



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: [www.elsevier.com/locate/apjtd](http://www.elsevier.com/locate/apjtd)

Document heading

## Evaluation of *in vivo* and *in vitro* anti-inflammatory activity of *Ajuga bracteosa* Wall ex Benth

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## ARTICLE INFO

## Article history:

Received 18 June 2012

Received in revised form 27 June 2012

Accepted 18 October 2012

Available online 28 October 2012

## Keywords:

Anti-inflammatory

*Ajuga bracteosa*

HRBC

Carrageenan

## ABSTRACT

Ethnopharmacological relevance: *Ajuga bracteosa* Wall Ex Benth. (Labiatae) is described in Ayurveda for the treatment of rheumatism, gout, palsy and amenorrhea. **Objective:** Present study was aimed to investigate the *in vivo* and *in vitro* anti-inflammatory activity of Neelkanthi (whole plant) and to support its traditional use. **Methods:** Methanolic extract of plant *Ajuga bracteosa* (ABE) was investigated for its anti-inflammatory activity in carrageenan induced rat paw oedema, egg albumin induced inflammation in rats and the study was further supported with *in vitro* anti-inflammatory study by using Human red blood cell membrane stabilization (HRBC) method. Three doses of the extract (ABE– 250, 500 and 750 mg/kg, i.p.) were used in the study and diclofenac sodium (5mg/kg, i.p.) was used as standard. **Results:** ABE (500 and 750 mg/kg, i.p.) significantly ( $P<0.05$ ) reduced increased in paw volume induced by carrageenan and egg albumin. ABE also showed significant stabilization toward HRBC membrane. **Conclusions:** ABE at the dose of 500 and 750 mg/kg showed potent action on comparison with the standard drug diclofenac sodium.

### 1. Introduction

Inflammation is a pathophysiological response of living tissue to injury that leads to local accumulation of plasmatic fluid and blood cells. Although it is a defence mechanism that helps body to protect it–self against infection, burns, toxic chemicals, allergens or other noxious stimuli, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases [1].

Drugs that are currently used for the management of pain are opioids or nonopioids and that for inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. All these drugs carry potential toxic effects. One study suggests that risk of gastrointestinal bleeding was significantly associated with acute use of non-steroidal anti-inflammatory drugs (NSAIDs) like regular-dose aspirin, diclofenac, ketorolac, naproxen or nimesulide.

Piroxicam increased the risk of bleeding in both acute and chronic therapy [2]. Opioids are the commonly used drugs for the management of acute postoperative pain [3].

*Ajuga bracteosa* (Family: Labiateae) commonly known as Neelkanthi is a perennial herb with diffused branching and aroma; flowers are hermaphrodite with white or pink colour. Plant is found in hilly areas and even on rock cervice upto 1500 m. and is distributed from Kashmir to Nepal, sub-Himalayan tract, plains of Punjab and the upper Gangetic plains. The herb is in use since ancient times and recommended in Ayurveda for the treatment of rheumatism, gout, palsy and amenorrhea. It is also credited with astringent, febrifugal, stimulant, tonic, and diuretic properties. Previous investigations on *Ajuga bracteosa* have reported the inhibition of acetylcholinesterase, butyrylcholinesterase and lipoxygenase[4]–[5], Calcium antagonistic property[6], cancer chemopreventive[7], antiplasmodial[8], anti-inflammatory effect through cyclooxygenase (COX) inhibition[9], analgesic activity[10], Antiarthritic activity[11] and cardiostimulant[12] actions.

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## 2. Material and Methods

### 2.1. Plant material

The whole plants of *Ajuga bracteosa* were collected from Chamoli Garhwal (Uttarakhand) and authenticated by Mrs. Sayyada Khatoon (Scientist) Pharmacognosy and Ethanopharmacology Division, National Botanical Research Institute, CSIR, Lucknow, UP.

