Effect of *Marrubium vulgare* L. Aerial Parts Aqueous and Ethanolic Extracts on Morphine Withdrawal Syndrome in Mice

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ABSTRACT

The effect of intraperitoneal injection of aqueous and ethanolic extracts of *Marrubium vulgare* L. (0.1, 0.5, 1.5, 2.5 g/kg) is studied on morphine withdrawal syndrome in mice. Dependence was induced using subcutaneous injections of morphine daily for three days. On the fourth day, morphine was injected two hours prior to the intraperitoneal injection of naloxone. The number of jumps during the 30 minute period after naloxone (5 mg/kg) injection was considered as measure of the withdrawal syndrome. Locomotion activity in open field test and mouse muscle relaxation and balance in Rotarod system were evaluated 30 and 60 minutes after injections. All doses reduced the number of jumping and mice balance. It also induced muscle relaxation. Only a dose of 0.1 g/kg could not affect locomotion activity. In conclusion, *M. vulgare* L. can decrease the withdrawal syndrome symptoms.

Keywords: *Marrubaium vulgar*e L., withdrawal syndrome, morphine dependence, muscle relaxation.

INTRODUCTION

Marrubium (horehound) is a genus of about 40 species of flowering plants in the family Lamiaceae, native to temperate regions of Europe and Asia [1]. Aerial parts and their aqueous and hydroalcoholic extracts of *Marrubium vulgare* L. are widely used in medicine as an antitussive [2], anticonvulsant [3] and analgesic [4]. Earlier phytochemical investigation of *M. vulgar* led to the characterization of several flavonoids including luteolin, apigenin and their 7-glycosides and 7-lactates, vitexin [5], labdane diterpenoids [6], phenyl propanoid esters as (E)-caffeoyl-L-malic acid and four glycoside derivatives as acteoside, forsythoside B, arenavioside and ballotetioside [7], genistein [8], caffeolcinic acid [9] and quercetin [10]. Marrubiin [11], a furan labdane diterpene, is the main component of this plant and exhibits potent antinociceptive properties and vasorelaxant activity [7, 12].

The potential to reduce morphine withdrawal signs in animals has been reported for some Lamiaceae plants such as *Rosmarinus officinalis* [13], *Nepeta glomerulosa* [14] and *Salvia leriifolia* [15]. Therefore, this study was initiated to investigate the effects of aqueous and ethanolic extracts of *Marrubium vulgare* as a Lamiaceae plant on morphine withdrawal syndrome in mice.

MATERIAL AND METHODS

Animals. Male albino mice (25-30 g) were housed at 22 ± 2°C under a 12 h light /12h dark cycle and with access to food and water ad libitum. The experiments were performed during the light phase of the cycle. The animal groups were acclimated to the laboratory for at least 2h before testing

and were used once throughout the experiments, each group consisted of 7 mice. All animal experiments were carried out in accordance with Mashhad University of Medical Sciences, Ethical Committee Acts.

Plant material. The plant material (Flowered aerial part of *Marrubium vulgare* L.) was collected in Ashkhaneh to Maraveh Tapeh road, in Eslam Ghaleh village, 920 m height, Iran, A voucher specimen was deposited at the Dr. Zargari herbarium, Department of Pharmacognosy, School of Pharmacy, Mashhad (153-1329-3).

Preparation of plant extract. The powder of flowered aerial parts was extracted using maceration with ethanol (70% v/v) or water for 48 h. and then filtered and concentrated under reduced pressure at 40°C. The extracts were dissolved in normal saline.

Hot plate test. The hot plate test was used to measure response latencies. The mice were treated with saline solution, naloxone (2 mg/kg), morphine (10 mg/kg, S.C.), *Marrubium vulgare* L. ethanolic and aqueous extracts (0.1, 0.5, 1.5 and 2.5 g/kg, i.p.), morphine plus naloxone or extracts plus naloxone placed individually on a hot plate maintained at 55 ± 0.2 °C and the time between placing the animal on the hot plate and the occurrence of either the licking of the hind paws, shaking the paw or jumping off the surface was recorded as response latency. The cut off time for the hotplate latencies was set at 40s. The latency was recorded before and 30, 60, 90 and 120 min following administration of the agents. Naloxone was injected every 45 min. [16-17].

Morphine dependence. Dependence was induced by subcutaneously injection of morphine (50, 50 and 75 mg/kg) three times a day (08.00, 11.00 and 14 h.) for three days.The higher dose at the daily 14 P.M. injection was aimed at minimizing any overnight withdrawal on day 4 they received a last dose of morphine (50 mg/kg) [18].

