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Sophora alopecuroides L. var. *alopecuroides* alleviates morphine withdrawal syndrome in mice: involvement of alkaloid fraction and matrine

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ABSTRACT

Objective(s): Evaluation of the *Sophora alopecuroides* var. *alopecuroides* seed effects on morphine withdrawal syndrome in mice and determination of the alkaloid composition of the seed total extract.

Materials and Methods: The effects of the seed total extract, alkaloid fraction and major compound matrine on the mice morphine withdrawal syndrome were compared to saline and methadone. Mice were made dependent on morphine by morphine sulfate injection 3 times a day for 3 days. The withdrawal jumping and diarrhea were induced by administration of naloxone 2 hr after the 10th injection of morphine sulfate on the day 4. The total extract (100, 200, 300 mg/kg), alkaloid fraction (5, 10, 20 mg/kg), matrine (5, 15, 30 mg/kg), methadone (10 mg/kg) or saline were injected 30 min before naloxone. All drugs were administered by subcutaneous injection. The total extract alkaloid composition was also determined by gas chromatography (GC) and GC-MS analysis.

Results: All doses of the total extract, alkaloid fraction and matrine as well as methadone decreased jumping and diarrhea significantly compared to the saline. The effects of the total extract and alkaloid fraction were not significantly different from methadone. But, there were significant differences between the effects of matrine and methadone. Matrine, cytisine, sophoridine, n-methyl cytisine, sophocarpine and sophoramine were the major alkaloids. There was no nicotine in the total extract.

Conclusion: *S. alopecuroides* var. *alopecuroides* suppresses opioid withdrawal with efficacy comparable to methadone. Matrine may be one of the alkaloids responsible for the effect of the plant.

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Introduction

Opioid dependence or addiction is a prevalent health and socioeconomic problem. There are 2 kinds of pharmacotherapies for opioid dependence: detoxification and opioid maintenance treatment (OMT). The purpose of detoxification is to provide a safe and comfortable withdrawal from drug. OMT aims to induce avoidance of illicit drug use. Many drugs are used for detoxification including tapered methadone, tapered methadone plus adjunctive medication, other opioid agonists, clonidine, lofexidine, other adrenergic agonists, buprenorphine, and symptomatic medications. Methadone is the most effective drug used in detoxification. Any opioid drug can be used in OMT which is also termed opioid replacement therapy or agonist substitution. Methadone, buprenorphine and injectable diamorphine (heroin) are the drugs usually used in OMT. Methadone maintenance is the gold standard of treatment. New and evolving therapies are compared to this standard (1-3). The current pharmacotherapies of opioid dependence have unfavorable efficacy and safety profile. A substantial number of patients do not respond adequately to the pharmacotherapies. Thus, novel more effective and safer pharmacological treatments for opioid dependence are needed (4-6).

Plants and their bioactive compounds are suitable sources for development of new opioid dependence pharmacotherapies (7, 8). *Sophora alopecuroides* L. var. *alopecuroides* (*S. alopecuroides*) (Leguminosae) is a plant widely distributed in Western and Central Asia. It is up to 1 meter tall perennial herb with rhizome. It has legumes 5-7 cm long, 7-8 mm diameter and cylindrical with spherical seeds (9). The plant seed is administered orally in the Iranian traditional medicine for the treatment of opium, heroin, methamphetamine and

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nicotine addiction, diarrhea and pain. Specifically, the Iranian therapists use the plant for the control of opioid withdrawal syndrome and OMT. The plant demonstrates good human safety profile (no adverse effects) in the usual oral doses (up to 5 g per day of the seed dried aqueous extract). Quinolizidine alkaloids are the main bioactive compounds of *S. alopecuroides*. More than 20 kinds of alkaloids have been isolated from the plant including matrine, oxymatrin, sophocarpine, oxysophocarpine, cytisine, sophoramine, sophorodine, nicotine and others. The alkaloids have shown anticancer, antimicrobial, immunological, nervous system, cardiovascular and gastrointestinal effects in the pharmacological studies (10, 11). Varenicline is an analogue of cytisine. Cytisine and varenicline are both effective aids to smoking cessation (12). The major alkaloid of *S. alopecuroides* matrine has a variety of biological activities including analgesic, anti-inflammatory, anti-arrhythmic, anti-tumor, anti-diarrhea, antiobesity and immunomodulatory effects (13-15). Matrine has been widely used in China for the treatment of cancer, hepatic, cardiac and skin diseases (16). Of note, the phytochemistry of the Iranian *S. alopecuroides* has not been examined so far. The only study relative to the biological effects of the Iranian *S. alopecuroides* has demonstrated that it has analgesic effect in the rat formalin test (17). Moreover, there has been no study concerning the effects of *S. alopecuroides* on drug dependence.

