

## Effects of *Otostegia persica* (Burm.) Boiss on morphine withdrawal syndrome in mice

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### Abstract

Effect of *Otostegia persica* on naloxone-induced morphine withdrawal syndrome was studied in male mice. Dependence was induced using daily subcutaneous injections of morphine for three days. Morphine was injected to mice at doses of 30 and 45mg/kg on day 1 and 60 and 90mg/kg on day 2 (8:00 am and 6:00 pm). On day 3, morphine (90mg/kg) was injected 1h before oral administration and 1.5h before intraperitoneal (i.p.) injection of hydroalcoholic and hexane extracts of the plant. Naloxone was injected (5mg/kg, i.p.) 2h after the final dose of morphine and the withdrawal signs including jumping, rearing, diarrhoea, piloerection, tremor and ptosis were recorded during a period of 30 minutes. While oral and i.p. administration of hydroalcoholic extract reduced the number of jumping and rearing, the hexane extract could not exert any significant change. Also the hydroalcoholic extract (1500mg/kg) significantly ( $p < 0.05$ ) reduced diarrhoea, piloerection, tremor and ptosis. The hexane extract only significantly ( $p < 0.05$ ) inhibited diarrhoea. Results of this study indicated that the extract of *Otostegia persica* contained component(s) that alleviate morphine withdrawal syndrome and the responsible constituent(s) is(are) found in polar fraction since the hexane extract had only a negligible effect.

**Keywords:** *Otostegia persica*; Morphine dependence; Naloxone withdrawal syndrome

### Introduction

Drug dependence is a serious problem in most regions of the world. Many investigators have worked on systems or drugs which alleviate morphine withdrawal syndrome. These include dopaminergic(1, 2), adrenergic(3), excitatory amino acids(4-6), purinergic(7,8), NMDA (9,10), nitric oxide (10) and serotonergic systems (11). Also some medicinal plants including ginseng (12), passion flower (13), *Salvia leriifolia* (14) and *Ferula gummosa* (15) have been studied in this regard. *Otostegia persica* (Labiatae) grows in different parts of Iran. In Sistan-Baluchestan province the plant is traditionally used to alleviate opium

withdrawal syndrome. This study was aimed to find pharmacological support for this usage.

### Experimental

#### Plant material

Aerial parts of *O. persica* were collected in summer 2003 from a region 25 km north of Zabul in Sistan-Baluchestan province (Iran). Voucher samples were preserved for further reference at the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Isfahan, Iran.

#### Preparation of hydroalcoholic and hexane extracts of *O. persica*

For preparation of hydroalcoholic extract, air-dried and powdered aerial parts of the plant

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**Table 1.** Effects of hydroalcoholic extract of *O. persica* on checked signs of morphine withdrawal syndrome.

Treatment	Dose (mg/kg)	Diarrhoea	Piloerection	Tremor	Ptosis
Vehicle	-	1 (0-1)	3 (3-3)	2 (1-3)	2 (0-3)
HE	500(p.o.)	0 (0-1)	1* (1-2)	2 (1-3)	0.5 (0-2)
	1000(p.o.)	0* (0-0)	2* (1-3)	1 (1-3)	0 (0-3)
	1500(p.o.)	0* (0-0)	0* (0-1)	0* (0-0)	0* (0-0)
Clonidine	0.2(p.o.)	0* (0-0)	0* (0-2)	1* (0-2)	0* (0-2)
Vehicle	-	1 (0-1)	3 (3-3)	2 (1-3)	2 (0-3)
HE	500(i.p.)	0* (0-0)	1.5* (1-3)	1.5 (1-2)	1.5 (1-2)
	1000(i.p.)	0* (0-0)	1.5* (1-3)	1.5 (0-2)	1* (0-2)
	1500(i.p.)	0* (0-0)	0* (0-1)	0* (0-0)	1* (0-1)
	Clonidine	0.2(i.p.)	0* (0-0)	0* (0-2)	1* (0-1)

The upper numbers show the medians and numbers in parentheses show the range of scores. HE; Hydroalcoholic Extract.

\*p<0.05 statistically significant difference between test and control groups.

(100g) were macerated with 400ml of ethanol-water (7:3) for 48 hours. After shaking and filtering, the solution was concentrated under reduced pressure (16). Hexane extract was prepared with the same procedure but the solvent was n-hexane. Evaporation and solvent removal of hydroalcoholic and hexane extracts gave semi-solid masses (yield 29% and 14% respectively).

