

## Effect of *Portulaca oleraceae* L. extracts on the Morphine Dependence in Mice

<sup>1</sup>\*Gholamreza Karimi, <sup>2</sup>Toktam Ziaee, <sup>3</sup>Azam Nazari

### Abstract

#### Objective

This study was initiated to investigate the effect of *Portulaca oleraceae* on morphine dependence in mice.

#### Materials and Methods

Dependence was induced using the subcutaneous injections of morphine for 3 days. On the day 4, morphine injected 2 h prior to intraperitoneal injection of naloxone. The plant extracts ethanolic or aqueous administered 0.5 h before the final dose of morphine. The number of jumping during the 30 min period after naloxone injection was considered as a measure of withdrawal syndrome.

#### Results

Both extracts reduced the jumping episodes dose-dependently. The maximum effect was observed at doses of 0.28 g/kg and 1.4 g/kg for the aqueous and ethanolic extracts, respectively. Clonidine and extracts decreased the total activity in locomotion test.

#### Conclusion

These findings indicated that *Portulaca oleraceae* extracts can decrease morphine dependence in mice.

**Keywords:** Morphine, Dependence, *Portulaca oleraceae*

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1- Department of Pharmacodynamics and Toxicology, School of Pharmacy and Medical Research Toxicology Center, Mashhad University of Medical Sciences, Mashhad, Iran.

\*Corresponding author: Tel: +98-511-8823255; Fax: +98-511-8823251; email: karimig@mums.ac.ir

2- School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

3- Pharmacist

## Introduction

Morphine is an important drug in the clinical treatment of severe pain. However, dependence which develops with chronic use of morphine, limits its usefulness. The alleviation of the withdrawal sign is a challenge to combat addiction.

*Portulaca oleraceae* L. (Portulacaceae) is a summer annual which is grown as a vegetable in many parts of the world. This half-hardy low growing plant has slightly succulent leaves and stems that are used raw or cooked (1).

It is used in Iranian folk medicine as a diuretic, vermifuge, antiscorbutic, antitussive, analgesic and in gastroesophageal reflux (2, 3). In traditional medicine, it is considered beneficial for bleeding, vomiting, urinary disorder and ulcer of mouth and stomach (4).

Recent pharmacological studies have showed muscle relaxant activity (5), increase in the onset time of PTZ induced convulsion (6), analgesic, anti-inflammatory (7) and gastric antiulcerogenic effects (8).

The hydroalcoholic extract of it exerted a significant antinociceptive activity in the tail flick test and this effect was inhibited by naloxone (6). Thus, there is a possibility of interaction with opioid receptors. Therefore, the aim of this study was to investigate the effect of *P. oleraceae* on morphine dependence.

## Materials and Methods

### Animals

Male albino Balb/c mice, weighing 25-35g housed in ventilated rooms at temperature of  $24 \pm 2^\circ\text{C}$  with a 12 h light/dark cycle and  $60 \pm 5\%$  humidity. They were provided with food and water ad libitum.

### Plant material

The leaves of plant were obtained from a cultivation located in Khaje-rabi (a village near Mashhad, Khorasan province) and identified by Botany Institute, University of Ferdowsi. A voucher specimen was deposited at the herbarium of Mashhad Pharmacy School, under the number 240-1615-12.

### Preparation of extract

The plant material was dried in shade, ground and extracted with 80% ethanol or distilled water by maceration at room temperature for 24 h. The extracts were filtered and dried at  $40^\circ\text{C}$  under the vacuum.

### Phytochemical screening

Phytochemical screening of the extracts was performed using the following reagents and chemicals (9): alkaloids with Dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds and hemolysis reaction.

### Morphine Dependence

Morphine was injected subcutaneously (S.C.) to mice at doses of 50, 50 and 75 mg/kg three times daily (08.00, 11.00 and 14.00 hrs, respectively) for 3 days. On the day 4, a single dose of morphine (50 mg/kg) was injected 2 hrs before the naloxone treatment (10).

### Morphine withdrawal

Withdrawal signs were elicited by the injection of naloxone (5 mg/kg S.C.) 2 hrs after the final administration of morphine. Following the naloxone challenge, mice were immediately placed in a glass cylinder (30 cm height, 20 cm in diameter). The number of jumping episodes was counted for 30 mins subsequent to the naloxone injection (10).

### Drug and extract treatments

The extracts were injected in grade doses to groups of five animals. After 24 hrs, the highest dose that did not induce any mortality was taken as the maximum tolerated dose (MTD). Doses in the range from 10% to 90% of MTD were chosen for tests.

The extracts administered 0.5 h before the final dose of morphine. Clonidine (0.3 mg/kg) was also injected 0.5 h before the last dose of morphine.

### Locomotor activity

The apparatus, made of white wood, had a floor of  $100 \times 100$  cm, divided by red lines into 25 squares of  $20 \times 20$  cm. The walls,

50 cm high, were also painted in white. The test room was illuminated at the same intensity as the colony room. Each mouse was placed in the center of the open field and its behavior was observed for 5 mins.

Total activity was taken as the parameter to evaluate the effect of extracts on the locomotion activity (11).

**Statistical analysis**

The results expressed as mean ± SEM. Data analyzed by one-way analysis of variance. Sequential differences among means calculated at the level of p<0.05, using the Tukey contrast analysis.

**Results**

The yields (w/w) of the ethanolic or aqueous extracts were 10.9% and 6.9%, respectively. The aqueous and ethanolic extracts showed positive reaction for flavonoids and tannins.

