Original Article

Evaluation of Antinociceptive Activity of *Berberis Vulgaris* L. Fruit's Hydroethanolic Extract in Male Mice

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Abstract

Herbal medicine has an old history with a broad application all over the world. Many researches have focused on the curative as well as antinociceptive effects of herbal extract. The aim of this study was to investigate the analgesic effects of *Berberis vulgaris* hydroethanolic extract (BVE) in male mice. 30 male mice were divided into 5 groups: control, treated by morphine, treated by BVE (150 & 300 mg/kg) and naloxane plus BVE (300 mg/kg) randomly. To assess the antinociceptive effects of BVE, the animals were examined by employing different pain models such as, tail-flick tests (for acute pain) and acetic acid-induced writhing (for chronic pain) after treatment with morphine, naloxane and BVE. The results indicate that the BVE showed an important antinociceptive effect at doses of 150 and 300 mg/kg ($P<0.01$ and $P<0.001$, respectively), administered intraperitoneally in mice, which significantly inhibited the abdominal constrictions (55.73% of inhibition) induced by acetic acid and increase tail-flick latency when compared to control group in the same dose, although they were less effective than morphine in the same assay. The antinociceptive models employed here reveal a potential analgesic effect of the *Berberis vulgaris* hydroethanolic extract. We suggest that this effect might be caused by anti-inflammatory effect and the stimulation of the opioid receptors.

Keywords: *Berberis vulgaris*, analgesia, tail flick, morphine, naloxone, mice.

1. Introduction

Pain is the most common reason for

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patients to seek advice from health professionals. It is one of the most frequent presenting symptoms of different pathologies and represents important medical and economic costs for the community [1]. Current analgesic therapies, despite their proven efficacy in alleviating symptoms and providing pain relief, all have considerable side effects including gastrointestinal

problems, renal damage, respiratory depression, emesis, tolerance and/or addiction [2]. In addition, many patients are not satisfied with their pain care and this makes the search for new analgesics that can treat pain more effectively, an important challenge to drug research. Medicinal plants are believed to be important sources of new chemical substances with potential therapeutic efficacy. The most important analgesic drugs (e.g., salicylic acid and morphine) were originally derived from plant sources. Although the plant species used as analgesics in traditional medicine were studied by researchers, their studies are of great interest yet, due to their properties and introduction of new analgesics.

Barberry, *Berberis vulgaris* (family Berberidaceae), grows in Asia and Europe. *B. vulgaris* called "*Zereshk*" in Persian is native to south-east of Iran. Different parts of this plant including root, leaf, bark, and fruit have been widely used as folk medicine for treatment and prevention of various diseases including cardiovascular, gastrointestinal, respiratory, skin, renal, and infectious ones [3]. Previous studies have been carried out on chemical composition of *B. vulgaris* and shown that the most important constituents of this plant are isoquinoline alkaloids such as berbamine, palmatine, and particularly berberine [4,5]. There are multiple pharmacological effects of berberine, such as antimicrobial [6,7], anti-tumor [8-10], and anti-inflammatory effects [5,11,12]. It also has effects on the gastro-intestinal [13], cardiovascular [14] and nervous systems [15].

The present study was undertaken to explore possible antinociceptive potential of the ethanolic fruit extract of *Berberis vulgaris* in mice so as to justify the traditional uses of this plant in folklore medicine.

2. Materials and Methods

2.1. Preparation of B. vulgaris extract

Samples of *Berberis vulgaris* L. fruits were collected from the Botanical Garden of Hamedan, Iran, and certified at the Botanical Laboratory, Jahad-e Keshavarzi, Hamedan, Iran. About 1 kg of the air-dried fruit of the herb was ground into fine powder. The powder was extracted twice, on each occasion with 1 L of 80% aqueous ethanol. The ethanol extract was filtered, and was concentrated until dry under reduced pressure in a rotary evaporator. The resulting ethanol extract was freeze-dried. *B. vulgaris* extract (BVE) was dissolved in normal saline to a stock concentration of 50% (w/v) and then stored at 4°C. The dosage calculations were based on body weight of animals.

2.2. Drugs

Drugs used were morphine sulfate and naloxone (Darupakhsh Co., Tehran, Iran). Morphine and naloxone was dissolved in saline. Morphine, was administered intraperitoneally at a dose of 5 mg/kg and naloxone was administered at a dose of 2 mg/kg (i.p.).

