

EFFECTS OF *BERBERIS VULGARIS* FRUIT EXTRACTS AND ITS ACTIVE COMPONENT, BERBERINE, ON MORPHINE DEPENDENCE, HYPNOSIS AND LOCOMOTOR ACTIVITY IN MICE

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Summary

The effects of aqueous and ethanol extracts of *Berberis vulgaris* (barberry) fruit, as well as its active constituent, berberine, were studied on morphine dependence, hypnotic potentiation, muscle relaxant and locomotor activity in mice. Dependence was induced using subcutaneous injections of morphine (50, 50, 75 mg/kg) daily for 3 days. On day 4, morphine was injected 2 h before the intraperitoneal injection of naloxone (5 mg/kg). The number of episodes of jumping during 30 min after the injection of naloxone was considered as the intensity of the withdrawal syndrome. Berberine (10- 20 mg/kg, i.p.) reduced the number of jumping episodes after the dependence significantly. In other hand, the intraperitoneal injection of both extracts of barberry in different doses decreased the number of jumping episodes significantly during the development of dependence and after dependence. The locomotion activity of animals reduced dose dependently. In the potentiation of pentobarbitone sleep test, berberine significantly increased sleeping time and decreased latency at the doses of 5 and 10 mg/kg in mice. Also the aqueous (100 mg/kg) and ethanol extracts (100- 200 mg/kg) of barberry increased sleeping time and decreased latency significantly in mice. In the traction test, berberine and both extracts couldn't show muscle relaxant activity. The present data support the hypothesis that the extracts of barberry and berberine have some potential role in potentiation inhibitory neural pathway and decreasing morphine dependence, locomotor activity and inducing hypnosis.

Key Words: *Berberis vulgaris*; berberine; morphine dependence, sedative-hypnotic, locomotion

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Introduction

Berberis vulgaris L. (Barberry) grows in Asia and Europe. The fruits of the plant have been used as food additive (1). In Iranian traditional medicine, several properties have been reported for all parts of the plant, including tonic, antimicrobial, antiemetic, antipyretic, antipruritic and cholagogue actions and it has been used extensively for treatment cholecystitis, cholelithiasis, jaundice, dysentery, leishmaniasis, malaria and gall stones (1, 2) Also, barberry root bark is used for morphine and opium withdrawal syndrome in the traditional medicine (3).

Berberine, an alkaloid isolated from rhizomes, roots, and stem bulk of the plants such as the Berberidaceae, Ranunculaceae families has long been known for its anti-inflammatory and anti-microbial activity and used to treat inflammation diseases and various infectious disorders like infectious diarrhea and dysentery in traditional Chinese traditional medicine (4- 7). Other bioactivities of berberine include antidiarrheal, antineoplastic, antiarrhythmic (8- 10).

Thus, the aim of this study was to confirm whether this study can support traditional uses of barberry and berberine by assessing their effect on morphine dependence, hypnosis and locomotor activity in mice.

Materials and Methods

Animals

Male Balb/c mice, 25–30 g were obtained from the animal house of Qazvin University of Medical Sciences. The animals were housed in colony rooms with 12/12 h light/dark cycle at $21 \pm 2^\circ\text{C}$ and had free access to food and water. All animal experiments were carried out in accordance with Qazvin University of Medical Sciences, Ethical Committee Acts.

Preparation of extracts

Berberis vulgaris was collected from Ghaen (a town in south Khorassan provinces) and authenticated by Qazvin Agriculture and National Resources Research Center, I.R. Iran (Voucher No: 1355). Fruits were dried in shade and followed by grinding. Then, the powder was extracted using aqueous decoction and maceration with the ethanol. In decoction method, 100 g of the powder of fruits was added to 1 liter of boiling water for 15 min and then filtered through a cloth.

The extract was then concentrated under reduced pressure to the desired volume. In the maceration method, 100 g of the powder was macerated in 1 liter ethanol (70%, v/v) for 3 days and, subsequently, the solution was filtered and concentrated in a rotary evaporator at 50°C. The yield of the both extract was 10% (w/w). Both extracts were diluted by saline.

