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Antiarthritic effects of *Ajuga bracteosa* Wall ex Benth. in acute and chronic models of arthritis in albino rats

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ABSTRACT

Objective: To evaluate the antiarthritic activity of *Ajuga bracteosa* using albino rats. **Methods:** The antiarthritic activity of 70% ethanolic extract of *Ajuga bracteosa* (EEAB) was evaluated against turpentine oil- and formaldehyde- induced acute non immunological and complete freund's adjuvant (CFA)-induced chronic immunological arthritis in albino rats. **Results:** EEAB showed a significant ($P<0.05$) and dose dependent inhibitory effect against acute and chronic models of arthritis. EEAB exhibited better antiarthritic activity than the standard aspirin. **Conclusions:** EEAB exhibits a significant and promising antiarthritic activity against acute and chronic arthritis and supports the traditional use of *Ajuga bracteosa* for rheumatism and other inflammatory diseases.

1. Introduction

Ajuga bracteosa (*A. bracteosa*) Wall ex Benth. (Labiatae) is a perennial herb with diffused branching and is distributed from Kashmir to Nepal, sub-Himalayan tract, plains of Punjab and the upper Gangetic plains of India. The herb is recommended in ayurveda for the treatment of rheumatism, gout, palsy and amenorrhea. The herb has also been credited for its astringent, febrifugal, stimulant, tonic, and diuretic properties[1]. The previous pharmacological investigations on *A. bracteosa* have reported the antifeedant activity against *Spodoptera littoralis* larvae[2], cancer chemopreventive[3], and antiplasmodial[4,5] activities. *A. bracteosa* was also reported to inhibit the acetylcholinesterase, butyrylcholinesterase and lipoxygenase activity[6].

Recently, we have reported the significant topical anti-inflammatory activity of 70% ethanolic extract of *A. bracteosa* (EEAB) using, terephthalic acid (TPA)-induced ear oedema assay. The topical anti-inflammatory activity was well supplemented with the *in vitro* cyclooxygenase (COX) inhibitory activity of EEAB and its isolated constituents *i.e.* ajugarin I, lupulin A, withaferin A, reptoside and 6-deoxyharpagide[7]. Based on information from previous literature and considering the topical anti-inflammatory along with *in vitro* COX inhibitory potential, the present study was undertaken to evaluate the plausible effect of EEAB against acute and chronic arthritis in albino rats as animal model. The aspirin was taken as a standard, which is regarded as the standard agent with which other drugs should be compared for the treatment of rheumatoid arthritis[8].

2. Materials and methods

2.1. Plant material and extract preparation

The whole plants of *A. bracteosa* were collected from adjoining areas of Palampur, Himachal Pradesh, India in October 2006 and were identified by Dr. Saklani A. A voucher specimen (NIP-134) was deposited in Herbarium of Department of Natural Products, NIPER. The collected plants were immediately shade dried and then powdered by a pulverizer. The powdered plants (0.3 kg) were extracted by maceration with 70% ethanol (3 times, 1.0 L each) at room temperature. It was pooled, filtered through a Whatman

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filter paper and the solvent was removed on a vacuum rotary evaporator under reduced pressure to get 70% dried EEAB (yield: 11.3%). The dried extract was stored at −20 ℃ till its further use.

2.2. Animals

Wistar strain of albino rats (100−150 g) were obtained from Central Animal House, Assam University. The animals were housed under standard conditions of temperature [(25±1) ℃] and humidity with 12 h light/dark cycle and had free access to commercial pellet diet and water. The animals were acclimatized to laboratory conditions for one week prior to experiments. All the experiments were performed according to the CPCSEA guidelines given on animal experimentation, Department of Animal Welfare, Government of India.

2.3. Drugs and chemicals

Turpentine oil and formaldehyde were purchased from S.D. Fine Chemicals Ltd. (Mumbai, India) and complete freund's adjuvant (CFA) from Difco Laboratories (USA). Aspirin was received as gift sample from Arbro Pharmaceuticals Limited (New Delhi, India). All other chemicals were of analytical grade.

