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Suppressive Effects of the Aerial Parts of *Datura Stramonium* L. Extract on Naloxone-Precipitated Morphine Withdrawal Signs in Mice

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ABSTRACT

Background & Objective: Datura stramonium L. is a medicinal herb from the family of Solanaceae. It has been used in herbal remedies for promoting health and treating several diseases. The current study was set up to compare the effects of Datura stramonium L. extract on the naloxone-precipitated opiate-withdrawal in mice.

Materials & Methods: Male BALB/c mice (30–35 g, n = 40) were arbitrarily separated into 4 groups. The control group received morphine and normal saline and other groups received three doses of D. stramonium extract (10, 20, or 30 mg/kg, intraperitoneally, i.p.). Physically dependent was made by the administration of morphine in increasing doses (50-75 mg/kg, i.p.). The withdrawal signs were elicited by intraperitoneal injections of naloxone (5 mg/kg) 2 h after the last injection of morphine.

Results: Administration of D. stramonium extract in doses of 20 and 30 mg/kg markedly diminished the jumping numbers compared to the control group ($P<0.05$). All three doses of D. stramonium extract could significantly suppress the increase in climbing ($P<0.05$, $P<0.001$, and $P<0.001$, respectively) and diarrhea ($P<0.001$). D. stramonium in higher doses (20 or 30 mg/kg) significantly decreased rearing and itching ($P<0.001$).

Conclusion: The study findings suggest that D. stramonium extract is effective in alleviating the signs of morphine withdrawal. Additional research is needed to determine the exact mechanisms underlying D. stramonium for inhibiting morphine withdrawal syndrome.

Keywords: Morphine Withdrawal Signs, Naloxone, Datura stramonium extract, Opioid Addiction

Introduction

Opioids, including morphine, are the most powerful medicines now available for pain management; however, morphine abuse often results in various behavioral and physiological effects. The phenomenon of withdrawal is one common feature of many abused drugs caused by the abrupt cessation of drug administration (1). Thus, the abstinence syndrome that results from finishing of the drug is called withdrawal abstinence (2).

Sudden cessation of morphine usage or precipitation via the systemic injection of an opioid receptor antagonist will lead to the appearance of signs and symptoms of withdrawal, such as diarrhea, rhinorrhea, abdominal cramping, increased blood pressure, sweating, insomnia, elevated heart rate, dysphoria, irritability, and hyperalgesia. The initiation and longevity of the withdrawal signs differ and they are believed to be associated with the pharmacokinetics of the opioid. Indeed, it has been demonstrated that withdrawal syndrome appeared in 24 h and lasted for 7–10 days (3). Despite considerable research to date, the complexity of opioid addiction and withdrawal is unclear and the prospects for easy solutions are lacking. Currently, buprenorphine, methadone, α 2-adrenoceptor agonists (e.g. clonidine) are appropriate for opioid detoxification. In addition, naltrexone (an opioid antagonist) is also consumed (4). However, different types of medications are used in the treatment of opioid dependence have main limitations in safety and efficacy. Therefore, the search for novel agents that are efficacious against opioid

dependence and withdrawal syndrome has become increasingly important. From this point of view, the natural extracts, which are optioned by medicinal plants, are already being used for the treatment of drug addictions and withdrawal management (5, 6). It has been previously reported that medicinal plants such as Hypericum perforatum, Avena sativa, Passiflora incarnata, and Valeriana officinalis have analgesic, antispasmodic, antianxiety, and hypnotic effects and can improve the symptoms of morphine withdrawal (7). Datura stramonium L. is an annual herb from a family of Solanaceae that grows wild in various regions, especially in temperate areas of the world. In traditional medicine, the flowers and leaves of the plant are applied in the treatment of Asthma. Interestingly, D. stramonium is used as a sedative and hallucinogenic agent (8). The analysis of the phytochemicals of the plant exhibited that D. stramonium comprised of atropine, hyoscyamine, and scopolamine. Previous studies reported that D. stramonium has diverse biological features including anti-inflammatory, antioxidant, and anticancer activities (9). As suggested by these properties, growing evidence indicates neuroprotective actions of Datura against a number of insults (10, 11). However, the behavioral effects of plant extracts of D. stramonium on the withdrawal syndrome are not clearly understood. Therefore, D. stramonium was hypothesized to reduce the intensity of morphine withdrawal syndrome in rats.

Materials and Methods

Materials

Morphine sulfate was provided by Temad Company, Tehran, Iran. Naloxone hydrochloride was kindly provided by Tolid Daru Company, Tehran, Iran.