Chemicals

The following reagents were used: morphine sulphate (Temad, I.R. Iran), naloxone hydrochloride (Tolid Daru, I.R. Iran), diazepam (Chemi Darou, I.R. Iran), berberine (Fluka), pentobarbital (Sigma).

Morphine dependence

Morphine was injected s.c. to mice at doses of 50, 50 and 75 mg/kg three times daily (8:00 a.m., 11:00 and 14:00 p.m., respectively) for 3 days. The highest dose at the third daily injection was aimed to minimize any overnight withdrawal. On day 4, a single dose of morphine (50 mg/kg) was injected 2 h before naloxone treatment (11).

Morphine withdrawal

Withdrawal signs were precipitated by injection of naloxone (5 mg/kg, s.c.) 2 h after their 10th injection of morphine on day 4. After the naloxone challenge, mice were immediately placed in a glass cylinder (30 cm high, 20 cm in diameter). The number of jumping episodes was counted for 30 min after naloxone injection (11).

Drug and extracts treatment

The aqueous and ethanol extracts of barberry, berberine and saline were injected 1h after the final dose of morphine. Diazepam was also injected 0.5 h after the final dose of morphine. In other study, the animals received the aqueous and ethanol extracts of barberry, berberine and saline during dependence 1 h before the daily dose of morphine (50, 50, 75 mg/kg).

Open field test

Locomotion activity was measured in the apparatus (100 × 100× 50 cm), made of white wood, divided by red lines into 25 squares of 20 × 20 cm. The walls were also painted in white and it was positioned in a quiet room. Each mouse was placed in the center of the open field, and its behavior was observed for 10 min. Total, peripheral, and central locomotion, respectively the total number of squares crossed, the number of outer squares (those adjacent to the walls) crossed and the number of inner squares crossed was measured (12). Experiments were carried out between 8 a.m.- 1p.m. Diazepam 3 mg/kg, normal saline (10 ml/kg) and berberine 2.5-20 mg/kg were

injected interaperitoneally (i.p.) to the 10 animal in each group, 30 min before the starting experiments. Both aqueous (100-300 mg/kg) and ethanol extracts (50-200 mg/kg) were injected (i.p.) 1 h before the experiments. Diazepam and normal saline were injected as positive and negative controls, respectively.

Potentialiation of sodium pentobarbitone sleep

Mice were divided into ten groups of animals. Berberine (1-20 mg/kg), aqueous extract (50-200 mg/kg), ethanol extract (50-200 mg/kg), diazepam (1mg/kg) and normal saline (10 ml/kg) were injected interaperitonally to separate groups. 1 h after receiving extracts and normal saline and 30 min after receiving berberine, diazepam each animal was injected sodium pentobarbitone (30 mg/kg, i.p). The sleeping time was noted by recording the interval between the loss and regaining of righting reflex (13).

Muscle relaxant activity

Traction test

Hind paws of a mouse were placed on a small twisted wire rigidly supported above a bench top. Normal mice grasped the wire with forepaws and when allowed to hang free, placed at least one hind foot on the wire within 5 seconds. Inability to put up at least one hind foot constituted failure to the traction (14). The test was conducted in groups of ten previously screened animals, 1 h after the injection of either saline (10 ml/kg) or aqueous extract (50- 200 mg/kg), ethanol extract, (50-200 mg/kg) and 30 min after berberine (1- 20 mg/kg) and diazepam (1 mg/kg).

Statistical analysis

The data were expressed as mean values \pm SEM. Analysis of variance followed by the multiple comparison test of Tukey- Kramer were used for comparison of data. Fischer's exact test (two sided) was used in traction test. Differences with a $p < 0.05$ were considered significant.

Results

Effect of berberine and extracts on morphine dependence

As illustrated in Fig. 1, administration of berberine (10- 20 mg/kg) 1 h before the naloxone reduced the number of jumping episodes significantly which this effect decreased with increasing the doses. However, administration of berberine (1-10 mg/kg) during development of dependence injection morphine as described in Material and Methods wasn't significant (Figure 2). In other hand, the ethanol extract (100- 200 mg/kg) of barberry, decreased the number of jumping episodes

significantly. But it was effective at the lower doses during development of dependence compared with control (Figure1, 2). Similar results were seen with aqueous extract of barberry. During development of dependence, mean jumping frequencies of aqueous extract (25- 50 mg/kg), were significantly less than administration after dependence (100- 200 mg/kg) (Figure1, 2).

