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Evaluation of analgesic activity of *Eremostachys laciniata* in mice

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Abstract

Eremostachys laciniata (L.) Bunge (family: Lamiaceae) is one of the fifteen endemic Iranian herbs of the genus *Eremostachys*, and also grows in other central and western Asian countries. In the Iranian traditional medicine, this plant has been used as a local analgesic and as an anti-inflammatory remedy. In the present study, the methanol (MeOH) extract of the rhizomes of *E. laciniata* as well as the solid-phase extraction (SPE) fractions have been evaluated for their analgesic property in mice using the hot-plate test. All doses of the SPE 20% aq. MeOH fraction ($P < 0.01$) and the MeOH extract at the doses of 0.5 mg/kg ($P < 0.01$) and 1 mg/kg ($P<0.05$) displayed considerable analgesic effects. The analgesic effect of the SPE 20% aq. MeOH fraction (2.5 mg/kg) was more potent than that of the positive control, morphine (9 mg/kg) at 45 min after injection ($P<0.001$). However, SPE 40% aq. MeOH fraction did not show any analgesic effects at test doses.

Key words: *Eremostachys laciniata*, Lamiaceae, analgesic, morphine, mice, hot-plate test.

1. Introduction

Eremostachys laciniata (L.) Bunge (family: Lamiaceae alt. Labiatae; subfamily: Lamioideae), a perennial herb with a thick root and pale purple or white flowers, is one of the fifteen endemic Iranian species of the genus *Eremostachys*, and also grows in other countries of the Central, Middle-East and Western Asia, and Caucasus [1, 2]. A decoction of the roots and flowers of

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E. laciniata has traditionally been taken orally for the treatment of allergies, headache and liver diseases [3]. Previous phytochemical studies on *E. laciniata* revealed the presence of various mono- and sesqui-tepenes [4], furanolabdane diterpenes and iridoid glycosides [5, 6]. The crude extract of this plant was reported to possess free-radical-scavenging property [7]. As part of our on-going studies on plants of Iranian flora [5, 6, 8-18], we now report on the analgesic property of the methanol extract of the rhizomes of *E. laciniata* in mice model.

2. Materials and methods

2.1 Plant material

The rhizomes of *Eremostachys laciniata* (L) Bunge, were collected during September-October 2005 from Ajabshir county in East Azarbaijan province in Iran (37° 36' 46.7''North latitude, 46° 11' 15.6''East longitude and altitude 1900 meters over sea level). A voucher specimen (TUM-ADE 0204) has been retained in the herbarium of the Faculty of Pharmacy, Tabriz University of Medical science, and in the herbarium of the Plant and Soil Science Department, University of Aberdeen, Scotland (ABD).

2.2 Extraction and solid phase extraction

The dried and ground rhizomes of *E. laciniata* (100 g) were Soxhlet- extracted, successively, with *n*-hexane, dichloromethane and methanol (1.1 L each). The MeOH extract (2 g) was subjected to solid phase extraction on a C_{18} silica cartridge (10 g) using a step gradient of MeOH-water mixture (10:90, 20:80, 40:60, 60:40, 80:20 and 100:0, 150 mL each).

2.3 Animals

Swiss Albino mice, weighing 25-30 g were used in this study. Mice were housed at controlled temperature (22 ± 2°C) with a 12 h light/dark cycle and with standard lab chow. The animals were habituated to the experimental room for at least 2 h before the experiments.

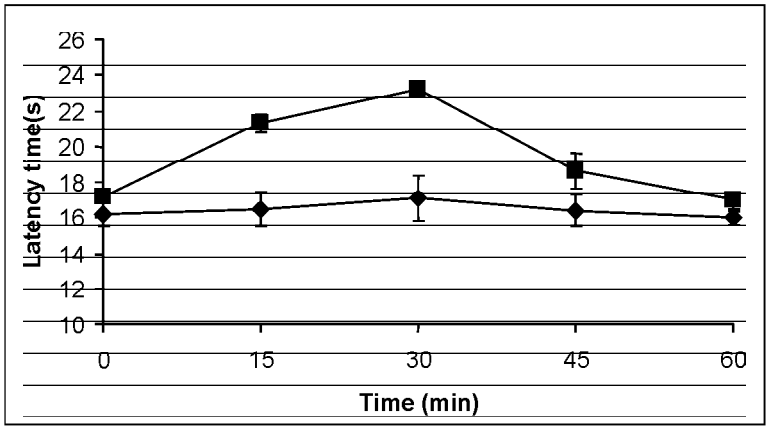

Fig. 1: Effects of morphine on tolerant and non tolerant mice.
Animals were injected morphine (30 mg/kg, i.p.) for 4 days. Antinociception of a test dose of morphine (9 mg/kg, i.p) was assayed 24 hr after the last dose of morphine (30 mg/kg, i.p). Tolerant (▾) and non tolerant (■) mice. Each value is the mean ± SEM of eight mice.**P*<0.05, ***P*<0.01, significantly different from the respective non tolerant control group.

