ANTAGONISTIC EFFECTS OF *POLYGALA PLATYPTERA* ON MORPHINE WITHDRAWAL SYNDROME IN MICE

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Summary

In this study, the effect of *Polygala platyptera* extracts on the withdrawal syndrome was evaluated in mice. After inducing morphine dependency, the mice were intraperitoneally injected by various concentrations of *P. platyptera* extracts (ethyl acetate and methanol). Morphine-withdrawal, induced by naloxone, was assessed by recording the incidence of escape jumps for 60 minutes. There was a significant difference between AcOEt group (the doses above 0.5 mg/ kg) and control in a dose-dependent manor. All the concentrations of the methanol extract (except of 5 mg/ kg) produced statistically significant decrease in developing of morphine dependence compared to the control group. Protective effect was not dose-dependent. Therefore, *P. platyptera* have a potency of using for improvement of withdrawal syndrome.

Keywords: Morphine, withdrawal, *Polygala platyptera*, Jumping

Introduction

Nowadays, detoxification and rehabilitation of opioid addicts is an important proposition because drug abuse has caused severe problems for both abusers and society (1). Physical dependence is characterized by a withdrawal syndrome when the opioid is abruptly discontinued, if an opioid antagonist (naloxone) is given, or when drug blood levels fall below a critical level (2). Dopaminergic, adrenergic, excitatory amino acids, purinergic, nitric oxide and serotoninergic systems are the systems involved in the withdrawal syndrome of morphine (3). Herbal drugs in the recent years have gained sufficient importance because of their safety, efficacy and cost effectiveness (4).

Polygala platyptera Bornm.& Gauba, named Shirafza in persian (milkwort), belongs to the Polygalaceae family and has been traditionally used as a remedy for enhancement of milk production in Iran (5). Several species of the genus *Polygala* contained telephiose G, telephiose D, flavonoids (quescetin 3-O-beta-D-glucopyranoside), xanthone glycosides (mangiferin, isomangiferin, polyhongkongenosides A and B), triterpenoid saponin and benzophenone c-glucoside (6-9). Until now, antidepressant components of sucrose ester have been reported from Polygala tenuifolia (10). Also, Tenuifolin, an extract derived from tenuigenin, could inhibit *in vitro* amyloid-beta secretion (11). In addition, Polygalasaponin G, a saponin extracted from *Polygala*, promoted neurite outgrowth of cultured neuron on myelin (12). There is no report about the chemical constituents and pharmacological activities of the plant, *P. platyptera*, which is growing exclusively in Iran. Because of a report about the antagonist effect of *P. telephioides* (growing widely in China) on morphine responses in mice (1), we aim to evaluate the antagonistic effect of *P. platyptera* on morphine withdrawal syndrome in order to support the usage of herbal based opiod withdrawal drugs.

Material and methods

Plant material

Aerial parts of *Polygala platyptera* were collected in July 2008 from Mazandaran province near to Zirab in north of Iran. A voucher specimen was preserved for further reference at the Herbarium of Medicinal Plants Research Center, Tehran University of Medical Sciences, Iran.

Extraction

Air-dried aerial parts of the plant (65 g) were cut in to small pieces and percolated consequently with ethyl acetate and methanol. After filtering, the solutions were concentrated under reduced pressure to gain ethyl acetate (1.5 g) and methanol (16 g) extracts.

Animals

Male albino mice (20-30 g) were prepared from Pasteur institute (Tehran, Iran). The mice kept in animal house under standard condition in 12h / 12h light dark cycle (25 ± 3 °C). The animals received standard pellet diet and water *ad libitum*. Animal handling was performed as per *Good Laboratory Practice*. Research proposal was prepared based on the CPCSEA (Committee for the Perpose of Control and Supervision of Experiments on Animal) and approved by IAEC (Institutional Animal Ethical Committee) of Tehran University of Medical Sciences.

Administration of the plant extracts

The male albino mice were randomly selected and divided in to 11 groups of six in each. In order to render the animals to morphine dependency, All the animals were injected subcutaneously (sc) with morphine at doses of 50, 50 and 7m (mg/ kg) three times daily for three days. Before treating with naloxone, one dose of morphine (50 mg/ kg) was injected to all groups on the forth day. When the morphine dependency was induced, normal saline (3 ml) injected intraperitoneally to control group. Various concentrations (0.5, 2, 5, 10 and 20 mg/ kg) of the AcOEt extract and (5, 25, 100 and 200 mg/ kg) of the MeOH extract were injected to test groups. On the next two hours (after the final administration of morphine), the withdrawal signs were observed by injection of naloxone (5 mg/ kg, sc). Immediately, the number of jumping episode was counted for 60 minutes (13, 14).

Statistical analysis

The data were expressed as Mean ± SEM. One-way ANOVA was used for comparison of the data followed by the multiple comparison Tukey-Kramer test and P values less than 0.05 were considered significant.

Results and Discussion

The effects of *Polygala platyptera* extracts (AcOEt and MeOH) on the morphine-withdrawal jumping in mice are shown in Figures 1, 2. Jumping is a sign of the development of dependence to opioid drugs.

