**Eur**opean **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2012; 16: 38-42

# Antinociceptive activity and effect of methanol extract of *Salvia limbata* on withdrawal syndrome in mice

Sh. ALEMY[1], M. KARAMI[2], E. HOSSINI[3], M.A. EBRAHIMZADEH[4], N. SHAHBI MAJD[5]

[1]Department of Physiology and Pharmacology, Islamic Azad University Sciences and Research Branc fars (Iran)
[2]Department of Toxico-Pharmacology and Pharmaceutical Sciences Research Center, School of Pharmacy, Mazandaran University of Medical Sciences, Sari (Iran)
[3]Department of Physiology and Pharmacology, Islamic Azad University Sciences and Research Branc fars (Iran)
[4]Departmnet of Medicinal Chemistry, School of Pharmacy, Mazandaran University of Medical Sciences, Sari (Iran)
[5]Department of Physiology and Pharmacology, School of Medicine, Mazandaran University of Medical Sciences, Sari (Iran)

**Abstract. – *Objectives:* *Salvia* (S.) is an important genus consisting of about 900 species in the *Lamiaceae* family. They are several reports that some Salvia spp. has effects on the central nervous system (CNS). The present experiments were undertaken to study the protective effect of *S. limbata* on the development of dependence to morphine in mice.**

***Material and Methods:* Antinociceptive activity of aerial parts of *S. limbata* was investigated using the hot plate method. In addition, the effect of its aerial parts on morphine dependence was investigated in mice. After induction of dependence by morphine, different concentrations of plant aerial parts extract were injected to treated groups. To assess morphine withdrawal, mice were injected naloxone (5 mg/kg) 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 frequency of escape jumps for 60 minutes.**

***Results:* Animal receiving acute treatment with morphine displayed dependence. The animals treated with different extract concentrations could decrease frequency of escape jumps in number or decrease development of morphine dependence. Addiction was observed following naloxone administration. Methanol extract of S. limbata produced a statistically significant inhibition of pain induced by hot plate latency at 500, 1000 and 1500 mg/kg i.p. A significant increase in pain threshold was observed after 30 and 60 min ($p < 0.001$). 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 ($p < 0.05$ compared to morphine).**

***Conclusions:* *S. limbata* extract produced statistically significant inhibition of pain and development of morphine dependence in mice.**

*Key Words:*

Morphine dependence, Antinociceptive activity, *Salvia limbata*, Jumping, Hot plate.

## Introduction

It is well clear that 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[1], serotonergic[2], adenosine receptor agonists[3], excitatory amino acid antagonists[4,5], protein kinase C inhibitors[6], glucocorticosteroids[7], benzodiazepines[8,9] and arachidonic acid[10] can modulate the morphine withdrawal syndrome.

Pain is still one of the main health problems of the world's populations[11]. Many bioactive substances are involved in the modulation of pain sensation[12]. Eclectic physicians relied upon herbal medicins and natural remedies to treat disease[13].

*Salvia* is an important genus consisting of about 900 species in the Lamiaceae family[14]. They are several reports that some *Salvia spp*. has effects on



*Corresponding Author:* Mohamamd Karami, Ph.D; e-mail: toxkarami@gmail.com

the central nervous system (CNS). *S. haematodes* has CNS depressant, antinociceptive and anticonvulsant activities[15,16]. The genus, *Salvia* (*Labiatae*), is generally known for its multiple pharmacological effects including analgesic and anti-inflammatory activities[17,18], *S. leriifolia* has an effect on morphine dependence[19] and hypoglycaemic effects morphine dependency as well[20]. Anti-nociceptive and anti-inflammatory activities have also been reported for this species[18].

Jumping is most suitable sign of measuring abstinence quantity as jumps are easily counted and jumping rate increases when dependence increases or dose of antagonist increased. Investigation on plant *S. limbata* revealed its beneficial effects to decrease 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 *S. limbata* extract on the development of dependence to morphine in mice.

## Materials and Methods

### Drugs

Morphine sulphate was prepared from Daru Pakhsh Co. (Iran) and naloxone hydrochloride was prepared from Tolid Daru Co. (Iran).

### Animals

Male albino mice 25-30 g was 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 12/12h light/dark cycle.

### Plant Material

Aerial parts (flowered browse) of *S. limbata* were collected from Tehran and was identified and confirmed by Department of Pharmacognosy (Dr. Gohari). A voucher specimen (No. 783) has been deposited in Tehran School of Pharmacy Herbarium. Aerial parts were dried at room temperature and coarsely ground before extraction. One hundred grams of the powdered sample was extracted at room temperature by percolation with methanol/water (80:20, 400 ml × 3). 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 tween 80 (4:1) for pharmacological studies.