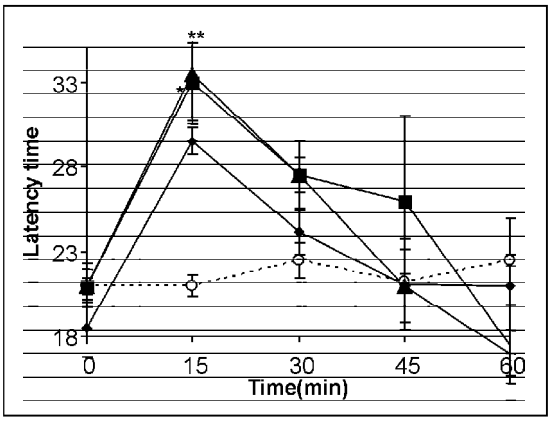

Fig. 2: Effect of the methanol extract of *E. laciniata* in the hot-plate test in mice. Dose 2.5 mg/kg (♦), 1 mg/kg (■) and 0.5 mg/kg (▲); DMSO (o). Each value is the mean ± SEM of eight mice.*P<0.05, **P<0.01, ***P< 0.001

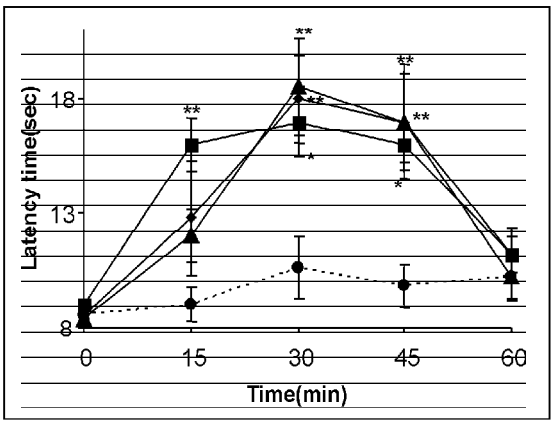

Fig. 3: Effect of SPE 20% aq. methanol fraction in the hot-plate test in mice. Dose 0.5 mg/kg (♦), 2.5 mg/kg (■) and 1.0 mg/kg (▲); DMSO (o). Each value is the mean ± SEM of eight mice.*P<0.05, **P<0.01, ***P< 0.001

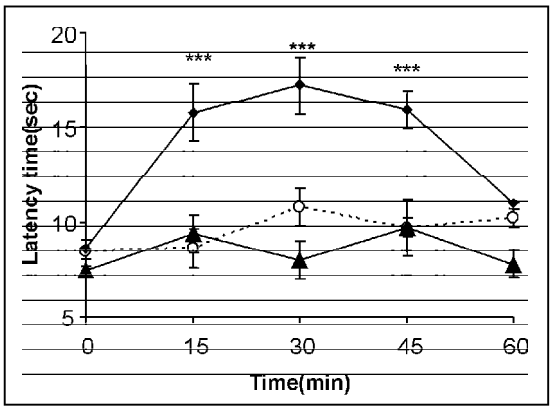

Fig. 4: Comparison of SPE 20% and SPE 40% aq. methanol fractions in the hot-plate test in mice. Dose SPE 20% 2.5 mg/kg (♦),and SPE 40% 2.5 mg/kg (▲); DMSO (o). Each value is the mean ± SEM of eight mice. *P<0.05, **P<0.01, ***P< 0.001

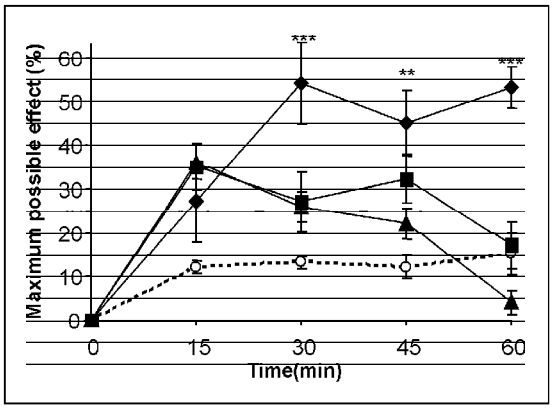

Fig. 5: Effect of the methanol extract of *E. laciniata* on morphine tolerance in the hot-plate test in mice. Dose 2.5 mg/kg (♦), 1 mg / kg (■) and 0.5 mg / kg (▲); DMSO (o).
Each value is the mean ± SEM of eight mice. *P<0.05, **P<0.01, ***P< 0.001

