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Ethanolic extract of anise (*Pimpinella anisum* L.) attenuates morphine physical dependence in mice

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ABSTRACT

Introduction: Previous studies revealed that anise (*Pimpinella anisum* L.) has several pharmacological effects including analgesic, antidepressant, anxiolytic, anti-inflammatory and antispasmodic activities. This study aimed to evaluate its effect on morphine physical dependence in mice.

Methods: In this experimental study, 40 male NMRI mice (25-30 g) were randomly divided into 5 groups of 8. Control group received morphine and normal saline (10 mL/kg, i.p.) and other groups received diazepam (5 mg/kg) plus one of three doses of *P. anisum* (50,100 or 200 mg/kg, i.p.). Dependence was induced by administration of increasing doses (50-75 mg/kg, i.p.) of morphine. A time of 30 minutes after naloxone injection was considered for the critical period of the withdrawal syndrome. The number of jumps and scores of 0 to 3 were given for incidences of wet dog shakes, teeth chattering, climbing, writing, diarrhea, grooming, and rearing during a 30-minute period.

Results: All doses of *P. anisum* ($P<0.01$) reduced the number of jumps. Additionally, all doses of the extract reduced the behaviors of grooming ($P<0.05$, $P<0.01$ and $P<0.01$, respectively) and writhing ($P<0.05$, $P<0.001$and $P<0.001$, respectively). None doses of the extract could reduce diarrhea ($P>0.05$). Climbing, rearing and wet dog shakes reduced only by the high dose of the extract ($P<0.05$). Teeth chattering reduced by 100 and 200 mg/kg of the extract ($P<0.05$).

Conclusion: These results obviously show that *P. anisum* ethanolic extract is effective in suppression of morphine physical dependence and further studies are needed to find out the responsible constituents and also the exact mechanisms of actions.

Implication for health policy/practice/research/medical education:
Opioid dependence is a major health problem in worldwide. The present study findings suggest that the plant anise (*Pimpinella anisum* L) is effective in suppression of some aspects of opioid (e.g. morphine) physical dependence in addicts related to morphine withdrawal and might be beneficial in these patients.

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Introduction

Opioid dependence is a major health problem worldwide that often requires long-term treatment and care. Morphine is one of the major opioid analgesics mainly used for alleviating pain and chronic diarrhea. But, its repeated use leads to physical dependence and tolerance (1). Furthermore, after abrupt cessation of morphine or an administration of opioid antagonist (e.g. naloxone), withdrawal signs will appear (2). Today, pharmacotherapies for opioid addiction include administration of opioid agonists (e.g. methadone), partial agonists (e.g. buprenorphine), opioid antagonists (e.g. naltrexone), and alpha-2-adrenergic agonists (e.g. clonidine), which are targeted toward either detoxification or long-term agonist maintenance (3). Several medicinal herbs including *Cymbopogon citratus, Avena sativa*, *Carthamus tinctorius, Rosa damascena, Otostegia percica, Carum copticum,* and *Rodmarinus officinalis* have been investigated for the treatment of some aspects of morphine dependence in animal models of drug dependence (4-11).

Pimpinella anisum L. form Apiaceae (Umbelliferae) family grows in different parts of Mediterranean countries and Iran. In Iranian traditional medicine, *P. anisum* is used for treating gastrointestinal and nervous system disorders. Previous phytochemical studies revealed that the prominent component of *P. anisum* is 1.5%–6.0% essential oil consisting primarily of *trans*-anethole. Other studies demonstrated the presence of eugenol,

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trans-anethole, methyl chavicol, anisaldehyde, estragole, coumarins, scopoletin, umbelliferone, estrols, terpene hydrocarbons, polyenes, and polyacetylenes as the other major components of the essential oil (12,13). *P. anisum* L. extract and essential oil showed different biological and pharmacological activities including antispasmodic, analgesic, anticonvulsant, antibacterial and laxative effects, as well as alleviating morphine dependence property (14-18). Furthermore, our recent study revealed the antidepressant - like effects of *P. anisum* in animal models of depression (19). Hence, the aim of the present study was to investigate the effect of *P. anisum* ethanolic extract on morphine physical dependence in mice.

Materials and Methods

Preparation of extract

Fruits of *P. anisum* were obtained from a local market in Urmia, Iran. *P. anisum* authenticated in the herbarium unit of the school of pharmacy, Tehran University of Medical Science, Tehran, Iran (Voucher No. 723.2). The fruits were dried in shadow and pulverized using a grinder-mixer. Then, 50 g dried fruits powder was macerated separately for 48 hours in 200 mL 70% (v/v) ethanol for extraction. The solvent of the extracts was removed at room temperature to be dried, and the dried extract was kept in clean vials in cool conditions.