### Morphine Dependence

Morphine was injected intraperitoneally (i.p.) to mice at doses of 50, 75,100 and 125 mg/kg 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 [21].

### 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[22].

### 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, 1500 mg/kg) were injected to 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 i.p.) to mice, as a single dose of 30 mg $kg^{-1}$ (as a positive control). Solvent was injected to the negative control group (10 mL $kg^{-1}$, i.p.). 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 Eddy and Leimbach[23].

### Statistical Analysis

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

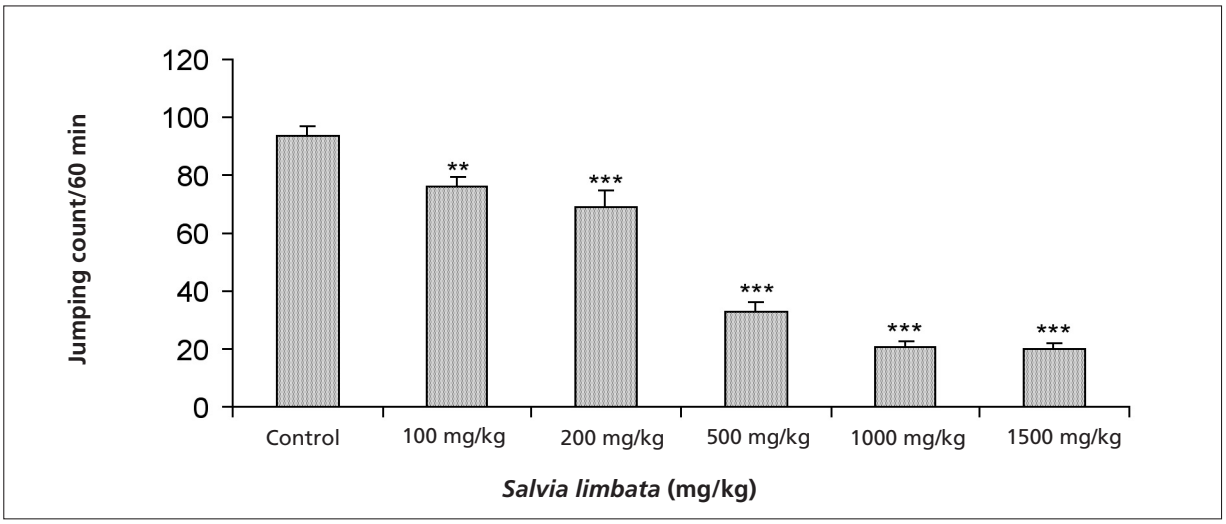


**Figure 1.** Relation between morphine withdrawal jumps and different concentration of plant methanol extract. Significant at $p < 0.0$, each value represents Mean ± SD.

## Results

Animal receiving acute treatment with morphine displayed dependency. The animals treated with different *Salvia limbata* extract concentration could decrease or increase frequency of escape jumps in number, following naloxone administration. Recently, we have shown that the high inhibition of morphine dependence in methanolic extract of *S. limbata* can decrease development of morphine dependence. However, mechanism of plant action to *S. 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 $kg^{-1}$. Results of the present study showed that the aqueous methanol extract of the aerial parts (flowered browse at 1000 mg $kg^{-1}$) of *S. limbata* produced a statistically significant increase in the pain threshold, after 30 min, in comparison to 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 60$^{th}$ min.

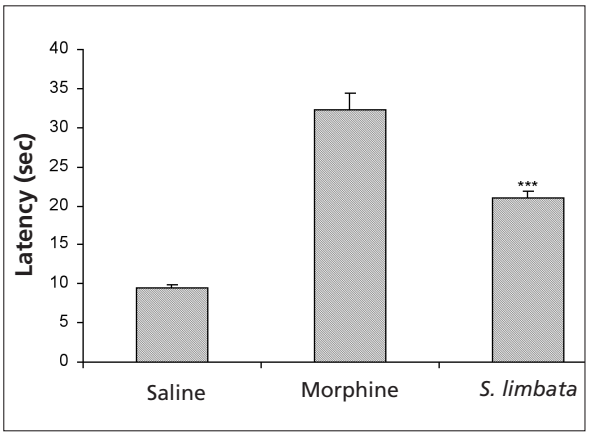


**Figure 2.** Anti-nociceptive activity of methanolic extract of *S. limbata* aerial parts after 30 min. Values are presented as Mean ± SD (n = 7), ***$p < 0.001$ with respect to control (ANOVA followed by Newman-Keuls multiple comparison test).

