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Comparison Effects of Methanolic Extracts of Salvia Macrosiphon and Withania Coagulans on Withdrawal Syndrome in Mice

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ABSTRACT

Background*:** It is well clear that repeated use of opioid drugs leads to physical dependence and tolerance. Dependance can be measured by evocation of abstinence sign by abrupt drug withdrawal or administration of a narcotic antagonist or both**.** Effect of Salvia macrosiphon aerial parts extract and Withania coagulans root extract on morphine dependence were investigated in mice. ***Methods: After induction of dependence by morphine, distilled water was injected to the control group and different concentrations of plants aerial and root parts extract were injected to the other groups. To assess morphine withdrawal, mice were injected naloxone (5mg/kg) i.p. on the5th day. After four consecutive days of morphine injection, withdrawal syndrome was assessed by placing each mouse in a 30cm high glass box and recording the incidence of escape jumps for 60 minutes. Animal receiving acute treatment with morphine displayed dependence. ***Results:*** The animals treated with different Salvia macrosiphon aerial (flowered browse) parts extracts concentration could decrease incidence of escape jumps in number or decrease development of morphine dependence and on the other hand, addiction was observed following naloxone administration. ***Conclusion:*** Results of the present study showed that the highest activity in methanolic extract of roots that at 200 mg/kg i.p. inhibited 97% incidence of escape jumps (for 60 minutes).

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.[1,2] Adenosine receptor agonists,[3] excitatory amino acid antagonists,[4,5] protein kinase C inhibitors,[6] glucocorticosteroids,[7] enzodiazepines[8,9] and arachidonic acid[10] can modulate the morphine withdrawal syndrome.

Salvia is an important genus consisting of about 900 species in the Lamiaceae family.[11] They are several reports that some Salvia genesis has effects on the CNS. S. haematodes has CNS-depressant, antinociceptive and anticonvulsant activities.[12,13] The genus, Salvia macrosiphon , is generally known for its multiple pharmacological effects including analgesic and anti-inflammatory activities,[14,15] S. leriifolia has an effect on morphine dependence[16] and hypoglycaemic effects morphine dependency as well.[17]

Investigation on plant, Withania revealed its beneficial effects to decrease dependence sign produced by morphine in comparison to the control. Administration of Withania somnifera *(Ws) (*family: Solanaceae, 100 mg/kg) for 9 days attenuated the development of tolerance to the analgesic effect of morphine (10 mg/kg). Ws (100 mg/kg) also suppressed morphine-withdrawal jumps, 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.[18,19]

The present experiments were undertaken to study the protective effects of Withania coagulan´s root and Salvia macrosiphon aerial methanolic extract on the development of dependence to morphine in mice.

Materials and Methods

Animals

Male albino mice 25-30 g were obtained from a random bred colony, maintained on a special diet in the animal house of Sari University of Medical Sciences.

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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.

Drugs

Distilled water and other drugs, 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.

Plant Material

Aerial parts (flowered browse) of Salvia macrosiphon and Withania coagulans root were collected from Tehran and were identified and confirmed by department of Pharmacognosy (Dr.Gohari). Aerial and root parts of plants 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 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 tween 80 (4:1) for pharmacological studies.

Morphine Dependence

Morphine was injected 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.

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 and the control group was injected distilled water. Then different concentrations of Salvia macrosiphon (100,200,500,1000,1500 mg/kg) and Withania coagulans extract (5,25,50,100,200 mg/kg) were injected respectively to the other groups i.p. 1/5 h after the final dose of morphine.

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 Salvia macrosiphon extract concentration could decrease or increase incidence of escape jumps in number, following naloxone administration. on the other hand , we have defined that the high inhibition of morphine dependence in methanolic extracts of Withania coagulans at 200 mg/kg i.p that can decrease development of morphine dependence. however, mechanism of plants action to Salvia macrosiphon and Withania coagulans to inhibit or decrease abstinence syndrome in dependent mice is unclear. Methanolic extract of Withania coagulans reduced the jumping episodes dose-dependently (Figure 1). The maximum effect of Salvia macrosiphon and Withania coagulans were observed at a dose of 1 g/kg and 200 mg/kg i.p respectively.

