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Full Length Research Paper

Effects of aqueous methanolic extract of *Salvia limbata* on antinociceptive activity and withdrawal syndrome in mice

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It is quite clear that the repeated use of opioid drugs leads to physical dependence and tolerance. Dependence can be measured by evaluation of self-restraint signs from abrupt drug withdrawal or administration of a narcotic antagonist or both. Effects of some *Salvia genesis*, of Salvia aerial parts extract on morphine dependence were investigated in mice. After induction of dependence by morphine, distilled water was injected into the control group and different concentrations of plants aerial extract were injected into the other five groups. To assess morphine withdrawal, mice were injected with naloxone (5 mg/kg) intraperitoneally (i.p.) on the 5th day. After four consecutive days of morphine injection, withdrawal syndrome was assessed by placing each mouse in a 30 cm high glass box and recording the incidence of escape jumps for 60 min. Animal receiving acute treatment with morphine displayed dependence. The animals treated with different *Salvia limbata* aerial (flowered browse) parts extracts concentration decreased incidence of escape jumps in number or decreased development of morphine dependence and on the other hand, addiction was observed following naloxone administration (P<0.001). Results from the present study showed that the methanolic extract from aerial parts of *Salvia limbata* produced a statistically significant inhibition of pain induced by hot plate latency at (500, 1000 and 1500 mg/kg) i.p. dose, as compared to the control groups. A significant increase in pain threshold after 30 and 60 min of i.p. injection of extract, compared with the control groups (P<0.001). The activity was comparable to that of morphine (30 mg kg-1 i.p., p> 0.05). The anti-nociceptive activity of *S. limbata* increased until the 60th min as compared to morphine (P<0.05).

Key words: Morphine dependence, anti-nociceptive activity, *Salvia limbata*, jumping, hot plate method.

INTRODUCTION

It is quite clear that the repeated use of opioid drugs brings physical dependence and tolerance. Based on evidence from neurochemical, neurophysiological and biochemical studies of opioid dependence, a variety of agents and systems such as noradrenergic system (Ambrosio et al., 1997; Baraban et al., 1995), adenosine receptor agonists (Michalska and Alec, 1993), excitatory amino acid antagonists (Belozertseva et al., 1996; Gonzalez et al., 1997), protein kinase C inhibitors (Tokuyama et al., 1995), glucocorticosteroids (Capasso et al., 1997), benzodiazepines (Suzuki et al., 1996; Puntillo et al., 1997) and arachidonic acid (Capasso and Sorrentino, 1997) can modulate the morphine withdrawal

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syndrome. Pain is still one of the main health problems of the world's populations (Basbaum and Field, 1984). Many bioactive substances are involved in the modulation of pain sensation (Ebrahimzadeh et al., 2006). Eclectic physicians relied upon herbal medicines and natural remedies to treat diseases (Winston, 2004).

Salvia is an important genus consisting of about 900 species in the Lamiaceae family (Rechinger, 1982). There are several reports that some Salvia geneses have effects on the CNS. *S. Haematodes* posses CNS-depressant, antinociceptive and anticonvulsant activities (Akbar et al., 1984, 1985). The genus, *Salvia Labiatae*, is generally known for its multiple pharmacological effects including analgesic and anti-inflammatory activities (Hernandez-Perez et al., 1995; Hosseinzadeh and Yavari, 1999), *Salvia leriifolia* has an effect on morphine dependence (Hosseinzadeh and Lari, 2000) as well as hypoglycaemic effects (Hosseinzadeh et al., 1998). Anti-nociceptive and anti-inflammatory activities have also been reported for this species (Hosseinzadeh and Yavari, 1999).

The most suitable sign of measuring abstinence quantity is jumping. Jumping rate increases when dependence decreases or dose of antagonism increases. Investigation on plant, "*Salvia limbata*" revealed its beneficial effects as decrease in dependence sign produced by morphine and increase in pain threshold after 60 min, in comparison to the control. The present experiments were undertaken to study the protective effects of Salvia limbata extract on the development of dependence on morphine in mice.

MATERIALS AND METHODS

Some male albino mice weighing 25 to 30 g were obtained from a random bred colony, maintained on a special diet in the animal house of Sari University of Medical Sciences. The animals had free access to a standard commercial diet and water *ad libitum* and were kept in rooms maintained at 25 ± 1°C with a 12/12 h light/dark cycle.

Distilled water and other drugs such as morphine sulphate (Daru Pakhsh, I.R. Iran), naloxone hydrochloride (Tolid Daru, I.R. Iran) and plant extracts were injected intraperitoneally in different doses and regimes.

