Effects of Aqueous and Ethanolic Leaf Extracts of *Mentha Longifolia* on Morphine Dependence in Mice

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ABSTRACT

The effects of aqueous and ethanolic leaf extracts of *Mentha Longifolia* on morphine dependence were examined in mice. Dependence was induced using subcutaneous injections of morphine daily for 3 days. On day 4, the last dose of morphine was injected 2 h perior the intraperitoneal injection of naloxone. The number of episods of jumping during 30 minutes after injection of naloxane was considered as the intensity of the withdrawal syndrome. Phytochemical study indicated that both the aqueous and ethanolic extracts have alkaloid and flavonoid. The extracts were injected at different doses 0.5 and 1 hour before and 0.5 hour after the last injection of morphine. The extracts reduced the number of jumping episods. In open field test, the ethanolic (lower doses) and aqueous extracts decreased locomotion activity. It is concluded that the aqueous and ethanolic leaf extracts of *M. longifolia* could diminish morphine withdrawal syndrome.

Key words: *Mentha longifolia*; morphine dependence; withdrawal syndrome; naloxone; opioid system; medicinal plants

INTRODUCTION

According to previous pharmacological studies, involvement of multiple systems and agents in opioid-induced dependence such as decreasing of plasma β-endorphin and cortisol level (1), raise of intracellular sodium (2), release of substance P(3), increasing of protein kinases (4), changes in galanin neuropeptid (5), involvement of K and δ receptors (6), NMDA receptors (7,8), α adrenergic receptors(8) CCK receptors (9) L-arginine/nitric oxide pathway(10,11) have been recognized.

Use of herbal drugs because of their effects on lessening signs of withdrawal syndrome has been evaluated in many studies such as *Salvia leriifolia*(12), *Rosmarinus officinalis* (13), *Crocus sativus* (14), *Berberis vulgaris*(15), *Nepeta glomerulosa*(16), *Marrubium vulgare* (17), *Zhumeria majdae* (18), *Stachys byzantine* (19), *Ferula gummosa*(20) and *Valeriana officinalis* (21).

Mentha Longifolia is a herbaceous plant, a species of genus *Mentha*. Some species of *Mentha* family are effective on the CNS. The leaf extracts of *S. lerrifolia* and rosemary can diminish withdrawal syndrome (12, 13).

The genus of *Salvia* may act on some benzodiazepine (BDZ) sites of GABA (22, 23, 24). Carvene, a substance in *Mentha* genus has sedative and CNS-depressant effects (25, 26). Anticonvulsant effects of some species of *Mentha* family have been shown (27).

MATERIALS AND METHODS

Bases on pharmacological activity of genus *Mentha* on CNS and withdrawal syndrome, in this study, the effects of *M. longifolia* on morphine withdrawal was investigated in mice.

Animals. Albino mice 25-30 g were obtained from a random bred colony (Animal house of Razi Vaccine and Serum Research Institute).

Plant material. The *M. longifolia* leaves were collected from Torbat Heydarieh a town in Razavi Khorasan province of Iran and dried in shade for 14 days and followed by griding. The *M. longifolia* was identified by Mr Joharchi, herbarium of Ferdowsi University.

Materials. The following reagents were used: Morphine sulphate (Daru Pakhsh, I.R. Iran), diazepam and naloxone hydrochloride (Tolid daru, I.R. Iran)

Preparation of plant extracts.

1. ***Preparation of aqueous extract.*** The leaves powder (100 g) were added to 100 ml boiling water and shaked for 15 minutes. The solution was filtered through filter paper. This process was repeated 4 times for getting clear solution. The extracts put into a water bath (40 °C) until the solvent was evaporated. The dried extract was removed and solved in normal saline.
2. ***Preparation of ethanolic extract.*** The dried leaves were extracted using a maceration method. The powdered leaves (100 g) were macerated in 500 ml ethanol (80 v/v) for 2 days. Then, the upper part was separated and added 500 ml ethanol. After 1 day, the extract was separated from wasting and this process repeated. All of the solutions concentrated in a rotary evaporator at 40 °C. The 80 percent of solvent was evaporated. The concentrated extract put into a water bath until was dried. The dried extract was diluted by tween-80 in saline.

Morphine dependence

Morphine was injected subcutanously (S.C) 3 times a day for 3 days at doses 50 mg/kg (11:00 a.m), 50 mg/kg (14:00 p.m) and 75mg/kg (17:00 p.m). On day 4, the last injection of morphine was injected (50mg/kg) 2 h before naloxone treatment (12).