Preparation of extract

Datura stramonium L. was purchased from a local herbal shop at Mashhad, Khorasan Razavi Province, Iran. Then was identified by the Herbarium of School of Pharmacy at Mashhad University of Medical Sciences, Mashhad (Voucher specimen: 13261). To prepare a hydro-alcoholic extract, 100g of the dried aerial parts of the plant including seed, calyx, and petal was powdered and mixed with 70% ethanol in a Soxhlet apparatus for 48h. The process is as follows: first, the dried and ground aerial parts of the plant were packed in a thimble whit filter paper. Solvent or 70% ethanol was added to balloon volume which was gradually heated through the heater. Finally, the solvent began to evaporate. The resulting vapors flow to the condenser and the distillation operation began in this part. Drops of solvent condensate from the condenser were poured on the plant sample. Under these conditions, the solvent was in direct contact with the plant and the extraction operation began. This cycle continued until the solvent color was clear inside the extraction chamber, or in other words, the condition for stopping the extraction process was that the solvent was clear in the extraction chamber. The obtained extract was then filtered and dehydrated in a water bath and kept at -20°C.

Animals

Male BALB/c mice (6–8 weeks of age, 30–35 g, from the animal house of Mashhad University of Medical Sciences, Mashhad, Iran) were kept in pathogen-free conditions, with a 12/12-h light/dark cycle with food and water available ad libitum. All experiments were approved by the Animal Research Group of Mashhad University of Medical Sciences (NO: IR.MUMS.MEDICAL.REC.1398.402.). All efforts were made to minimize both the number of animals used and the suffering caused.

Induction of morphine dependence

Continual subcutaneous injections of morphine in doses of 50, 50, and 75 mg/kg three times daily at 9 a.m. (50 mg/kg), 1 p.m. (50 mg/kg), and 5 p.m. (75 mg/kg) for three days were done for the generation of morphine dependence in animals. On the fourth day, to prevent overnight withdrawal syndrome in mice only a single dose (50 mg/kg) of morphine was used 2 hours before injection of naloxone (12).

Naloxone-precipitated withdrawal scoring

Two hours after the injection of the last dose of morphine on the fourth day, a single intraperitoneal dose of naloxone (5mg/kg, i.p.) was injected into all mice. Following naloxone administration, animals were located individually in a plexiglass cylinder (30 cm diameter, 30 cm high). Then withdrawal signs in animals including jumping, climbing, itching, rearing, and the number of times of diarrhea were recorded with a camera for 30 minutes and analyzed by the researcher. The number of symptoms of opioid withdrawal for each mouse was summed over 30 min (12).

Experimental design and animal grouping

40 adult male mice were randomly assigned to the four groups (10 per group): the control group, Datura stramonium L. (10 mg/kg), Datura stramonium L. (20 mg/kg), and Datura stramonium L. (30 mg/kg) (13). In the Datura treatment groups, Datura stramonium L. was administered 30 min before the injection of the last dose of morphine (50mg/kg, i.p.) on the fourth day, whereas the control group was treated with an equal among of normal saline.

Statistical analysis

Data were expressed as the mean ± SEM. All analysis was carried out using SPSS software version 19 for Windows. Mean values were appropriately calculated and compared using a one-way analysis of variance (ANOVA) followed by the Bonferroni test. $P<0.05$ was considered statistically significant.

Ethical Considerations

Ethical approval of our study was gotten by the ethical committee of Mashhad University of Medical Sciences (MUMS) with ethic code number: IR.MUMS.MEDICAL.REC.1398.402.

Results

In morphine-dependent mice, i.p. injection of naloxone elicited withdrawal signs. As shown in Figure 1, the jumping sign was induced when naloxone was applied, however, treatment with different doses of D. stramonium extracts showed a significant decrease in naloxone-induced jumping. In doses of 20 and 30 mg/kg of the extract, the occurrence of jumping significantly diminished compared to the control group (P<0.05). In addition, Datura extract in doses of 20 and 30 mg/kg significantly reduced the jumping more compared to 10 mg/kg of extract (P<0.05), (Figure 1). All doses of the extract significantly reduced climbing in comparison with control (P<0.05, P<0.001, and P<0.001, respectively). Just 20 and 30 mg/kg of the extract significantly reduced climbing compared with 10 mg/kg of Datura extract (P<0.05 and P<0.001, respectively), (Figure 2). Only 20 and 30 mg/kg of the extract significantly decreased rearing versus Control (P<0.001). In addition, both 20 and 30 mg/kg showed a significant difference revealing more decrease (P<0.001), (Figure 3). Itching was also similar to rearing, and 20 and 30 mg/kg of Datura were lesser than control (P<0.001) and 10 mg/kg of Datura extract (P<0.001), (Figure 4). The rate of diarrhea was lower in three doses of Datura extract versus control (P<0.001). There are no significant differences between doses of 20 and 30 mg/kg in comparison to 10 mg/kg of extract, (Figure 5).