Aerial parts (flowered browse) of Salvia limbata were collected from Tehran and was identified and confirmed by the department of Pharmacognosy by Dr. A. R. Gohari. After a voucher number specimen (436) was deposited in "Tehran School of Pharmacy Herbarium" aerial parts were dried at room temperature and coarsely ground before extraction . Thereafter One hundred grams of the powdered sample was extracted at room temperature by percolation with methanol/water (80:20, 400 ml 3 times). The resulting extract was concentrated over a rotary vacuum evaporator, until a solid extract sample was obtained. The resulting crude extract was freeze-dried. The extract was prepared in phosphate buffer (pH 7.4) and between 80 (4:1) for pharmacological studies.

Morphine dependence

Morphine was injected i.p. to mice (n = 7) at doses of 50, 75, 100 and 125 mg/kg (total= five groups) a control group three times daily (8:00 a.m, 12:00 and 16:00 p.m, respectively) for 4 days. On day 5, a single dose of morphine (50 mg/kg) was injected 2 h before naloxone treatment (Marshal and Grahame, 1994).

Morphine withdrawal

Withdrawal signs were precipitated by injection of naloxone (5 g/kg, i.p.) 2 h after the final administration of morphine. After the naloxone challenge, mice were immediately placed in a glass cylinder (30 cm high, 20 cm in diameter). The number of jumping episodes was counted for 60 min after naloxone injection.

Extract treatment

After induction of dependence by morphine, mice were divided into 10 groups. Then the control group was injected distilled water and different concentrations of plant extract(100, 200, 500, 1000 and 1500 mg/kg) were injected on the other groups i.p. 1.5 h after the final dose of morphine.

Antinociceptive study

The hot-plate test was assessed on male mice. The temperature of the metal surface was maintained at 55 ± 0.2°C. Latency to a discomfort reaction (licking paws or jumping) was determined before and after drug administration. The cut-off time was 55 s.

Morphine was injected intraperitoneally (i.p.) on mice, as a single dose of 30 mg kg^{-1} (as a positive control). Thereafter, Solvent was injected on the negative control group (10 mL kg^{-1}, i.p.). Then an aqueous methanolic extract of the aerial parts of S. limbata was given at doses of 500,1000,1500 mg kg^{-1} i.p. to the animals. Antinociceptive activity was assessed by measuring the hot plate latency to heat, as described by Leimbach and Eddy (Eddy and Leimback, 1953).

Statistical analysis

Statistical analysis was performed using the SPSS software for Windows (Ver.10, SPSS Inc., Chicago, USA). Data were analyzed by one-way analysis of variance (ANOVA) and presented as mean±sem. Student-Newman-Keuls test was used for statistical analysis and $p<0.05$ was considered to be significant.

RESULTS

Animal receiving acute treatment with morphine displayed dependency. The animals treated with different *S. limbata* extract concentration could decrease or increase incidence of escape jumps in number, following naloxone administration. Recently, we have shown that the high inhibition of morphine dependence in aqueous methanolic extracts of *S. limbata* can decrease development of morphine dependence. However, mechanism of plant action on Salvia limbata to inhibit or decrease abstinence syndrome in dependent mice is unclear. The extract reduced the jumping episodes dose-dependently. The maximum effect was observed at a dose of 1 g/kg.

