC lysis and protein denaturation. **Method:** The extract at different concentrations was incubated with HRBC and egg albumin in controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. **Results:** The present findings exhibited a concentration dependent inhibition of lysis of HRBC and protein (albumin) denaturation by the *A.lanata* extract. The effect of diclofenac sodium was found to be less when compared with the ethanol extract. **Conclusions:** From the present study it can be concluded that *A.lanata* possessed marked in vitro anti-inflammatory effect against the HRBC lysis and denaturation of protein. The effect was possibly due to the polyphenols and flavanoid contents of *A.lanata*.

**Key words:** *Aerva lanata*, anti-inflammatory, stabilization, protein denaturation.

### INTRODUCTION

Inflammation is defined as local response of living mammalian tissue to injury due to any agent. It is the body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissue<sup>1</sup>. A variety of molecules are released from cells and plasma proteins during acute inflammation whose net overall effect is to increase vascular permeability, resulting in tissue edema. The released molecules includes histamine, PGs, eicosanoids, PAF, bradykinin and serotonin<sup>2</sup>. The most commonly used drug for management of inflammatory conditions are nonsteroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers<sup>3</sup>. For this reason, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is lack of scientific evidence.

*Aerva lanata* (Amaranthaceae) is a woody, prostrate or succulent, perennial herb common weed which grows wild everywhere in the plains of India. The root has a camphor-like aroma. The dried flowers, which look like soft spikes, are sold under the commercial names as Buikallan or Boor. It is one of the plants included in Dasapushpam, the ten sacred flowers of Kerala<sup>4</sup>. *A. lanata* has been reported to have the following pharmacological properties anthelmintic activity, hepatoprotective activity, hypoglycemic effect, anti-microbial activity, anti asthmatic effect, urolithiatic activity, invivo anti-inflammatory activity, anti-diarrheal activity, diuretic activity, anti-oxidant activity and anti-nociceptive effect<sup>5</sup>.

### MATERIALS AND METHODS

#### *Plant material*

The plant *Aerva lanata*, were collected during October 2013 from the premises of Academy of Pharmaceutical Sciences, Pariyaram, Kannur, Kerala, India. The plant material was taxonomically identified by Dr.P G Radha, Associate Professor, HOD, Department of Botany, Government Brennen College, Thalassery, Kannur, Kerala, India. Herbarium specimen bearing voucher number A.P.S.C 528 have been deposited in the Department of Pharmacology, Academy of Pharmaceutical Sciences, Pariyaram, Kannur, Kerala, India for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an airtight container.

#### *Preparation of plant extracts*

The dried powdered material was successively extracted with petroleum ether, chloroform, methanol and water by cold maceration process. The solvent was distilled off in reduced pressure using rotary flash evaporator to yield a solid residue and the percentage extractive values were accordingly 1.4% w/w, 3.62% w/w, 4.7% w/w and 9.2% w/w respectively. The preliminary phytochemical analysis was performed for all four extracts to identify the phytoconstituents present in the extracts<sup>6</sup>.

#### *Chemicals and instruments*

All chemicals used in the estimation were of analytical grade. Shimadzu 1700 UV visible spectrophotometer, Weiber-Acmas Technocracy BOD incubator, Superfit rotavapour and Remi mechanical stirrer was used for the *in vitro* study.

#### *Screening of Anti-inflammatory activity*

##### *HRBC method*

The human red blood cell (HRBC) membrane stabilization method was used as one of the methods for the determination of anti-inflammatory activity invitro<sup>7</sup>. The

Table.1: *In vitro* anti-inflammatory activity of *Aerva lanata* activity by HRBC method

Concentration µg/ml	Diclofenac sodium	Petroleum ether extract	Chloroform extract	Methanol extract	Aqueous extract
31.25	40.93±0.46	11.35±0.70	13.16±0.47	43.52±0.42	23.35±0.86
62.50	45.48±0.38	13.01±0.92	15.85±0.89	47.83±0.96	25.69±0.48
125	49.85±1.06	16.15±1.02	18.20±0.66	52.31±0.81	26.99±1.05
250	54.17±0.26	18.72±0.64	20.39±1.10	58.99±0.23	29.42±0.62
500	59.44±1.07	20.19±0.59	23.65±0.75	66.57±0.77	32.84±1.09
1000	63.06±0.58	22.21±0.91	30.34±0.73	71.84±0.61	38.47±0.78

Values are expressed as mean ± S.D(n=6)

Table.2: *In vitro* anti-inflammatory activity of *Avera lanata* activity by protein denaturation method

