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### Original article

## A Study on *Ajuga bracteosa* wall ex. Benth for analgesic activity

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#### ABSTRACT

Ethnopharmacological relevance: *Ajuga bracteosa* wall ex. Benth (Labiatae) is traditionally used medicine in the treatment of malaria and gout. The plant is substitute of cinchona. Its allied species *Ajuga Parviflora* is also found sporadically. In Ayurvedic preparation the aqueous extract of the leaves part showed diuretic activity. Aim of the study: The present study was carried out to investigate analgesic activity of *Ajuga bracteosa* wall ex. Benth aerial part extracts. Materials and methods: *A. bracteosa*, a widespread medicinal plant traditionally used in the disease, was collected from Hamirpur district of Himachal Pradesh. Aerial part was extracted with petroleum ether, chloroform, methanol, ethanol and water. Analgesic activity of these extracts was assessed in swiss albino mice with acetic acid-induced writhing test and tail immersion test. Results: At the doses used (200 and 400 mg/kg, i.p.) chloroform and water extracts showed significant and dose-dependent analgesic effects. Conclusion: Our results indicate that extracts *Ajuga bracteosa* wall ex. Benth obtained from demonstrate an analgesic effect probably mediated by opioid receptors.

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### 1. Introduction

Historically, men have used medicinal plants as a traditional form of providing relief to several diseases. Some of the used plants demonstrate effects comparable to the ones obtained from allopathic medicines. It is very well known that many plant-derived compounds present significant analgesic properties. Based on this, agents derived from medicinal plants with very little side effects are required as substitute therapeutics [1]. Analgesics are the substances which decrease pain sensation by increasing pain threshold to external stimuli. Noxious pain stimuli can be developed by thermal, chemicals and physical pressure [2]. Two neurotransmitters are released by the nociceptive afferent fibers in the dorsal horn of the spinal cord. These neurotransmitters, which stimulate the second-order sensory neurons, include: Glutamate and Substance P.

The amino acid glutamate is the major neurotransmitter released by A-delta fibers and C fibers. Glutamate binds to the AMPA-type glutamate receptor on the second-order sensory neuron to elicit action potentials and continue transmission of the

*Ajuga bracteosa* wall ex. Benth (Labiatae) named Neelkanthi is a perennial herb found in hilly areas and even on rock cervice upto 1500 m. The flowers are hermaphrodite with white or pink colour. The juice of the leaves is used as a blood purifier and powdered form for burns and boils [4, 5].

Few chemical studies dedicated to this species had evidenced the presence of beta-sitosterol, Y-sitosterol and ceryl alcohol. It was possible to identify glycoside and tannin. Whereas palmitic acid, cerotic acid and glucosidic constituents were also characterized in the plant. Based on our continuous interest for the search of medicinal plants with analgesic activity and with a lower cost, the purpose of this paper was to investigate the analgesic activity of the pet-ether extract, chloroform, methanol, ethanol and water extract, obtained from aerial part of *Ajuga bracteosa* wall ex. Benth. mainly due to the fact that the in Ayurvedic formulation widely use this plant as an analgesic medicine [6,7]. The present study is an attempt to evaluate the analgesic activity of *Ajuga bracteosa* wall ex. Benth.

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## 2. Materials and methods:

### 2.1. Plant Material

The plant material *Ajuga bracteosa* wall ex. Benth aerial part was collected from Hamirpur district of Himachal Pradesh in January 2009. The aerial parts were authenticated by a Botanist Dr. Zia Ul Hassan, Department of Botany, Safia Science College, Bhopal. The Voucher specimen No. is 131/Bot/Safia/2010.

### 2.2. Preparation of extract of *Ajuga bracteosa*

The aerial part were dried under airflow at 37 °C and powdered in a mill of knives. The extraction was performed by successive solvent extraction with pet-ether, chloroform, methanol, ethanol and water. After filtration, the crude pet-ether, chloroform, methanol ethanol and water extract was concentrated under reduced pressure yielding 0.89, 15.32, 15.45, 2.85, 2.85, 16.18 g, respectively, after solvent evaporation.

### 2.3. Animals

Healthy Swiss albino mice weighing 25-30 gm. were utilized for the analgesic activity. The animals were obtained from VNS institute of pharmacy, Bhopal. All the animals were stored in standard cages and maintained at 27±2 °C under 12 hrs dark/light cycle. Animal study was performed in VNS institute of pharmacy Bhopal with due permission from institutional animal ethical committee (Registration No. 778/03/C/CPCSEA).