Morphine withdrawal syndrome. Opioid receptor antagonist, naloxone (5 mg/kg), was used inducing withdrawal syndrome after morphine physical dependence. Mice were placed individually in glass cube boxes (28 cm diameter, 50 cm height). The number of jumps (as a main sign of withdrawal syndrome in mice) was recorded over a 20 min., beginning immediately after the injection of naloxone, 2 h after the final administration of morphine [15, 19].

Drug and extracts treatment. The following substances were used: *M. vulgare* L. ethanolic (70% v/v) and aqueous extracts, in doses of 0.1, 05, 1.5, 2.5 g/kg, diazepame 5 mg/kg, intraperitoneally injection 1.5 h before naloxone injection in each group. Control group was received normal saline (10 mL/kg) containing tween 80 (1% v/v).

Rotarod test. Balance, muscle relaxation and motor coordination were checked using accelerating Rotarod (TES Rotarod system). Mice were placed on a horizontal metal coated rod with rubber (3 cm diameter) rotating at an initial speed of 10 rpm/min.

Terminal speed of the rod was 20 rpm in accelerated studies and rotational velocity of the rod was linearly increased from 10 to 20 rpm within 20s. The time each animal was able to maintain its balance walking on top of the rod was measured. Mice were given two trails with a maximum time of 300s and a 30 to 60 min. intertrial rest interval. Before the beginning of all experiments, the riding ability of the animals in the Rotarod was checked. Thus, the mice were initially put on a rotating rod, and mice that immediately dropped off (within 30s) were removed from the experience [20].

Open field test. The test room was illuminated at the same intensity at the colony room. Each mouse was placed in the

center of the open field, made of white wood had a floor of 100 × 100 cm divided by red lines into 25 squares of 20 × 20 cm and walls (50 cm high) were also painted in white, and mice behaviors were observed for 5 min. The parameters evaluated were the total number of squares crossed, the number of outer squares (those adjacent to the walls) crossed, and the number of inner squares crossed; the three measures were referred to as total, peripheral, and central locomotion, respectively [21].

Statistical analysis. The results are presented as mean ± S.E.M. The statistical significance of the differences between the groups was detected by ANOVA, followed by Tukey-Kramer comparison test. P-values of less than 0.05 were considered as indicative of significance.

RESULTS

Hot plate test. Naloxone (2 mg/kg) diminished antinociceptive effect of morphine 10 mg/kg, at 30, 60 and 90 min. after injection (Figure 1). Naloxone decreased only the antinociceptive effect of extracts at dose of 1.5 g/kg, in 60 and 90 min after injection (Figures 2 and 4). Naloxone reduced the analgesic effects of extracts at doses of 1.5 and 2.5 g/kg at 30, 60 and 90 min after injection (Figures 3 and 5).

Withdrawal syndrome. All of doses, the ethanolic and aqueous extracts, decreased jumping factors , dose dependently (Figures 6 and 7).

Rotarod test. In accelerod performances, the aqueous and alcoholic extracts (except dose of 0.1 g/kg) showed a decline in motor function relative to control at 30 and 60 min after injection, dose dependently (Figures 8 and 9).

Open field test. Except dose of 0.1 g/kg, the ethanolic and aqueous extracts, decreased open field test factors, central, peripheral and total locomotion activities (Figures 10 and 11).

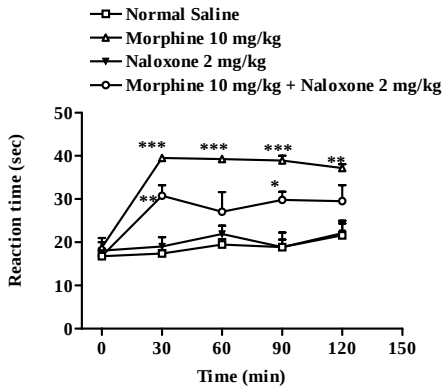


Figure 1. Effect of naloxone (2 mg/kg) on antinociceptive effect of morphine (10 mg/kg) in hot plate test, 30, 60, 90 and 120 min. after injections in 7 male mice. Data was reported as mean + SEM, *P<0.05, **P<0.01, ***P<0.001 *vs* saline, Tukey-Kramer test.

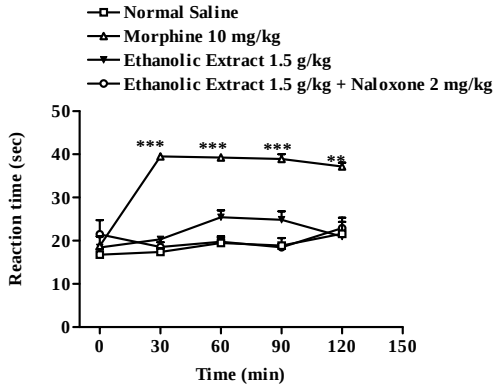


Figure 2. Effect of naloxone (2 mg/kg) on antinociceptive effect of ethanolic extract of *Marrubium vulgare* L. (1.5 g/kg), in hot plate test, 30, 60, 90 and 120 min. after injections in 7 male mice. Data was reported as mean + SEM, **P<0.01, ***P<0.001 *vs* saline, Tukey-Kramer test.