2.4. Acute non-immunological arthritis

2.4.1. Turpentine oil-induced joint oedema in rats

Wistar albino rats were fasted for 24 h before experimentation with free access to water. Animals were divided into five groups of six animals each. Group I served as control and received normal saline (3 mL/kg, i.p.), group II−IV received EEAB (5, 10, 20 mg/kg, i.p.), respectively, and group V received a standard drug aspirin (100 mg/kg, i.p.). Acute non-immunological inflammatory joint oedema was produced by injecting 0.02 mL of turpentine oil into the synovial cavity of the right knee joint, 30 min after the drug administration. Diameter of the joint was monitored at hourly intervals for 6 h, using a micrometre screw gauge[9].

2.4.2. Formaldehyde-induced arthritis in rat

Wistar albino rats were divided into five groups of six animals each. Group I (control) received normal saline (3 mL/kg, i.p.), group II−IV received EEAB (5, 10, 20 mg/kg, i.p.) and animals of the group V received aspirin (100 mg/kg, i.p.) for 10 days, respectively. On day 1, 30 min after the drug administration chronic non-immunological arthritis was induced by sub plantar injection of 0.1 mL of 2% formaldehyde solution and repeated on day 3. Arthritis was assessed by measuring the mean increase in paw diameter over a period of 10 days using micrometre screw gauge[9].

2.5. Chronic immunological CFA-induced arthritis in rats

Experimental immunological arthritis was induced in rats according to the method of Newbould[10]. The left footpad of each rat was injected subcutaneously with 0.05 mL (0.5% w/v) of CFA. Different groups of animal (n=6) received control vehicle (normal saline, 3 mL/kg), EEAB (5, 10, 20 mg/kg, i.p.) and standard drug (aspirin, 100 mg/kg, i.p.), 1 day before the CFA injection and daily treatment continued for 14 days. The oedema of the left and right hind paws was evaluated at 5, 10 and 14 days post injection of CFA using micrometre screw gauge. After the 14th day, animals were killed with ether, and their legs were amputated at knee joints and immediately fixed straight on cardboard for X-ray photograph.

2.6. Statistical analysis

All the data were presented as mean ± SD and analyzed by one way ANOVA followed by Student Newans Keuls Test for the possible significance identification between the various groups. P<0.05 was considered statistically significant. Statistical analysis was carried out using Graph pad prism 5.0 (Graph pad software, San Diago, CA).

3. Results

The effect of EEAB on turpentine oil induced joint oedema was depicted in Table 1. EEAB exhibited a dose-dependent protection against the swelling observed in the synovial cavity after administration of turpentine oil. The effect of EEAB at 20 mg/kg was found to be slightly more than that of aspirin after 6 h of treatment (Table 1).

Table 1 also summarized the effect of EEAB (5, 10, 20 mg/kg) and aspirin on formaldehyde induced arthritis. EEAB significantly suppressed the joint oedema when compared with control between day 2 and day 10 post formaldehyde

Table 1
Effect of EEAB on acute non-immunological arthritis in albino rats (mean±SD) (n=6).

Treatment	Turpentine-induced joint oedema						Formaldehyde-induced arthritis				
	Increase in joint diameter (cm)						Increase in joint diameter (cm)				
	1 h	2 h	3 h	4 h	5 h	6 h	Day 2	Day 4	Day 6	Day 8	Day 10
Control (3 mL/kg, i.p.)	0.84±0.02	1.23±0.14	2.17±0.21	2.88±0.18	3.23±0.33	4.01±0.31	0.44±0.03	0.57±0.08	0.79±0.07	0.96±0.07	1.11±0.11
EEAB (5 mg/kg, i.p.)	0.81±0.13 (3.57)	1.19±0.18 (3.25)	1.83±0.19* (15.66)	1.41±0.11* (51.04)	1.23±0.12* (61.91)	0.99±0.04* (75.31)	0.41±0.04 (6.81)	0.37±0.05 *(35.09)	0.28±0.02 *(64.55)	0.27±0.04 *(71.88)	0.23±0.02 *(79.28)
EEAB (10 mg/kg, i.p.)	0.47±0.09* (44.04)	0.42±0.07* (65.85)	0.34±0.03* (84.33)	0.26±0.01* (90.97)	0.15±0.01* (95.35)	0.11±0.01* (97.26)	0.35±0.02* (20.45)	0.27±0.06 *(52.63)	0.21±0.04 *(73.42)	0.19±0.03 *(80.21)	0.18±0.01 *(83.78)
EEAB (20 mg/kg, i.p.)	0.13±0.01* (84.52)	0.36±0.04* (70.73)	0.23±0.01* (89.40)	0.11±0.01* (96.18)	0.09±0.01* (97.21)	0.07±0.01* (98.25)	0.23±0.06* (47.72)	0.19±0.06 *(66.67)	0.15±0.01 *(81.01)	0.33±0.02 *(65.63)	0.12±0.01 *(89.19)
Aspirin (100 mg/kg, i.p.)	0.47±0.02* (44.05)	0.34±0.03* (72.36)	0.22±0.01* (89.86)	0.18±0.02* (93.75)	0.11±0.02* (96.59)	0.10±0.02* (97.51)	0.19±0.08* (56.82)	0.26±0.03 *(54.39)	0.47±0.03 *(40.51)	0.45±0.04 *(53.12)	0.41±0.05 *(63.03)