Results of the present study showed that the aqueous

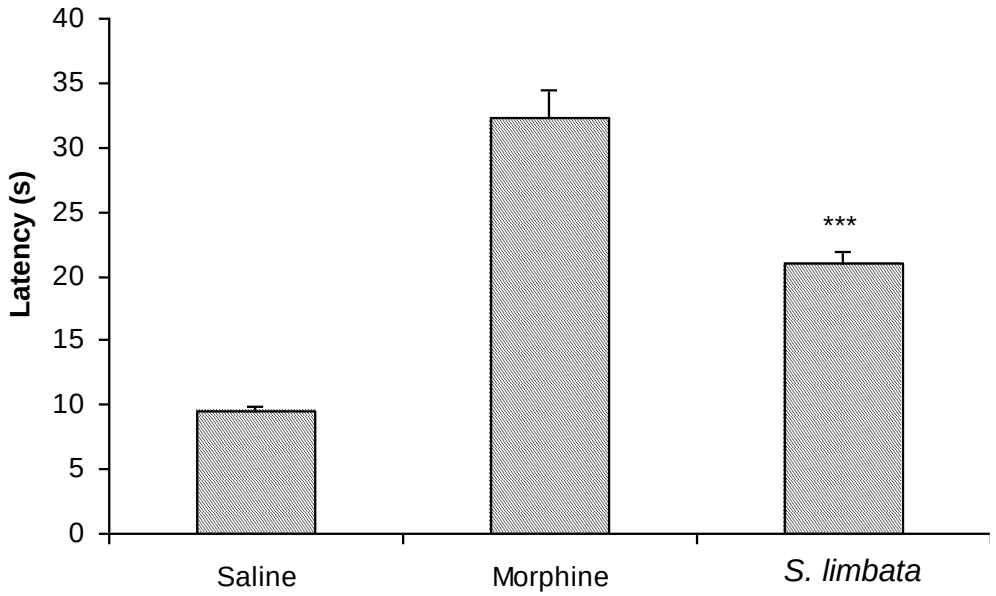

Figure 1. Anti-nociceptive activity of aqueous methanolic extract of *S. limbata* aerial parts after 30 min. Values are presented as mean ± SEM (n = 7), ***P < 0.001 with respect to control (ANOVA followed by Newman–Keuls multiple comparison test).

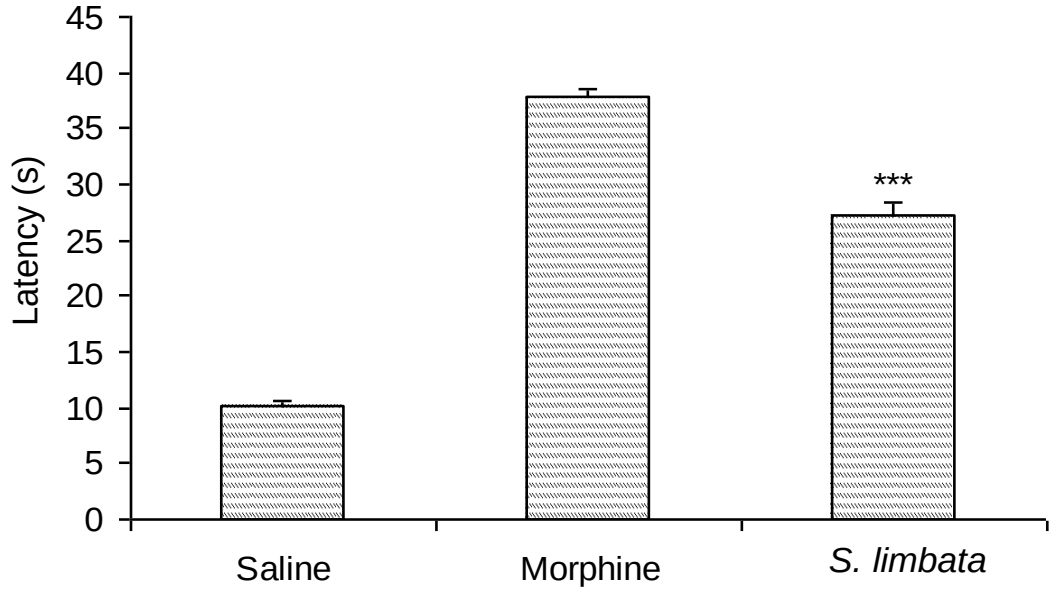

Figure 2. Anti-nociceptive activity of aqueous methanolic extract of *S. limbata* aerial parts after 60 min. Values are presented as mean ± SEM (n = 7), ***P < 0.001 with respect to control (ANOVA followed by Newman–Keuls multiple comparison test).

methanolic extract of the aerial parts (flowered browse at 1000 mg kg^{-1}) of *S. limbata* produced a statistically significant increase in the pain threshold (MPE = 65%), after 30 min, in comparison with the control (Figure 1). The effect or activity was rather low, however enough for treatment and blocking the pain. This activity was comparable to that of morphine (30 mg kg-1 i.p., p> 0.05). The anti-nociceptive activity of extract increased until the 60th min (Figure 2).

DISCUSSION

The present results indicate that the macerated methanolic extract of *S. limbata* leaves reduced the with-

