Volatile oil of *Artemisia santolina* decreased morphine withdrawal jumping in mice

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ABSTRACT

Introduction: Flowered aerial parts of *Artemisia santolina* Schrenk (Asteraceae), which is found in the central and western regions of Iran were collected from Khorasan province and the volatile oil extracted by hydro distillation. **Materials and Methods:** The oil (0.5% v/w) was analyzed by GC and GC/MS using DB-5 column. The effect of this oil on the withdrawal syndrome was determined in mice. After induction of dependency by morphine, mice were intraperitoneally administered different concentrations of the oil. Morphine-withdrawal inducing by naloxone was assessed by recording the incidence of escape jumps for 60 min. **Results:** The results indicated that a significant difference between the essential oil received group (at dose of 3.6 mg/kg) and control group was shown but the lower doses were not effective. Essential oil analysis showed that there were forty-six components, representing 95.4% of the oil. **Conclusion:** The oil of *A. santolina* which is rich in oxygenated monoterpenes with the major components, *trans*-verbenol (34.6%) and *p*-mentha3-en-8-ol (13.1%), can decreased the number of withdrawal jumping in addicted mice.

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INTRODUCTION

Artemisia genus (Worm Wood), with the common Persian name of Dermane, is a fairly large genus within the family of the Asteraceae (Compositae), with 200 individual species known, which are usually found in dry areas. They are invariably found as small fragrant shrubs or herbs and most yield essential oils. Some of these oils have found uses in perfumery and medicine. There are approximately 34 native *Artemisia* spp. in Iran.[1-4]

Artemisia and *Achillea* species (both plants belong to the tribe Anthemideae, the largest tribe of the family Asteraceae) have been reported to be effective as anti-diabetic herbal medicines.[5-7] These plants have been used in many countries of middle east and Iran as a herbal medicine for treatment of diabetes, high blood pressure, anti-migraine, anti-fungal, digestive, anti-helminic, tonic, mycolytic, stomachic, and lipolytic.[6,8,9] Essential oils of various species of *Artemisia* have exhibited antibacterial activity.[10,11] *In vitro* antibacterial activity was also reported from the ethanolic extract of *A. herba alba*.[12] In the literature, the chloroform extract of *A. maciverae* (whole plant) have been revealed the antimalarial activity.[13] Furthermore, the antifungal activity of the essential oil of *A. sieberi* has been evaluated against four soil born fungi and it was highly effective against *Rhizoctonia solani*.[14] In another study, the *in vitro* activity of *A. cina* was investigated against *Ascaridia galli* adult worms (one of the most common parasitic round worms of poultry), which was resulted in a weak potency for destruction of lips and damage of buccal cavity of the worm.[15]

Artemisia santolina Schrenk is a perennial herb with woody shoots which covered with brown splitting bark. It found throughout the North-East of Iran, especially Khorasan province and prefers dry, partially shaded stream sides or riverbanks on most geologic formations.[3,16] The chemical composition of *A. santolina* oil, collected from North part of Iran, has already been studied and the main components found to be lavandulol (37.2%), 1,8-cineole (15.9%), linalool (13.6%) and lavandulyl acetate (9.5%).[17] *A. santolina* oil, gathered from-central parts of Iran (Semnan province) is reported to consist of neryl acetate (13.4%), bornyl acetate (10.9%), *trans*-verbenol (9.9%), lavandulol (8.8%), linalool (6.9%) and 1,8-cineole (6.5%).[18]

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The withdrawal jumping behavior under the influence of narcotic analgesics is usually used in order to estimate the degree of morphine-like physical dependence in mice. It is suggested that this method is suitable to evaluate the degree and property of morphine-like physical dependence in mice.[19] In the present study we have explained the antagonistic activity of the volatile oil of *A. santolina* on morphine withdrawal syndrome in mice.

MATERIALS AND METHODS

Plant material

Aerial parts of *A. santolina* were collected in September 2003, during the full flowering stage, from Birjand (Khorasan province). The plant was identified by Prof. Valiallah Mozaffarian. A voucher specimen has been deposited at the herbarium of the Dr. Zargari, Faculty of Pharmacy, Mashhad University of Medical Sciences.

Oil isolation

Aerial parts of *A. santolina* were dried at room temperature and pulverized in electric mill. Powdered plant (90 g) was hydro distilled using a Clevenger-type apparatus for 3 h. The oil was dried over anhydrous sodium sulfate and stored at 4°C before analysis.

