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Original Article

Effect of *Nepeta glomerulosa* Boiss. Aerial Parts Aqueous Extract on Morphine Withdrawal Syndrome in Mice

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Abstract

The effect of aqueous extract of *Nepata glomerulosa* Boiss. aerial parts on morphine withdrawal syndrome was investigated in mice. Dependence was induced by subcutaneous injections of morphine for 3 consecutive days. On day 4, morphine was injected 2 h prior to intraperitoneal injection of naloxone. The number of jumps during a 20 min. period after naloxone injection was considered as a measure of the withdrawal syndrome. Open field and rotarod tests were also performed. The results indicated that the aqueous extract (0.1 to 2.8 g/kg, s.c.) and diazepam (5 mg/kg) reduced the number of jumps (58±2.3 to 10±0.82, $p<0.001$ *vs* normal saline, 87±3.4). The extracts also reduced motion balance and locomotion activity. It is concluded that the aqueous extract of *N. glomerulosa* aerial parts could diminish morphine withdrawal syndrome.

Keywords: Diazepam; Morphine dependence; *Nepeta glomerulosa*; Withdrawal syndrome.
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1. Introduction

The withdrawal syndrome is typically observed following abrupt termination of morphine intake or by the administration of a narcotic antagonist such as naloxone that has been shown to cause a specific set of behavioral effects in morphine dependent rodents. Stereotyped jumping behavior is the most dominant syndrome among them [1] and is widely considered the most sensitive and reliable index of withdrawal intensity [2-4]. It is suggested that jumping is the most suitable sign of measuring abstinence quantitatively because jumps are easily counted [5]. This behavioral phenomenon has a central origin and a direct relation to the intensity of the abstinence syndrome due to morphine withdrawal [6].

Nepeta glomerulosa (Lamiaceae) is a medicinal plant found in Asia (widely distributed in Iran, especially areas of Khorasan and Isfahan provinces), America and Europe. The aerial parts of *N. glomerulosa* are empirically used in folk medicine as antitussive. Some *Nepeta* species have been reported to have antinociceptive (*N. italica; N. cataria*), antimicrobial (*N. crispa*.) and antiviral (*N. cataria*) effects [7-10]. The

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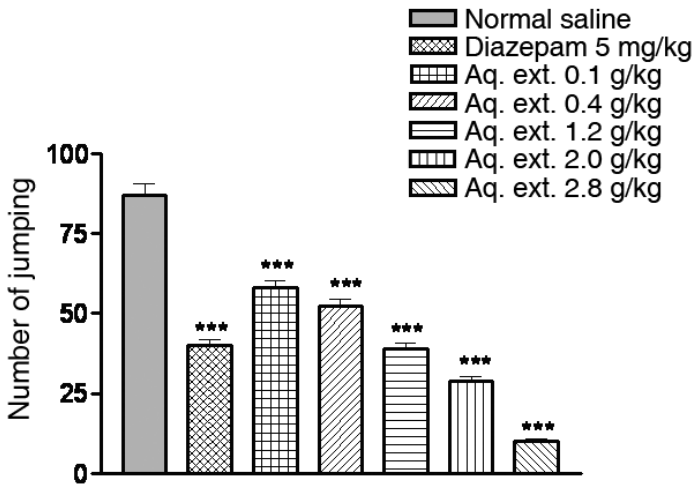

Figure 1. Effects of aqueous extracts of *Nepeta glomerulosa* Boiss. on withdrawal syndrome in male mice (10 mg/kg morphine s.c. injected, 3 times a day, during 3 days and one dose injected on day 4). Withdrawal syndrome was induced by the i.p. injection of naloxone (5 mg/kg) 90 min. after the i.p. treatments of extracts, diazepam or normal saline. Data are reported as Mean±SEM. ***p<0.001 *vs* NS, Tukey-Kramer test.

ingredients of *N. glomerulosa* extract are alkaloid, saponin and oils [11]. It's oil contains over 35 components. The major components were α-pinene (18.3%), 1,8-cineole (13.9%), limonene (9.7%), gernyl acetate (9.3%), caryophyllene oxide (8.0%), linalool (4.8%), trans-β-ocimene (4.7%), humulene epoxide (4.2%), trans-α-bergamotene (3.5%), α-humulene (3.2%) and camphene (3.1%) [12, 13].

Because of some the Lamiaceae plants such as *Rosmarinus officinalis* [14] and *Salvia leriifolia* [15] have been reported to decrease morphine withdrawal syndrome symptoms, therefore, this study was conducted to investigate the effect of *N. glomerulosa* on morphine dependence.

