

Full Length Research Paper

Antidepressant screening and flavonoids isolation from *Eremostachys laciniata* (L) Bunge

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Accepted 27 September, 2010

Eremostachys laciniata (L) Bunge (*Lamiaceae*), a rich source of flavonoids, has been investigated for chemical constituents and *in vivo* antidepressant property using forced swim test (FST) model. Five important compounds were isolated, including luteolin (1), apigenin (2), 5,8-dihydroxy-6,7-dimethoxyflavone (3), 5,7-dihydroxy-6,8-dimethoxyflavone (4) and luteolin 7-O- β -glucoside (5). Compound 3 and 4 were isolated for the first time from the plant. A profound antidepressant action was observed for the crude extract at low doses, followed by a state of depression at higher doses. The initial antidepressant-like property of the plant may be attributed to the presence of apigenin like compounds; where as, an increase in immobility time observed at higher doses of the extract may be due to the sedative and calming effect of luteolin present in the plant. *E. laciniata* may be a potential source for the isolation of important natural products with antidepressant-like properties.

Key words: *Eremostachys laciniata*, antidepressant, apigenin.

INTRODUCTION

Depression is considered as a serious health care problem globally. One of the most popular reasons for its pathogenesis is its neurotransmitter deficiency (Farvolden et al., 2003). Basic and clinical studies demonstrated that alteration in the hypothalamic-pituitary-adrenal (HPA) axis system were characteristic of depression as e by increased release of corticotropin-releasing factor (CRF) and cortisol (Arborelius et al., 1999; Holsboer, 2003). Reduction in HPA axis activity may contribute to antide-

pressant actions of some treatments, at least partly by reducing CRF and cortisol levels (Fadda et al., 1995). Although a number of synthetic drugs are being used as standard treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatment and also provide an opportunity for alternative remedies based on natural products (Dhingra and Sharma, 2006).

In order to explore the rich biodiversity of the country and discover natural products of medicinal importance, *Eremostachys laciniata* (L) Bunge (*Lamiaceae*) was investigated for antidepressant property due to its rich flavonoid contents (Tomas-Barberan and Gil, 1992). The plant grows naturally at high altitude of about 2200 m, from East Mediterranean region, Central and South-West Asia, Afghanistan and isolated areas of Pakistan (Hedge, 1990; Stewart, 1862; Jamzad et al., 2003). *E. laciniata* has been reported to possess significant antioxidant

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Abbreviations: HPA, Hypothalamic-pituitary-adrenal; CRF, corticotropin-releasing factor; EtOAc, ethylacetate; TLC, thin layer chromatography; UV, ultraviolet; IR, infrared; NMR, nuclear magnetic resonance; FST, forced swim test.

activity (Erdemoglu et al., 2006).

MATERIALS AND METHODS

Plant material

Aerial parts of *E. laciniata* were collected February 2002 from at Malakand division of Khyber Pakhtunkhwa, Pakistan. After its identification by Prof. Dr. Jehandar Shah, a voucher specimen (EL-102) was submitted to the Department of Botany, University of Peshawar.

Extraction and fractionation

Shade dried powdered plant material (4 kg) was extracted with methanol (80%) by percolation at room temperature. Combined extract was dried *in vacuo* at ambient temperature to afford 250 g of crude methanol extract (EI). After removing a portion of the crude extract, the remaining was subjected to fractionation with different organic solvents (Khan et al., 2010).

Preliminary screening and toxicity study

Crude methanol extract was screened to ascertain the presence of different families of organic compounds including alkaloids, flavonoids, saponins, terpenes and tannins (Evans, 1996; Parekh and Chanda, 2007). LD₅₀ value was also determined for the extract as described by Nayak et al. 2004.

Isolation

Ethyl acetate (EtOAc) fraction was subjected to flash chromatography using EtOAc-methanol (9.5:0.5 v/v) as solvent system. Five yellow to yellowish white compounds were isolated from the same fraction. The isolated compounds were then subjected to different physical and spectroscopic techniques, including melting point, thin layer chromatography (TLC), ultraviolet (UV) light, infrared (IR) light, mass and nuclear magnetic resonance (NMR) for structure elucidation.

Animals and treatments

Albino mice (NMRI) weighing 20 - 30 g were obtained from the animal house of the International Center for Chemical and Biological Sciences (ICCBS), University of Karachi. Animals were distributed into nine groups, each comprising of five test animals. Samples were prepared in doses of 0.1, 1, 3, 5, 7, 10, 12 and 15 mg/kg by suspending the given extracts in normal saline. Samples, standard drug (phenazine) and vehicle were administered intraperitoneally. All tests were performed in triplicate in accordance with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences (National Research Council, 1996).

Forced swim test (FST)

For the determination of antidepressant activity, FST protocol was employed (Porsolt et al., 1977). During the test, animals were individually placed in a glass cylinder (20 cm in height, 14 cm in diameter) filled 10 cm high with water at 25 ± 2°C. All animals were forced to swim for 6 min and the duration of immobility was observed and measured during the final 4 min interval of the test.

Immobility period was regarded as the time spent by the mouse to float in water with no struggle and making only those movements necessary to keep its head above the water. In order to check the fitness level of each test animal, a pre-test was carried out 24 h before the FST by subjecting each test animal to a session of 15 min swimming.