2.3. Experimental animals

All experiments were carried out on male Swiss mice, weighing 25-35 g (Pasteur Institute, Tehran, Iran), that were housed four per cage in a temperature-controlled room (22 ± 2°C) on a 12-h light/dark cycle with free access to food and water. Animals were handled daily (between 9:00 and 10:00 a.m.) for 3 days, before the experiment days in order to adapt them to manipulation and minimize nonspecific stress responses. Mice were divided randomly into several experimental groups control, treated by morphine (5 mg/kg), treated by BVE (150 & 300 mg/kg) and naloxone (2 mg/kg) plus BVE (300 mg/kg), each comprising 6 animals. Animals were handled in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health (NIH) publication 86-23; revised 1985). All the protocols were also approved by the institutional ethics committee of Bu-Ali Sina University. Each mouse was used only once.

2.4. Measurement of antinociception responses

2.4.1. Tail-flick test

Antinociception was assessed by tail-flick test [16]. Radiant heat (power intensity = 7) was focused on 4–7 cm from the tail distal end. The tail-flick latency for each animal was determined three times and the mean was designated as baseline latency before drug injection. The intensity of the beam was adjusted to produce mean control reaction time between 2 and 4 seconds. The cut-off time was fixed at 10 seconds in order to avoid any damage to the tail. Tail-flick latencies were measured at 20 min after injection of drugs or vehicles in experimental groups.

2.4.2. Acetic acid-induced writhing

The abdominal constriction was induced in mice by intraperitoneal injection of acetic acid (0.6%), as described by Collier et al. (1968) with minor modifications [17]. Animals were pre-treated intraperitoneally (150 & 300 mg/kg, 30 min before) with the BVE. Control animals received a saline solution. The number of abdominal constrictions (full extension of both hind paws) was cumulatively counted over a period of 20 min. Antinociceptive activity was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with BVE.

2.5. Statistical analysis

Data are expressed as mean ± SEM (n=6). Analysis of data was performed using one-way ANOVA followed by a significant *P*-value, *post hoc* analysis (Tukey test) was performed for multiple comparison in each experiment. P<0.05 was considered statistically significant in all tests. The percent of inhibition by an antinociceptive agent was determined for each experimental
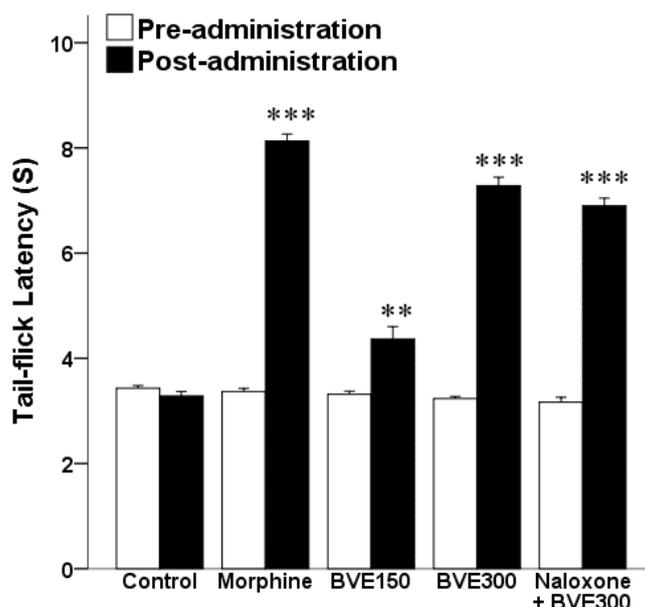
Figure 1. The effect of BVE (*Berberis vulgaris* extract) on pre-administration, and, post-administration tail-flick latency time (s). Values represent mean ± SEM (n=6). **P<0.01, ***P<0.001 significantly different vs. control group.

group using the following formula [18]:

Inhibition%=100[(control experiment)/control]

3. Results and Discussion

3.1. Tail-flick test

As shown in Figure 1, *B. vulgaris* extracts (both 150 & 300 mg/kg) had potent antinociceptive effects on the tail-flick test (P<0.01 and P<0.001, respectively) compared to the control group. In addition, naloxone administration did not reverse the antinociception induced by the *B. vulgaris* extract. The reference drug morphine (5 mg/kg, i.p.) significantly (P<0.001) increased

Table 1. Effect of BVE (*B. vulgaris* extract) on acetic acid-induced writing test.