#### Phytochemical screening

The hydroalcoholic extract of *O. persica* was screened for alkaloids, anthraquinones, cardiac glycosides, saponins, steroids, tannins and triterpenoids (17).

#### Animals

Male albino mice weighing 25-35g were obtained from Pasteur institute (Tehran, Iran) and maintained in animal house of Isfahan university of medical sciences in 12/12h

light/dark cycle at 21±2 °C. They had free access to food and water.

#### Grouping of animals and administration of extracts

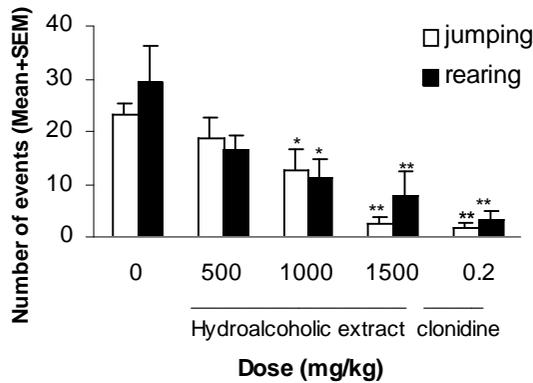
Ninety male mice were randomly divided in groups of six in each. All animals were rendered dependent on morphine. One series of animals orally received either saline (10 ml/kg), different doses of hydroalcoholic extract of *O. Persica* (500, 1000 and 1500 mg/kg) or clonidine (0.2 mg/kg) as a reference drug one hour prior to naloxone injection. The second series were given i.p. injections of either saline (10ml/kg), the same doses of hydroalcoholic extract of *O. Persica* (500, 1000 and 1500 mg/kg) or clonidine (0.2 mg/kg) thirty minutes before naloxone injection. The third series orally received either saline (10ml/kg), hexane extract of *O. Persica* (500, 1000 and 1500 mg/kg) or clonidine (0.2 mg/kg) one hour prior to naloxone injection.

**Table 2.** Effects of hexane extract of *O. persica* on checked signs of morphine withdrawal syndrome.

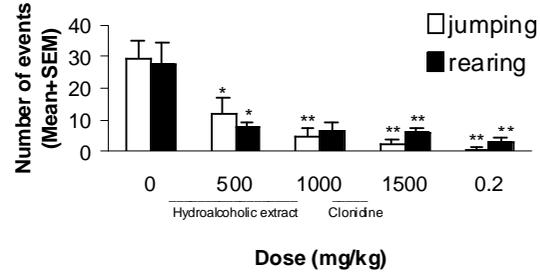
Treatment	Dose (mg/kg)	Diarrhoea	Piloerection	Tremor	Ptosis
Vehicle	-	1 (0-2)	3 (3-3)	2 (1-3)	3 (3-3)
Hexane extract	500(p.o.)	0.5* (0-1)	1.5 (1-3)	2 (2-3)	1 (0-2)
	1000(p.o.)	0* (0-1)	2 (1-3)	2 (1-2)	1.5 (1-3)
	1500(p.o.)	0* (0-1)	2 (1-3)	2 (1-3)	2 (1-3)
Clonidine	0.2(p.o.)	0* (0-0)	0* (0-2)	0* (0-1)	0* (0-2)

The upper numbers show the medians and numbers in parentheses show the range of scores.

Significant differences between test and control groups is shown as \*p<0.05.



**Figure 1.** Effect of oral administration of hydroalcoholic extract of *O. persica* on naloxone-induced jumping and rearing in morphine dependent mice. Hydroalcoholic extract and clonidine were administered orally 1 h prior to naloxone injection (5mg/kg, i.p.). Number of jumping and rearing was counted during a 30min period starting just after naloxone injection. The data are expressed as mean ± S.E.M. (n=6). Significant differences between test and control groups are shown as \*p<0.05 and \*\*p< 0.01 (one-way ANOVA followed by Duncan).



**Figure 2.** Effect of i.p. administration of hydroalcoholic extract of *O. persica* on naloxone-induced jumping and rearing in morphine dependent mice. Hydroalcoholic extract and clonidine were administered i.p. 30min prior to naloxone injection (5mg/kg, i.p.). Number of jumping and rearing was counted during a 30min period starting just after naloxone injection. The data are expressed as mean ± S.E.M. (n=6). Significant differences between test and control groups are shown as \* p<0.05 and \*\* p< 0.01 (one-way ANOVA followed by Duncan).

### Morphine dependence

Morphine was injected subcutaneously to mice at doses of 30 and 45mg/kg on day 1 and 60 and 90mg/kg on day 2 (8:00 am and 6:00 pm). On day 3, a single dose of morphine (90mg/kg) was injected at 8:00 am (18).