Considering the above data, in the present study, the effects of a standardized extract of the Iranian *S. alopecuroides*, its alkaloid fraction and major compound matrine on the mice morphine withdrawal syndrome were evaluated and compared with saline and methadone.

Materials and Methods

Plant materials

The seeds of *S. alopecuroides* L. var. *alopecuroides* (Leguminosae) were collected at fruiting stage from Raviz, 60 km Northwest of Rafsanjan, province of Kerman, Iran. The voucher specimen (number 27235) was deposited in the Tehran University Central Herbarium.

Total extract

The seeds of plant were dried, powdered (500 g) and macerated with a 90% ethanol solution for 3 days with three changes of the solution. The resulting extract was filtered and evaporated under vacuum into a dried powder extract (30 g, 6%).

Extraction of alkaloids

Alkaloid extraction was carried out as described by Kamada *et al* (1986) (18): 200 ml of $CHCl_3$- Me OH-NH_4OH (15: 5: 1) was added to 600 mg of total extract, sonicated for 10 min. After filtration, the residue was washed twice with 200 ml of solution. The pooled filtrate was evaporated to dryness. To the residue, 5 ml of $CHCl_3$ and 2 ml of 1 N H_2SO_4 were added and then the solution was mixed. The $CHCl_3$ phase was removed and the H_2SO_4 phase was adjusted to pH 10 with 28% NH_4OH. From the solution, alkaloids were extracted once with 2 ml and twice with 1 ml of $CHCl_3$. The combined extracts were filtered after adding anhydrous Na_2SO_4. The combined filtrates were evaporated to dryness at 40 ˚C (265 mg).

GC (gas chromatography) and GC-MS (gas chromatography-mass spectrometry) analysis

The extraction of alkaloids was analyzed on a Younglin Acm 600 instrument with an FID detector operated with a split/splitless injector (Younglin, Korea) and DB-5 capillary column, 30 m × 0.25 mm i.d., 0.25 µm film thicknesses (Agilent, USA).

Carrier gas: He, linear velocity (u): 30 cm/sec, flow: 0.8 ml/min Injection temperature: 290 ˚C. Injection volume: 1.0 µl. Injection mode: Split (1:50). Temperature program: 50 ˚C for min, rising at 3 ˚C /min to 240 ˚C, then rising at 15 ˚C/min to 300 ˚C, held at 300 ˚C for 3 min. FID (290 ˚C): H_2 flow: 50 ml/min; air flow: 400 ml/min.

GC/MS analysis was performed on an Agilent 6890/5973 N instrument and DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Carrier gas: He, Linear velocity (u): 32.4 cm/sec, flow: 0.8 ml/min. Injection temperature: 290 ˚C. Injection volume: 1.0 µl. Injection mode: split (1:10). Temperature program: 50 ˚C, for 5 min, rising at 3 ˚C/min to 240 ˚C, then rising at 15 ˚C/min to 300 ˚C, held at 300 ˚C for 3 min. MS interface temperature: 290 ˚C, MS mode: EI, Ionization voltage: 70 eV; mass range: 40-500 u; scan speed: 3.18 scans/sec; interval: 0.50 sec (2 Hz). Data handling was conducted using a Chem. Station (Agilent).

Identification of the components

The compounds of the extraction of alkaloids were identified by comparison of their retention indices, which were calculated by using the retention times of injected n-alkanes (C8–C28) (obtained from Fluka) with the same chromatographic conditions, along with the fragmentation patterns of the mass spectra with those reported in the literatures and the published mass spectra or WILEY library (19-22). The percentage of the identified compounds was calculated based on GC peak areas without any correction factors.

Drugs

Morphine sulfate and methadone hydrochloride were purchased from the Darou Pakhsh Pharmaceutical Company (Tehran, Iran). Naloxone hydrochloride and matrine were obtained from the Sigma-Aldrich and Ningxia Zijinghua companies respectively. For dilution, all drugs and extracts were dissolved in normal saline. The drugs and extracts were

prepared immediately before use and injected subcutaneously in a volume of 5 ml/kg. The doses of total extract, alkaloid fraction, matrine and methadone were as follows. Total extract: 100, 200 and 300 mg/kg; alkaloid fraction: 5, 10 and 20 mg/kg; matrine: 5, 15 and 30 mg/kg; methadone: 10 mg/kg.