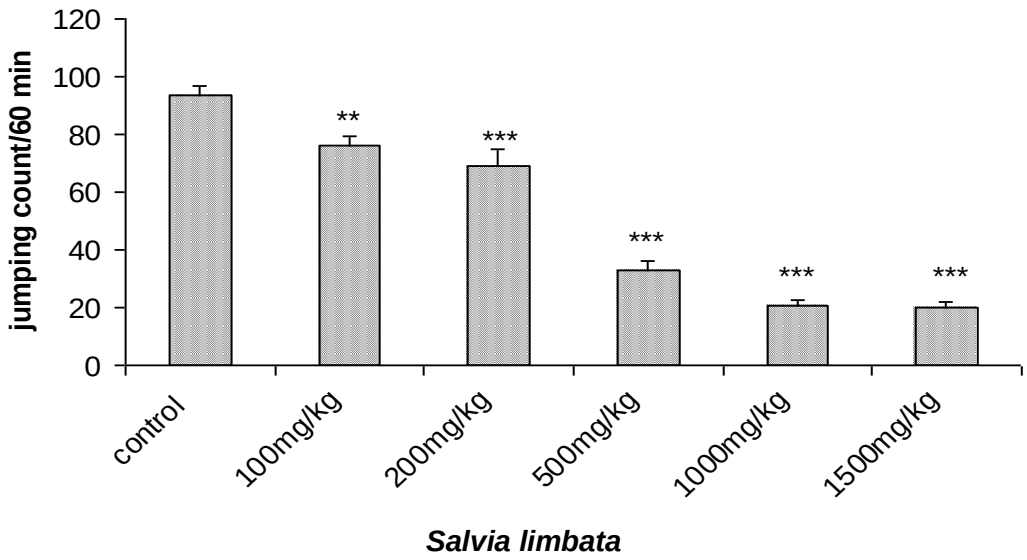

Figure 3. Relation between morphine withdrawal jumps and different concentration of plant of aqueous methanolic extraction. Significant at $p<0.01$. Each value represents mean ± S.E.M.

drawal signs of morphine, dose-dependency.

Aqueous methanol produced statistically significant, decreased development of morphine dependence and other hand addiction following naloxone administration when compared with the control groups (Figure 3). Protective effect was generally dose-dependent. The highest activity showed in aqueous methanolic extract of aerial parts (flowered browse) that at 1000 mg/kg i.p. inhibited 80% incidence of escape jumps (for 60 min). These activities were comparable after induction of dependence by morphine at 30 mg /kg i.p. and assess morphine withdrawal by injection of naloxone (5 mg/kg) i.p. as treatment protocol in mice.

Adenosine A1 receptor agonists such as 2-chloroadenosine and R-phenylisopropyladenosine suppressed the withdrawal syndrome of morphine. Adenosine receptor antagonists such as caffeine and theophylline increased the jumping episodes and blocked the effects of adenosine analogues (Michalska and Malec, 1993). *Salvia miltorrhiza* extract increased the ATP level in the brain (Wang et al., 1995). As ATP is broken down to adenosine (Hossein zadeh and Stone, 1996), it might be possible that the extract decreased morphine dependence by an adenosine mechanism. Further study is required to confirm this mechanism. Benzodiazepines, via GABAA receptors had an inhibitory effect on the dependence to morphine (Suzuki et al., 1996; Puntillo et al., 1997). As some binding sites were found on the GABA/benzodiazepine receptor complex for some *Salvia* species (Lee et al., 1991; Rutherford et al., 1992), there is also a possibility that *S. limbata* acts through this complex to affect morphine dependency.

The involvement of other mechanisms may also be considered. *S. miltorrhiza* via danshen, a constituent in the root, inhibited adenylate cyclase activity in rat brain (Kohda et al., 1989). It also inhibited the phosphatidylinositol system in acute myocardial ischaemia (Tao, 1993). Therefore, some Salvia genus may potentially have inhibitory effects on the withdrawal syndrome of morphine via these second messenger systems, which have modulatory effects on morphine dependency (Fundytus and Coderre, 1994; Thomas et al., 1995).

In conclusion, the methanolic extract of *S. limbata* can suppress the morphine withdrawal syndrome. The results of this study are valuable as a step towards the search for different mechanism of actions, which may be involved in the inhibitory effect of the extract on morphine dependency. It is difficult to speculate on the exact mechanism of action at this time.

The present results indicate that the aqueous extract of *S. limbata* has central antinociceptive activity, because it showed a significant antinociceptive effect in the hot-plate test and also its effect was inhibited by naloxone, a specific antagonist of opioid receptors. The inhibitory effect of naloxone on the antinociceptive activity of extract suggests a morphine-like activity profile for *S. limbata*. With regard to the LD50 value and in comparison with a toxicity classification (Loomis, 1968), the extract was of low toxicity.Antinociceptive and/or anti-inflammatory activities have been reported for some Salvia genera such as *Salvia hemaematodes* (Akbari et al., 1984), *Salvia aethiopis* (Hernandez-Perez et al., 1995), *Salvia* leriifolia leaf (Hosseinzadeh and Yavari, 1999) and other genera (Zargari, 1990). This study and other research on aerial parts of *S. limbata* also confirm that Salvia genera are good candidates for anti-inflammatory and analgesic uses. It can be concluded

that the methanolic extract of *S. limbata* has a central (no spinal) antinociceptive effect and this may be mediated by opioid receptors.

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