EFFECT OF WITHANIA COAGULANTS ROOT EXTRACT ON THE WITHDRAWAL SYNDROME IN MICE

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Summary

Effect of *withania coagulans* root extract on the withdrawal syndrome was determined in mice. After induction of dependence by morphine, mice were intraperitoneally administered different concentrations of *withania coagulans* root extract. Morphine-withdrawal, induced by naloxone, was assessed by recording the incidence of escape jumps for 60 minutes Administration of *Withania coagulants* root extract significantly (P<0.05) suppressed morphine-withdrawal jumps and decreased development of morphine dependence. We can therefore conclude this plant can be used successfully in treatment of addiction.

Keywords: Morphine, *Withania coagulans*, withdrawal, Jumping.

Drug abuse is a serious social problem in most parts of the world with the opium addiction being the most widespread type in Asia and Europe. The alleviation of the withdrawal syndrome is a challenge to combat addiction. Many different pharmacological categories have been used to alleviate the morphine withdrawal syndrome. These include noradrenergic system, adenosine receptor agonists, excitatory amino acid antagonists, protein kinase C inhibitors, glucocorticosteroids, benzodiazepines, arachidonic acid, tetrahydrocannabinoids and cholecystokinin antagonists (1). Withania coagulans Dunal belongs to family Solanaceae. It is distributed in the East of the Mediterranean region and extend to South Asia (2). Different parts of this plant have been reported to possess a variety of biological activities. Its berries are used for milk coagulation. The fruits are reported to be sedative, emetic and diuretic. Fruit has been shown to exert hepatoprotective, anti-inflammatory activity and hypoglycemic activity (3-5). They are useful in dyspepsia, flatulent colic and other intestinal infections. They are employed for the treatment of asthma, biliousness and stranguary. The berries are used as

a blood purifier. It is well known in the indigenous system of medicine for the treatment of ulcers, rheumatism, dropsy, consumption and sensile debility (6). Antifungal and antibacterial properties have also been demonstrated in extract of the whole plant and leaves (7-8). Investigation on plant, *Withania coagulans* revealed its beneficial effects to decrease dependence sign produced by morphine (9). The present experiments were undertaken to study the protective effect of *Withania coagulan´*s root extract on the development of dependence to morphine in mice.

Materials and methods

Animals

Swiss Albino mice of either sex (n = 10, 25-30g) were used. Animals were housed under standard condition of temperature (25 ± 2°C), 12h/12h light dark cycles and fed with standard pelleted diet and water was given *ad libitum.* Animal handling was performed as per *Good Laboratory Practice*. A research proposal was prepared according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional Animal Ethical Committee (IAEC) of Mazandaran University of medical sciences approved the proposal.

Plant material

Roots were collected from Mazandaran in Nov 2005 and dried in shade followed by grinding. The *withania coagulans* was identified by Dr Gohari, Herbarium of Tehran University and voucher samples were preserved for reference at the herbarium in Department of Pharmacognosy, School of Pharmacy, Tehran, Iran (0506-15).

Preparation and fractionation

Air-dried root powder was fractionated by solvent extraction at 48 hrs. (3 times) by percolation with ethyl acetate, chloroform and finally methanol successively. The mixture was filtered and the filtrate was concentrated to dryness. For pharmacological studies the residue was prepared in phosphate buffer (pH=7.4) and Tween 80 (4:1).

Morphine dependence

Morphine was injected subcutaneous (s.c.) to mice at doses of 50, 50 and 75 mg/kg three times daily (10:00, 13:00 and 16:00 h, 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 elicited by the 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 height, 20 cm in diameter). The number of jumping episodes was counted for 60 min after naloxone injection.

Drug and fractions treatment

After induction of dependence by morphine, mice are divided in to 7 groups. To the control group distilled water was injected (10 ml/ kg, ip.) and to the other groups different concentrations of root extract were injected (5, 25, 50, 100, 200 mg/kg, ip.) 0.5 h after the final dose of morphine. Withdrawal syndrome was assessed by counting the number of jumping episodes for 60 min after naloxone injection (1, 10-11).

Statistical analysis

Statistical analysis was performed using SPSS for Windows (ver.10, SPSS Inc., Chicago, USA). Data were analyzed by one-way analysis of variance (ANOVA) followed by the multiple comparison test of Tukey–Kramer and presented as Mean ± Standard error (SEM). In all cases $p < 0.05$ was taken as statistically significant.

Results

Effect of *withania coagulans* root extracts on the morphine-withdrawal jumps, a sign of the development of dependence to opiate is shown in figures 1, 2 and 3. All extracts (ethyl acetate, chloroform and methanol extracts) produced statistically significant decrease in development of morphine dependence compared to the control groups. Protective effect was generally dose-dependent. The highest activity showed in methanol and chloroform extract of roots that at 200 mg/kg i.p. inhibited 97% and 95% incidence of escape jumps (for 60 minutes), respectively.

