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Effect of *Salvia leriifolia* Leaf Extract on Morphine Dependence in Mice

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The effect of *Salvia leriifolia* leaf extract on morphine dependence was investigated in mice. Dependence was induced using subcutaneous injections of morphine daily for 3 days. On day 4, morphine was injected 2 h before the intraperitoneal injection of naloxone. The number of episodes of jumping during the 30 min after injection of naloxone was considered as the intensity of the withdrawal syndrome. The ethanol extract reduced the number of jumping episodes dose-dependently. The extract at a dose of 500 mg/kg was as effective as a dose of 5 mg/kg of diazepam in reducing the number of jumping episodes. The effect of the extract was blocked by aminophylline (20 mg/kg), a non-selective antagonist of adenosine receptors. It is concluded that the ethanol extract of *S. leriifolia* leaves could diminish the withdrawal syndrome of morphine. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords: Salvia leriifolia; morphine dependence; diazepam; aminophylline; medicinal plants.

INTRODUCTION

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 Malec, 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), arachidonic acid (Capasso and Sorrentino, 1997) can modulate the morphine withdrawal syndrome.

Salvia leriifolia is a perennial herbaceous plant that grows exclusively in south and tropical regions of Khorassan and Semnan provinces, I.R. Iran. The species of genus *Salvia*, Labiatae, are generally known for their multiple pharmacological effects such as antimicrobial (Dentali and Hoffmann, 1992), and hypoglycaemic actions (Jimenez *et al.*, 1986; Zarzuelo *et al.*, 1990).

They are several reports that some *Salvia* genus have effects on the CNS. *S. haematodes* has CNS-depressant, antinociceptive and anticonvulsant activities (Akbar *et al.*, 1984; Akbar *et al.*, 1985). A subcutaneous injection of *S. triloba* increased the hypnotic effect of hexobarbital (Todoros *et al.*, 1984). Benzodiazepine binding sites have been introduced for some constituents of *S. miltiorrhiza* roots (Lee *et al.*, 1991) and *S. officinalis* leaves (Rutherford *et al.*, 1992). Diazepam pretreatment suppressed morphine withdrawal signs in the mouse (Puntillo *et al.*, 1997). Therefore, this study was initiated to investigate the effect of *S. leriifolia* on morphine dependence.

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MATERIALS AND METHODS

Animals. Male albino mice 25–30 g were obtained from a random bred colony maintained on a special diet (Khorassan Javane co, Mashhad, I.R. Iran) in the animal house of Mashhad University of Medical Sciences. Animals were housed in a colony room 12/12 h light/dark cycle at $21° \pm 2°C$. Animals had free access to water and food.

Plant material. Leaves were collected in Brone (a town in Khorassan province) and dried in shade followed by grinding. The *S. leriifolia* was identified by Mrs Safavy, Herbarium of Ferdowsi University and voucher samples were preserved for reference in the herbarium of Department of Pharmacognosy, School of Pharmacy, Mashhad (153-1912-1).

Preparation of plant extract. The dried leaves were extracted using a maceration method. Powdered leaves (20 g) were macerated in 400 mL alcohol (70%, v/v) for 3 days and, subsequently, the solution was filtered and concentrated in a rotary evaporator at 50 °C. The yield of the extract was 10% (w/w). The ethanol extract was diluted by Tween-80 in saline.

Morphine dependence. Morphine was injected s.c. to mice at doses of 50, 50 and 75 mg/kg three times daily (11:00 a.m., 14:00 and 17:00 p.m., respectively) for 3 days. On day 4, a single dose of morphine (50 mg/kg) was injected 2 h before naloxone treatment.

Morphine withdrawal. Withdrawal signs were precipitated by injection of naloxone (5 mg/kg, s.c.) 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 30 min after naloxone injection.