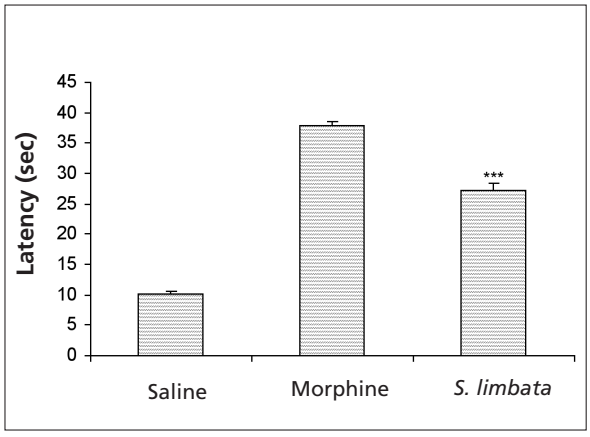


**Figure 3.** Antinociceptive activity of aqueous methanol extract of *S. limbata* aerial parts after 60 min. Values are presented as Mean ± SD (n = 7), ***$p < 0.001$ with respect to control (ANOVA followed by Newman-Keuls multiple comparison test).

## Discussion

The present results indicate that the macerated methanolic extract of *S. limbata* reduced the withdrawal signs of morphine, dose-dependency. 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[3]. *S. miltorrhiza* extract increased the ATP level in the brain[24]. As ATP is broken down to adenosine[25], it might 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[8,9]. As some binding sites were found on the GABA/benzodiazepine receptor complex for some *S. spp.*[26,27], 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[28]. It also inhibited the phosphatidylinositol system in acute myocardial ischaemia[29]. 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[30,31].

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 this plant. With regard to the $LD_{50}$ value and in comparison with a toxicity classification[32], the extract was of low toxicity.

Antinociceptive and/or anti-inflammatory activities have been reported for some *Salvia genera* such as *S. hemaematodes*[15], *S. aethiopis*[17], *S. leriifolia*[18] other genera[33]. 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 is concluded that the methanol extract of *S. limbata* has a central (no spinal) antinociceptive effect and this may be mediated by opioid receptors.

### Acknowledgements

This work was supported by a grant from the Research Council of the Medical Sciences University of Mazandaran/Iran.

## References

1) Ambrosio E, Iglesias V, Garcia-Lecumberri C, Orensanz L, Alguacil LF. Effect of yohimbine on the development of morphine dependence in the rat: lack of involvement of cortical beta-adrenoceptor modifications. Pharmacol Biochem Behav 1997; 56: 487-491.

2) Harris GC, Aston JG. Augmented accumbal serotonin levels decrease the preference for a morphine associated environment during withdrawal. Neuropsychopharmacol 2001; 24: 75-85.

3) Michalska E, Malec D. Agonist and antagonists of adenosine receptors and morphine withdrawal syndrome in rats. Pol J Pharmacol 1993; 45: 1-9.

4) Belozertseva I, Zuartav E, Bespalov A. Behavioral effect of MK-801 in morphine dependent and non-dependent mice. Life Sci 1996; 58: 55-61.

5) Gonzalez P, Cabello P, Germany A, Norris B, Contreras E. Decrease of tolerance to, and physical dependence on morphine by glutamate receptor antagonists. Eur J Pharmacol 1997; 332: 257-262.

6) Tokuyama S, Feng Y, Wakabayashi H, Ho IK. Possible involvement of protein kinases in physical dependence on opioids: study using protein kinase C inhibitors, H7 and H8. Eur J Pharmacol 1995; 284, 101-107.

7) Capasso A, Pinto A, Sorrentino L, Cirino G. Dexamethasone inhibition of acute opioid physical dependence in vitro is reverted by anti-lipocortin-1 and mimicked by anti-type II extracellular PLA2 antibodies. Life Sci 1997; 61: 127-134.

8) Suzuki T, Tsuda M, Narita M, Funada M, Mizoguchi H, Misawa M. Diazepam pretreatment suppresses morphine withdrawal signs in the mouse. Life Sci 1996; 58: 349-357.

9) Puntillo K, Casella V, Reid M. Opioid and benzodiazepine tolerance and dependence: application of theory to critical care practice. Heart Lung 1997; 26: 317-324.

10) Capasso A, Sorrentino L. Arachidonic acid and its metabolites are involved in the expression of morphine dependence in guinea-pig isolated ileum. Eur J Pharmacol 1997; 9: 330: 199-204.

11) Basbaum AI, Field HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Ann Rev Neurosci 1984; 7: 309-338.