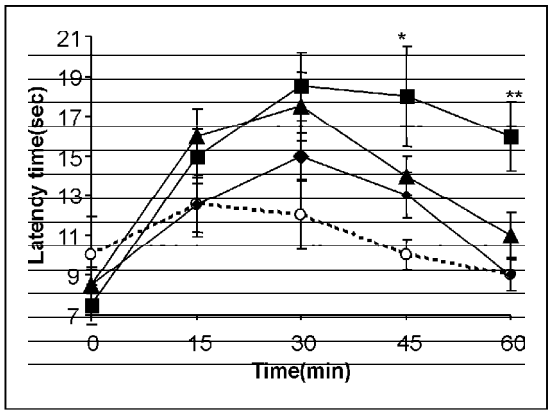

Fig. 6: Effect of SPE 20% aq. methanol fraction on morphine tolerance in the hot-plate test in mice. Dose 0.5 mg/kg (♦), 2.5 mg/kg (■) and 1.0 mg/kg (▲); DMSO (o).
Each value is the mean ± SEM of eight mice.*P<0.05, **P<0.01, ***P< 0.001

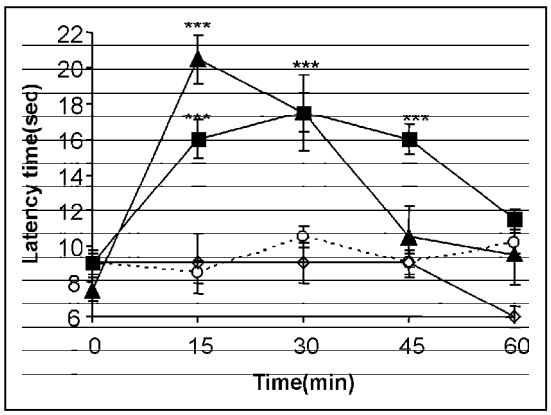

Fig. 7: Comparison of SPE 20% aq. methanol fraction (2.5 mg/kg) with morphine (9 mg/kg) in the hot-plate test in mice. Dose SPE 20% aq. methanol fraction 2.5 mg/kg (■) and morphine 6.0 mg/kg (▲); DMSO (o)., saline (<>)
Each value is the mean ± SEM of eight mice. *P<0.05, **P<0.01, ***P< 0.001

Each animal was used only once. The animals were divided into following groups, 8 animals in each group: MeOH extract + DMSO (0.5, 1 and 2.5 mg/kg, i.p.) SPE 20% aq. MeOH fraction + DMSO (1, 2.5 and 5 mg/kg, i.p.), SPE 40% aq. MeOH fraction + DMSO (1, 2.5 and 5 mg/kg, i.p.), negative control DMSO for analgesia test, and morphine + DMSO, morphine + MeOH extract + DMSO and morphine + SPE 20% aq. MeOH fraction + DMSO for tolerance test. As the SPE 20% and the SPE 40% aq. MeOH fractions were the main fractions (>88% of the MeOH extract), and they are known to contain iridoid glycosides and phenylethanoids, only these two SPE fractions were subjected to the analgesic test. Morphine in DMSO was used as the positive control.

2.4 Hot-plate test

The hot-plate analgesic test [19, 20] was employed to evaluate the analgesic effect and morphine tolerance of the MeOH extract as well as the SPE fractions of *E.laciniata* in mice. Animals were habituated twice to the hot plate in advance. For testing, the mice were placed on a hot-plate maintained at 55 ± 2°C. The time that elapsed until the occurrence of either a hind paw licking or a jump-off the surface was recorded as the reaction time. Mice with baseline latencies of <5 s or >30 s were eliminated from the study. The cut off time was 30 s. After the determination of baseline response latencies, hot plate latencies were re-determined at 15, 30, 45, 60 min after intraperitoneally (i.p) administration of test samples.

2.5.1 Evaluation of analgesic activity

The MeOH extract of plant + DMSO was tested at doses of 0.5, 1 and 2.5 mg / kg i.p., and the SPE 20% aq. MeOH fraction + DMSO and the SPE 40% aq. MeOH fraction + DMSO were tested at doses of 1, 2.5 and 5 mg/kg i.p. The control group received DMSO only.

2.5.2 Evaluation of morphine tolerance

The MeOH extract of plant + DMSO + morphine (30 mg / kg i.p.) [21] was tested at doses of 0.5, 1, 2.5 mg / kg, i.p., and the SPE 20% aq. MeOH fraction + DMSO + morphine (30 mg / kg i.p.) was tested at doses of 1, 2.5 and 5 mg/kg i.p. The control group received DMSO + morphine (30 mg/kg i.p.). After 24 h of the last dose of morphine (fifth day), one dose of morphine (9 mg/kg i.p.) was administrated for control and test groups for evaluation of morphine tolerance as previously described in the literature [21].