Concentration µg/ml	Diclofenac sodium	Petroleum ether extract	Chloroform extract	Methanol extract	Aqueous extract
32.25	56.41±0.51	12.84±0.34	15.23±1.02	60.26±0.53	25.38±0.31
65.50	58.82±0.84	15.06±0.93	16.39±1.28	65.92±0.82	26.62±1.06
125	60.56±1.21	17.44±1.15	19.74±0.65	68.84±0.58	30.58±0.63
250	63.71±0.87	20.79±0.88	25.64±0.67	73.02±0.86	33.97±0.76
500	66.36±0.45	26.85±0.49	32.98±0.36	78.41±0.98	37.86±0.35
1000	78.25±0.79	33.68±0.26	37.79±1.08	83.67±0.54	41.52±0.72

Percentage protection is a mean of six readings ± S.D(n=6)

blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution and centrifuged at 3,000 rpm and the packed cells were separated. The packed cells were washed with isosaline and a 10% suspension was made with isosaline. This HRBC suspension was used for the estimation of anti-inflammatory property. Different concentration of extract, reference sample and control were separately mixed with 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. All the assay mixtures were incubated at 37°C for 30 min in a BOD incubator and sufficiently centrifuged at 3,000-rpm. The supernatant liquid was decanted and the hemoglobin content was estimated spectrophotometrically at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

% protection =  $[1 - (\text{OD sample}/\text{OD control})] \times 100$

#### Protein denaturation method

The protein denaturation method was the other method of assay for the assessment of anti-inflammatory activity invitro with slight modification as described by Elias<sup>8</sup>. The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the test extract so that final concentrations become 31.25, 62.5, 125, 250, 500, 1000 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 minutes and then heated at for 5 minutes. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the final concentration of (31.25, 62.5, 125, 250, 500, 1000 µg/ml) was used as reference drug and treated similarly for determination of absorbance of test extract. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition =  $100 \times [Vt / Vc - 1]$

Where, Vt = absorbance of test sample, Vc = absorbance of control.

The extract/drug concentration for 50% inhibition (IC<sub>50</sub>) was determined from the dose response curve by plotting percentage inhibition with respect to control

#### Statistical analysis

Statistical analysis was done with one way analysis of variance followed by dunnets *t* test. P value <0.05 were considered significant.

## RESULT

### Phytochemical analysis

The qualitative chemical screening for the identification of various classes of active chemical constituents of the ethanolic was carried out. The ethanolic extract showed the presence of flavanoids, terpenoids, phenol, tannins, alkaloids and proteins.

### Anti-inflammatory activity by HRBC method

The leaf extracts of *Aerva lanata* at different concentration showed significant stabilization towards HRBC membranes. The percentage protection of methanolic extract showed significant anti-inflammatory activity in a concentration dependent manner. The results are tabulated in Table.1

### Anti-inflammatory activity by protein denaturation method

The denaturation of proteins is a well-documented cause of inflammation. Phenylbutazone, salicylic acid, flufenamic acid etc, have shown dose dependent ability to thermally induced protein denaturation. As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation at different concentrations as shown in Table 2. The methanol extract of *Avera lanata* showed more inhibition than the standard drug Diclofenac sodium.

**DISCUSSION**

The *Avera lanata* of the family *Amaranthaceae* is a commonly found weed all over India. The plant is a phytotherapeutic plant used in folk medicine that is believed to have active components that help to treat and manage various diseases. Chemical evaluation of the methanolic extract of leaf reveals the presence of flavanoids, terpenoids, phenol and tannins. These phytochemicals are capable in preventing free radical attack and stabilizing the lysosomal membrane thereby preventing inflammatory process<sup>9</sup>. Here anti-inflammatory activity invitro was performed based on folk lore information and invivo anti-inflammatory study<sup>10</sup>. In HRBC method the erythrocytic membrane is similar to lysosomal membrane and its stabilization entails that the extract may stabilize lysosomal membranes. This stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents, which causes further tissue inflammation and damage upon extracellular release<sup>11</sup>. The result indicated that the methanolic extract of *Avera lanata* had significant anti-inflammatory in collation with diclofenac sodium. Denaturation of proteins is one of the conductive causes for the production of autoantigens in certain rheumatic diseases invivo. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding<sup>12</sup>. From the present study it can be stated that *Avera lanata* is capable of controlling the production of autoantigens due to denaturation of proteins and stabilize the lysosomal membranes *invivo*.

**CONFLICT OF INTEREST**

The authors declare that are no conflicts of interest.

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