Morphine withdrawal

Withdrawal syndrome was induced by injection of naloxone (5 mg/kg, SC) 2 h after the last injection of morphine. Then immediately mice were placed in a glass cylinder (30 cm high, 20 cm in diameter). The number of jumping episodes during 30 minutes was counted (12).

***Determination of the maximum tolerated dose:* (MTD)** (28)

The extract treatment

The extracts were injected at different doses and times. The most effective dose of aqueous extract (0.194 g/kg) and ethanolic extract (0.2 g/kg) were injected 0.5 and 1 hour before and 0.5 after the last injection of morphine. After 2 h naloxone (5 mg/kg) was injected and counted the number of episodes of jumping. The most effective time was considered for the next experiments. Then, 1 hour before the last injection of morphine, the aqueous extracts were injected at doses (0.194, 0.776, 1.358 g/kg) and the ethanolic extracts (0.029, 0.12, 0.2 g/kg) intraperitoneally. After 2 h naloxone was injected and counted the number of episodes of jumping.

Phytochemical research

1. Flavonoid test: Shinoda test for flavonoids: To 2–3 ml of methanolic extract, a piece of

magnesium ribbon and 1 ml of concentrated hydrochloric acid were added. (pink red or red coloration of the solution indicate the presence of flavonoids in the drug) (29).

2. Alkaloid test: Alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (potassiomercuric iodide solution) to give a cream coloured precipitate (30).

Open field activity test. Extracts were injected at different doses (the aqueous extracts at doses 0.194, 0.766 and 1.358 g/kg and the ethanolic extracts at doses 0.029, 0.12, and 0.2 g/kg). 5 mice in each group were considered. The extracts were injected 1 hour before the beginning of test. Positive control (diazepam) and negative control (normal saline) were injected. Open field activity box has 25 squares. The mice were placed in the central square. After 1 hour some factors were counted for 10 minutes. TL (total locomotion), CL (Central locomotion), PL (peripheral locomotion) (16).

Statistical analysis. The data were presented as mean value ± SEM. Analysis of variance followed by the multiple comparison test of Tukey-Kramer were used for comparison of data. Differences with a $p < 0.05$ were considered significant.

RESULTS

Phytochemical study indicated both aqueous and ethanolic extracts have alkaloid and flavonoid.

The injection of aqueous extracts 0.5 and 1 hour before and 0.5 hour after the last injection dose of morphine reduced the number of episodes of jumping and the ethanolic extracts reduced the number of episodes of jumping 0.5 and 1 hour before the last injection dose of morphine (Figures 1 and 2). The aqueous and ethanolic extracts (except one dose) reduced the number of episodes of jumping (Figures 3 and 4). In open field test, the aqueous and ethanolic extracts (except the high dose) reduced locomotion in mice (Figures 5 and 6).

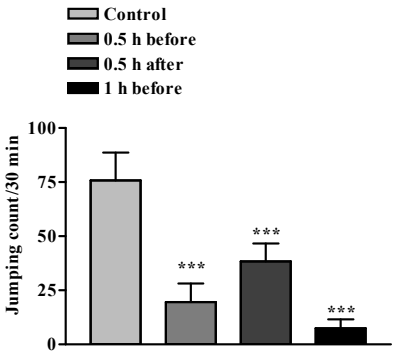

Figure 1. Effects of different treatment schedules of the intraperitoneal dose of *M. longifolia* aqueous leaf extract on naloxone-induced jumping in morphin-dependent mice. Each point represents the mean ± SEM, for 8 mice.***p<0.001 compared with saline,Tukey-Kramer test.

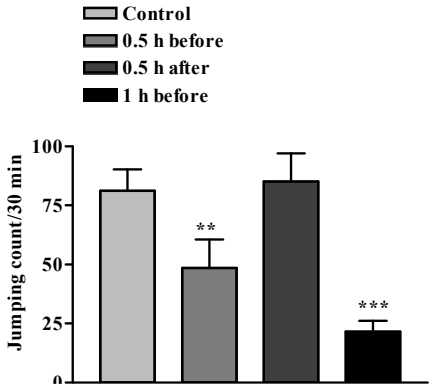

Figure 2. Effects of different treatment schedules of the intraperitoneal dose of *M. longifolia* ethanol leaf extract on naloxone-induced jumping in morphin- dependent mice. Each point represents the mean ± SEM, for 8 mice.**p<0.01,***p<0.001 compared with saline,Tukey-Kramer test.

