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Antinociceptive and anti-inflammatory properties of the hydroalcoholic extract of the Melilotus indicus (L) in male mice

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Abstract. In current study, the antinociceptive and anti-neuropathic pain effects of hydroalcoholic extract of Melilotus indicus in male mice were assessed using formalin and hot plate tests, respectively. Cotton pellet- induced granuloma model was employed for anti- inflammatory assessments. Melilotus indicus hydroalcoholic extract (50 and 100 mg/kg, oral gavage) reduced the time and the number of lickings during the late phase of the formalin test. Anti-neuropathic pain properties were obtained with the dose of 100 mg/kg. Anti-inflammatory effect of this extract (100 mg/kg) was confirmed by a significant decrease in Cotton pellet weight.

Keywords: Antinociceptive, Melilotus indicus, Neuropathic pain, Formalin test, inflammati

1. INTRODUCTION

Melilotus Indicus (family: Fabeaceae (Leguminosae), subfamily: Papilionoideae) found in Asia, Europe and throughout Arabia as a weed of cultivation; and has been introduced to many regions of the world. Antibacterial, anticoagulant, astringent, emollient, laxative and narcotic properties of Melilotus Indicus have been studied previously. It has been shown that seasonal variations affect biosynthesis of different types of antioxidant and free radical scavenging compounds in Melilotus indicus. Flavonoid glycosides, coumarins, terpenoids, and steroids have been determined in the plant in phytochemical evaluations. A $_D$-galacto-$_D$-mannan was isolated from the seeds of Melilotus indica [1-4]. Current study was conducted in order to investigate the antinociceptive, anti-neuropathic pain and anti-inflammatory potentials of Melilotus indicus hydroalcoholic extract in male mice.

2. MATERIALS AND METHODS

Animals: Male albino mice (25-30 g) from the Faculty of Pharmacy, Zabol University of Medical Sciences were used in this research. In current study, all animal manipulations were carried out according to the guidelines for the care and use of laboratory animals of Zabol University of Medical Sciences. All animals were maintained under controlled conditions (12-h light/dark cycle at room temperature of 20-22^{o}C) with free access to food and water. All animal experiments were done during the light cycle.

Drugs: Morphine (Darou Pakhsh Co), imipramine (Sobhan Darou Co), and diclofenac sodium (Darou Pakhsh Co) were dissolved in saline and were injected intraperitoneally (i.p.). Ketamine (alfasan, Holland) and xylazine (Pantex Holland B.V.) were used for surgical anesthesia.

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Plant material and Preparation of the hydroalcoholic extract: The Melilotus indicus (L) Griseb in Zabol Medicinal Plants Research Center was collected in May 2014, chopped, dried in the open air and stored in 4-8 ºC in dark well closed containe. The plant was taxonomically identified by the Ferdowsi University Of Mashhad Herbarium, Iran (voucher no:29959) The extraction was done by maceration method in ethanol 80% at room temperature. After filtration with Whatman No. 1 filter paper, the resulting solutions were concentrated by a rotary evaporated to dryness under vacuum.

3. ANTINOCICEPTIVE STUDIES

Formalin test: Melilotus indicus hydroalcoholic extract (25, 50 and 100 mg/kg), were orally administered via gavage needles once a day for one week. On day 8th, diclofenac sodium (10 mg/kg) or morphine (9 mg/kg) were administered intraperitoneally (i.p.) 30 min before subcutaneous injection of 50 µl formalin (0.5%) into the right hind paw of mice. The time spent (in seconds) and the number of lickings the injected paw by the mouse was recorded between 0-5 min (first phase) and 15-60 min (second phase) after formalin injection. Control animals received saline the same as extract- treated animals.

Hot-plate test: Pain sensitivity in sciatic nerve ligated mice (a model of neuropathic pain) was evaluated using the hot-plate test. At first, animals were anesthetized with ketamine (80 mg/kg) and xylazine (20 mg/kg) and then the animal's right sciatic nerve was ligated by a copper wire. All nerve ligated animals, received Melilotus indicus hydroalcoholic extract (25, 50 and 100 mg/kg) for 14 consecutive days via gavage needles once a day. Reaction time (latency for licking the hind feet or jumping from the hot-plate surface; 55 ± 0.2 °C) was determined 14 days after sciatic nerve ligation (cut-off time was restricted on 45 sec) at intervals of 30, 60, 90 and 120 min. Control animals received saline via gavage needles for the same period of time. Positive control group, received imipramine (40 mg/kg, i.p.) at test day.

4. ANTI-INFLAMMATORY STUDY

Cotton pellet- induced granuloma formation in mice:One sterilized 20 mg adsorbent cotton pellet was implanted subcutaneously in male mice. Animals, received Melilotus indicus hydroalcoholic extract (25, 50 and 100 mg/kg) for 7 days via gavage needles once a day. Control animals received saline via gavage needles and positive control group received diclofenac (10 mg/kg, i.p.) for the same period of time. On day 8th after cotton pellet implantation, the mouse was sacrificed and the implanted pellet was removed carefully and the dried weight of the pellet (24 h later) was assessed.