Animals

Male albino mice weighing between 25-30 g respectively from our own breeding colony were used. The mice were maintained at a temperature of 22-25 ˚C on a 12 hr dark-light cycle. The animals had access to standard rodent feed and water *ad libitum*. The number of animals used for each dose of the extracts or drugs was 10. All animals were used only once.

Animal study

The effects on opioid withdrawal were evaluated using a method described previously (23). To induce morphine dependence in the mice, morphine was given with the following dosage schedule. Morphine was injected thrice a day at 9:30, 13:30 and 17:30 hr using the doses 50, 50 and 75 mg/kg respectively for 3 days. The higher afternoon dose was aimed to minimize overnight withdrawal. Moreover, a 50 mg/kg dose of morphine was given in the 4th day morning (2 hrs before naloxone injection). Hyperactivity and Straub tail effect were noted after morphine injection. Weight loss of 10% without any animal death was also observed.

Naloxone (2 mg/kg) was given 2 hrs after the last injection of morphine in the 4th day. Subsequently, the animals were placed singly on a piece of blotting paper in a cylindrical glass (25 cm in diameter, 40 cm height) for 30 min. Naloxone immediately caused morphine withdrawal signs as jumping and diarrhea. The number of jumps and feces weight during the 30 min period was recorded for each animal. The total extract, alkaloid fraction and matrine as active treatments, methadone (positive control) or saline (negative control) were injected 30 min before naloxone. This study was conducted according to the European Community Guidelines (EEC Directive of 1986; 86/609/EEC) for laboratory animal use and care.

Statistical analysis

The animal study results were presented as mean +standard error of the mean (SEM). One way analysis of variance (ANOVA) followed by Tukey *post hoc* test was used for data analysis. Significance level was set at 0.05.

Results

GC and GC-MS analysis

Alkaloid extraction was analysed by capillary GC and GC-MS and the main compounds were determined via areas under the peaks. Matrine (23.2%), cytisine (20.9%), sophoridine (17.2%), n-methyl cytisine (13.4%), sophocarpine (9.1%) and sophoramine (1.2%), were identified as the main components in the alkaloid extract (Table 1). There was no nicotine in the alkaloid extract.

Animal study

All doses of the total extract, alkaloid fraction and matrine as well as methadone decreased jumping and diarrhea significantly compared to the saline (Figures 1-6). The effects of the total extract and alkaloid fraction were not significantly different from methadone. But, there were significant differences between the effects of matrine and methadone.

Table 1. The major compounds (in terms of percentage of the total alkaloid content) identified in the alkaloid extraction of *Sophora alopecuroides* var. *alopecuroides*

No.	Compounds[a]	R_t[b]	K_I[c]	Percentage of the total alkaloid content
1	n- methyl cytisine	55.3	1965	13.4
2	cytisine	56.1	1994	20.9
3	sophocarpine	64.5	2235	9.1
4	sophoridine	65.1	2246	17.2
5	matrine	66.2	2266	23.2
6	sophoramine	69.8	2451	1.2

[a] Compounds listed in order of elution from the HP-5 MS column; [b] Retention times (as min); [c] Kovats indices on a DB-5 column in reference to n-alkanes

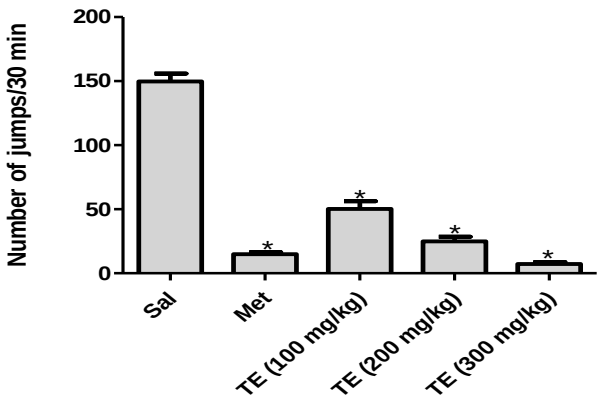

Figure 1. Effects of saline (Sal), methadone (Met) (10 mg/kg, SC), *Sophora alopecuroides* var. *alopecuroides* total extract (TE) (100 mg/kg, 200 mg/kg, 300 mg/kg, SC) on jumping induced by naloxone (2 mg/kg, SC) in groups of 10 morphine dependent mice. Data are given as mean+SEM. *P*-value<0.001 for all groups compared to the saline. The asterisked columns are significantly different from the saline