Values in the parenthesis indicate percentage inhibition; *: P<0.05 as compared with the control.

Table 2
Effect of EEAB on CFA-induced chronic immunological arthritis in albino rats (mean±SD) (n=6)

Treatment	Increase in joint diameter (cm)		
	Day 5	Day 10	Day 14
Control (3 mL/kg, i.p.)	1.73±0.09	2.21±0.04	2.43±0.43
EEAB (5 mg/kg, i.p.)	1.54±0.07* (10.98)	1.23±0.22* (44.34)	1.11±0.28* (54.32)
EEAB (10 mg/kg, i.p.)	1.44±0.07* (16.73)	1.09±0.19* (50.68)	0.96±0.21* (60.49)
EEAB (20 mg/kg, i.p.)	1.12±0.05* (35.26)	0.89±0.17* (59.73)	0.77±0.17* (68.31)
Aspirin (100 mg/kg, i.p.)	0.71±0.02* (58.96)	0.81±0.11* (63.35)	0.96±0.28* (60.49)

Values in the parenthesis indicate percentage inhibition; *: P<0.05 as compared with the control.

treatment. Treatment with EEAB (5, 10 and 20 mg/kg) exhibited 64.55%, 73.42% and 81.01% of protection against joint oedema in comparison to standard aspirin (*i.e.* 40.51% inhibition) after 6 days of treatment.

Table 2 showed the time course of oedema due to CFA and inhibition rate after the administration of EEAB. The right hind paw also developed oedema in addition to the left footpad confirming the immunologic nature of these lesions. Three different doses of EEAB exhibited antiarthritic activity which was maintained until the experiment was terminated on day 14. The secondary inflammatory response observed from 9th-14th day was also inhibited much more effectively by EEAB at a dose of 20 mgk/kg (68.31%) when compared with aspirin (60.49%). (Figure 1).

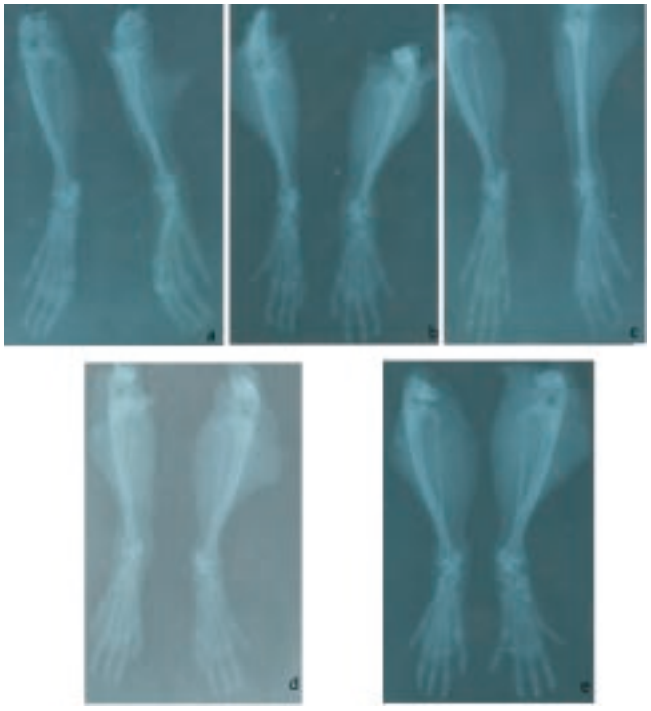

Figure 1. Effect of EEAB on CFA-induced arthritis in rats (X-ray photographs).
a: Toxic arthritic control; b: EEAB 5 mg/kg; c: EEAB 10 mg/kg; d: EEAB 20 mg/kg; e: Aspirin, 100 mg/kg.

