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Anti-nociceptive and antiinflammatory effects of the hydroalcoholic extract of the Spergularia Marina (L). Griseb in male mice

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Abstract. In this study, the anti-nociceptive properties of hydroalcoholic extract of Spergularia Marina (L). Griseb in male mice were carried out using chemical (writhing and formalin) and thermal (hot plate) models of nociception. Cotton pellet- induced granuloma model was used for anti- inflammatory investigations in male mice. Spergularia Marina hydroalcoholic extract (12.5, 25 and 50 mg/kg, oral gavage) reduced time and number of paw licks during the second phase of the formalin test and the number of writhings. This extract did not show any significant analgesia on acute pain in first phase of the formalin test. Also, the effect of the extract (25 and 50 mg/kg, p.o.) in sciatic nerve ligation model provides a confirmation of its probable antineuropathic pain properties. Anti-inflammatory effect of this extract (50 mg/kg) was confirmed by a significant decrease in Cotton pellet weight in male mice.

Keywords: Antinociception, Spergularia Marina, Sciatic Nerve ligation, Formalin test, Inflammation

1. INTRODUCTION

Spergularia (Caryophyllaceae) includes 25 species distributed especially in cosmopolitan [1].Hypoglycemic, diuretic, antihypertensive, antibacterial, antioxidative and cholesterol-lowering properties of several species of Spergularia have been studied from the pharmacological points of view [2].β-sitosterol glycoside and tricin, dihydroferulic acid, vanillic acid, 4-hydroxybenzoic acid, uracil and 8-hydroxy cuminoic acid were isolated from different extracts of the Spergularia marina (SM) [1].

The purpose of this study was to evaluate the effect of oral administration of the hydroalcoholic extract of Spergularia marina in different (acute, chronic and neuropathic) pain conditions and inflammatory related disorders to open a new view in the control of these suffering conditions.

2. MATERIALS AND METHODS

2.1.Animals

Male albino mice (25-30 g) from the Faculty of Pharmacy, Zabol University of Medical Sciences were used throughout this study. All animal manipulations and experimental protocols were carried out according to the ethical guidelines of Zabol University of Medical Sciences for use and care of laboratory animals. The subjects used in this study were housed randomly in

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groups of five per appropriate stainless-steel cages at 22 ± 2 °C on a 12 h light/dark cycle with free access to food and water. All animal experiments were done during the light cycle.

2.2. 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 employed for surgical anesthesia.

2.3.Plant material and Preparation of the hydroalcoholic extract

Spergularia Marina (L). Griseb in Zabol Medicinal Plants Research Center was collected in February 2013, chopped, dried in the open air and stored in 4-8 ºC in dark well closed containe. The plant was identified by the Ferdowsi University Of Mashhad Herbarium, Iran (voucher no:29960). The extraction was done by maceration method in ethanol 80% at room temperature. After filtration, the ethanol was evaporated to dryness under vacuum.

2.4. Anti-nociceptive studies

2.4.1.Formalin test

Different concentrations of Spergularia marina hydroalcoholic extract (12.5, 25 and 50 mg/kg), were orally administered via gavage needles once a day for one week. On day 8^{th}, diclofenac sodium (10 mg/kg) or morphine (9 mg/kg) were given intraperitoneally 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.

2.4.2. Acetic acid-induced writhing test in mice

Mice (n = 7) were pretreated 30 min before the nociceptive agent, acetic acid 0.6 % (v/v, 10 ml/kg). Vehicle (saline), Spergularia marina hydroalcoholic extract (12.5, 25 and 50 mg/kg, oral gavage), diclofenac sodium (10 mg/kg) and morphine (9 mg/kg) were administered before acetic acid injection. The number of writhes following the injection of acetic acid was evaluated between 5 and 30 min after stimulus injection.

