

Analgesic activity of methanol extract of *Eupatorium adenophorum* Spreng. leaves

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The methanol extract of the leaves of *E. adenophorum* (100, 200 and 300 mg/kg, po) showed significant analgesic activity, as compared to standard drugs diclofenac sodium and pentazocine, employing acetic acid-induced writhing test, tail immersion test and tail flick test models.

Keywords: Acetic acid-induced writhing test, Analgesic activity, *Eupatorium adenophorum*, Leaves, Tail flick test, Tail immersion test

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Eupatorium adenophorum Spreng. (Asteraceae), commonly known as Banmara (Nepali), Banmarmukh (Lepcha), is the prominent species found in Sikkim, Darjeeling in West Bengal, Meghalaya, Himachal Pradesh and Nagaland. It is a perennial erect herb of about 1 meter height. It grows on open sunny slopes, on roadside, in abandoned fields and wastelands. The juice of fresh leaves when applied to cuts and wounds protected infection effectively⁷. The plant has cadinene group of sesquiterpenes⁹ and a new bicyclic sesquiterpene eupatorone¹. It also contains eupatorin, a triterpenoid saponin and tremetol. The aerial parts of the plants have been claimed to be used as antimicrobial, antiseptic, blood coagulant, potentiator of phenobarbitone-induced sleep and as an analgesic². The present study has been carried out to evaluate the analgesic activity of the methanolic extract of *E. adenophorum* leaves.

Preparation of extract—Fresh leaves of *E. adenophorum* were collected during the month of

December from Gangtok, Sikkim, India. The plant was authenticated by Dr. S Mahapatra, Scientist and a voucher specimen (No.CHN/1-(32)/2000-Tech II/846) was deposited in the Central National Herbarium, Botanical Survey of India, Shibpur. The leaves were dried (under shade), powdered and passed through 40 mesh sieve and stored in a closed vessel for further use. The dried and powdered leaves (500 g) were extracted with 90% v/v methanol in a Soxhlet apparatus for 9 hr. Methanol was removed under vacuum and a semisolid mass (11.5% w/w, with respect to dry starting material) was obtained. The extract was stored in a refrigerator.

Phytochemical studies—In preliminary phytochemical analysis the extract showed positive response for flavonoids, tannins, steroids, triterpenoids^{4,11}. This was confirmed by qualitative analysis and also by thin layer chromatographic studies.

Animals—Adult albino mice (18-25 g) of either sex obtained from M/s B.N. Ghosh & Co., Kolkata were used for the experiment.

Dosage and treatment—Three different sets of rats were randomized into 5 groups of 6 each for acetic acid-induced writhing test, tail immersion test and tail flick test respectively. The Group 1 served as vehicle control and received gum acacia 4% solution. Group 2 served as positive control and received either diclofenac sodium (for acetic acid induced writhing test) or pentazocine (for tail immersion test and tail flick test). Groups 3-5 served as test groups and received 100, 200 and 300 mg/kg, po doses of the extract, respectively. All test drugs were suspended in 4% gum acacia as per Shivamurthy and Srinivasa⁸. Diclofenac sodium was used at 10 mg/kg, po suspended in 4% gum acacia and pentazocine used at 10 mg/kg, ip.

Analgesic activity

Acetic acid induced writhing response in mice⁶—Acetic acid (0.6%) was administered ip at a dose of 10 ml/kg volume. The test drugs were administered 1 hr before administering acetic acid. The number of writhes were recorded 10 min after administering acetic acid for next 10 min. Diclofenac sodium was used as positive control.

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Table 1—Analgesic activity of methanol extract of *E. adenophorum* leaves (MEEAL) in various animal models. [Values are mean \pm SE from 6 animals in each group]

Treatment	Dose	Reaction time (sec)		
		Acetic acid induced Writhing test	Tail Immersion test	Tail flick test
Vehicle control	10 ml/kg	38.3 \pm 2.09	4.7 \pm 0.35	4.6 \pm 0.55
Positive control	10	12.0 \pm 2.1 ^b	9.1 \pm 0.51 ^b	8.6 \pm 0.72 ^b
MEEAL	100	29.3 \pm 2.23 ^a	5.9 \pm 0.52	5.8 \pm 0.25
MEEAL	200	16.0 \pm 1.84 ^b	7.5 \pm 0.55 ^b	6.9 \pm 0.35 ^b
MEEAL	300	14.2 \pm 2.5 ^b	8.2 \pm 0.69 ^b	7.8 \pm 0.30 ^b

P values; ^a< 0.01, ^b< 0.001, significantly different from vehicle control

Tail immersion test in mice¹²—The distal 5 cm of the tail was immersed in the water maintained at 55°C. Time taken for tail withdrawal response was recorded 1 hr after administering the test drugs. The cutoff time was fixed at 15 sec to prevent injury to the tail. Pentazocine was used as positive control.

Tail flick test in mice³—The tail received radiant heat from a wire heated by passing a current of 6 mA. The time taken for the withdrawal of the tail was recorded as tail flicking latencies in seconds. Tail flicking response was recorded 1 hr after administration of the test drugs. The cutoff time for determination of latent period was taken as 40 sec to avoid injury to the skin⁵. Pentazocine was used as positive control.

Statistical analysis—The values are expressed as mean \pm S.E. Statistical significance was evaluated on the values of all the tests by the student's *t*-test¹³. *P* < 0.05 was considered significant.

The results are presented in Table 1. The extract of *E. adenophorum* leaves significantly increased the induction time required to produce the writhing movements. The extract showed significant analgesic activity by tail flick and tail immersion tests respectively.

Under acetic acid induced writhing reflex model of analgesic activity, the result were comparable to that of positive control group. The number of writhing

movements were significantly less in the mice treated with the methanol extracts of *E. adenophorum* leaves when compared to that of vehicle treated control group thereby suggesting its analgesic effect. In tail flick and tail immersion tests the reaction time were significantly increased in animal treated with the methanol extracts of *E. adenophorum* leaves. From these results it is clear that the extract posses both peripheral and central analgesic activity. Further research would be of interest to explain the exact mechanism of this analgesic effect.

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