### 2.2. Preparation of extracts

The collected plants were immediately shade dried and then powdered by a pulverizer. The powdered plant material (0.5 kg) were defatted with petroleum ether and extracted by maceration with methanol (3 times, 1litre each) at room temperature. The extract was pooled, filtered through a Whatmann filter paper and the solvent was removed on a vacuum rotary evaporator under reduced pressure to get the dried extract (yield: 15.3%).The dried extract was stored at  $-20^{\circ}\text{C}$  till its further use.

### 2.3. Animals

Swiss albino mice of either sex (20–30g) were procured from Central Drug Research Institute (CDRI) Lucknow and were randomly distributed into different experimental groups. The mice were kept in the departmental animal house at an ambient temperature of  $25 \pm 1^{\circ}\text{C}$  and 45–55% RH, with a 12-h light/dark cycle. The animals had free access to standard pellet chow and tap water ad libitum. Experiments were conducted between 9:00 and 16:00 h. The behavioural testing was done during the light phase. Animals were acclimatized for at least one week before using them for experiments and exposed only once to every experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) 1279/ac/09/CPCSEA.

### 2.4. Evaluation of anti-inflammatory activity of the extract

#### 2.4.1. In vitro Anti-inflammatory activity

HRBC method was used for the estimation of anti-inflammatory activity *in vitro*<sup>[13]</sup>. 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  $37^{\circ}\text{C}$

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 haemolysis was estimated by assuming the haemolysis produced in the control as 100%.

Percentage protection=  $100 - (\text{OD sample} / \text{OD control}) \times 100$

#### 2.4.2. In vivo Anti-inflammatory activity

Paw oedema was induced on each rat by injecting 0.1 mL of carrageenan on physiological saline to the left hind paw<sup>[14]</sup>. The extracts at different concentrations were administered orally 30 minutes prior to carrageenan administration. Paw volumes were measured at 60, 120, 180 and 240 minutes by mercury displacement method using plethysmograph. The percentage inhibition of paw volume in extract treated groups was compared with control. Diclofenac sodium (5 mg/kg) was used as the standard.

#### 2.4.3. Egg albumin induced inflammation

Inflammation was induced in rats by the injection of egg albumin (0.1 mL, 1% in normal saline) into the sub plantar tissue of the right hind paw<sup>[15]</sup>. Paw volumes were measured at 60, 120, 180 and 240 minutes by mercury displacement method using plethysmograph. The percentage inhibition of paw volume in extract treated groups was compared with control. Diclofenac sodium (5 mg/kg) was used as the standard.

### 2.5. Statistical analysis

Statistical analysis was done using one way analysis of variance followed by Dunnett's test.  $P < 0.05$  were considered as significant.

## 3. Results

### 3.1. In vitro anti-inflammatory activity

ABE at all concentration showed significant stabilization towards HRBC membranes. The percentage protection of ABE at concentration 500 mg/ml was higher than that of 250mg/ml concentrations. However the percentage protection was found to be decreased at higher concentration of 750 mg/ml. The results were tabulated in Table 1.

**Table 1**

*In vitro* anti-inflammatory activity of ABE in HRBC method.

| S.No. | Extract type (mg/ml)       | Percentage protection |
|-------|----------------------------|-----------------------|
| 1.    | Diclofenac sodium (5mg/ml) | 38.72                 |
| 2.    | ABE (250mg/ml)             | 25.27                 |
| 3.    | ABE (500mg/ml)             | 37.01                 |
| 4.    | ABE (750mg/ml)             | 34.32                 |

**Table 2**

Anti-inflammatory activity of ABE against carrageenan induced paw oedema in rats

| Treatment group | Dose (mg/kg) | Carrageenan induced paw oedema in rats (Volume in ml) |            |            |            |             |
|-----------------|--------------|---|------------|------------|------------|-------------|
|                 |              | 00 min  | 60min      | 120min     | 180 min    | 240min      |
| Control         | 10ml/kg      | 0.13±0.69   | 0.38±0.098 | 0.43±0.18  | 0.45±0.097 | 0.45±0.18   |
| Diclofenac      | 05           | 0.11±0.31*  | 0.19±0.12* | 0.21±0.39* | 0.18±0.67* | 0.18±0.56*  |
| ABE 250         | 250          | 0.13±0.37   | 0.22±0.91  | 0.31±0.76  | 0.35±0.28  | 0.35±12     |
| ABE 500         | 500          | 0.12±0.093  | 0.18±0.32  | 0.25±0.98* | 0.23±0.56* | 0.22±0.93*  |
| ABE 750         | 750          | 0.12±0.97   | 0.20±0.85* | 0.21±0.48* | 0.20±0.38* | 0.17±0.31*a |

Values are expressed in mean ± SEM (n=6); \*- P<0.05 with control, a-P<0.05 with standard.