2.4.3. Hot-plate test

Pain sensitivity in sciatic nerve ligated mice (a model of neuropathic pain) was evaluated using the hot-plate test [3].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 Spergularia marina hydroalcoholic extract (12.5, 25 and 50 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.

2.5. Anti-inflammatory study

2.5.1. Cotton pellet- induced granuloma formation in mice

The details of the Cotton pellet- induced granuloma formation was described in previous studies with minor modifications. One sterilized 20 mg adsorbent cotton pellet was implanted subcutaneously in male mice. Animals, received Spergularia marina hydroalcoholic extract (12.5, 25 and 50 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 8^{th} 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 determined.

2.6. 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.

3. RESULTS

3.1. Effects of Spergularia marina hydroalcoholic extract on licking response in first and late phases of the formalin test

As shown in figure 1, the assessment of the licking response in the late phase of the formalin test showed (Figs. 1C and 1D) that oral administration of Spergularia marina (12.5, 25 and 50 mg/kg) for 7 consecutive days caused a significant decrease on licking response in comparison with the control group. In the late phase of the formalin test, morphine and diclofenac (***$p<0.001$) exerted significant decrease on licking response compared to control animals. These concentrations of the Spergularia marina did not change the licking responses in the first (acute pain) phase of the formalin test (Figs. 1A and 1B).

3.2. Effect of Spergularia marina hydroalcoholic extract on acetic acid- induced abdominal constrictions in mice

As a part of this pharmacological evaluation (Fig. 2), a single oral dose of Spergularia marina hydroalcoholic extract (12.5 (*$p<0.05$), 25 (**$p<0.01$) and 50 (***$p<0.001$) mg/kg, p.o.) decreased the number of abdominal constrictions in comparison with corresponding control values.

3.3. Analgesic effects of Spergularia marina hydroalcoholic extract in sciatic nerve ligated mice

Our findings in this part of study indicated that there was a significant hyperalgesia between control (sham-operated) and sciatic nerve ligated animals at 14 days after surgery and sciatic nerve ligation (Fig. 3).

As shown in figure 4 (A-E), 2-weeks oral administration of Spergularia marina hydroalcoholic extract (25 and 50 mg/kg, p.o.) showed significant analgesic effects in sciatic nerve ligated animals in comparison with control animals. The analgesic effect of this extract (50 mg/kg) was remained high until 120 min in hot-plate test. The analgesic effect of imipramine as a positive control was started at 30 min after i.p. injection and remained high until 120 min.

3.4. Anti-inflammatory effects of Spergularia marina hydroalcoholic extract in Cotton pellet- induced granuloma formation in mice

Seven days pre-treatment with Spergularia marina hydroalcoholic extract (50 mg/kg, p.o.) showed a significant anti-inflammatory effect in the Cotton pellet- induced granuloma model in mice (Fig. 5, *$p<0.05$). Diclofenac, at a dose of 10 mg/kg significantly inhibited the inflammatory responses (**$p<0.01$).

4. DISCUSSION

The results of the present research indicate that Spergularia marina (SM) hydroalcoholic extract showed anti-nociceptive activity in the late phase of formalin test, acetic acid- induced abdominal writhings and sciatic nerve ligated model in mice. Also, it had anti-inflammatory activities in cotton pellet-induced granuloma model.

The formalin test, a continuous pain induction in injured tissues, is applied to assess the central and/or peripheral nervous system's pain conditions [4-6]. The late phase of formalin test due to inflammatory responses in the peripheral tissues [7], histamine, serotonin and bradykinin involvements [4, 6] can be inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), steroids and centrally acting drugs [8, 9]. In this study, morphine and diclofenac and all administered doses of SM, induced anti-nociceptive effects in late phase of formalin test. Diclofenec exhibits its anti-nociceptive effects by affecting various pathways [10, 11].