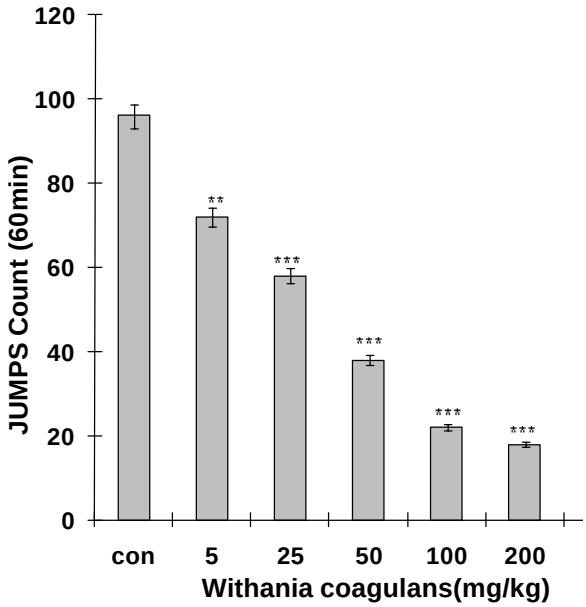

Figure 1. Relation between morphine withdrawal jumps and different concentration of Methanolic withania coagulan extraction
Significant at $p<0.01$(***), $p<0.05$(**). Each value represents mean± S.E.M

Results of the present study showed that the methanolic extract of the aerial parts (flowered browse of S. macrosiphon produced a statistically significant decrease incidence of escape jumps or abstinence syndrome in dependent in mice, in comparison to the control (Figure 2).

Discussion

The present results indicate that the macerated methanolic extract of S.limbata leaves 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. As ATP is broken down to adenosine,[20] it might be possible that the extract decreased morphine dependence by an adenosine mechanism. Further study is needed to confirm this mechanism.

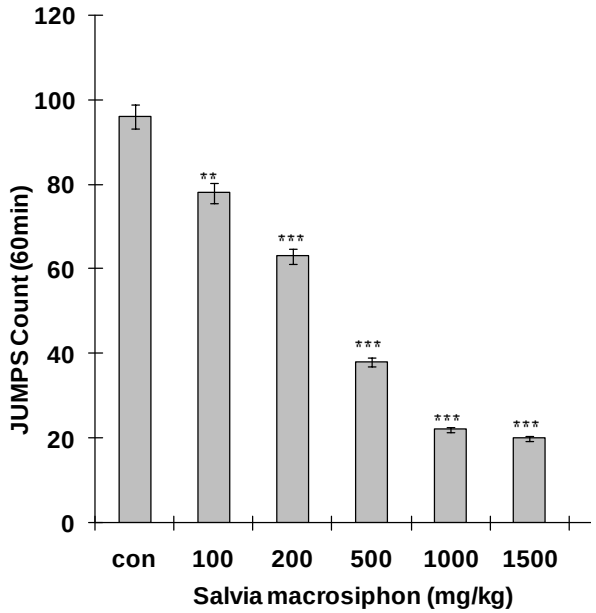

Figure 2. Relation between morphine withdrawal jumps and different concentration of Methanolic S. *Macrosiphon* mbata extraction
Significant at p<0.01(***), p<0.05(**).Each value represents mean± S.E.M

Benzodiazepines, via GABAA 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 Salvia species,[21,22] there is also a possibility that S. macrosiphon 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.[23] It also inhibited the phosphatidylinositol system in acute myocardial ischaemia.[24] 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.[25,26]

In conclusion, the methanolic extract of S. macrosiphon 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 inhibitory effect of naloxone on the antinociceptive activity of extract suggests a morphine-like activity profile for S. macrosiphon. With regard to the LD50 value and in comparison with a toxicity classification,[27] the extract was of low toxicity.

fruit and root of Withania coagulans are used in treating burns and infectious wounds, arthritis, inflammation and rheumatism.[28] According to a study from India Administration of aqueous extract of fruits of Withania coagulans Dunal significantly lowered the blood sugar, serum cholesterol, serum LPO, and hepatic LPO levels at the highest concentration of 1 g/kg; po in streptozotocin induced diabetic rats. In normal rats as well, the blood sugar levels were significantly decreased following treatment with the above drug. Withania coagulans also exhibited free radical scavenging activity in an in vitro system.[29] Two new withanolides, epoxywithanolide I and 17β-hydroxywithanolide K have been isolated from the whole plant of Withania coagulance and their structures were elucidated by spectroscopic techniques to be active against a number of potentially pathogenic fungi.[30] The identification of Polyphenols as biologically active compounds has been previously reported.[31] Withania coaggulans has shown protection against development of dependence by morphine as seen in experiment carried out by Kulakami with another species of some family named.[18,32,33] Recently we have shown the high inhibition of morphine dependence in chloroform and Ethyl acetate extracts of roots of Withania coaggulans. Alcoholic extract of roots of Withania coaggulans was safe and showed a high protective effect. These portions, chloroform and Ethyl acetate extract were moderate protective effect on the development of dependence to morphine in mice.

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