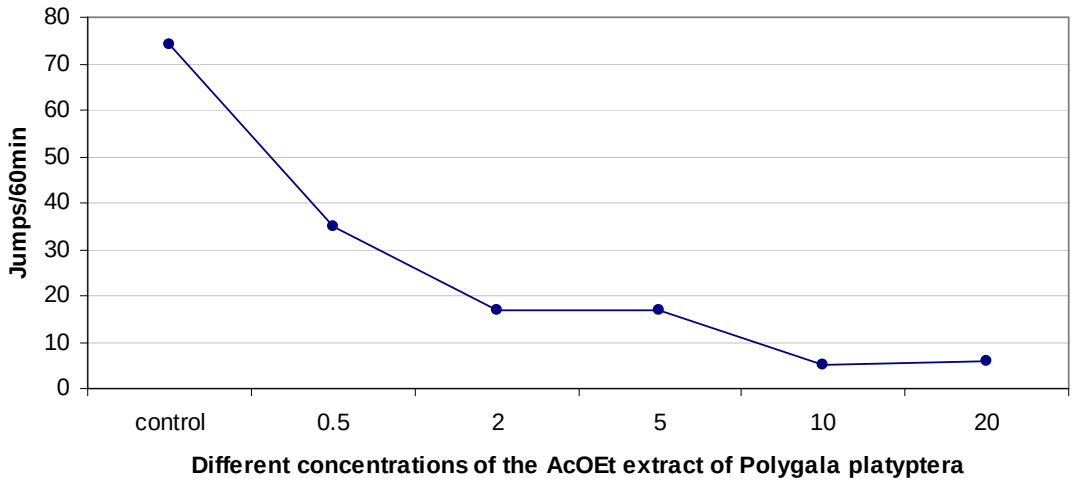

Fig.1. Correlation between morphine withdrawal jumps per 60 min and different concentrations of the plant AcOEt extract. Significant differences between all test (except at dose of 0.5 mg/ kg) and control groups are shown (P value<0.05).

Based on the data resulted from jumping test, there was a significant difference between AcOEt group (at the doses above 0.5 mg/ kg) and control (**Fig.1**). Protective effects were generally dose-dependent as a significant decrease ($p < 0.05$) was observed in the morphine withdrawal jumps by decreasing the concentration of the AcOEt extract. All the concentrations of the methanol extract (except of 5 mg/ kg) produced statistically significant decrease in developing of morphine dependence compared to the control group. Protective effect was not dose-dependent (**Fig.2**). The highest activity was observed at 10 mg/ kg of ethyl acetate extract of *Polygala platyptera* which inhibited more than 93% incidence of escape jumps (for 60 minutes).

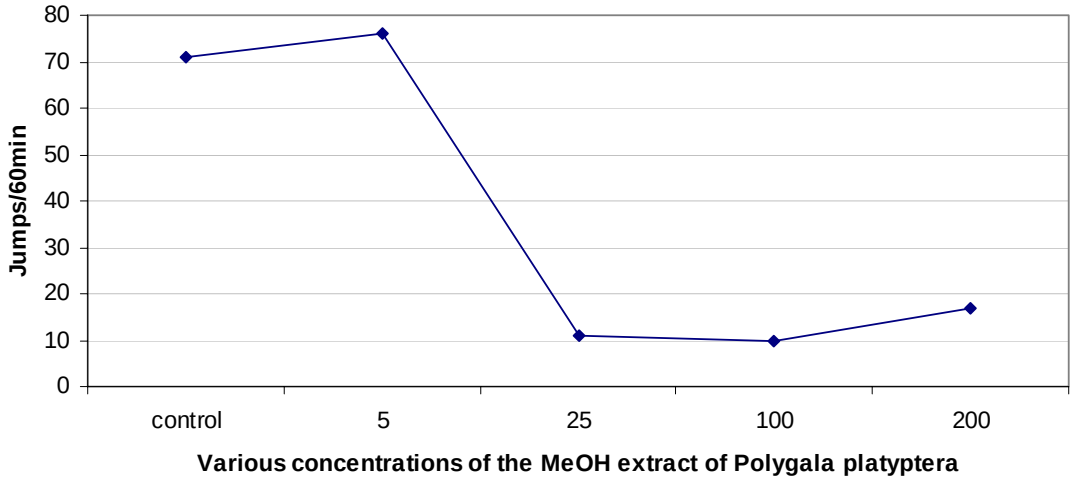

Fig.2. Relation between morphine withdrawal jumps per 60 min and different concentration of the plant methanol extract. Significant differences between test (at all doses except 5 mg/kg) and control groups are shown (P value<0.05).

Previous studies on *P. telephioides* (PT) in China revealed that single administration of PT tended to antagonize the morphine-induced analgesia in a hot-plate test. Moreover, PT (300 mg/ kg, p.o.) improved the morphine-induced memory impairment in an elevated plus maze test. The effects of PT on naloxone-induced jumping, as withdrawal sign, were studied and found that the naloxone-induced jumping was not affected by a single large administration of PT. It seems that the inhibitory effect of PT on the jumping was due to the development of dependence rather than expression of withdrawal sign. Regarding to falling the morphine levels down in plasma by single administration of PT (300 mg/ kg, p.o.), *P. telephioides* may be useful in facilitating narcotic detoxification (1).

There are several documents indicating the involvement of the central glutamatergic system in morphine dependence (15) for example benzodiazepines showed inhibitory effect on the dependence on morphine via $GABA_A$ receptors (16). Recently, *Polygala tenuifolia* has been reported to prevent cocaine-induced behavioral effects via the activation of the adenosine A_{2A} receptor. A_{2A} receptors are almost exclusively located on striatopallidal GABAergic neurons. The stimulation of A_{2A} receptors increases the release of GABA in the striatum and globus pallidus and counteracts the dopamine D2 receptor-mediated effects (17). In our study, it is possible that the effect of *P. platyptera* on morphine dependence has a relation with GABA receptors.

In conclusion, the ethyl acetate and ethanol extracts of *P. platyptera* could suppress morphine withdrawal syndrome. The results of this study are the first steps towards finding the exact mechanism of action which might be involved in the inhibitory effect of the extracts on morphine dependency.

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