Statistics:One-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison post–hoc test was used for comparison of findings of this study in Graphpad Prism 5. A p-value of 0.05 or less was considered statistically significant.

5. RESULTS

Effects of Melilotus indicus hydroalcoholic extract on licking response in formalin test:

As shown in figure 1, the evaluation of the licking response in the late phase of the formalin test showed that Melilotus indicus (50 and 100 mg/kg, oral gavage) for 7 consecutive days caused a significant decrease (**$p<0.01$ and ***$p<0.001$, respectively) on licking response in comparison with the control group (Figs. 1C and 1D). In the late phase of the formalin test, morphine and diclofenac (***$p<0.001$) exerted significant decrease on licking response compared to control animals. All administered doses of Melilotus indicus (25, 50 and 100 mg/kg) did not change the licking responses in the first phase of the formalin test (Figs. 1A and 1B).

6. ANTINOCICEPTIVE EFFECTS OF MELILOTUS INDICUS HYDROALCOHOLIC EXTRACT IN SCIATIC NERVE LIGATED MICE:

As shown in figure 2 (A-E), 14 days oral administration of Melilotus indicus hydroalcoholic extract (100 mg/kg, oral gavage) induced significant antinociception in sciatic nerve ligated animals compared to control group. The analgesic effect of this extract (100 mg/kg) was comparable with imipramine as a positive control group until 120 min in hot-plate test.

Anti-inflammatory effects of Melilotus indicus hydroalcoholic extract in Cotton pellet-induced granuloma formation model in mice

Treatment with Melilotus indicus hydroalcoholic extract (100 mg/kg, oral gavage for seven consecutive days) showed a significant anti-inflammatory effect in the cotton pellet- induced granuloma formation model in mice (Fig. 3, *$p<0.05$). The anti-inflammatory effect of this extract (100 mg/kg) was comparable with diclofenac sodium as a positive control group.

7. DISCUSSION

Findings of this experimental study showed that Melilotus indicus hydroalcoholic extract induced antinociceptive activity in the late phase of the formalin test and sciatic nerve ligated mice as amodel of neuropathic pain. Also, it showed anti-inflammatory activities in cotton pellet-induced granuloma model.

The formalin test, a continuous pain induction in injured tissues, is submitted and applicated for assessment of the central and/or peripheral nervous system's pain conditions [5-8]. Non-steroidal anti-inflammatory drugs (NSAIDs), steroids and centrally acting drugs can reduce or inhibit inflammatory responses [6-8] in the peripheral tissues in the second phase of the formalin test [9-11]. In this study, morphine, diclofenac and high administered doses of Melilotus indicus induced analgesia in late phase of formalin test. Diclofenec exhibits its anti-nociceptive effects by affecting several different pathways [12-13].

It has been shown in our previous studies that sciatic nerve ligation as a model of neuropathic pain caused significant hyperalgesia, 14 days after ligation in the hot plate test. Oral administration of the hydroalcoholic extract of Melilotus indicus (100 mg/kg, oral gavage for 14 days) caused significant antinociception. Considerable analgesic effects of flavonoids have been well documented in previous studies [14-17]. Thus, one of the underlying mechanisms of its analgesic properties in chronic pain conditions may be related to its flavonoid source [18].

Transudative, exudative and proliferative are three important phases of the chronic inflammations. Cotton pellet- induced granuloma as a model of inflammation has been extensively used to evaluate the transudative and proliferative phases of the chronic inflammation [6, 19].

The absence of antinociception for Melilotus indicus in acute pain, in addition to its analgesia and anti-inflammatory effects in sciatic nerve ligated and cotton pellet-induced granuloma models, suggests that these antinociceptive and anti-inflammatory effects may be induce due to some interactions with the synthesis and/or action of the neuropeptides or prostanoids and is probably explained by its effect on peripheral nervous system. Further evaluations will be required to clarify the main antinociceptive and anti-inflammatory effects of the Melilotus indicus.