Treatment	No. of writings	% Inhibition
Control (1 ml/kg)	26.66 ± 0.55	-
Morphine (5 mg/kg)	10.83 ± 0.6[b]	59.37
BVE (150 mg/kg)	23.0 ± 0.57[a]	13.72
BVE (300 mg/kg)	11.8 ± 0.47[b]	55.73
Naloxane plus BVE (300 mg/kg)	28.16 ± 0.61[a]	0

Results are presented as mean ± SEM of 6 mice in each group. One-way ANOVA followed by the Tukey's *post hoc* multiple comparison test was used to analyze the data. All treatment were administered i.p. at times and doses scheduled from the writing test.

[a] *P*<0.01 compared with control (normal saline) group.

[b] *P*<0.001 compared with control (normal saline) group.

the tail flick latency, as compared to the control group.

3.2. Acetic acid-induced writhing

The data on the effect of BVE on the number of writhing movements induced by intraperitoneal injection of acetic acid in mice are presented in Table 1. BVE at doses of 150 and 300 mg/kg, significantly ($P<0.01$ and $P<0.001$, respectively) inhibited the writhing counts. Naloxone administration reversed the antinociception induced by the *B. vulgaris* extract in the writhing movements when compared with control. The reference drug morphine (5 mg/kg, i.p.) also significantly ($P<0.001$) inhibited the writhing counts, compared to the control.

3.3. Possible Mechanisms

The present study was carried out to evaluate the possibility of BVE in alleviating pain. The antinociceptive activity of the BVE was investigated using experimental models that employed chemical- or thermal-induced nociception, which at the same time were used to determine the effectiveness of the extract on inflammatory-mediated nociception (abdominal writhing test), non-inflammatory-mediated nociception (tail-flick test) and provides some evidence on the mechanism implicated in this effect.

Our study demonstrated that *Berberis vulgaris* L. hydroalcoholic extract can exert significant antinociceptive effects. As can be seen, the mean tail-flick latency (TFL) values increased significantly in the extract-receiving groups. The levels of antinociception showed a certain dose

dependence in the tail-flick tests. In group BVE (300 mg/kg), since *B. vulgaris* at the dose of 300 mg/kg showed the best analgesic results, this dose was selected at the first step and naloxone was injected in the second step. According to the results, naloxone had no effect on *B. vulgaris* analgesic activity (Figure 1). As *B. vulgaris* analgesic effects are not via opioid receptors, therefore they are blocked by naloxone.

In the writhing test, clear antinociceptive effects were observed in all extract- and drug injected groups. Thus, the effects of the *B. vulgaris* extract in the case of visceral pain differed noticeably from those in the tail-flick test (thermonociception-induced somatosensory pain). There were significant differences between the naloxone-injected and control group; it seems that, in the case of visceral pain, the *B. vulgaris* extract may affects some different afferent and CNS pathways. Naloxone (an opioid antagonist) suppresses the antinociceptive effects of the extract under these conditions (in contrast to what was observed in the tail-flick test). Interactions between multiple compounds present in the extract and involved in the antinociception effects of the latter and differences between the pathways of pain modulation in the case of somatic and visceral pain may be the reasons for some of the contradictions in our observations.

Many researchers previously reported anti-inflammatory and antinociceptive activities for flavonoids and polyphenolic compounds of other plants [19-22]. It seems that the pharmacological effects observed in

the present study may be partially due to flavonoids and polyphenoilc contents of *B. vulgaris* extract. Chemical analysis of the extract samples by HPLC showed 1.24% and 2.5% Berberine and Berbamin respectively [5]. In this research, the most effective curing of *B. vulgaris* is attributed to Berberine. Several pharmacological effects of berberine, such as antimicrobial [6,7], anti-tumor [8-10], and anti-inflammatory effects, [5,11,12] for Berberine are reported. The antinociceptive and anti-inflammatory effects of flavonoids and polyphenolic compounds may be attributed to their antioxidant activity [23], inhibition of histamine release from mast cells and inhibition of arachidonic acid metabolism [24]. In general, inflammation is a complex process which results from involvement of many mediators and further studies are required to find out the exact mechanism of *B. vulgaris* fruit extract.

4. Conclusion

The present study demonstrated the antinociceptive activity of hydroethanolic extracted of *B. vulgaris*. Further studies are needed in order to know the mechanism behind the observed antinociceptive action. By considering of the need for new, safe and effective therapies, and the adverse effects associated with the drugs currently used, the results showed that *B. vulgaris* can be an important and promising source of herbal medicine for the treatment of pathologies for which no efficacious treatment exists, such as chronic pain. Finally, the antinociceptive action demonstrated in the present study supports, at least in part, the ethno-medical uses of this plant.

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