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Short Communication

Evaluation of micronutrients level and antinociceptive property of *Eremostachys laciniata* (L) Bunge

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Eremostachys laciniata **(Lamiaceae) a rich source of structurally different flavonoids is ignored for its micronutrients level and many biological activities. In this study, the plant was investigated for different micronutrients including, phosphorus (P), nitrogen (N), boron (B), zinc (Zn) and manganese (Mn) and compared with adequate level of micronutrients for orchid crops. The crude methanol extract and different fractions of *E. laciniata* were also investigated for *in vivo* antinociceptive activity using Tail-flick model. Except for boron, all other micronutrients investigated were well in the limits recommended for orchid crops. Crude extract and different fraction thereof showed excellent increase in latency time along with certain central effect of causing sedation, ataxia and hind limb extension. This increase in latency time is not only due to analgesia but may be a combined effect of sedation, ataxia and analgesia. The plant may be a useful source in future for the isolation of natural product with analgesic property.**

Key words: Extract, fractions, nitrogen, orchid, analgesic.

INTRODUCTION

Pain is an unpleasant subjective experience that is the net effect of a complex interaction of the ascending and descending nervous systems involving biochemical, physiological, psychological, and neocortical processes (Chisholm-Burns et al. 2008). Pain is the most common symptom prompting patients to seek medical attention and is reported by more than 80% of individuals who visit their primary health care provider (American Geriatrics Society, 2002). Pain can affect all areas of a person's life including sleep, thought, emotion, and daily activities. Since there are no reliable objective markers for pain, the patients are the only ones to describe the intensity and quality of their pain. Looking to the importance of natural products in human healthcare and rich biodiversity of our country, Pakistan, *Eremostachys* (L) Bunge was selected for *in vivo* antinociceptive property investigation. *E. laciniata* (Lamiaceae) recognized by yellow flowers, grows only naturally in sunny and sandy soils and shades at an altitude of about 2200 M (Adylov et al., 1987). This is supported by reports from the east mediterranean region (Hedge, 1990), central and south-west Asia (Jamzad, et al. 2003), Afghanistan and Pakistan (Stewart, 1863). The plant is reported as an excellent source of structurally varying flavonoids with medicinal importance (Navaei and Mirza, 2006). Volatile oils isolated from the plant possess significant pharmacological activities (Edris, 2007). Anti-oxidant activity has also been reported for the crude extract of the plant (Erdemoglu et al., 2006).

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MATERIALS AND METHODS

Plant material

The aerial parts of *E. laciniata* were collected in February 2002 at

Chakdara, Malakand division of Pakistan. The plant was identified by a plant taxonomist and a voucher specimen (EL-102) was submitted to the department of botany, University of Peshawar, Pakistan.

Extraction and fractionation

The collected parts were dried in shades and then powdered (4 kg). A portion of powdered material was separated for micronutrients determination, while the rest was subjected to extraction by percolation with methanol (80%) at room temperature. The resulting combined extract was dried through rotary evaporator and 250 g of crude methanol extract was obtained. After removing a portion of the crude extract, the rest was subjected to fractionation with different organic solvents and various fractions including, hexane (Elh), chloroform (Elc), ethyl acetate (Ele), butanol (Elb) and water (Elw) were obtained.

Preliminary screening and toxicity study

Crude methanol extract was screened to ascertain the presence of different families of organic compounds including alkaloids, terpenes, flavonoids, saponin and tannins (Trease and Evan, 1983). LD_{50} value was also determined for the crude extract and different fractions by employing the method reported by Nayak et al. (2004).

Micronutrients assay

Phosphorus

The method described by Tadon et al. (1968) was used for the determination of phosphorus contents. Dried plant (0.5 g) was digested with 4 ml of concentrated nitric acid at 145°C for about 1 h. Then 4 ml of conc. perchloric acid was added and heated up to 210°C and allowed to digest for another 1 h. Finally the digest was analyzed colorimetrically at 460 nm by using vanadate-molybdate yellow method.

Nitrogen (N)

The aliquot was put into a stream, carrying helium and first passed through a copper column at high temperature- where oxygen was removed and the NO_x gasses were reduced to N_2 and then through a reagent tube, in order to scrub the remaining H_2O and CO_2 from the stream. The percentage weight of N_2 was then measured by a thermal conductivity cell against a helium background (Lee et al., 1996).

Boron (B)

Dried plant material 10 g was boiled under reflux condenser with 20 ml of 0.01 M $CaCl_2$ for 5 min using fiber digestion apparatus (Watson, 1998). The mixture was then filtered and the filtrate obtained was analyzed by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy) (Fassel and Kniseley, 1974).

Zinc (Zn) and Manganese (Mn)

For the determination of Zn and Mn reported spectroscopic technique was employed. These components were first extracted by vigorous shaking of 10 gm of dried plant material with 20 ml of 0.005 M diethylenetriamine penta acetic acid (DTPA) for 2 h and filtered. The resulting filtrate was then analyzed for Zn and Mn by ICP-AES (Baker and Amacher, 1982).

Antinociceptive activity

Animals

Male NMRI mice of 20 - 25 g were used. Animals were kept at 21 ± 2°C on a 12 h light/dark cycle with free access to standard laboratory food pellets and water (Simjee et al., 2007). Animals were handled for at least 2 to 3 days before the experiment was conducted. For thermal nociception experiments, animals were transported to the testing room and acclimated to the environment for 1 h. Mice were maintained in accordance with the guidelines of SRI International and of the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003). The ethical guidelines of the International Association for the Study of Pain in conscious animals were followed. Mice were randomly distributed to each treatment group of six animals to avoid the individual variation. The group size was determined as the minimum number of animals for valid statistical analysis based on a pilot study conducted; the group size of six has an 80% power to detect differences in the means (Simjee et al., 2007).