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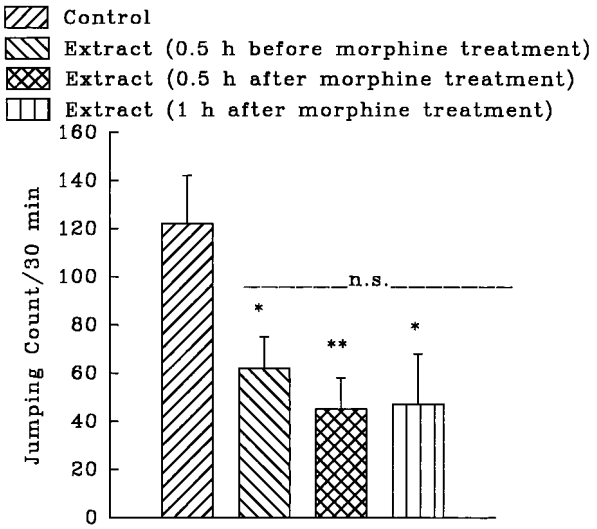

Figure 1. Effect of different treatment schedules of the intraperitoneal dose of *S. leriifolia* ethanol leaf extract on naloxone-precipitated jumping in morphine-dependent mice. Each point represents the mean ± SEM, for $n = 6$–7 mice. $*p < 0.05$, $**p < 0.01$, compared with saline, Tukey–Kramer test, n.s., non-significant.

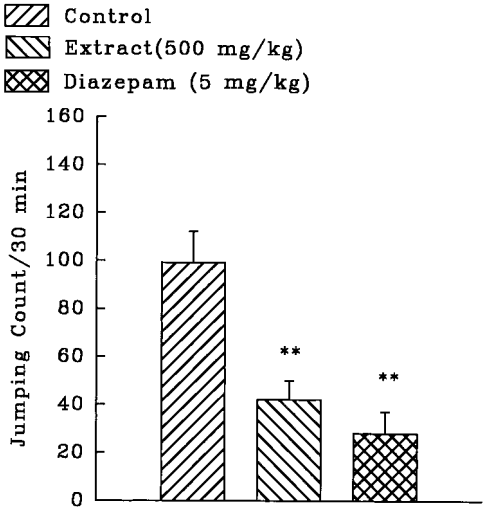

Figure 3. Effect of intraperitoneal doses of *S. leriifolia* ethanol leaf extract and diazepam on naloxone-precipitated jumping in morphine-dependent mice. Each point represents the mean ± SEM for $n = 6$–7 mice. $**p < 0.01$, compared with saline, Tukey–Kramer test.

Drug and extract treatment. Initially the extract of leaves (500 mg/kg) was injected i.p. 0.5 h before and 0.5 and 1 h after the final dose of morphine. In an other study, the extract only administered 0.5 h after the final dose of morphine. Diazepam (5 mg/kg) and aminophylline (20 and 75 mg/kg) were also injected i.p., 0.5 h after the last dose of morphine.

Materials. The following reagents were used: morphine sulphate (Daru Pakhsh, I.R. Iran), diazepam and naloxone hydrochloride (Tolid Daru, I.R. Iran), aminophylline (Iran Hormon, I.R. Iran).

Statistical analysis. The data were expressed as mean values ± SEM. Analysis of variance followed by the multiple comparison test of Tukey–Kramer were used for comparison of data. Differences with a $p < 0.05$ were considered significant.

RESULTS

Administration of the extract 0.5 h before and 0.5 h and 1 h after the last dose of morphine significantly reduced the jumping episodes. There was no significant difference among these three treatments (Fig. 1). The extract reduced the jumping episodes dose-dependently. The maximum effect was observed at a dose of 1 g/kg (Fig. 2).

Pretreatment with diazepam (5 mg/kg) 0.5 h before the

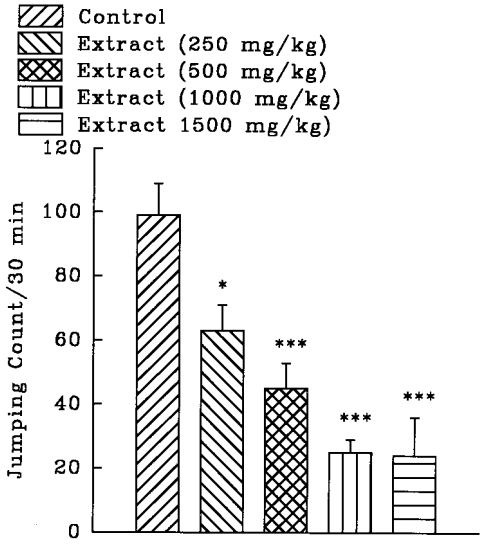

Figure 2. Effect of different intraperitoneal doses of *S. leriifolia* ethanol leaf extract on naloxone-precipitated jumping in morphine-dependent mice. Each point represents the mean ± SEM for $n = 6$–7 experiments on mice. $*p < 0.05$, $***p < 0.001$, compared with saline, Tukey–Kramer test.