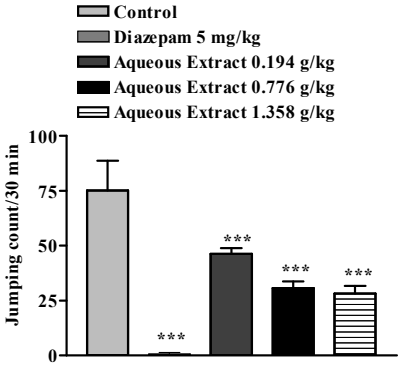

Figure 3.Effect of different intraperitoneal doses of *M. Longifolia* aqueous leaf extract on naloxone-induced jumping in morphine- dependent mice. point represents the mean ± SEM for 8 mice. ***p<0.001, compared saline. Tukey-Kramer test.

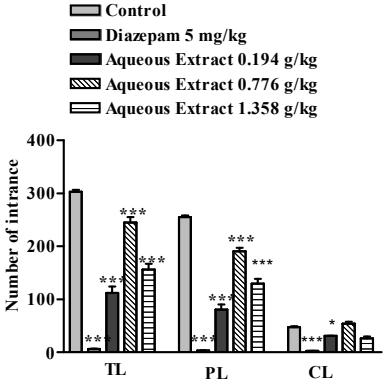

Figure 6. Effects of *M. longifolia* aqueous leaf extract on open field test factors in 8 male mice, 60 min. after injection of extracts, diazepam or normal saline. Data were reported as Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001, compared with saline.Tukey-Kramer test

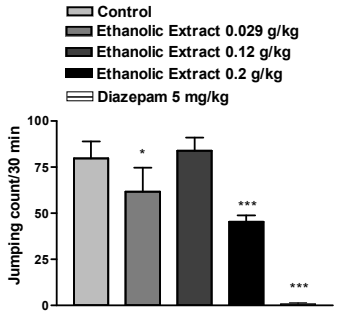

Figure 4. Effect of different intraperitoneal doses of *M. longifolia* ethanol leaf extract on naloxone-precipitated jumping in morphine- dependent mice. Each point represents the mean ± SEM for 8 mice. .*p<0.05, ***p<0.001, compared with saline.Tukey-Kramer test.

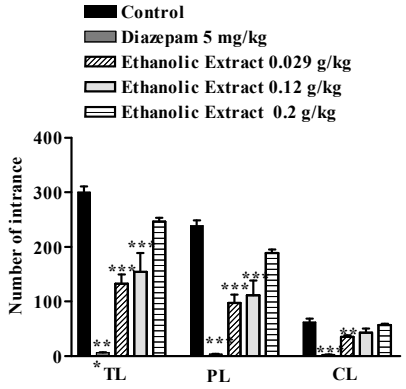

Figure 5.Effects of *M. longifolia* ethanol leaf extract on open field test factors in 8 male mice, 60 min after injection of extracts, diazepam or normal saline. Data were reported as Mean ± SEM, **p<0.01, ***p<0.001, compared with saline.Tukey-Kramer test

DISCUSSION

The results of this study indicated that ethanolic and aqueous leaves extracts can reduce the number of jumping episods. The ethanolic leaf extract of *M. longifolia* was more effective than aqueous extract in reduction of withdrawal signs.

Phytochemical research confirmed presence of alkaloid and flavonoid in ethanolic and aqueous extracts of *M. Longifolia*. Previous studies have been proved that flavon and 10 hydroxy flavon have antinociceptive activity and reducecd acetic acid-induced pain (31). Flavon and flavon derivatives are effective on opioid systems and their effetcs antagonized by naloxone. Equamin and dihydroequamin (monoterpen alkaloids) can be bind to Κ and μ receptors (32). Isoquinoline alkaloids can reduce morphine withdrawal in guinea ileum (33). The aqueous extract of *Nepeta glomerulosa* Boiss. aerial prts containing alkaloid, saponin and oils inhibited morphine withdrawal syndrome in mice (16). Due to the presence of alkaloids and flavonoids in aqueous and ethanolic of *M.longifolia*,it is suggested that these extracts may be effective on opioid systems.

In open field test, the aqueous and ethanolic extracts (except a dose of 0.2 g/kg) reduced the locomotor activity. It is possible this activity is involved in the reduction of withdrawal syndrome.

In conclusion, the aqueous and ethanolic leaf extracts of *M. longifolia* could reduce morphine withdrawal syndrome partially through the reduction of locomotor activity.

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