**Table 3**

Anti-inflammatory activity of ABE against egg albumin induced paw oedema in rats.

| Treatment group | Dose (mg/kg) | Egg albumin induced paw oedema in rats (Volume in ml) |            |             |             |             |
|-----------------|--------------|---|------------|-------------|-------------|-------------|
|                 |              | 00 min  | 60min      | 120min      | 180 min     | 240min      |
| Control         | 10ml/kg      | 0.11±0.69   | 0.28±0.098 | 0.32±0.18   | 0.38±0.097  | 0.39±0.18   |
| Diclofenac      | 05           | 0.12±0.31*  | 0.13±0.12* | 0.17±0.39*  | 0.18±0.67*  | 0.18±0.56*  |
| ABE 250         | 250          | 0.12±0.37   | 0.17±0.91  | 0.22±0.76   | 0.25±0.28   | 0.30±12     |
| ABE 500         | 500          | 0.12±0.093  | 0.18±0.32  | 0.20±0.98*  | 0.21±0.56*  | 0.22±0.93*  |
| ABE 750         | 750          | 0.11±0.97   | 0.15±0.85* | 0.16±0.48*a | 0.17±0.38*a | 0.17±0.31*a |

Values are expressed in mean ± SEM (n=6); \*- P<0.05 with control, a-P<0.05 with standard

### 3.2. In vivo anti-inflammatory activity

#### 3.2.1. Carrageenan induced paw oedema in rat

The ABE at different concentrations showed significant reduction in the paw volume of rats. The ethanolic extract at concentration of 500mg/mg showed potent activity compared with the reference standard Diclofenac sodium. The results were tabulated in Table 2.

#### 3.2.2. Egg albumin induced oedema in rat paw

The ABE at different concentrations showed significant reduction in the paw volume of rats. The ethanolic extract at concentration of 500mg/mg showed potent activity compared with the reference standard Diclofenac sodium. The results were tabulated in Table 3.

## 4. Discussion

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. *Ajuga bracteosa* is plant of family labiatae and well known for its medicinal values. Plant has been used extensively used for the treatment of gout, arthritis and fever. Recent studies suggested that *Ajuga bracteosa* plant having withanolides which are responsible for anti-inflammatory activity by inhibiting Cyclooxygenase (COX) enzyme, which essential for prostaglandin synthesis and further inflammation<sup>[9]</sup>. Other studies also revealed that plant

is useful in treatment of arthritis<sup>[11]</sup>, nociceptive pain<sup>[10]</sup>. HRBC method was selected for the *in vitro* evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane<sup>[16]</sup> 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. The result indicted that the methanolic extract of plant *Ajuga bracteosa* (ABE) at various concentrations has significant anti-inflammatory property. Carrageenan induced inflammation is a useful model for the estimation of anti-inflammatory effect. The development of oedema in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin, prostaglandin and the like <sup>[17-19]</sup>. ABE showed significant anti inflammatory activity. This significant anti-inflammatory effect may be due to the inhibition of any inflammatory mediators by the glycosides or steroids <sup>[20]</sup> present in the extract. The present result indicates the efficacy of *Ajuga bracteosa* as an effective therapeutic agent in the treatment of acute inflammations.

The result of present study authenticifies the folk lore information on the anti-inflammatory property of the *Ajuga bracteosa* extract.

### Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgements

The authors are thankful to the management, director and faculties of Sree Dev Bhoomi Institute, Dehradun for rendering the necessary requirements in this work.

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