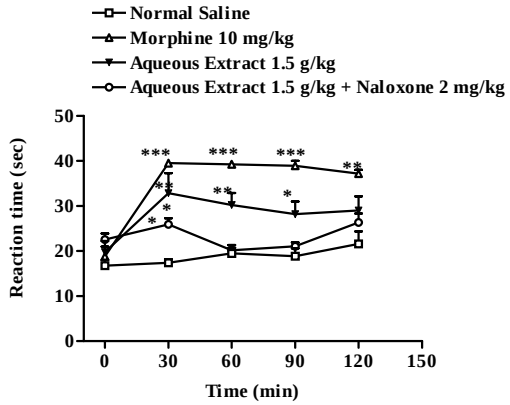


Figure 3. Effect of naloxone (2 mg/kg) on antinociceptive effect of aqueous extract of *Marrubium vulgare* L. (1.5 g/kg), in hot plate test, 30, 60, 90 and 120 min. after injections in 7 male mice. Data was reported as mean + SEM, *P<0.05, **P<0.01, ***P<0.001 *vs* saline, Tukey-Kramer test.

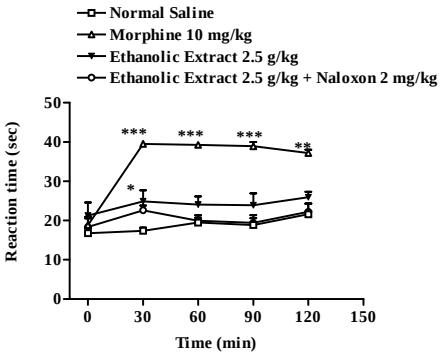


Figure 4. Effect of naloxone (2 mg/kg) on antinociceptive effect of ethanolic extract of *Marrubium vulgare* L. (2.5 g/kg), in hot plate test, 30, 60, 90 and 120 min. after injections in 7 male mice. Data was reported as mean + SEM, *P<0.05, **P<0.01, ***P<0.001 *vs* saline, Tukey-Kramer test.

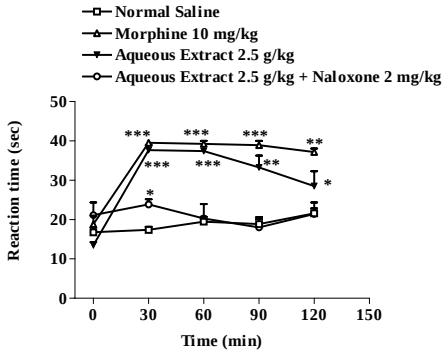


Figure 5. Effect of naloxone (2 mg/kg) on antinociceptive effect of aqueous extract of *Marrubium vulgare* L. (2.5 g/kg), in hot plate test, 30, 60, 90 and 120 min. after injections in 7 male mice. Data was reported as mean + SEM, *P<0.05, **P<0.01, ***P<0.001 *vs* saline, Tukey-Kramer test.

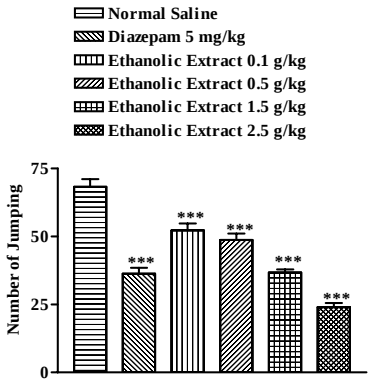


Figure 6. Effect of ethanolic extract of *Marrubium vulgare* L. on withdrawal syndrome in 7 male mice. Morphine (10 mg/kg, S.C.) was injected, 3 times a day, during 3 days and one dose injected on day 4. Withdrawal syndrome was induced by the i.p. injection of naloxone (5 mg/kg) 90 min fter the i.p. treatments of extracts, diazepam or saline. Data was reported as mean + SEM. ***P<0.001 *vs* saline, Tukey-Kramer test.

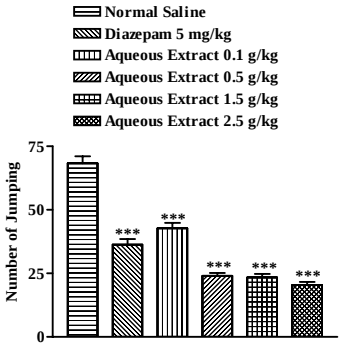


Figure 7. Effect of aqueous extract of *Marrubium vulgare* L. on withdrawal syndrome in 7 male mice. Morphine (10 mg/kg, S.C.) was injected, 3 times a day, during 3 days and one dose injected on day 4. Withdrawal syndrome was induced by the i.p. injection of naloxone (5 mg/kg) 90 min after the i.p. treatments of extracts, diazepam or saline. Data was reported as mean + SEM. ***P<0.001 *vs* saline, Tukey-Kramer test.