### 2.4. Drugs and reagents

The chemicals used in this study include: acetic acid, diclofenac sodium, pentazocine, Sterile normal saline, carboxy methyl cellulose was used as control in all studies and the crude pet-ether, chloroform, methanol, ethanol and water extract used in various studies were prepared in normal saline.

### 2.5. Acute toxicity studies

The albino mice were divided into 5 groups each containing 4 animals (for each extract). The mice were fasted for 18 h with water ad libitum. Crude extracts (PEE, ME, EE, CE and AE) were dissolved in 0.1% CMC aqueous solution. Test solution was administered orally at 4 different doses of 500, 1000, 1500, and 2000mg/kg body weight, respectively, to different groups of rat. A separate set of control mice were given the vehicle only (0.1% CMC aqueous solution). The animals were observed for 24 h and the toxicity was determined [8].

### 2.6. Experimental protocol of analgesic activity

#### 2.6.1. Acetic acid-induced writhing test

This test was done using the method described by (Collier et al.) Muscle contractions were induced in mice by intra peritoneal injection of 0.6 % solution of acetic acid (10 ml/kg). Thirty minutes before this administration the animals were treated with diclofenac sodium (50 mg/kg), different extract orally at doses of (200 and 400 mg/kg) and 0.1 % CMC (5 ml/kg). Immediately after administration of acetic acid, the animals were placed in glass cages, and the number of 'stretching' per animal was recorded during the following 15 min.

Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs. There was significant reduction in the number of writhes by drug treatments as compared to vehicle treated animals. This was

considered a positive analgesic response and the percentage inhibition of writhing was calculated [9].

#### 2.6.2. Tail immersion

Male Swiss albino mice weighing 25-30 gm were divided into six groups each containing five animal's Adult albino mice of either sex (25-30 g) were selected for the study and were divided into following groups of 5 animals each. The reaction time was measured at 0, 15, 30, 60 and 90 mins. The group-1 was served as solvent control which received the vehicle saline (5 mg/kg) through oral route, the group-2 was served as reference control which received Pentazocin (10 mg/Kg) and group-3 to 7 were received in a dose of 200 and 400 mg/Kg each the extracts of petroleum-ether, methanol, ethanol, chloroform and aqueous. After administration of above drug, the basal reaction time was measured after in a regular interval of 30 minutes, by immersing the tail tips of the mice (Last 1-2 cm) in hot water heated at temperature of temperature (55 ± 1) °C. The actual flick responses of mice i.e. time taken in second to withdrawn it's from hot water source was calculated and result were compared with control group [10].

#### 2.7. Statistical analysis

Data were recorded as mean±SEM. The statistical significance of differences between groups was determined by analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons among groups. Differences of p<0.05 were considered statistically significant.

## 3. Results and discussion

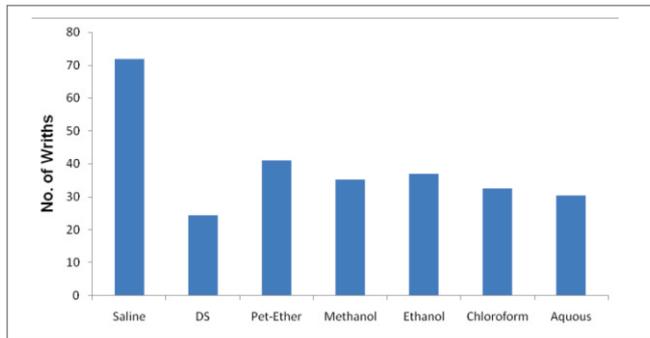
The present study was conducted to assess the analgesic properties of different extract of *Ajuga bracteosa*. The methods selected were chemical nociception in the test model of acetic acid-induced writhing and tail immersion test. These methods were selected to evaluate both centrally and peripherally mediated effects of different extract. The acetic acid induced abdominal constriction is believed to show the involvement of peripheral mechanisms, whereas the tail immersion tests are believed to do same by central mechanisms. The results of the present study demonstrated that different extract-possessed analgesic activity is evident in all the nociceptive models, suggesting the involvement of both central and peripherally mediated activities.

In acetic acid-induced writhing test, the results showed that the chloroform and aqueous extract (400 mg/kg) potently and significantly reduced the number of abdominal writhing in a dose dependent manner with 54.754% and 57.77% of inhibition, respectively as compared to control animals (fig.1.1 ,1.2). The positive control group treated with diclofenac sodium (50 mg/kg) also showed significant reduction in the number of writhes (65.9%).