12) Yang D, Yang S, Zhang Y, Liu Y, Meng X, Liang Z. Metabolic profiles of three related *Salvia species*. Fitoterapia 2009; 80: 274-278.

13) Winston D. The use of botanicals in eclectic pediatrics. J Am Herbalists Guide 2004; 3: 59-64.

14) Rechinger KH, 1982. Salvia. In: Flora Iranica, Rechinger KH and IC Hedge Akademische Druck and Verlagsanstalt, Graz, Austria, p. 439.

15) Imanshahidi M, Hosseinzadeh H. The pharmacological effects of *Salvia species* on the central nervous system. Phytother Res 2006; 20: 427-437.

16) Baricevic D, Sosa S, Della LR, Tubaro A, Simonovska B, Krasna A, Zupancic A. Topical anti-inflammatory activity of *Salvia officinalis* L. leaves: the relevance of ursolic acid. J Ethnopharmacol 2001; 75: 125-132.

17) Hernandez-Perez M, Rabanal RM, de la Torre MC, Rodriguez B. Analgesic, antiinflammatory, antipyretic and haematological effect of aethiopinone, ano-naphthoquinone diterpenoid from *Salvia aethiopis* roots and two hemisynthetic derivatives. Planta Med 1995; 61: 505-509.

18) Hosseinzadeh H, Yavari M. Anti-inflammatory effects of *Salvia leriifolia* Benth. leaf extract in mice and rats. Pharm Pharmacol Let 1999; 9: 60-61.

19) Hosseinzadeh H, Lari P. Effect of *Salvia leriifolia* extract on morphine dependence in mice. Phytother Res 2000; 14: 384-387.

20) Hosseinzadeh H, Haddadkhodaparast MH, Shokohizadeh H. Antihyperglycemic effect of *Salvia leriifolia* Benth. leaf and seed extract in mice. Irn J Med Sci 1998; 23: 74-80.

21) Marshall L, Grahame-Smith DG. Evidence against a role of brain 5-HT in the development of physical dependence upon morphine in mice. J Pharmacol Exp Ther 1994; 197: 63-64.

22) Harborne JB. Phytochemical methods: A guide to modern technique of plant analysis. Chapman and Hall, Harborne JB. 1998. 3rd ed. Chapman and Hall.

23) Eddy NB, Leimbach D. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. J Pharmacol Exp Ther. 1953 Mar; 107: 385-393.

24) Wang L, Milne B, Jhamandas K. Involvement of excitatory amino acid pathway in the expression of precipitated opioid withdrawal in the rostral ventrolateral medulla: an *in vivo* voltametric study. Brain Res 1995; 697: 130-142.

25) Hosseinzadeh H, Stone TW. Adenosine in the central nervous system. Med J Isl R Iran 1996; 9: 361-368.

26) Lee CM, Wong HN, Chui KY Coang TF, Hon PM, Chang HM. (1991). Miltrione, a central benzodiazepine receptor partial agonist from a chinese medicinal herbs Salvia militorrhiza. Neurosci Lett 1991; 127: 241-273.

27) Rutherford DM, Nelson MP, Hansen SK, Witt MR, Bergendroff O, Sterner O. Isolation and identification from Salvia officinalis of two diterpenes which inhibit t-butylbicyclophosphoro [35S] thionate binding to chloride channel of rat cerebrocortical membranes *in vitro*. Neurosci Lett 1992; 135: 224-226.

28) Zarrindast MR, Mohajeri S. Influence of ATPdependent $K^+$ channels on nicotine-induced inhibition of withdrawal in morphine-dependent mice. Eur J Pharmacol 2006; 552: 90-98.

29) Tao Y. Effect of Salvia miltiorrhiza compositae on phosphoinositides metabolism in acute myocardial ischemia. Chang Kvo Chang His Chieh Ho Iso Chin 1993; 13: 354-355.

30) Fundytus ME, Coderre TJ. Effect of activity at metabotropic, as well as ionotropic (NMDA), glutamate receptors on morphine dependence. Br J Pharmacol 1994; 113: 1215-1220.

31) Thomas JM, Frazier JS, Hu ZW, Hoffman BB. Phosphorylation of cyclic AMP response element binding protein and induction of c-fosgene expression on withdrawal from chronic treatment with carbachol in NG108-15 cells. Mol Pharmacol 1995; 48: 593-600.

32) Loomis TA. Essential of Toxicology. Lea and Febiger: Philladelphia, 1968; pp. 67-78.

33) Zargari A. Medicinal Plants, Vol. 4. Tehran University Press: Tehran, 1990; pp. 1-57.