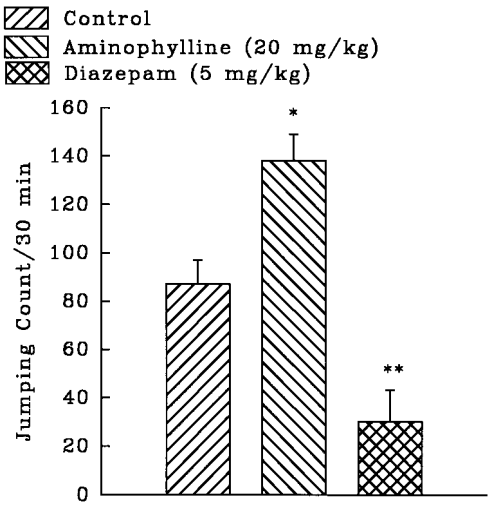

Figure 4. Effect of intraperitoneal doses of aminophylline and diazepam on naloxone-precipitated jumping in morphine-dependent mice. Each point represents the mean ± SEM for $n = 6$–7 mice. $*p < 0.05$, $**p < 0.01$, compared with saline, Tukey–Kramer test.

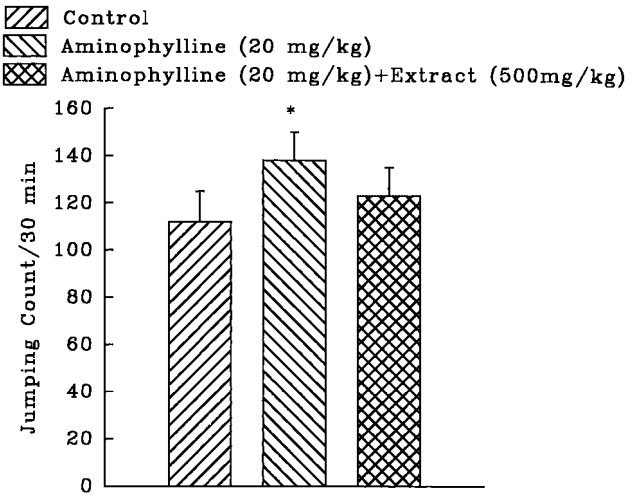

Figure 5. Effect of intraperitoneal co-administration doses of *S. leriifolia* ethanol leaf extract and aminophylline on naloxone-precipitated jumping in morphine-dependent mice. Each point represents the mean ± SEM for $n = 6–7$ mice. $^*p < 0.05$, compared with saline, Tukey–Kramer test.

last dose of morphine reduced the number of jumping episodes. This effect was equal to 500 mg/kg of extract treatment (Fig. 3).

Aminophylline (20 mg/kg) increased the withdrawal signs (Fig. 4). Co-administration of aminophylline (20 mg/kg) and the ethanol extract significantly suppressed the expression of naloxone-precipitated jumping compared with that induced by treatment of the extract (Fig. 5).

DISCUSSION

The present results indicate that the macerated ethanol extract of *S. leriifolia* leaves reduced the withdrawal signs of morphine, dose-dependently.

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 also blocked the effects of adenosine analogues (Michalska and Malec, 1993). In this study, aminophylline increased the jumping episodes and blocked the inhibitory effect of the extract on the withdrawal episodes. *S. miltorrhiza* extract increased the ATP level in the brain (Wang *et al.*, 1995). As ATP is broken down to adenosine (Hosseinzadeh and Stone, 1996), it may be possible that the extract decreased morphine dependence by an adenosine mechanism. Further study is needed to confirm this mechanism.

Benzodiazepines, via $GABA_A$ 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. leriifolia* 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 ethanol extract of *S. leriifolia* 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.

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