2.6 Statistical analyses

The results were expressed as mean ± SEM and evaluated by one way ANOVA followed by Tukey test to assess the level of significance of the differences between the test and control group data means. Statistically p-value of less than 0.05 was considered to be significant and p-value less than 0.01 was considered to be very significant.

3. Results and discussion

Animals received morphine (30 mg/kg, i.p.) for 4 days. In each group antinociceptive response of a test dose of morphine (9 mg/kg, i.p) was assayed 24 h after the last dose of morphine (30 mg/kg, i.p.). Animals that became tolerant to effects of morphine exhibited only a negligible antinociceptive effect (Figure 1).

When mice were treated with three doses (0.5, 1, 2.5 mg/ kg i.p.) of the MeOH extract of the rhizomes of *E. laciniata*, a considerable increase in the animal reaction time to the heat stimulus was observed. Values were found to be significant ($p< 0.01$) at 15 min after i.p. injection of doses of 0.5 and 1 mg/kg. The extract with dose of 2.5 mg/kg had analgesic effect but its value was not found to be significant compared with the negative control group (Figure 2). This might be due to the presence of any compounds, which bacame significant at a higher dose, anatagonising the effect of the analgesic compounds present in the extract.

SPE 20% aq. MeOH fraction + DMSO (1, 5 mg/kg, i.p.) produced significant ($p< 0.01$) analgesic activity at 30 and 45 min after injection, in comparison with the negative control group, whereas the dose of 2.5 mg/kg, i.p showed significant ($p< 0.01$) analgesic effect at 15, 30 and 45 min after injection (Figure 3). Figure 4 shows the comparison of SPE 20% and SPE 40% aq. MeOH fractions at dose of 2.5 mg/kg, i.p. The SPE 20% aq. MeOH fraction produced significant ($p< 0.001$) analgesic effect but the SPE 40% fraction did not show any analgesic effect at the test dose.

When mice were treated with three doses (0.5, 1, 2.5 mg/ kg, i.p.) of MeOH extract for 4 days, 30 min before receiving morphine (30 mg/kg i.p.), an increase in the animal reaction time to the heat stimulus was observed compared with the control group (DMSO + morphine) after receiving a dose of 9 mg/kg, i.p, of morphine on fifth day. Values were found to be significant at 30 and 60 ($p< 0.001$) and 45 min ($p< 0.01$) after i.p. injection of doses of 2.5 mg/kg. The dose of 1 mg/kg of extract showed significant (p, 0.05) effect at 45 min after injection in comparison with the control group (Figure 5).

When mice were treated with three doses (1, 2.5, 5 mg/ kg i.p.) of SPE 20% aq. MeOH fraction for 4 days, 30 min before receiving morphine (30 mg/kg i.p.), an increase in the animal reaction time to the heat stimulus was observed compared with the control group (DMSO + morphine) after receiving a dose of 9 mg/kg i.p of morphine on fifth day. Values were found to be significant at 60 min ($p< 0.01$) and significant 45 min ($p< 0.05$) after i.p. injection of doses of 2.5 mg/ kg in comparison with the control group (Figure 6). The SPE 20% aq. MeOH fraction at 2.5 mg/kg, i.p. in comparison with morphine at 9 mg/kg, i.p., produced significant ($p<0.001$) effect at 45 min after injection (Figure 7).

The present study assessed the analgesic effect of the rhizomes of *E. laciniata*, an Iranian medicinal plant reputed for its analgesic properties. The hot-plate method used for investigating the analgesic effect and the morphine tolerance is believed to be associated with the central mechanism of pain [22]. Previous phytochemical studies on this plant, specially the SPE 20% fraction, revealed the presence of iridoid glycosides. Iridoid glycosides are known to produce inhibitory effects on glutamate (simulative neurotransmitter) activation process associated with pain [23, 24]. The SPE 40% fraction was previously shown to contain predominantly phenylethanoid glycosides, and this fraction did not show any analgesic property at test concentrations. Thus it is reasonable to assume that the anlagesic activity of the extract of *E. laciniata* was due to the presence of iridoid glycosides in the methanol extract. The results obtained in the morphine tolerance test might lead to assume that the iridoid glycosides in MeOH extract and 20% fraction of Sep-Pak act as NMDA receptors´ antagonist and prohibit the activation process of glutamate. Clinical investigations showed that the block of NMDA receptors inhibit the defined type of pain. C fibers, interfering in nociceptice process, are responsible of pain transition and stimulation because of release of glutamate neurotransmitter. Glutamate stimulates the NMDA receptors in spinal cord and increasing response of neurons to all forms of pain [25, 26]. Chronic use of opioids is known to cause the over activity of NMDA receptors leading to an increase of intercellular calcium concentration. In fact, use of NMDA receptors' antagonist is a way for inhibiting calcium entrance into cells. Therefore, the MeOH extract, which is rich in iridoid glycosides, might be able to inhibit NMDA receptors as well as calcium entrance in neurons.

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