Statistical analysis

The inhibition activity on immobility time by the inter peritoneal administration of the extract is given as mean ± S.E.M. Statistical significance was determined using the student's t-test (Olajide et al., 2004). Values with $p \leq 0.05$ were considered significantly different from control.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of crude methanol extract (EL) demonstrated strong positive test for flavonoids, additionally, alkaloids and saponins were also present. LD₅₀ value for the crude extract was found to be greater than 2.0 g/kg (i.p.). The structure of compounds deduced on the basis of physical and spectroscopic data showed that all five compounds isolated were known flavonoids, including, luteolin, apigenin, 5,8-dihydroxy-6,7-dimethoxyflavone, 5,7-dihydroxy-6,8-dimethoxyflavone and luteolin 7-O-β-glucoside.

The effect of increasing dose of crude methanol extract of *E. laciniata* on immobility time is presented in Table 1, whereas plot of percentage inhibition of immobility against standard and increasing doses of extract are shown in Figure 1. Crude methanol extract of *E. laciniata* induced significant inhibition of immobility time, that is, 31 s at a dose of 0.1 mg/kg, compared to 33 s of standard at 10 fold greater dose. Other doses including 1, 3 and 5 mg/kg elicited significant ($p \leq 0.05$) decrease in immobility time, that is, 66, 91 and 107 s, respectively. At doses above 5 mg/kg, a reduction in the decreasing effect of crude extract on immobility time, that is, 102, 52 and 28 s for 10, 13 and 15 mg/kg doses, respectively, was noted.

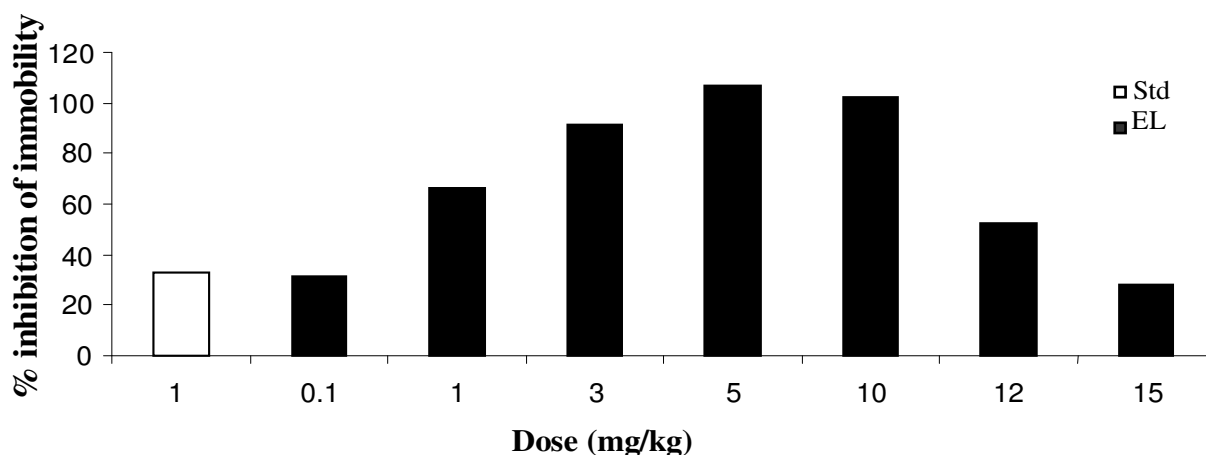
The antidepressant-like effect of the extract was found to be significant at low doses, followed by an increase in the immobility time at doses above 5 mg/kg. The initial antidepressant action followed by the appearance of depression-like symptoms may be attributed to the active flavonoid contents of the plant. Apigenin isolated from the plant has been reported to decrease the immobility duration during forced swim test (Nakazawa et al., 2002), whereas, luteolin similar to benzodiazepines increases the immobility time by inducing sedative and calming effect (Coleta et al., 2008; Terry et al., 2009). The presence of such structurally similar flavonoids with opposing effects on motor activity may be the cause of antidepressant-like action with low doses followed by an increase in immobility time at higher doses of the extract. *E. laciniata* possesses profound antidepressant-like property at low doses and may be explored for developing antidepressant drugs derived from natural products.

Table 1. Effect of crude methanol extract of *E. laciniata* on immobility time of mice during forced swim test (FST).

| Treatment | Dose (mg/kg) | FST (s) | Decrease in immobility (s) |
|-----------|--------------|----------|----------------------------|
| Control | - | 198 ± 8* | 0 |
| Std | 1 | 165 ± 9* | 33 |
| EL | 0.1 | 167 ± 9* | 31 |
| | 1 | 132 ± 8* | 66 |
| | 3 | 107 ± 8* | 91 |
| | 5 | 91 ± 9* | 107 |
| | 10 | 96 ± 8 | 102 |
| | 12 | 146 ± 8* | 52 |
| | 15 | 170 ± 8* | 28 |

Std, Phenalzine; EL, crude methanol extract of *E. laciniata*.

*Significantly different from the control group ($p \leq 0.05$). Values are mean ± SEM (n = 5).

**Figure 1.** Effect of increasing concentration of crude methanol extract of *E. laciniata* on immobility in mice.

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