Tail-Flick assay

Acute nociception was assessed using the tail-flick assay with an analgesia instrument (The 37360 - Tail-Flick Unit with Data Acquisition Software Package, Cat. 52050-09 was used and company is UGO BASILE, Comerio VA - Italy) that uses radiant heat. The cut off time (that is, the maximum time allowed for the animal to flick its tail away from the heat source before the lamp automatically switches off) was decided to be 20 sec in order to avoid damage to the tail. The heat from an infrared beam on the tail flick unit was focused on the tail and the time taken for the animal to remove its tail (tail flick latency) from the source of heat was recorded. Base line latency response values induced by thermal stimulus were recorded for tail-flick latency before drug administration in each animal, enabling the change in latency responses to be calculated post injection. Observations were taken thrice at each time point in order to minimize chances of error during the study. After baseline measurement, animals received injections (i.p.) of their assigned sample and were tested for tail-flick latencies at 15, 30, 60, 90 and 120 min post injection. Same route was used for all the treatments to avoid the pharmacokinetic differences between drugs. Control group received vehicle, where standard group was administered with indomethacine. All samples and standard were dissolved in 0.01 N NaOH.

RESULTS AND DISCUSSION

Preliminary gross phytochemical screening of crude methanol extract (El) gave strongly positive test for flavornoids. LD_{50} value for crude extract and all the fractions was found to be greater than 3.5 g. Micronutrients analysis given in Table 1 showed that except for boron all other micronutrients including, P, N, Zn and Mn were well in the adequate level recommended for orchid crops. The antinociceptive profile of crude methanol extract and different fractions are listed in Table 2. In control animals, the latency period remained almost at the base line throughout the experiment. Animals treated with reference

Table 1. Micronutrients level of *Eremostachys laciniata*.

Micronutrient	Unit	I	II	III	Mean	*Adequate
P	g/kg	0.45	0.44	0.40	0.43	0.08 - 18.0
N	g/kg	2.4	3.1	3.5	3.0	1.50 - 10.0
B	mg/kg	37.0	35.6	36.5	36.4	50.0 - 100
Zn	mg/kg	56.0	77.0	59.0	64.0	20.0 - 150
Mn	mg/kg	171	161	126	152.6	60.0 - 500

*Adequate level is with reference to orchid crops.

Table 2. Time of measurement of Latency of Tail Flick (Sec).

Gp	Treatment	0 min	15 min	30 min	60 min	90 min	120 min
1	Control	2.99 ± 0.29	3.08 ± 0.33	3.15 ± 0.24	2.89 ± 0.31	2.98 ± 0.26	3.04 ± 0.14
2	Stand (5 mg/kg)	2.39 ± 0.31*	2.76 ± 0.47*	5.78 ± 0.58*	6.59 ± 0.88	5.56± 0.54*	4.34 ± 0.67*
3	El (800 mg/kg)	2.36 ± 0.36*	3.56 ± 0.49*	5.28 ± 0.35*	5.88 ± 0.78	5.34 ± 0.77	3.99 ± 0.22*
5	Elc (800 mg/kg)	3.04 ± 0.34**	3.63 ± 0.67**	5.24 ± 0.69	5.29 ± 0.22**	6.7 ± 0.64**	6.63 ± 0.28**
6	Ele (1500 mg/kg)	2.07 ± 0.24*	4.34 ± 0.23*	5.35 ± 0.23*	6.22 ± 0.25*	5.55 ± 0.14*	4.19 ± 0.21*
7	Elb 1500 mg/kg)	2.40 ± 0.33***	4.69 ± 0.23***	5.50 ± 0.28***	5.74 ± 0.58***	7.20 ± 0.77	4.82 ± 0.54***

Values are mean ± SEM (n = 6), *($p < 0.01$), ** ($p < 0.017$), *** ($p < 0.03$).

drug, that is, indomethacine showed the peak effects between 30-90 min post injections. Animals treated with El and Elc showed a significant increase in their latency of tail flick ($p < 0.001$ and $p < 0.017$ respectively) at 30 min after administration of the same dose that is, 800 mg/kg, however, in case of El, the activity started to decline after 90 min whereas, the Elc showed a persistent analgesic activity until the end of the experiment. In contrast, the animals injected with either Ele or Elb showed a significant analgesic activity at 15 min ($p < 0.01$ and $p < 0.03$, respectively) with a dose of 1500 mg/kg, which persist until 90 min post injection. An interesting observation was made during the experiment that the animals injected with all test samples showed not only sedation but severe ataxia and hind-limb extension.

On the basis of increase in latency time observed in first 15 - 30 min, it may be suggested that the plant has an excellent analgesic property. However, the appearance of ataxia and sedation with hind limb extension showed some central effect of the plant. One of the basis of these observations revealed that observed increase in latency time, induced by crude methanol extract and sub-crude fractions of *E. laciniata*, is not only due to analgesic activity, but may be the combined effect of sedation, ataxia and analgesia. Looking at these results, it may be concluded that *E. laciniata* may be a potential source in the future for the isolation of important natural products with a variety of effects including analgesia.

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