2. Materials and methods

2.1. Plant material

The aerial parts of *N. glomerulosa* Boiss. were collected from Torbat Jam Bazd mountains (26.5.2005, at an altitude of 1850 m). The plant was identified by Mr. Ahi, and voucher samples (152-1407-2) are preserved for reference at the herbarium of the department of Pharmacognosy, Faculty of Pharmacy, Mashhad University of Medical Sciences.

2.2. Animals

Adult male albino mice weighing 25-30 g were obtained from the animal house of Mashhad University of Medical Sciences. Animals were housed in plastic cages in an animal colony room 12/12 h light/dark cycle at 21±2 °C with access to food and water *ad libitum*. Groups of animals of 8 mice were chosen randomly for the studies.

2.3. Preparation of extract

The powder of aerial parts was extracted using the aqueous decoction. In the decoction method, 100 g of powder was added to 1 L of boiling water for 15 min. and then filtered through a cloth. The extract was then concentrated under reduced pressure (in a rotary evaporator at 40 °C) to the desired volume. The yield of the extract was 10% (w/w).

2.4. Morphine dependence

The mice were treated with subcutaneous injection of morphine three times a day (08.00, 11.00 and 14.00 h) for three consecutive days. The doses of morphine were 50, 50 and 75 mg/kg, respectively. The higher dose at the daily 14 h injection was aimed at minimizing

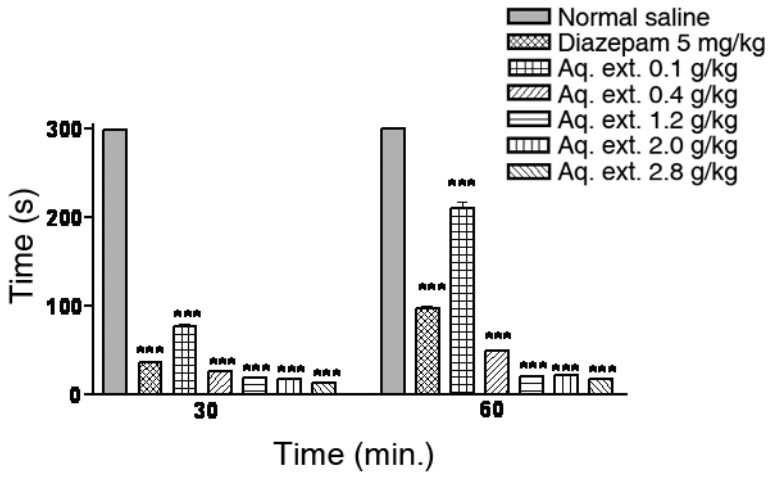

Figure 2. Effects of aqueous decoction extracts of *Nepeta glomerulosa* Boiss. on motor function in Rotarod system in 8 male mice, 30 and 60 min. after injection of extract, diazepam or normal saline, Data was reported as Mean±SEM. ***p<0.001 *vs* NS, Tukey-Kramer test.

any overnight withdrawal. On day 4, they received a last dose of morphine (50 mg/kg) [16].

2.5. Morphine withdrawal

Physical dependence was inferred if a withdrawal syndrome could be precipitated with the opioid receptor antagonist, naloxone. Groups of mice were tested for the occurrence of jumping after 10 injections of morphine and two hours after the last administration of morphine, animals were given naloxone (5 mg/kg) ip. and placed individually in glass cube boxes (28 cm diameter, 50 cm height). The number of jumps was recorded over a 20 min. period beginning 1 min. after the injection of naloxone (2 h after the final administration of morphine) [16].

2.6. Drug and extracts treatment

The extracts (0.3 ml) were injected inter-aperitoneally 0.5 h after the final dose of morphine. Diazepam (5 mg/kg) and normal saline (10 ml/kg) containing Tween 80 (1%, v/v) were injected i.p. as positive and negative controls, respectively.

2.7. Rotarod test

Motor coordination and balance were tested using accelerating rotarod (TSE 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 20 s. The time each animal was able to maintain its balance walking on the top of the rod was recorded. Mice were given two trials with a maximum time of 300 s and a 30 to 60 min. inter-trial rest interval [18]. Before the beginning of all experiments, the riding ability of the animals on the rotarod was checked. Thus, the mice were initially put on a rotating rod, and mice that immediately dropped off (within 30 s) were removed from the experiment [18].