4. Discussion

The acute inflammatory response in the knee joint of rat induced by turpentine oil was significantly checked by EEAB in a dose dependent manner. The turpentine oil-induced joint oedema is reported to be mediated by the sequential release of mediators, *i.e.* histamine and serotonin in early phase; kinin-like substances in intermediate phase; and prostaglandins in late phase[11]. Inhibition of turpentine oil-induced joint oedema, suggests the possible effect of EEAB on different phases of inflammation, which in turn could either be due to lipoxygenase and /or cyclooxygenase inhibition as reported previously[6,7].

The inhibition of formaldehyde-induced joint oedema is one of the most suitable methods to evaluate antiproliferative activity and screen antiarthritic agents[12]. The injection of formaldehyde into rat paw produced localized inflammation and pain which is biphasic in nature *i.e.* an early neurogenic component followed by a later tissue mediated response[13]. EEAB inhibited the proliferative global oedematous response to formaldehyde in dose dependent manner, suggesting that it may alter certain aspects of the inflammatory response better than aspirin with possible antiarthritic potential.

The antiarthritic potential of EEAB was further confirmed against CFA-induced immunological, chronic, cellular and proliferative arthritis, which is more akin to the clinical situation. The CFA-induced arthritis was characterized by pronounced swelling in the hind paw which persisted for weeks (primary reaction). The primary reaction was followed by swelling in the contralateral and front paws along with appearance of arthritic nodules in ear and tail *i.e.* delayed systemic response (secondary reaction). EEAB was found to significantly decrease the humoral immune response plausibly by inhibiting the acute inflammatory reaction by reducing vascular permeability and /or inhibiting other mediators. The secondary lesions are reported to presume due to a delayed hypersensitivity reaction[10] and EEAB exerted a marked effect on this. The aforementioned observations were also supported by the X ray pictures showing significant reduction in the inflammation, by EEAB, comparable to aspirin. These observations suggest the possible efficacy of EEAB in therapy of clinical conditions like rheumatoid arthritis.

The key mediators for inflammation are the n-6 eicosanoids *e.g.* prostaglandin E2 (PGE2), prostaglandin D2 (PGD2), prostacycline (PGI2) and thromboxane A2 (TxA2). Eicosanoids are derived from polyunsaturated fatty acid arachidonic acid (AA; 20:4, n-6) by action of COX-1 and COX-2[14-20]. PGE2 depresses the humoral antibody response by inhibiting the differentiation of B-lymphocytes into antibody secreting plasma cells. PGE2 also acts on T-lymphocytes to inhibit mitogen stimulated proliferation and lymphokine release from sensitized cells. PGD2 is a major product of mast cells and is a potent chemoattractant for eosinophils and induce chemotaxis and migration of

lymphocytes[21]. Together PGE2 and PGI2 markedly enhance oedema formation and leukocyte infiltration by promoting blood flow to the inflamed region. Both of these have been found to be associated with inflammatory pain and potentiate the pain producing activity of bradykinin, histamine and other autacoids. PGE2 also potentiates the effect of bradykinin by sensitizing afferent C fibres to increase pain associated with arthritis[22]. Previously authors have reported the significant topical anti-inflammatory activity of EEAB. Anti-inflammatory activity of EEAB and its isolated constituents *i.e.* ajugarin I, lupulin A, withaferin A, reptoside and 6-deoxyharpagide was further confirmed using *in vitro* inhibition assay for COX-1 and COX-2. It was proposed that the anti-inflammatory activity of EEAB is mediated through the inhibition of COX-1 and COX-2[7]. Thus, reduction in eicosanoids synthesis due to COX inhibition is assumed to account for the beneficial effects of EEAB in the present experiment. It is noteworthy to mention that EEAB showed no toxicity and exhibited better antiarthritic activity than the standard drug aspirin.

In conclusion, we would like to say that the significant and promising antiarthritic activity of EEAB is possibly mediated through COX-1 and COX-2 inhibition. The isolated constituents *i.e.* ajugarin I, lupulin A, withaferin A, reptoside and 6-deoxyharpagide could be responsible, at least in part, for the antiarthritic activity. The present observation is the first experimental data providing the scientific evidence to rationalize and support the traditional use of *A. bracteosa* for the treatment of rheumatism and other inflammatory disorders.

Conflict of interest statement

We declare that we have no conflict of interest.

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