ORIGINAL ARTICLE

Evaluation of antinociceptive activity of *Ajuga bracteosa* wall ex benth

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Objective: The present study was aimed to investigate the *in vivo* anti-nociceptive activity of Neelkanthi (whole plant) and to support its traditional use. **Materials and Methods:** Methanolic extract of plant *Ajuga bracteosa* (ABE) was investigated for its anti-nociceptive activity in hot-plate method, tail flick method and formalin induced hind paw licking test in mice. Three doses of the extract (ABE - 250 mg/kg, 500 mg/kg and 750 mg/kg, i.p.) were used in the study and codeine (5 mg/kg, i.p.) was used as standard. **Results:** ABE (500 mg/kg and 750 mg/kg, i.p.) significantly ($P < 0.05$) increased the reaction time in both hot plate method and tail flick method. ABE also showed significant decrease in the paw licking response in formalin induced hind paw licking test in mice. **Conclusions:** ABE at the dose of 500 mg/kg and 750 mg/kg showed potent anti-nociceptive activity on comparison with the standard drug and supported its traditional use as analgesic.

Key words: Anti-nociceptive, codeine, formalin, tail-flick

INTRODUCTION

Pain transmission is a mechanism that involves a very complex interaction of peripheral and central structures from the skin surface to the central cerebral cortex.[1] Drugs that are currently used for the management of pain are opioids or no opioids 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 NSAIDs such as 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 cervices up to 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 (LOX),[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] cardiac stimulant[12] and *in vivo* and *in vitro* anti-inflammatory activity.[13]

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MATERIALS AND METHODS

Plant Material

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

Preparation of Extracts

The collected plants were immediately shade dried and then powdered by a pulveriser. The powdered plant material (0.5 kg) were defatted with petroleum ether and extracted by maceration with methanol (3 times, 1 l each) at room temperature. The extract was pooled, filtered through a Whatman 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°C till its further use.

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Animals

The animals were kept in the departmental animal house at an ambient temperature of 25 ± 1°C and 45-55% relative humidity, 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 carried out 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.

Evaluation of Anti-Nociceptive Potential of ABE

Thermally induced pain in mice (hot plate method)

The effect of extract on hot plate induced pain was investigated in adult mice. The hot plate was used to measure the response latencies according to the method reported by Mbagwu *et al*.[14] In these experiments, the hot-plate was maintained at 45 ± 1°C, each animal was placed into a glass beaker of 50 cm diameter on the heated surface, and the time (s) between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 30-sec cut-off was used to prevent tissue damage.

The mean reaction time for each treated group was determined and compared with that obtained for each group before treatment. Percentage increase in reaction time (I%), was derived, using the formula $I\% = \{(I_t - I_0)/I_0\} \times 100$, where I_t = reaction time at time, t, and I_0 = reaction time at time zero (0 h).[15] The animals were subjected to the same test procedure at +30, +60, +120, and +180 min after the administration of test/standard/control drug.

Radiant heat tail-flick method

Antinociceptive effect of the test samples was determined by the tail-flick method described by Sewell and Spencer (1976). One to two centimeter of the tail of experimental mice was immersed in warm water kept constant at 50°C. The pain reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as a mean of the next two readings. The latent period of the tail-flick response was taken as the index of antinociceptive activity and was determined before and at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h after the administration of drugs.[16] The maximum reaction time was fixed at 0.5 h (30 min). The maximum possible analgesia was calculated according to the method of Idid *et al* (1998).

Formalin induced hind paw licking in mice

The procedure was essentially similar to that described by Okokon and Nwafor.[17] The animals were used to analyse the first phase of formalin-induced licking and 20 µl of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution concentration: NaCl 137 mM, KCl 2.7 mM and phosphate buffer, 10 mM) was injected subcutaneously under the surface of the right hind paw. The amount of time spent licking the injected paw was timed and considered as indication of pain. The first of the nociceptive response normally peaks 5 min after injection and the second phase 15-30 min after formalin injection, representing the neurogenic and inflammatory pain responses, respectively.[18] Adult albino mice (23-27 g) of either sex randomised into five groups of 6 mice each were used for the experiment. The mice were fasted for 24 h before being used but allowed access to water. The responses were measured for 5 min after formalin injection (first phase) and 15-30 min after formalin injection (second phase).

Statistical Analysis

The data of anti-nociceptive activity was expressed as mean ± SEM of six animals in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's *t*-test. The difference in values at $P < 0.01$ was considered as statistically significant.

RESULTS

Effect of ABE on Thermally Induced Pain in Mice (Hot-Plate Method)

In hot plate method codeine caused significant increase ($P < 0.001$) in the reaction time. The increase in latency period at different time points significantly differed ($P < 0.01$) compared to baseline values within the same drug treated groups. ABE at 500 mg/kg and 750 mg/kg dose showed significant increase ($P < 0.001$) in the reaction time while ABE 250 mg/kg was not found to increase the reaction time significantly. Table 1 showing the effect of ABE at different does on reaction time at different time interval.