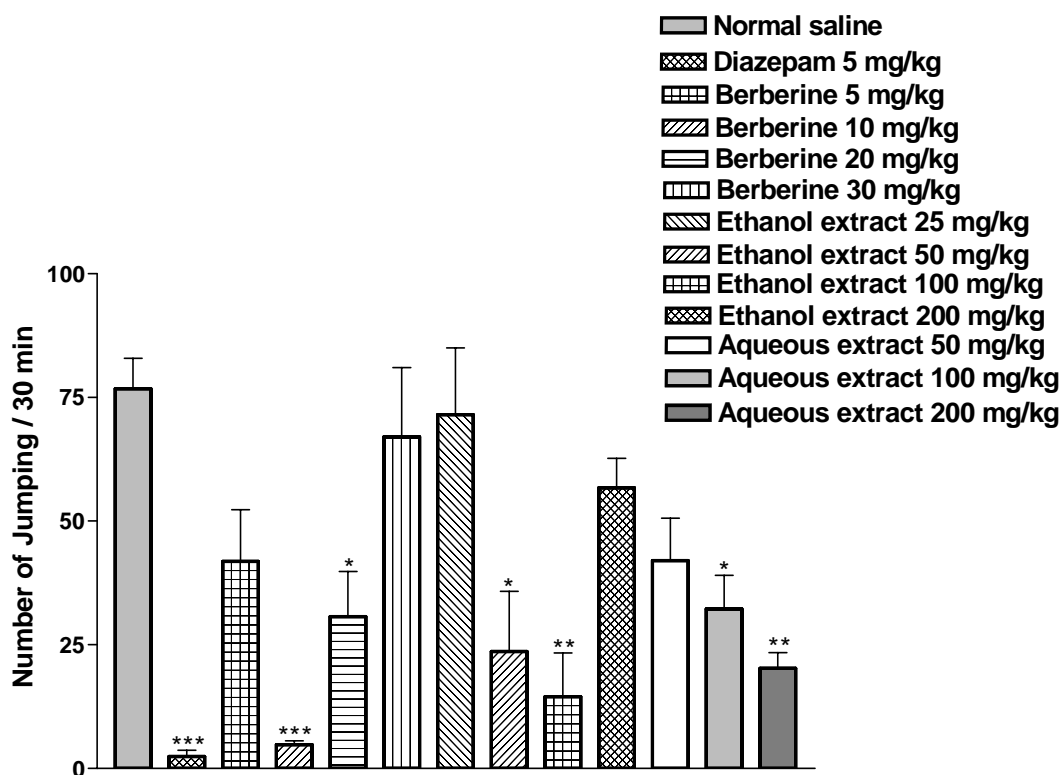


Fig. 1. Effect of different doses of aqueous and ethanol extracts of barberry, berberine and diazepam on naloxone-precipitated jumping in morphine-dependent mice. The animals received (i.p) saline (10 ml/kg), diazepam (5 mg/kg), aqueous extract (50-200 mg/kg), ethanol extract (25-200 mg/kg), berberine (5-30 mg/kg) 1h before naloxone (5 mg/kg, i.p) administration. Each group represents the mean \pm SEM for n = 10 mice. *p < 0.05, **p < 0.01, ***p < 0.001, compared with saline, Tukey- Kramer test.