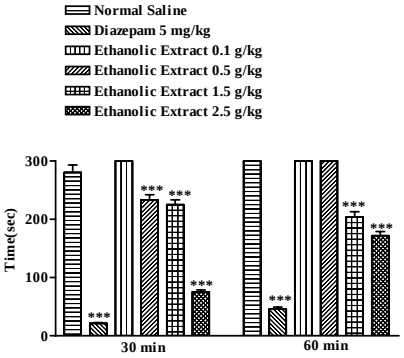


Figure 8. Effect of ethanolic extract of *Marrubium vulgare* L. on motor function in Rotarod system in 7 male mice, 30 and 60 min after injection of extract, diazepam or saline. Data was reported as mean ± SEM. ***P<0.001 *vs* saline, Tukey-Kramer test.

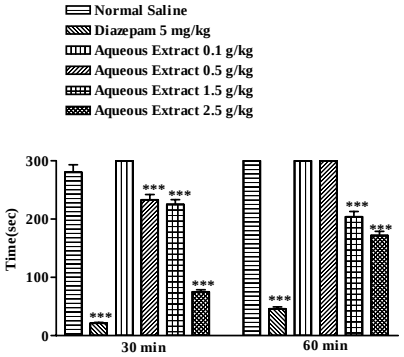
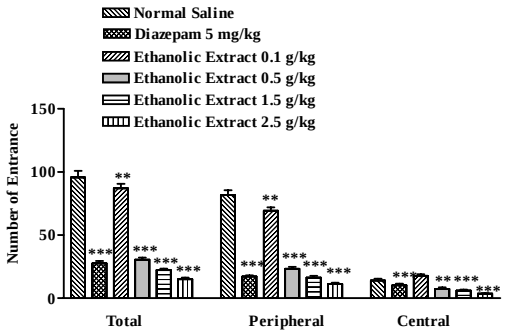
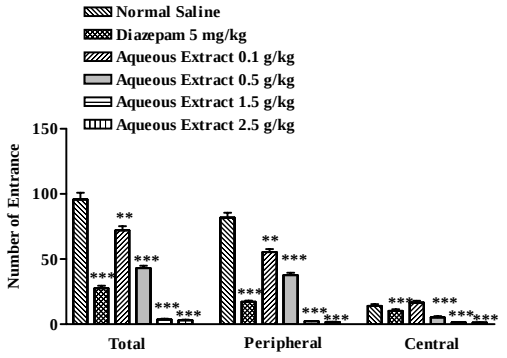


Figure 9. Effect of aqueous extract of *Marrubium vulgare* L. on motor function in Rotarod system in 7 male mice, 30 and 60 min after injection of extract, diazepam or saline. Data was reported as mean ± SEM. ***P<0.001 *vs* saline, Tukey-Kramer test.



Figures 10. Effect of *Marrubium vulgare* L. ethanolic extract on open field test factors in 7 male mice, 60 min after injection of extracts, diazepam or saline. Data was reported as mean + SEM, **P<0.01, ***P<0.001 *vs* saline, Tukey-Kramer test.



Figures 11. Effect of *Marrubium vulgare* L. aqueous extract on open field test factors in 7 male mice, 60 min after injection of extracts, diazepam or saline. Data was reported as mean + SEM, **P<0.01, ***P<0.001 *vs* saline, Tukey-Kramer test.

DISCUSSION

The results of this study showed that the ethanolic and aqueous extracts of *M. vulgare* L. aerial parts decreased the morphine withdrawal syndrome dose-dependently.

The mechanism of action of this effect is not clear. *M. vulgare* L. has been used as an antinociceptive, anti-inflammatory [7, 12], muscle relaxant and antitussive [11] in folk medicine. *M. vulgare* L. constituents as marrubiin showed antinociceptive effect via opioid receptors. Marrubiinic acid and luteolin were indicated to be as a muscle relaxant component [12, 17]. The antitussive effect of *M. vulgare* L. may be mediated via opioid receptors [22]. In this study, naloxone inhibited the antinociceptive activity of the ectracts in hotplate test. Thus, there is an interaction between the extract and opioid receptors. This may help that the extracts reduce withdrawal syndrome. However, our results showed that the extracts decreased locomotion activity and motor coordination as well. These activities may also involve in the reduction of withdrawal syndrome

It is concluded that *M. vulgare* L. extracts could decrease morphine withdrawal syndrome symptoms via interaction with opioid receptors and indirectly via reduction motor activity and coordination.

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