Animals

Male NMRI mice 25-30 g were used for the study. They had free access to food and water and maintained at 25 ± 1°C with a 12-h light/12-h dark cycle. All experiments were carried out in accordance with guidelines of National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Induction of morphine physical dependence

Morphine was injected i.p. into mice at doses of 50, 50 and 75 mg/kg three times daily (9:00 AM, 13:00 and 17:00 PM, respectively) for 3 days. On the fourth day, a single dose of morphine (50 mg/kg) was injected 2 hours before naloxone treatment (20).

Naloxone-precipitated withdrawal syndrome

Two hours after the last injection of morphine (50 mg/kg), withdrawal signs were elicited by i.p. injection of naloxone hydrochloride (5 mg/kg). The signs were recorded during a 30-minute period starting immediately after naloxone injection. Jumping's were counted, but other checked signs including grooming, rearing, diarrhea, climbing, teeth chattering, writing and wet dog shakes were evaluated with one point given for the presence of each sign during each period (range of scores: 0-3) (7,21).

Experimental design and animal grouping

Mice were randomly divided into 5 groups (8 mice per group):

1- One chronically i.p. injected morphine (as a negative control group): In this group normal saline (10 mL/kg) was administered i.p. Thirty minutes after the last dose of morphine and 30 minutes later, naloxone was injected.

2- One chronically i.p. injected morphine (as a positive control group): In this group diazepam (5 mg/kg) was administered i.p. Thirty min after the last dose of morphine and 30 minutes later, naloxone was injected.

3- Three chronically i.p. injected morphine (as a treatment group): In this group, different doses of *P. anisum* L. (50, 100 or 200 mg/kg, i.p.) ethanolic extract were administered i.p. Thirty minutes after the last dose of morphine and 30 minutes later, naloxone was injected (19).

Statistical analysis

Statistical analysis was performed using SPSS version 19 software for Windows. The results were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey 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.

Results

The results demonstrated that acute administration of *P. anisum* hydro-ethanolic extract at doses of 50, 100 and 200 mg/kg significantly reduced the number of jumping episodes compared to control group ($P<0.01$) (Figure 1). Diazepam, as a standard drug, significantly reduced the number of jumping episodes ($P<0.01$) (Table 1). All doses of the extract significantly decreased grooming ($P<0.05$, $P<0.01$ and $P<0.01$, respectively) and writhing ($P<0.05$). Only 100 and 200 mg/kg of the extract significantly reduced teeth chattering behavior ($P<0.05$). Diazepam group compared to control group significantly reduced other signs such as grooming ($P<0.01$), teeth chattering ($P<0.05$), climbing ($P<0.05$), rearing ($P<0.05$), writhing ($P<0.05$), wet dog shakes and diarrhea ($P<0.01$) (Table 1).

Discussion

The aim of the present study was to evaluate the effect of the *P. anisum* hydro-alcoholic extract on morphine

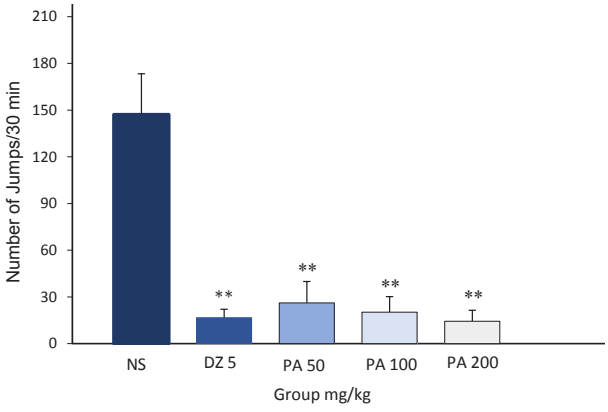

Figure 1. The effect of different doses of *Pimpinella anisum* on number of jumping during 30 min. Normal Saline: NS (10 mL/kg, i.p.); DZ: Diazepam (5 mg/kg, i.p.); *P. anisum*: PA (50-200 mg/kg, i.p.) (n = 8, mean ± SEM, ** $P<0.01$ compared to NS group, Tukey test).

Table 1. The effect of different doses of *Pimpinella anisum* on morphine withdrawal signs

Treatment/Group	Grooming	Teeth chattering	Climbing	Rearing	Wet dog shakes	Writhing	Diarrhea
Control (NS)	2 ± (2-3)	2 ± (1.5-3)	2 ± (1-2.5)	3 ± (1-3)	1 ± (1-2)	2 ± (1.5-3)	3 ± (2-3)
Diazepam 5 mg/kg	1 ± (0-1)**	1 ± (0.5-1)*	1 ± (0-1)*	1 ± (0-1)*	1 ± (0-1.5)	0 ± (0-1)*	1 ± (0-1)**
Mann-Whitney U	0.0	2.0	3	3.0	6.5	1	0
PA 50 mg/kg	1 ± (0.5-1.5)*	1 ± (1-2)	1 ± (1-2)	1 ± (1-2)	1 ± (0.5-2)	1 ± (0-1.5)*	2 ± (2-2.5)
Mann-Whitney U	0	7	9	7	10	3	7.5
PA 100 mg/kg	1 ± (0.5-1)**	0 ± (0-1.5)*	1 ± (1-1)	1 ± (1-1)	1 ± (0.5-1)	1 ± (0-1.5)*	2 ± (1.5-2.5)
Mann-Whitney U	1.5	2.5	5	5	6	3	6.5
PA 200 mg/kg	1 ± (1-1)**	1 ± (0-1.75)*	1 ± (0.5-1)*	1 ± (0.5-1)*	1 ± (0.5-1)*	1 ± (0.5-1.5)*	2 ± (2-2.5)
Mann-Whitney U	0	3	4	4	4	3.5	7.5