In the present study, Spergularia marina induced inhibition of acetic acid-induced abdominal constrictions in writhing test. 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 SM induced significant analgesia. Significant anti-nociceptive activity of flavonoids has been well documented in previous studies [12-15]. Thus, one of the underlying mechanisms of anti-nociceptive activity of SM in chronic pain may be related to its flavonoid source [16]. Further investigations will be required to evaluate the main anti-nociceptive mechanism of antinociceptive and anti-inflammatory effects.

Chronic inflammation consists of transudative (increase of the pellet wet weight during the first 3 h), exudative (plasma leaking from the bloodstream around the granuloma between 3 and 72 h) and proliferative (increase of the granuloma dry weight between 3 and 6 days) phases. This inflammatory- induced method (Cotton pellet- induced granuloma) has been extensively used to evaluate the first and third phases of chronic inflammation [4, 17].

The absence of antinociception for SM in the first phase of the formalin test, in addition to its antinociceptive and anti-inflammatory effects in writhing, 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.

Since SM was proved to induce anti-inflammatory effects in cotton pellet implantation, its analgesia in the late phase of formalin test and sciatic nerve ligation model in hot-plate test is probably explain by its effect on peripheral nervous system.

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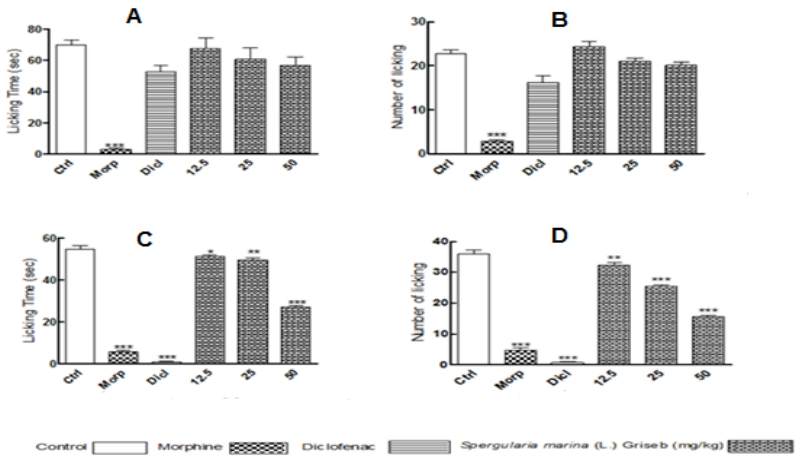


Figure 1. Effects of Spergularia marina, morphine and diclofenac in the early (A and B) and late phase (C and D) of the formalin test in mice by assessing the licking time and the number of lickings. Each value represents the mean ± S.E.M. for 7 mice. *p<0.05, **p< 0.01 and ***p<0.001 significantly different from the control animals.

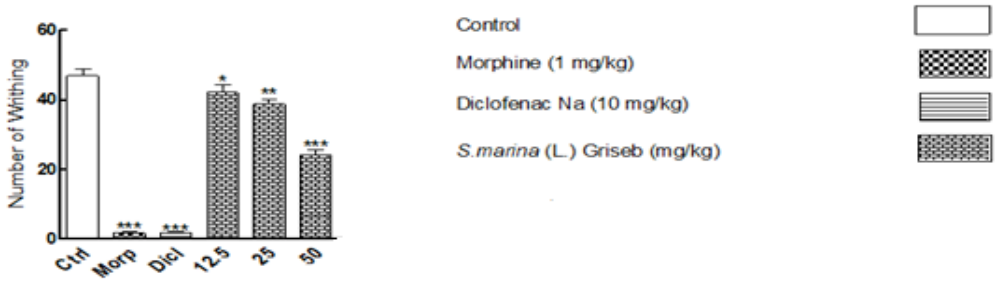


Figure 2. Effects of treatment with Spergularia marina on acetic acid- induced abdominal constrictions in mice. Each value represents the mean ± S.E.M. for 7 mice. *p<0.05, **p< 0.01 and ***p<0.001 significantly different from the control animals.