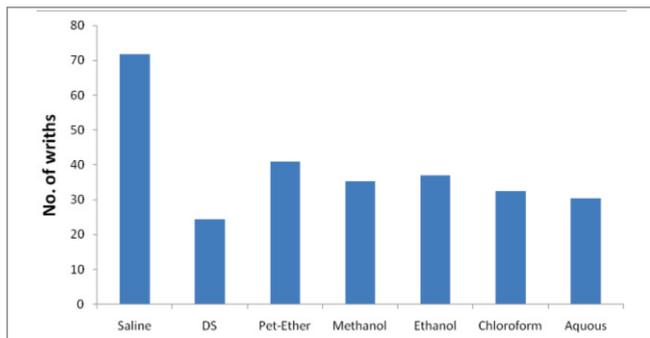
It has been postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, such as PGE2 (prostaglandin E2) and PGE2α in peritoneal fluids as well as lipooxygenase products, which stimulate the nociceptive neurons sensitive to NSAIDs [11, 12]. Therefore, the results of the acetic acid induced writhing strongly suggests that the mechanism of this extract may be linked partly to inhibition of lipooxygenase and/or cyclooxygenase in peripheral tissues, reducing in PGE2

synthesis and interfering with the mechanism of transduction in primary afferent nociceptor.

**Fig.1.1 Effect of different extract (200mg/kg) of *Ajuga bracteosa* on Acetic acid induced writhing method.**

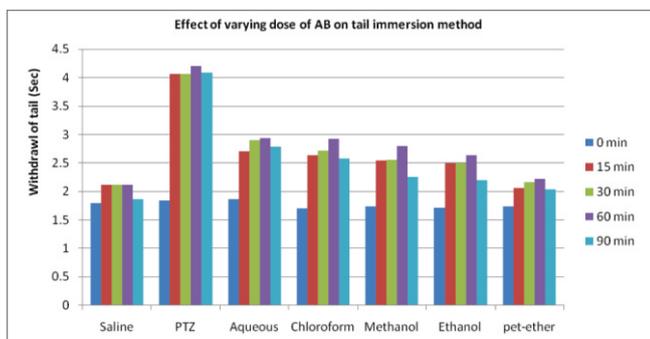


**Fig.1.2 Effect of different extract (400mg/kg) of *Ajuga bracteosa* on Acetic acid induced writhing method**



The central analgesic effect of the chloroform and aqueous extract may be supported by the results recorded in the tail immersion tests, a selective method used to screen centrally acting opiate analgesic drugs. It was demonstrated that administration by intraperitoneal route of chloroform and aqueous extract (400 mg/kg) significantly effective (Fig. 2.1, 2.2).

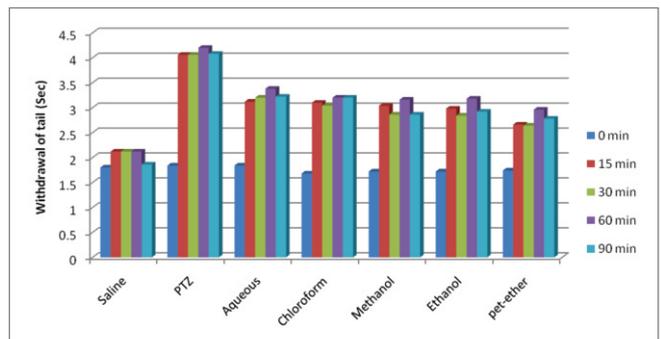
**Fig.2.1 Effect of different extract (200mg/kg) of *Ajuga bracteosa* on Tail immersion method**



This effect began early at 30 min after administration of extract and persisted until the following 90 min. Analgesic effect against thermal noxious stimuli may be elicited through opoid receptors or through modulation of several neurotransmitters involved in relevant phenomena. But the extend of activity shown by the crude extracts are less than that of the standard drug Pentazocin

(10mg/kg) which justifies its activity. It could be concluded that the plant *Ajuga bracteosa* wall ex. Benth is having analgesic activity and better result are obtaining from extract of aqueous. This further study needed to identify the chemical constituents present in extract of this herb that may elicit analgesic activity.

**Fig.2.2 Effect of different extract (400mg/kg) of *Ajuga bracteosa* on Tail immersion method**



#### 4. Conclusion

In conclusion, this study demonstrated that the opioid dependant central effect of the *Ajuga bracteosa* has synergistic effect by enforcing the peripheral analgesic effects and thus substantiated the traditional claim of the plant in the treatment of painful conditions. As in other cases, it is necessary to fully isolate and identify the active compounds and obtain a comprehensive pharmacological profile. This study also confirms the validity of the ethnopharmacological approach in the search for new drugs.

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