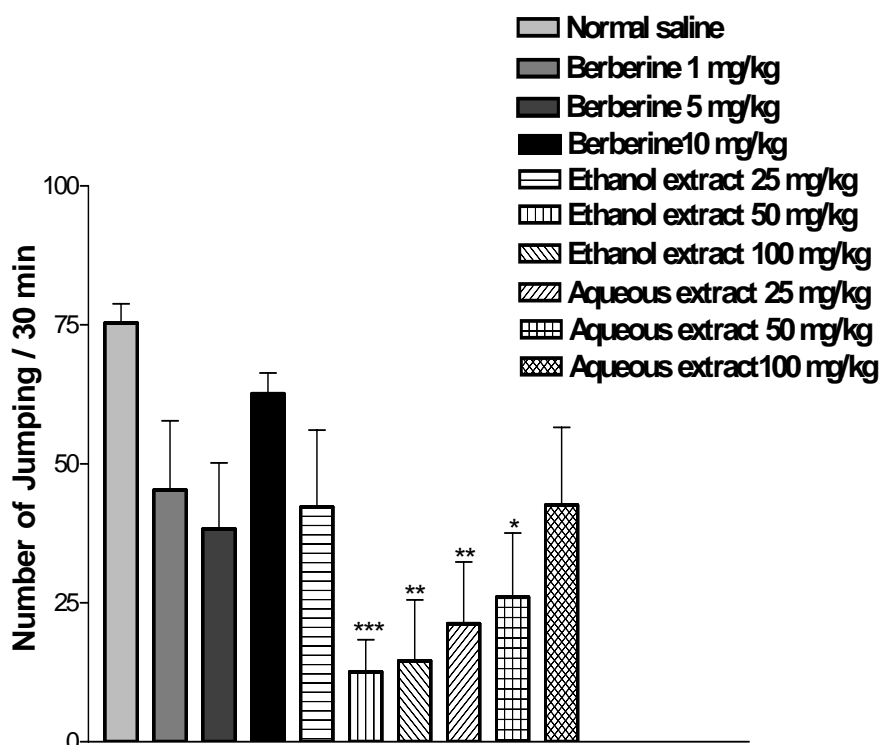


Fig. 2. Effects of aqueous and ethanol extracts of barberry, berberine and diazepam on the development of morphine dependence in mice. The animals received (i.p) saline (10 ml/kg), aqueous extract (25-100 mg/kg), ethanol extract (25-100 mg/kg), berberine (1-10 mg/kg) 1 h before daily dose of morphine. Each group represents the mean \pm SEM for $n = 10$ mice. ** $p < 0.01$, *** $p < 0.001$, compared with saline, Tukey- Kramer test.

Effect of berberine and extracts on locomotion

Berberine reduced total locomotion as well as peripheral locomotion and central locomotion (Fig. 3). Also, both extracts reduced total locomotion as well as peripheral locomotion and central locomotion. Diazepam (3 mg/kg) as the positive control decreased total locomotion, peripheral and central locomotion compared with the normal saline group significantly (Fig. 3).