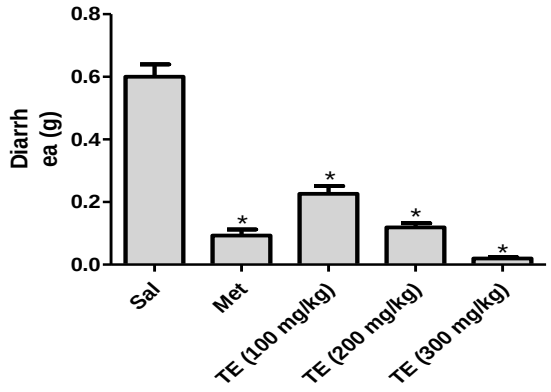

Figure 2. Effects of saline (Sal), methadone (Met) (10 mg/kg, SC), *Sophora alopecuroides* var. *alopecuroides* total extract (TE) (100 mg/kg, 200 mg/kg, 300 mg/kg, SC) on diarrhea induced by naloxone (2 mg/kg, SC) in groups of 10 morphine dependent mice. Data are given as mean ± SEM. *P*-value<0.001 for all groups compared to the saline. The asterisked columns are significantly different from the saline

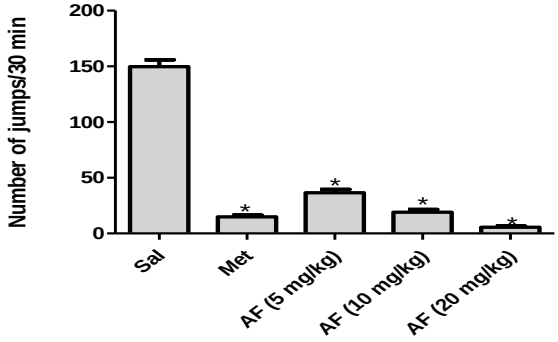

Figure 3. Effects of saline (Sal), methadone (Met) (10 mg/kg, SC), *Sophora alopecuroides* var. *alopecuroides* alkaloid fraction (AF) (5 mg/kg, 10 mg/kg, 20 mg/kg, SC) on jumping induced by naloxone (2 mg/kg, SC) in groups of 10 morphine dependent mice. Data are given as mean±SEM. *P*-value<0.001 for all groups compared to the saline. The asterisked columns are significantly different from the saline

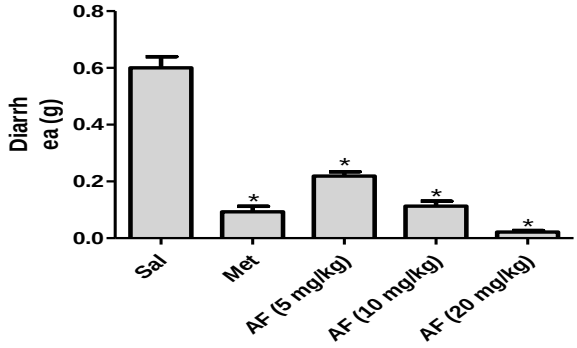

Figure 4. Effects of saline (Sal), methadone (Met) (10 mg/kg, SC), *Sophora alopecuroides* var. *alopecuroides* alkaloid fraction (AF) (5 mg/kg, 10 mg/kg, 20 mg/kg, SC) on diarrhea induced by naloxone (2 mg/kg, SC) in groups of 10 morphine dependent mice. Data are given as mean±SEM. *P*-value < 0.001 for all groups compared to the saline. The asterisked columns are significantly different from the saline

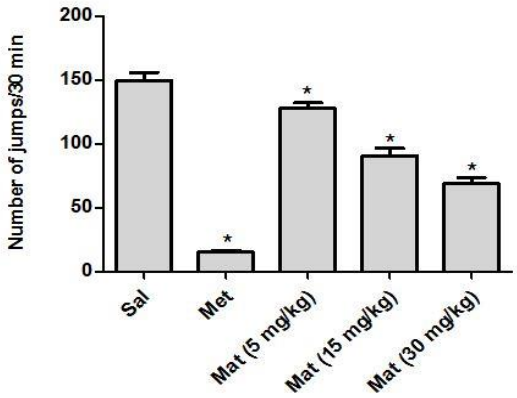

Figure 5. Effects of saline (Sal), methadone (Met) (10 mg/kg, SC), matrine (Mat) (5 mg/kg, 15 mg/kg, 30 mg/kg, SC) on jumping induced by naloxone (2 mg/kg, SC) in groups of 10 morphine dependent mice. Data are given as mean ± SEM. *P*-value=0.018 for Mat (5 mg/kg) and *P*-value<0.001 for the other groups compared to the saline. The asterisked columns are significantly different from the saline