2.8. Open field test

The apparatus, made of white wood, had a floor of 100×100 cm divided by red lines into 25 squares of 20×20 cm. The walls (50 cm high) were also painted in white. The test room was illuminated at the same intensity at the colony room. Each mouse was placed in the center of the open field, and its behavior was 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

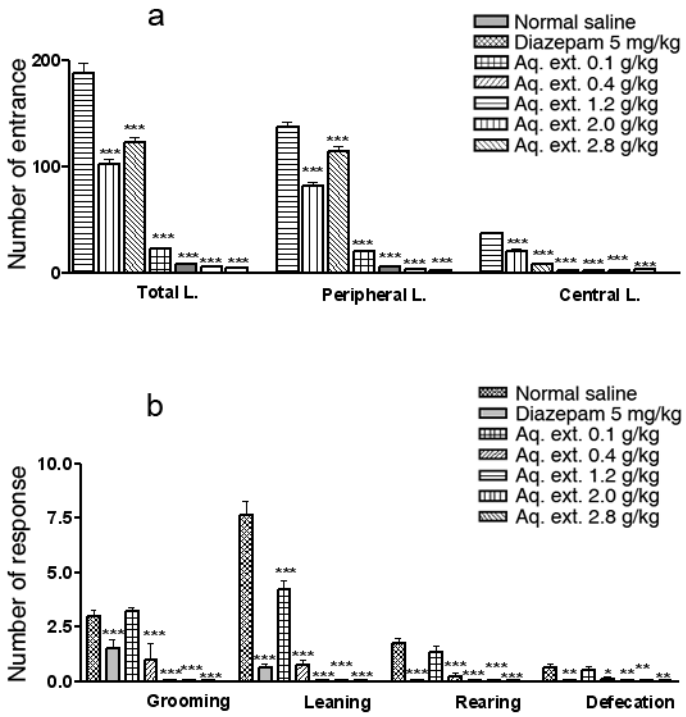

Figure 3 (a and b). Effects of *Nepeta glomerulosa* Boiss. aqueous decoction extracts on open field test factors in 8 male mice, 60 min. after injection of extracts, diazepam or normal saline. Data were reported as Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001 *vs* NS, Tukey-Kramer test.

of inner squares crossed; the three measures were referred to as total, peripheral, and central locomotion, respectively.

The numbers leanings (one or two paws in contact with the wall), rearing, grooming (face cleaning, paw licking, fur liking, head scarping and rubbing), and defecations were also recorded. At the end of each test, the whole area was cleaned with a wet sponge and a dry paper towel [19].

2.9. Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey-Kramer test for multiple comparison. Differences with a p<0.05 were considered significant.

3. Results

The administration of *N. glomerulosa* aqueous extracts (0.1 to 2.8 g/kg) 90 min. after the last dose of morphine on the 4th day, significantly reduced the jumping episodes. The maximum effect was observed at a dose of 2.8 g/kg (Figure 1). Furthermore, in confirmation to withdrawal syndrome declining, extracts (0.1 to 2.8 g/kg) decreased motion balance and function in rotarod system at the 30th min. after injections (Figure 2). The extract also decreased open field test factors (Figure 3a and 3b). Diazepam also decreased withdrawal signs, open field factors and motion balance (Figures 1, 2, 3a and b).

4. Discussion

The present results indicate that the boiling water extract of *N. glomerulosa* aerial parts reduced the withdrawal signs of morphine, dose-dependently. Benzodiazepines have been shown to have an inhibitory effect on the dependence to morphine which has been suggested to be via $GABA_A$ receptors [20, 21]. It has been reported that morphine caused an increase in the whole brain GABA concentration in mice. Increase of GABA in

discrete parts of the thalamus and the spinal cord of the rats have been also reported [22, 23].

N. glomerulosa has some saponins which have depression effects on CNS [11, 24]. There is also a possibility that *N. glomerulosa* acts through complex ways to affect morphine dependence. It is well recognized that open field behavior is under the influence of several processes [19]. Our results showed that the extracts decrease locomotion activity factors like total, peripheral and central entrances with all doses. The extracts reduced the number of leaning, rearing, grooming and defecation by all doses except a dose of 0.1 g/kg. In our study, the reduction in locomotion activity with the extract is similar to diazepam effects. Drugs that depress spinal neurons can cause relaxation and decrease muscle spasmic reflexes and motion balance [25, 26]. Diazepam and possibly the extracts showed this effect via $GABA_A$ receptors in spinal neurons [26, 27].

The results of this study showed that *N. glomerulosa* aqueous extracts decreased the morphine withdrawal syndrome. The mechanism of action of this effect is not clear. Besides the induction of muscle relaxations and decrease of motor functions, other mechanisms are possibly involved in the mechanism of action of the extract in decreasing morphine withdrawal syndromes.

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