### Naloxone- precipitated withdrawal syndrome

Withdrawal signs were elicited by i.p. injection of naloxone hydrochloride (5mg/kg) 2h after the last injection of morphine. Counted and checked signs were evaluated during a 30min period starting just after naloxone injection. Jumping and rearing were counted and checked signs including diarrhea, ptosis, tremor and piloerection were evaluated over 3x10 min periods with one point given for the presence of each sign during each period ( maximum score : 3) (18).

### Statistical analysis

The data were expressed as mean ± S.E.M. One-way ANOVA followed by Duncan test was used for comparison of data and P values less than 0.05 were considered significant. The Mann-Whitney U test was used for comparison of checked signs data. All statistical calculations were done with SPSS for windows (SPSS 10) software.

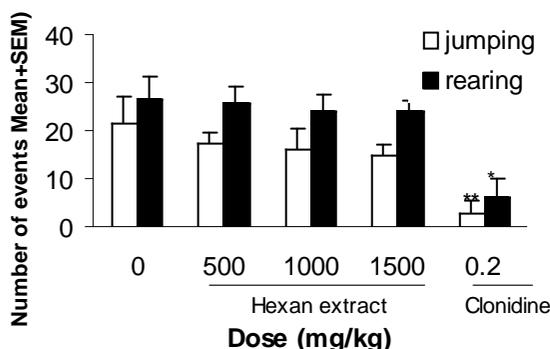
## Results and discussion

### Phytochemical screening

Preliminary phytochemical screening of hydroalcoholic extract of *O. persica* showed the presence of flavonoids, steroids, tannins and triterpenoids.

### Pharmacological study

Both oral and i.p. administration of the hydroalcoholic extract reduced the number of jumping episodes in a dose dependent manner (fig. 1 & 2 ). Oral doses of 500, 1000 and 1500mg/kg of hydroalcoholic extract produced 18, 45 and 89% reduction of jumps, respectively. Intraperitoneal injection of the same doses decreased the number of jumps by 58, 82 and 91% respectively. Oral and i.p. administration of hydroalcoholic extract also significantly (P<0.05) reduced the number of rearing (fig. 1 & 2). Clonidine as a reference drug significantly (p<0.01) reduced the number of jumping and rearing. The effects of the high dose of hydroalcoholic extract on these signs were comparable to those of clonidine. Hexane extract at doses of 500, 1000 and 1500mg/kg could not exert any significant effect on number of jumping and rearing episodes (figure 3). Diarrhea, piloerection, ptosis and tremor were



**Figure 3.** Effect of oral administration of hexane extract of *O. persica* on naloxone-induced jumping and rearing in morphine dependent mice.

Hexane extract and clonidine were administered orally 1h. prior to naloxone injection(5mg/kg, i.p.). Number of jumping and rearing was counted during a 30min period starting just after naloxone injection. The data are expressed as mean S.E.M. (n=6).

also suppressed by clonidine and the hydroalcoholic extract. The hexane extract at applied doses only inhibited diarrhea (Table 1 and 2).

The results of the present study indicate that hydroalcoholic extract of *O. persica* has component(s) that could alleviate the morphine withdrawal syndrome. Hexane extract of this plant, which contains non-polar substances, could not produce any significant decrease in number of jumping and rearing episodes and therefore it seems that hexane-extractable constituents are not involved in alleviation of morphine withdrawal syndrome. The results of phytochemical study showed that flavonoids are found in the plant and probably these compounds may have some role in observed pharmacological effects. There is also a report that flavonoids could suppress opioid withdrawal syndrome (19). In comparison with intraperitoneal injection, oral route of the hydroalcoholic extract at doses of 500 and 1000mg/kg had less effect on jumping. In general this could be due to incomplete oral absorption (19) or high first pass effect of the active compounds (20).

In addition to jumping which is a very specific behavior of the morphine withdrawal syndrome (18) the other withdrawal signs including ptosis, piloerection, diarrhea and tremor were also significantly reduced by the hydroalcoholic extract of *O. persica* and according to our results it seems that this extract

contains active constituent(s) which merit further works. As mentioned in introduction section, various systems including dopaminergic (1,2), adrenergic (3), excitatory amino acids (4-6), purinergic (7,8), NMDA (9,10), nitric oxide (10) and serotonergic (11) are involved in suppression of opioid withdrawal syndrome. However, the mechanism of action of this plant is not known and further investigations are needed to clarify it.

### Acknowledgement

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