GC-MS analysis

The analytical gas chromatography (GC) was carried out using a Thermoquest-Finnigan Trace GC-MS chromatograph equipped with a DB5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Carrier gas was Helium at a flow rate of 1:1 ml/min with a split ratio equal to 1/50. Temperature programming was performed from 60°C (2 min) to 250°C at a rate of 5°C/min and held for 10 min. Transfer line and detector temperatures were 250°C and 260°C, respectively. The quadrupole mass spectrometer was scanned over the 35-465 amu with an ionizing voltage of 70 eV and ionization current of 150 μA.

Identification of the oil composition

The retention indices for all the components were calculated by using retention times of n-alkenes (C_8-C_{25}) that injected after the essential oil at the same temperature and conditions. The components were identified by comparison of retention indices (RRI, DB-5) with those reported in the literatures and also by comparison of their mass spectra and fragmentation patterns with the published mass spectra or Wiley and NIST libraries.[20]

Animals

Male albino mice (20-30 g) were prepared from Pasteur institute (Tehran, Iran) and kept in animal house under standard condition (12 h/12 h light dark cycle at 25 ± 3°C). The animals received standard pellet diet and water *ad libitum*. Animal handling was performed as per *Good Laboratory Practice*. Research proposal was prepared based on the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animal) and approved by IAEC (Institutional Animal Ethical Committee) of Tehran University of Medical Sciences.

Administration of the extracts

The mice were randomly divided in three groups of six in each. All animals were rendered dependent on morphine. All the animals were injected subcutaneously (*sc*) with morphine at doses of 50, 50 and 7 m (mg/kg) three times daily for three days. On the fourth day, one dose of morphine (50 mg/kg) was injected to all groups before treatment with naloxone. After induction of morphine dependence, normal saline was injected to control group (3 ml, *ip*) and various concentrations (3.6, 1.8 and 0.9 mg/kg) of the essential oil were injected to the test groups. Two hours after the final administration of morphine, the withdrawal sign were appeared by injection of naloxone (5 mg/kg, sc). Immediately, the number of jumping episode was counted for 60 minutes.[21,22] The data were expressed as Mean ± SEM. One-way ANOVA was used for comparison of the data and *P* value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Hydro distillation of air-dried aerial parts of *A. santolina*, from Iran, yielded 0.5% (v/w) of the oil based on dry weight of sample. The oil was transparent and yellow with lower density than water. Analysis of the oil by GC and GC-MS resulted in forty-six constituents, representing 95.4% of the total oil [Table 1]. The main components were *trans*-verbenol (34.6%), *p*-mentha-3-en-8-ol (13.1%), davana ether (7.6%), 1,8-cineole (7.3%), *cis*-verbenol (6.3%) and thuja-2,4 (10)-diene (3.4%). The analysis of the essential oil of this plant material was previously published[23] and our results are in agreement with their results except in some minor and trace components.

The results of withdrawal jumping test indicated that a significant difference between the essential oil received group (at dose of 3.6 mg/kg) and control group was shown but the lower doses were not effective. Statistical significant difference between morphine withdrawal jumps per 60 min in the test group (which received 3.6 mg/kg of essential oil as 40.7 ± 28.8) and control (109.0 ± 77.3) was observed. This is the first report of the essential oil of this plant which demonstrated antagonistic effect on morphine withdrawal syndrome in mice. Because the opioid dependence is an important problem in the world and the addict patients are

Table 1: The main chemical composition of the volatile oil of *Artemisia santolina*

Compounds*	RRI**(DB-5)***	Percent
thuja-2,4 (10)-diene	979	3.4
1,8-cineole	1058	7.3
dehydro linalool	1108	1.5
β-thujone	1142	1.6
cis-verbenol	1170	6.3
trans-verbenol	1178	34.6
p-mentha3-en-8-ol	1194	13.1
4-terpineol	1204	1.1
verbenone	1244	2.2
nor davanone	1260	1.5
cis-chrysanthenyl acetate	1294	1.1
davana bis ether	1484	1.2
davana ether	1491	7.6
C_xH_y		8.9
$C_xH_yO_z$		86.5
C_{10}		82.3
C_{15}		0.6
Unknown		4.6
Total		95.4

*Only the main components with percentage more than 1 are summarized in this table; **RRI = Relative Retention Indices; ***DB-5 = Phenyl methyl silicon capillary column chromatography

widespread in Asia and Europe, finding the new source of antagonists is necessary to improve the treatment. The oil of *A. santolina* could suppress morphine withdrawal syndrome, so this study is the first step in the research for finding the main mechanism of action mainly involved in the inhibitory effect on morphine dependency.

CONCLUSION

The oil of *A. santolina* which is rich in oxygenated monoterpenes with the major components, trans-verbenol and p-mentha3-en-8-ol, can decreased the number of withdrawal jumping in addicted mice.

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