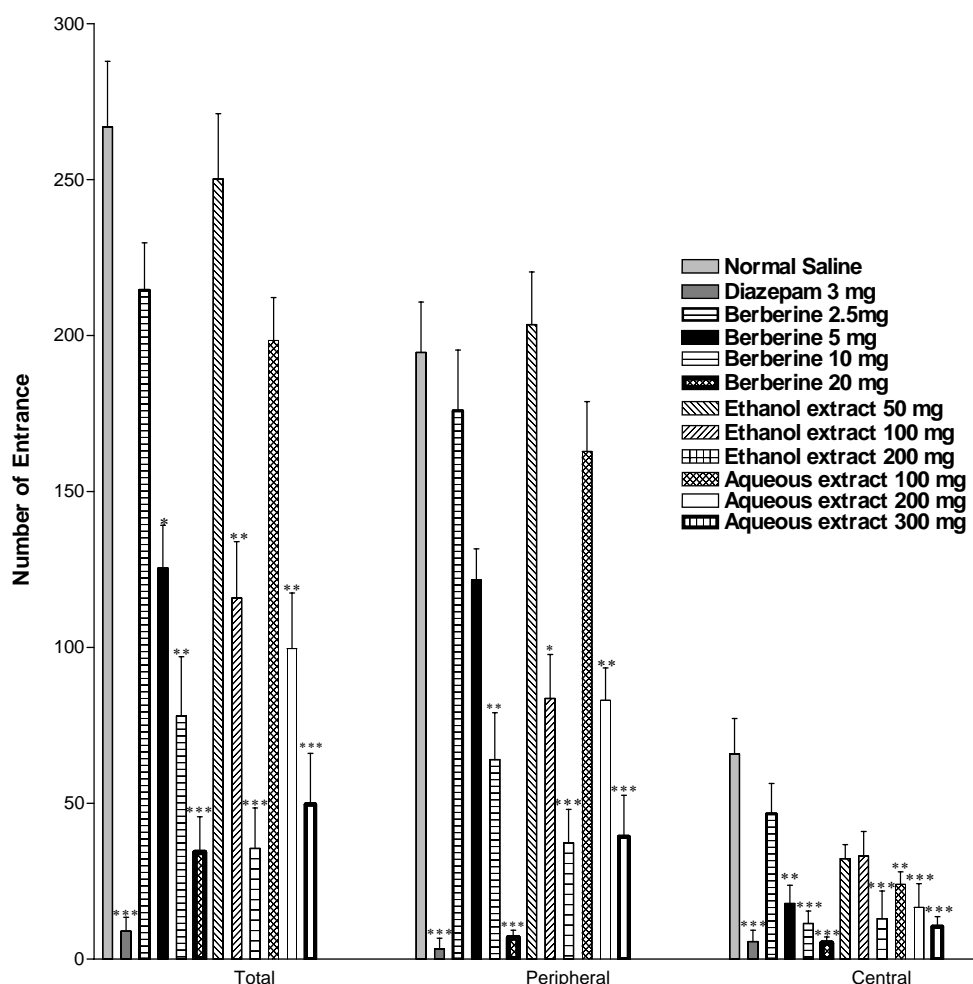


Fig. 3. Effects of aqueous, ethanol extracts of *Berberis vulgaris* and berberine on open field test. Data were reported as mean ± SEM, n= 10, *P<0.05, **P<0.01, ***P<0.001, Tukey-Kramer test.

Effects of berberine and extracts on potentiation of pentobarbitone sleep test

Berberine significantly increased sleeping time and decreased latency at the doses (5- 10 mg/kg) in mice. The ethanol extract at a 100 mg/kg dosage increased sleeping time and in the doses 100- 200 mg/kg decreased latency. Moreover, lengthening of the sodium pentobarbital-induced hypnosis time and latency was observed at a 100 mg/kg dosage with aqueous extract. Diazepam (1 mg/kg) was used as a positive control and significantly increased sleeping time and decreased latency (Table 1).

Table 1: Potentiation of the pentobarbital sleep with aqueous and ethanol extracts of *Berberis vulgaris* as well as berberine in mice. Berberine and diazepam (i.p.) were injected 30 minutes, ethanol and aqueous extracts 1 h, before pentobarbital (30 mg/kg), respectively. Mean latency and duration of sleep in minutes \pm SEM from 10 mice in each group. **P < 0.01, ***P < 0.001, compared to control, Tukey-Kramer test.

Treatment	Dose	Latency (min)	Duration (min)
Normal saline	10 ml/kg	8.27 \pm 1.92	20.32 \pm 1.6
Diazepam	1 mg/kg	2.4 \pm 0.24**	95.71 \pm 6.31**
Berberine	1mg/kg	5.28 \pm 0.22	29.18 \pm 3.80
	2.5 mg/kg	3.34 \pm 0.74	37.53 \pm 4.98
	5 mg/kg	2.06 \pm 0.33*	137.66 \pm 33.28***
	10 mg/kg	2.39 \pm 0.17**	248.26 \pm 49.44***
	20 mg/kg	5.21 \pm 0.55	103.7 \pm 3.70*
Ethanol extract	50 mg/kg	5.37 \pm 0.76	63.57 \pm 3.9
	100 mg/kg	2.86 \pm 0.23*	112.90 \pm 18.10*
	200 mg/kg	2.7 \pm 0.24*	47.09 \pm 7.98
Aqueous extract	50 mg/kg	7 \pm 2.09	34.28 \pm 13.44
	100 mg/kg	2.42 \pm 0.29**	108.12 \pm 16.06**
	200 mg/kg	3.51 \pm 0.45	37.96 \pm 17.34

Effects of berberine and extracts on muscle relaxant activity

The aqueous and ethanol extracts of fruit as well as berberine couldn't induce muscle relaxant effect in a dose-dependent manner compared to the saline group in traction test. Diazepam (1 mg/kg) was used as a positive control and significantly showed muscle relaxant activity (Table 2).