Table 1: Effect of ABE on reaction time in hot plate method

Treatments	Dose	Reaction time in sec. (mean±SEM)				
		Basal (0)	30 min	60 min	120 min	180 min
Control	10 ml/kg i.p.	1.78±0.24	1.82±0.28	2.31±0.11	2.32±0.21	2.34±0.17
Codeine	5 mg/kg i.p.	7.14±0.18**	8.18±0.22**	8.98±0.31**	10.13±0.21**	11.87±0.21**
ABE 250	250 mg/kg i.p.	2.76±0.22	3.65±0.21	4.12±0.27	4.61±0.17*	5.14±0.12*
ABE 500	500 mg/kg i.p.	4.56±0.12	5.85±031*	6.32±0.21*	7.67±0.27**	7.89±0.21**
ABE 750	750 mg/kg i.p.	4.96±0.23	6.61±0.27*	7.89±0.24**	8.61±0.17**	9.18±0.17**

ABE – *Ajuga bracteosa*; *P<0.01; **P<0.001 when compared with control group

Table 2: Effect of ABE on reaction time in tail flick method

Treatments	Dose	Reaction time in sec. (mean±SEM)				
		Basal (0)	30 min	60 min	120 min	180 min
Control	10 ml/kg i.p.	1.78±0.24	1.82±0.28	2.31±0.11	2.32±0.21	2.34±0.17
Codeine	05 mg/kg i.p.	4.24±0.18	7.18±0.22**	9.98±0.31**	11.13±0.21**	11.87±0.21**
ABE 250	250 mg/kg i.p.	2.16±0.22	3.85±0.21	4.82±0.17	5.61±0.17*	5.87±0.12*
ABE 500	500 mg/kg i.p.	5.56±0.12*	5.95±031*	6.87±0.21**	8.97±0.27**	10.89±0.21**
ABE 750	750 mg/kg i.p.	5.76±0.23	6.69±0.27*	8.89±0.24**	10.67±0.17**	11.101±0.17**

ABE – *Ajuga bracteosa*; *P<0.01; **P<0.001 when compared with control group

Table 3: Effect of ABE in hind paw licking in the formalin test in mice

Treatments	Dose	Early phase	% protection	Late phase	% protection
Control	10 ml/kg i.p.	38.12±0.87	–	51.23±0.59	–
Codeine	05 mg/kg i.p.	15.12±0.65*	54.32	17.32±0.73*	61.08
ABE 250	250 mg/kg i.p.	32.03±0.23	05.12	38.71±0.71	07.81
ABE 500	500 mg/kg i.p.	24.21±0.19*	20.21	25.32±0.32*	25.41
ABE 750	750 mg/kg i.p.	18.26±0.21*	48.64	19.31±0.72*	51.57

ABE – *Ajuga bracteosa*; *P<0.01 when compared to standard group

Effect of ABE on Radiant Heat Tail-Flick Method

In the tail flick method, the increase in latency period at different time points significantly differed ($P < 0.01$) compared to baseline values within the same drug treated groups. As shown in Table 2 codeine significantly ($P < 0.001$) increases the reaction time and ABE 500 and 750 also showed significant increase in reaction time while ABE increases the reaction time in late phase.

Effect of ABE on Formalin Induced Hind Paw Licking in Mice

Codeine significantly ($P < 0.001$) suppressed the licking activity in either phase of the formalin induced pain in mice. As shown in Table 3, ABE 500 and 750 significantly reduces the pain while ABE 250 showed more licking activity against both phases of formalin induced pain that of standard drug codeine.

DISCUSSION

To evaluate for a possible central anti-nociceptive effect of ABE the hot plate and tail-flick methods were used for evaluation of the central pain at the supraspinal and spinal levels respectively,[19] possibly acting on a descending inhibitory pain pathway.[20] The tail-flick response is believed to be a spinally mediated reflex and the paw-licking hot plate response is more complex supraspinally organized behavior.[21] The effectiveness of analgesic agents in the tail-flick pain model is highly correlated with relief of human pain perception.[22] In the two models used, though the data showed that ABE dose-dependently increased the pain threshold, the increase in the pain threshold/tail flick latency profiles of the extract were less than that of the standard drug, codeine. The μ receptor stimulation is generally associated with pain relief and has been shown to be potent in regulating thermal pain.[23] Non analgesic effects through the μ receptors include respiratory depression and most importantly for therapeutic considerations is its induction of physical dependence. Activation of μ2 opioid subtype leads to spinal analgesia and commonly causes constipation as adverse effect.[24] Therefore, taking all these data together we believe that the Antinociceptive activity of ABE is most likely to be mediated by central action (spinally and supraspinally)[19] and indicates a like mechanism by binding with opioid receptors. Although opioids possess dependence and abuse liabilities, new drugs producing less euphoria at onset and withdrawal symptoms as the medication wear off would be more beneficial. *Ajuga bracteosa* could be a better substitute for the opioid drugs like methadone is an excellent choice over morphine for the management of chronic severe pain like in cancer. Methadone is an orally active, slow-onset opioid with a long duration of action.[25] In the tail-flick and hot plate methods, both the doses (500 and 750 mg/kg body wt.) of ABE increased the stress tolerance capacity of the animals and hence indicate the possible involvement of a higher centre.[26]

The formalin model normally postulates the site and the mechanism of the analgesic.[27] The biphasic model is represented by neurogenic (0-5 min) and inflammatory pain (15-30 min), respectively.[28] Drugs that acts on primarily on central nervous system such as narcotics inhibits both as steroids and NSAIDs suppresses mainly the late phase.[29] ABE at dose of 500 and 750 mg/kg were found to suppress late phase as well as the early phase.

On the basis of these findings, it may be concluded that ABE has analgesic activity, this may be due to the chemical constituents it possess (Aajugarin I, lupulin A, withaferin A, reptoside and 6-deoxyharpagide) which are found to have inhibitory effect on COX and LOX.[10] COX and LOX plays

key role in production of pain peripherally. There may be another mechanism behind the activity, which might be through interaction with Opioid receptors exists centrally. These activities were related to the dose and these results corroborate the past finding and potential traditional use of the plant in folk medicine.

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