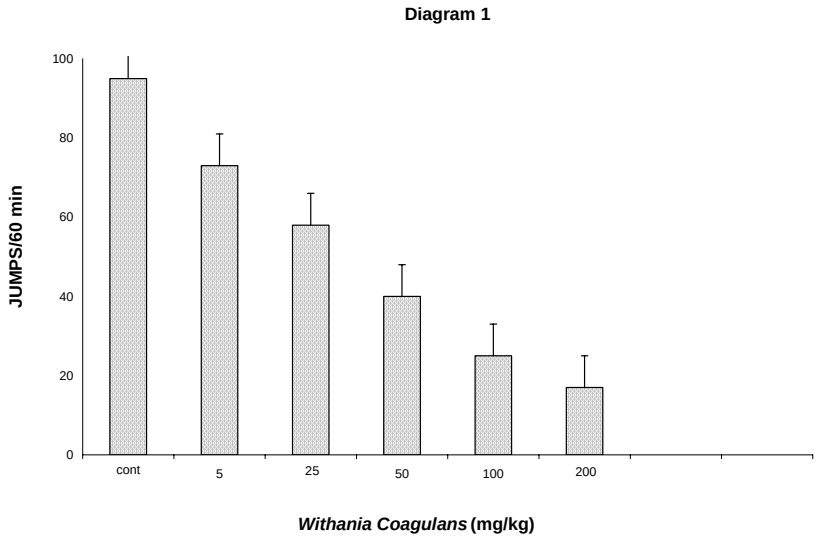

Figure 1: Relation between morphine withdrawal jumps and different concentration of plant methanolic extraction. A significant decrease ($p < 0.05$) was observed in the morphine withdrawal jumps by decreasing the concentration of root extract. Error bars indicate the standard error on the mean.

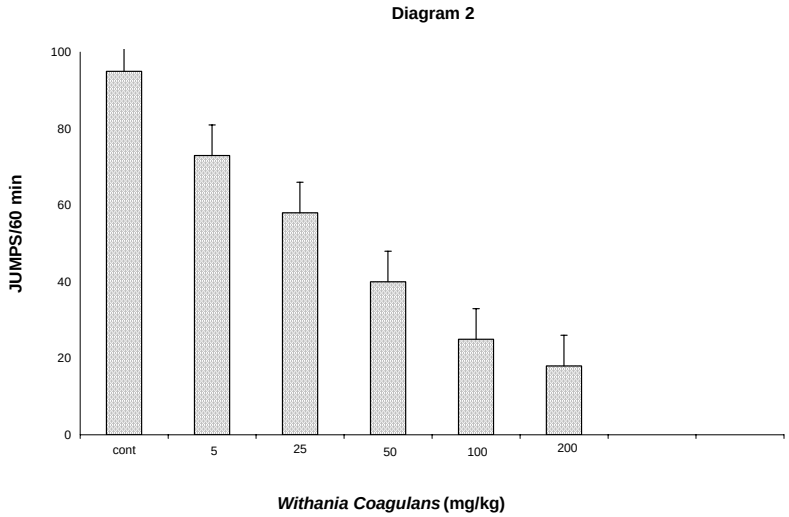

Figure 2: Relation between morphine withdrawal jumps and different concentration of plant chloroform extraction. A significant decrease ($p < 0.05$) was observed in the morphine withdrawal jumps by decreasing the concentration of root extract. Error bars indicate the standard error on the mean.

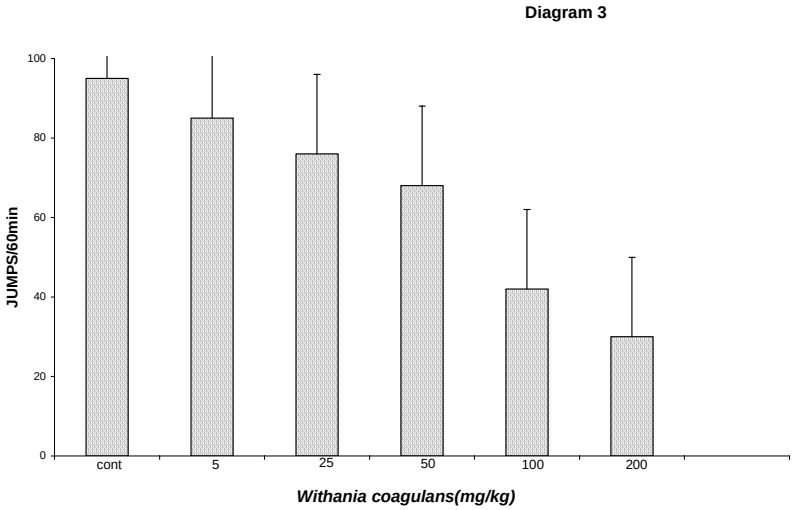

Figure 3: Relation between morphine withdrawal jumps and different concentration of plant Ethyl acetate extraction. A significant decrease ($p < 0.05$) was observed in the morphine withdrawal jumps by decreasing the concentration of root extract. Error bars indicate the standard error on the mean.

Discussion

The present results indicate that the Withania coagulants extract has component(s) that could alleviate the morphine withdrawal syndrome. The activity-guided fractionation showed that the some active principle(s) were polar as the highest activity was observed in the methanol fraction and some nonpolar components as the latter nonpolar fraction (chloroform) still showed high activity. Nearly all extracts produced statistically significant decrease development of morphine dependence when compared to the control groups. Protective effect was generally dose-dependent. The highest activity showed in methanolic and chloroform extract of roots that at 200 mg/kg i.p. inhibited 97% and 95% incidence of escape jumps (for 60 minutes) respectively. There is one report about protective effect of *Withania somnifera* against development of dependence by morphine (12). Recently we have shown the high inhibition of morphine dependence by *Withania coaggulans* (9). The results of this study might be valuable in searching components having inhibitory effect on morphine dependency. The structural characterization, potency evaluation and detailed mechanistic studies of compounds present in Withania coagulants methanolic fraction will be carried out later.

Acknowledgments

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