Table 2: Effects of aqueous, ethanol extracts of *Berberis vulgaris* and berberine on traction test performance. Berberine and diazepam (i.p.) were injected 30 minutes; ethanol and aqueous extracts (i.p.) were injected 1h before the test. Values are the mean \pm SEM for 10 mice, ***P < 0.001, Compared to control, Fisher's exact test (two sided).

Treatment	Dose	% Failure
Normal saline	10 ml/kg	0
Diazepam	1 mg/kg	100***
Berberine	1mg/kg	0
	2.5 mg/kg	0
	5 mg/kg	0
	10 mg/kg	0
	20 mg/kg	0
Ethanol extract	50 mg/kg	10
	100 mg/kg	20
	200 mg/kg	30
Aqueous extract	50 mg/kg	10
	100 mg/kg	20
	200 mg/kg	20

Discussion

In the present study jumping frequencies in the two treatment conditions displayed some differences in the responses. As the results, it seems that the effect of berberine during development of dependence is less than after dependence to reduce jumping. But both extracts of fruit were effective in reducing the number of jumping episodes in the two treatment conditions and these effects were greater in lower doses during development of dependence. It seems that berberine and extracts of barberry could diminish the withdrawal syndromes of morphine but it appears that berberine is more effective to reduce withdrawal syndrome after dependence but aqueous and ethanol extracts are useful in both conditions.

Berberine was shown to have anti-inflammatory and antinociceptive. It also has possessed significant inhibitory activity against serotonin-induced hind paw edema both on oral and topical applications (15, 16). Recently, it has shown that isoquinoline alkaloids were able to produce significant influence on the opiate withdrawal *in vitro* via both mu and kappa opioid receptors

(17). Moreover, potential role for treatment of drug abuse has been discussed for them (18, 19). Thus, it is possible that berberine and other isoquinoline alkaloids of barberry interact with opioid system and reduce jumping frequency.

In other hand, the effects of *Berberis vulgaris* extracts and its active component, berberine on the locomotion activities were evaluated by the open field test. This test is used extensively for examining the behavioral effects of drugs and anxiety (20). Locomotion is related to the motor reactivity. As the results, it seems that berberine significantly reduced motor reactivity in the mice, dose dependently. Similar results could be observed with the aqueous and ethanol extracts. In the previous study berberine at the dose of 10 mg/kg decreased catecholamine levels in both plasma and left ventricular tissue of rats (21). Meanwhile we could observe antianxiety effect of berberine on the mice by decreasing their total locomotion which may be related to decrease in the serotonin levels of the brain stem which was explained in previous study (22).

It was shown that phenolic compounds in barberry increased potassium channels activity (23-25). This effect was inhibited by potassium channel blockers (24). In other hand, berberine could modulate potassium channel during brain ischemia and liver damage (26) It also blocked L-type calcium current ($I_{Ca,L}$) in guinea pig ventricular myocytes and hepatocytes(27, 28) So, two possibilities may exit: First, an inhibitory effect on cell excitability by augmentation of potassium currents which caused by the extracts and berberine, may contribute to its hypnotic effects and reducing locomotion and emotional activity. Second, the inhibitory effects of berberine on calcium current could explain the above effects. Because it was suggested that neuroprotective mechanism of berberine was by inhibiting serotonin induced calcium elevation (29). Also, morphine inhibits calcium influx and calcium channel blocker like verapamil decrease tolerance to morphine (30-31).

In conclusion, our data showed that the both extracts of *Berberis vulgaris* and berberine, could induce hypnosis, decrease jumping and locomotion. It is possible that partial mechanism of them related to interaction with opioid system. In addition, inhibitory effects on calcium channels or augmentations of potassium channels activity may be other mechanisms. But further study, is needed to investigate these effects.

Acknowledgment

The authors are thankful to the Vice Chancellor of Research, Qazvin and Mashhad University of Medical Sciences for financial support.

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