The upper numbers show the median, and the numbers in the parenthesis show the range of scores (n = 8, Median, *$P < 0.05$ and **$P < 0.01$; statistically significant between test groups and NS group, respectively).
NS, normal saline; PA, *Pimpinella anisum*: PA

physical dependence in mice. In the present study, the increasing doses of morphine over a four-day schedule followed by naloxone injection produced full-blown withdrawal signs. This method was used to evaluate the ability of the essential oil to inhibit morphine withdrawal signs such as jumping, grooming, teeth chattering, climbing, wet dog shakes, writing and diarrhea. In the naloxone-precipitated withdrawal signs, acute injection of *P. anisum*, 1 hour after the last injection of morphine significantly reduced withdrawal signs. Jumping is one of the main opioid dependence signs. The results indicate that different doses of *P. anisum* and diazepam (as a positive control) are able to reduce the number of jumping in morphine-dependent mice. Also, in line with other findings, the present results showed that diazepam (as a positive control) reduced the number of jumping (20,22). Results showed that the extract reduced grooming behavior. In agreement with these results Bekar et al showed that the aqueous extract of *P. anisum* reduced the grooming behavior in open field test (23). In line with other findings that revealed the antispasmodic effects of *P. anisum*, the present results demonstrated that all doses of extract could reduce the writing behavior in morphine-dependent mice (24). Additionally, all doses of the extract reduced the behaviors of grooming, climbing, writhing, and teeth chattering. None of the used doses of the extract could reduce diarrhea significantly. In this regard, previous studies revealed the significant laxative effect of *P. anisum* when compared with placebo (17). Wet dog shakes reduced only by high dose (200 mg/kg) of the extract. Diazepam significantly suppressed other checked signs except for wet dog shakes.

Several investigations revealed that anethole is a major compound of *P. anisum* (15,25). On the other hand, Anethole is a terpenoid, found in the essential oil of anise, fennel (*Foeniculum vulgare*) and several other plants. In previous studies the antidepressant-like activity of the aqueous and ethanolic extract of *P. anisum* was attributed to anethole (19). In this regard, previous studies revealed that antidepressants might directly or indirectly decrease the abuse potential of opioids by affecting their rewarding effect or improving depressed mood resulting from opioid withdrawal, respectively (26). In line with these findings, Sahraei et al showed that essential oil of *P. anisum* might induce conditioned place aversion (CPA) in morphine-dependent mice. This may indicate that the essential oil of this plant has some aversive effects as investigated by place conditioning method. Also, they suggested that this oil has a GABAergic action. In support of this claim, pretreatment of animals with bicuculline (a GABA-A receptor antagonist) but not CGP35348 (a GABA-B receptor antagonist) inhibited the suppressive effect of the essential oil on morphine-induced conditioned place preference (CPP) (16).

Gamma-aminobutyric acid (GABA) is one of the major inhibitory neurotransmitters in the central nervous system (27). Previous studies have shown that GABAergic system is able to play a key role in the development of morphine-induced physical dependence (28,29). It has been reported that morphine causes an increase in the whole brain GABA concentration in mice. Previous studies have reported that in separate parts of the thalamus and the spinal cord of the addicted rats, GABA is increased (30,31). In support of this hypothesis, pretreatment of mice with benzodiazepines (e.g. midazolam and diazepam) suppresses morphine withdrawal signs (23,32).

Apart from anethole, *P. anisum* contains essential oil and different polyphenols including flavonoids. In line with our findings; previous studies revealed the suppressive effect of polyphenolic compounds on morphine withdrawal signs (10,33). Some flavonoids including quercetin, 3-glucuronide, rutin, and luteolin 7-glucoside were isolated from *P. anisum*. Previous studies revealed that some flavonoids decrease the morphine physical dependence (10,34). However, it may be suggested that anethole, the major component of *P. anisum*, is implicated in reducing the withdrawal syndrome in morphine-dependent mice.

Conclusion

In conclusion, the results of this study revealed that *P. anisum* L. could prevent some major signs of morphine

withdrawal signs and might be beneficial in morphine dependence. Nevertheless, further studies need for determining its exact mechanism of action.

Author's contributions

SAM had a role in hypothesis making, designing the study, data analysis and preparation of the manuscript. DSH contributed to data acquisition and data collection. All authors read and approved the final manuscript.

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Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

In the present study, all experiments were carried out in accordance with guidelines of National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

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