Both extracts reduced the jumping episodes dose-dependently. The maximum effect was observed at doses of 0.28 g/kg and 1.4 g/kg for the aqueous and ethanolic extracts, respectively. Pretreatment with clonidine reduced the jumping number similar to maximum dose of extracts (Figs 1, 2). All doses of both extracts and clonidine decreased the total activity in locomotion test. The highest dose of extracts reduced the total activity similar to clonidine (Figs 3, 4).

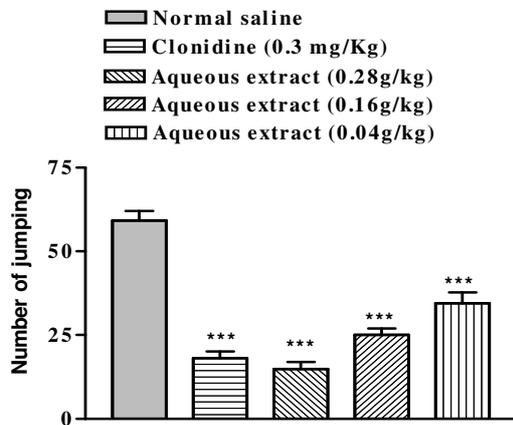


Figure 1. Effect of aqueous extract of *P. oleraceae* L. on naloxone-precipitated jumping in morphine-dependent mice (n=6, Mean±SEM, \*\*\*p<0.001 compared to control, Tukey-Kramer test).

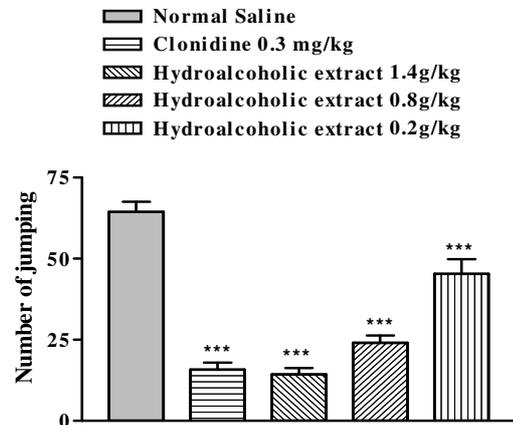


Figure 2. Effect of hydroalcoholic extract of *P. oleraceae* L. on naloxone-precipitated jumping in morphine-dependent mice (n=6, Mean±SEM, \*\*\*p<0.001 compared to control, Tukey-Kramer test).

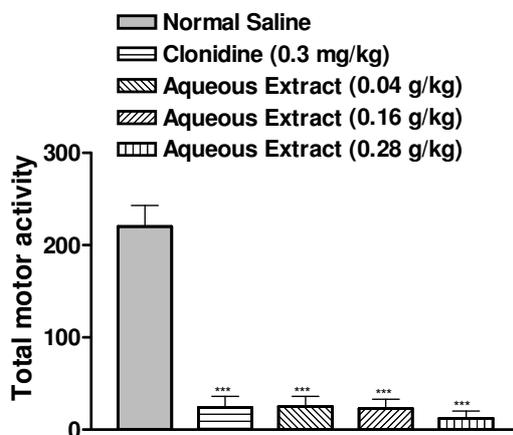


Figure 3. Effect of aqueous extract of *P. oleraceae* L. on total locomotion activity (n=6, Mean±SEM, \*\*\*p<0.001 compared to control, Tukey-Kramer test).

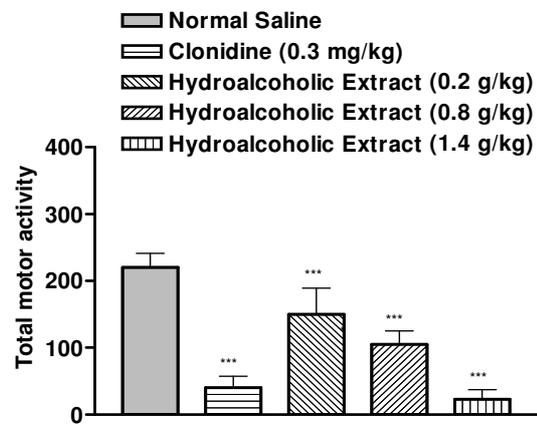


Figure 4. Effect of hydroalcoholic extract of *P. oleraceae* on total locomotion activity (n=6, Mean±SEM, \*\*\*p<0.001 compared to control, Tukey-Kramer test).

## Discussion

The present results indicate that the aqueous and ethanolic extracts of *P. oleraceae* reduced the morphine dependence. It seems that the active principles were polar as the activity was observed in the both extracts. The better results of aqueous extract indicate that the active components were more soluble in water. The phytochemical tests showed positive reaction to flavonoid. It is reported that some flavonols decrease the dependence of morphine (12). So, it may be suggested that these constituents implicated in reducing the withdrawal syndrome.

The reduction in locomotion activity could be due to inhibitory effects of the extracts on the CNS or the peripheral muscle relaxant activity which was reported in other studies (5, 6). Therefore, the reduction in jumping may be influenced by these effects of extracts.

The ethanolic extract of *P. oleraceae* indicates anticonvulsant effect which may be

involves the activation of inhibitory neurotransmitters such as GABA in the CNS (6). Benzodiazepines, via GABA receptors had an inhibitory effect of morphine dependence (5, 13).

Thus, the extracts may modulate dependence via potentiation of the GABA system. It has been demonstrated that dopamine is one of the major bioactive components of *Portulaca oleraceae* (14). Activation of dopamine receptor suppresses the naloxone induced jumping in morphine-dependent mice (15). It is possible that *P. oleraceae* affects the morphine dependence by this mechanism.

Further studies using different models are needed to clarify the *P. oleraceae* effect on morphine dependence.

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