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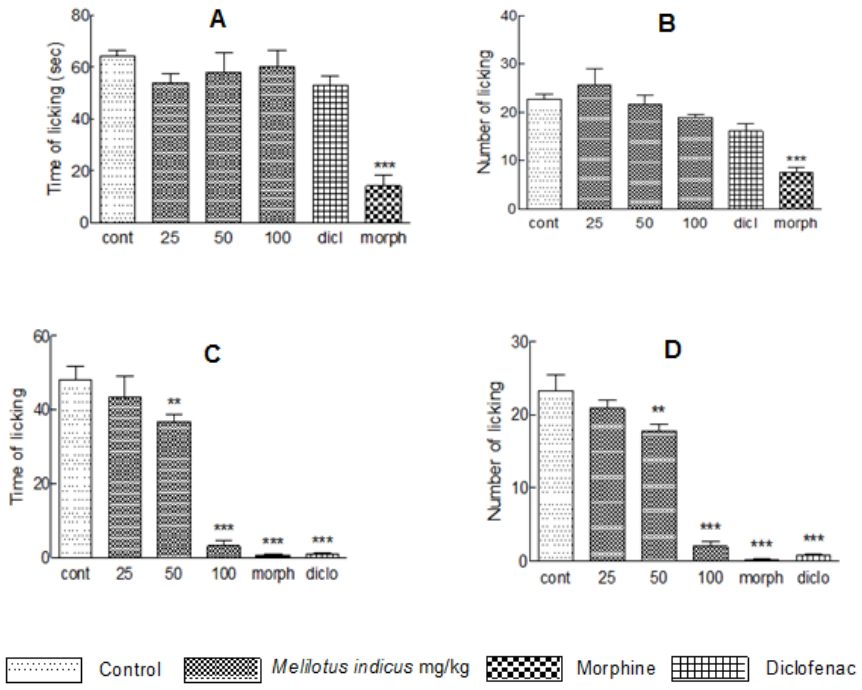

Figure 1. Effects of melilotus indicus, morphine and diclofenac in the first (A and B) and second phase (C and D) of the formalin test. Each value represents the mean ± S.E.M. (n=7). **p< 0.01 and ***p<0.001 significantly different from the control animals.

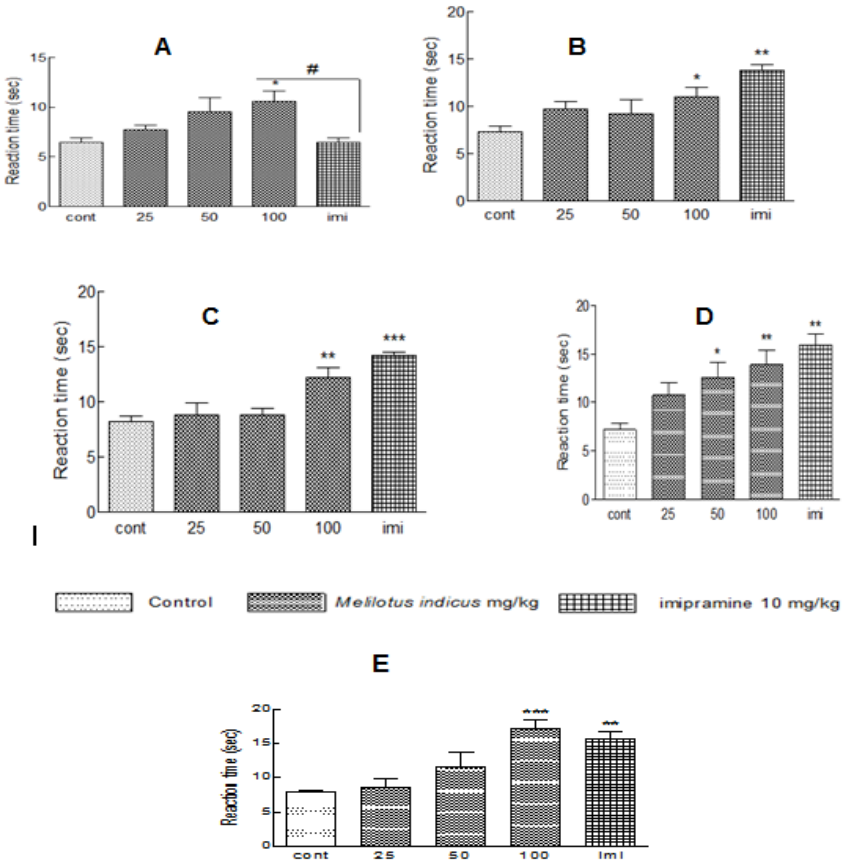

Figure 2 A-E. Latency response of the melilotus indicus- treated animals in comparison with the control and imipramine- treated animals (n = 7, mean ± S.E.M.).

Figs A, B, C, D and E represent assessment of anti-nociception at 0, 30, 60, 90 and 120 min, respectively. *p<0.05, **p< 0.01 and ***p<0.001 significantly different from the control animals. #p<0.05 significantly different from the imipramine- treated animals.

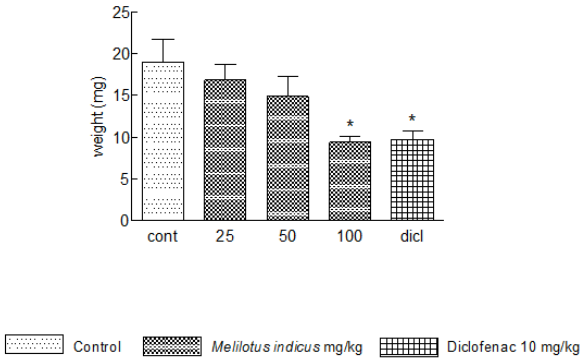

Figure 3. Anti-inflammatory activity of melilotus indicus hydroalcoholic extract in cotton pellet- induced granuloma formation (n = 7, mean ± S.E.M.). *p<0.05 significantly different from the control animals.