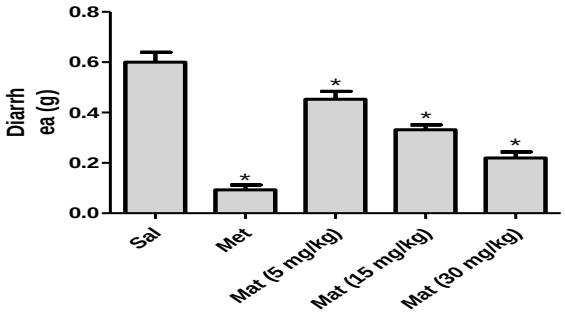

Figure 6. Effects of saline (Sal), methadone (Met) (10 mg/kg, SC), matrine (Mat) (5 mg/kg, 15 mg/kg, 30 mg/kg, SC) on diarrhea induced by naloxone (2 mg/kg, SC) in groups of 10 morphine dependent mice. Data are given as mean±SEM. *P*-value=0.004 for Mat (5 mg/kg) and *P*-value<0.001 for the other groups compared to the saline. The asterisked columns are significantly different from the saline

Discussion

The present study is the first study evaluating the alkaloid composition of the Iranian *S. alopecuroides* seeds. The results indicate that matrine, cytisine, sophoridine, n-methyl cytisine, sophocarpine and sophoramine are the major constituents of the Iranian *S. alopecuroides* seeds. The Iranian *S. alopecuroides* has no nicotine. This is in contrast to the Chinese *S. alopecuroides* which contains nicotine (24). Also, the *S. alopecuroides* total extract and alkaloid fraction profoundly reduce the signs of morphine withdrawal. The inhibitory effect of the plant total extract and alkaloid fraction on the opioid withdrawal syndrome is comparable to methadone. Matrine alleviates the morphine withdrawal syndrome markedly less than the total extract and alkaloid fraction. The significant difference between the effects of matrine and methadone shows that the effect of the plant cannot be

totally attributed to matrine and the other alkaloids besides matrine may also be involved in the plant effect. Moreover, the results explain the traditional use of the plant for the treatment of opioid dependence in Iran. The effects of plants from numerous families have been examined in opioid dependence and withdrawal syndrome so far (7, 8). However, there has been no study regarding the family Leguminosae and the genus *Sophora*. Further, the effects of the constituents of *S. alopecuroides* in the animal models and clinical trials of opioid dependence have not been evaluated. The mechanisms of the inhibitory effects of the *S. alopecuroides* total extract and alkaloid fraction and matrine on the morphine withdrawal syndrome were not investigated in the present study. Of note, cytisine is an agonist of nicotinic receptors (25-28). Agonists of nicotinic receptors can suppress opioid withdrawal (27, 28). Thus, the nicotinic receptor agonistic action of the *s. alopecuroides* cytisine may have some role in the morphine withdrawal inhibitory effect of *S. alopecuroides*. Several studies have reported involvement of μ and κ opioid receptors in the analgesic effect of matrine in mice (29-31). However, a study concluded that matrine has no affinity for μ, κ and δ opioid receptors *in vitro* and its analgesic effect in mice may be through cholinergic activation rather than acting on opioid receptors directly (32). Therefore, the matrine present in *S. alopecuroides* may alleviate morphine withdrawal by activation of nicotinic receptors. Moreover, in another study, the plant total extract did not cause Straub tail effect in mice. This indicates that it does not activate μ_2 opioid receptors and thus may not produce the opioid adverse effects caused by μ_2 receptor activation (physical dependence, respiratory depression and constipation) (17). The just-mentioned study suggests that the plant can be regarded as a non-opioid agent. In practice, there has also been no report showing the addictive potential of the plant despite its use for several thousand years. Notably, the plant may be of significance given the paucity of medications approved for opioid detoxification and relapse prevention, particularly non-opioid medications. Also, the effectiveness of the plant comparable to methadone in the present study shows the possible clinical value of the plant. In conclusion, the promising results of the current study warrant conduction of clinical trials regarding the efficacy and safety of the plant in opioid detoxification and maintenance treatment. Further, more studies addressing identification of the components and mechanisms mediating the inhibitory effect of the plant on the morphine withdrawal syndrome are needed.

Conclusion

S. alopecuroides controls morphine withdrawal syndrome to a degree comparable to methadone. Matrine may be one of the alkaloids involved in the effect of *S. alopecuroides*.

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