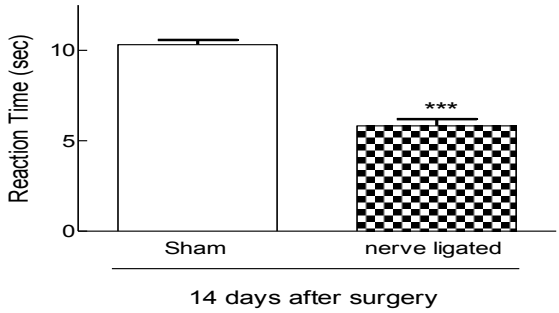
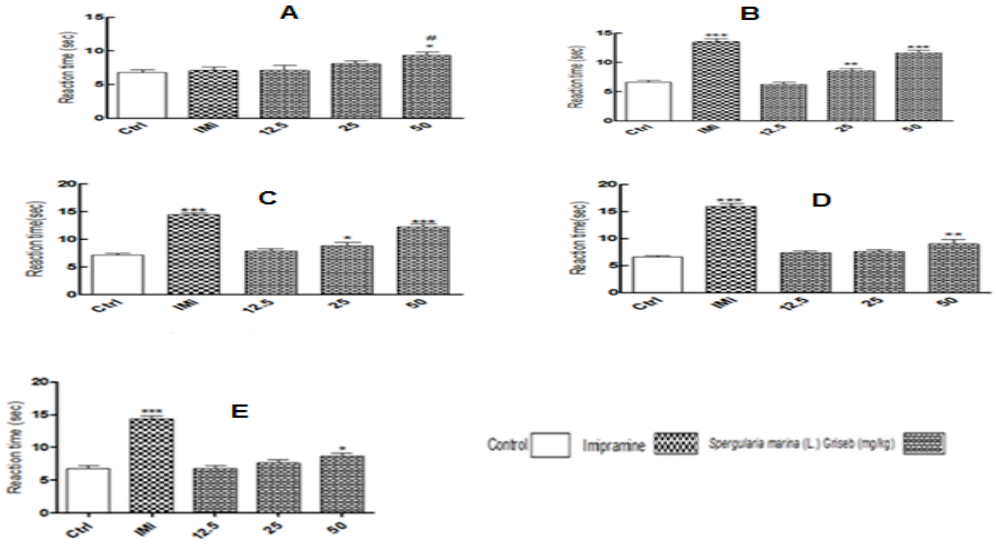


Figure 3. Changes in reaction time in sham-operated and nerve ligated mice, 14 days after the sciatic nerve ligation. Each value represents the mean ± S.E.M. for 7 mice. ***p<0.001 significantly different from the control (sham) - treated animals.



Figures 4. A-E. Latency response of the Spergularia marina 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.

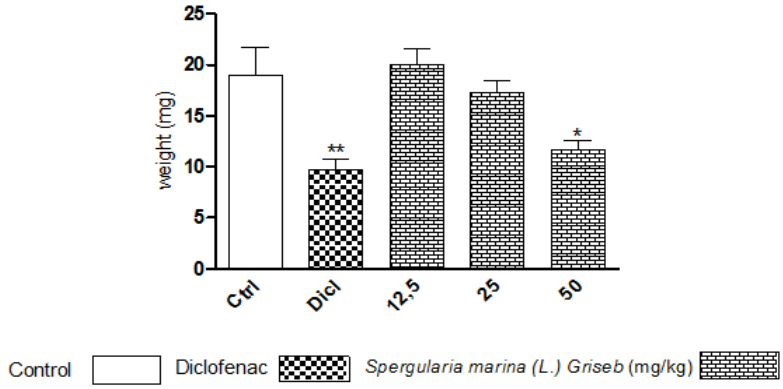


Figure 5. Anti-inflammatory activity of Spergularia marina in cotton pellet- induced granuloma formation (n = 7, mean ± S.E.M.). *p<0.05 and **p< 0.01significantly different from the control animals.