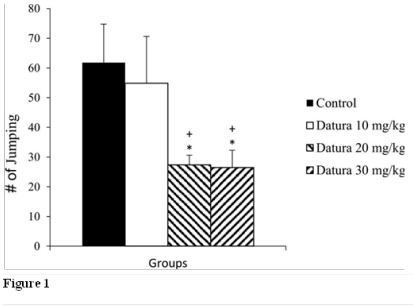

Figure 1. Effect of the various doses of D. stramonium on naloxone-precipitated jumping in morphine-dependent mice. The results were expressed as mean ± SEM (N = 10), *P<0.05 versus the control group and + P<0.05 versus D. stramonium 10 mg/kg group.

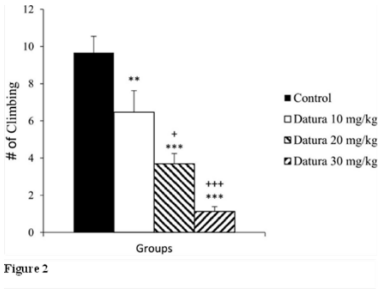

Figure 2. Effect of the various doses of D. stramonium on naloxone-precipitated climbing in morphine-dependent mice. The results were expressed as mean ± SEM (N = 10), **P<0.01, ***P<0.001 versus control group and +P<0.05, +++P<0.001 versus D. stramonium 10 mg/kg group.

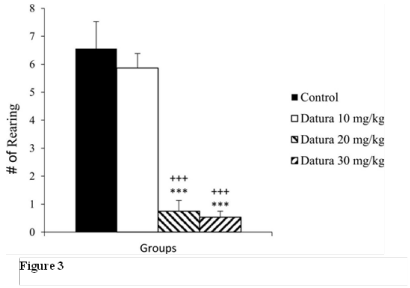

Figure 3. Effect of the various doses of D. stramonium on naloxone-precipitated rearing in morphine-dependent mice. The results were expressed as mean ± SEM (N = 10), ***P<0.001 versus control group and +++P<0.001 versus D. stramonium 10 mg/kg group.

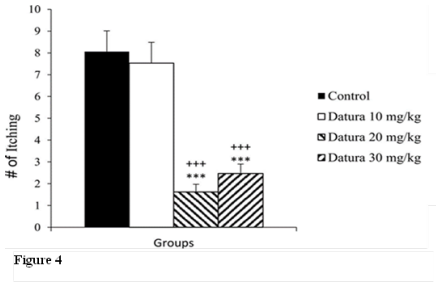

Figure 4. Effect of the various doses of D. stramonium on naloxone-precipitated itching in morphine-dependent mice. The results were expressed as mean ± SEM (N = 10), ***P<0.001 versus control group and +++P<0.001 versus D. stramonium 10 mg/kg group.

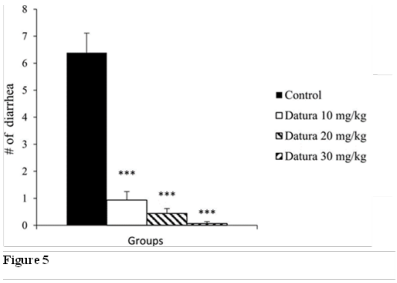

Figure 5. Effect of the various doses of D. stramonium on naloxone-precipitated diarrhea in morphine-dependent mice. The results were expressed as mean ± SEM (N = 10), ***P<0.001 versus the control group.

Discussion

The current study is the first report describing the effect of D. stramonium extract on the withdrawal signs in morphine-dependent mice. This study indicates escalating doses of morphine over a four-day period followed by injection of naloxone induces some morphine abstinence behavior in mice. This animal

model was conducted to investigate the potency of the extract to diminish morphine withdrawal behaviors such as jumping, rearing, climbing, itching, and diarrhea. In the present investigation, all doses of D. stramonium extract 1 hour after the last dose of morphine exhibited an inhibitory effect against withdrawal symptoms of morphine in mice. The cause and the mechanisms underlying the beneficial effects of the D. stramonium extract on the withdrawal signs in morphine-dependent mice were not examined in the current study and need to be explored in details in future studies. Nevertheless, several possible mechanisms could be postulated to explain these findings. The phytochemical studies showed that D. stramonium had atropine, tropane alkaloids, and scopolamine **(14)**. It has been revealed that the alcoholic extract of D. stramonium extract has analgesic properties, which was probably mediated by reinforcing the opioid system **(15)**. This suggests that the extracts of D. stramonium may cooperate with the opioid system, a theory that is confirmed by the evidence that the antinociceptive effects of the extracts were suppressed by the opioid blocker naloxone.

Some studies revealed that alkaloids like tannins, saponins, and glycosides, which are principal bioactive constituents of most plants, could be responsible for several plant biological activities. Phytochemical investigations revealed that the extract of D. stramonium included saponins, alkaloids, steroids, tannins, phenols, flavonoids, and glycosides **(11)**. The findings of our study are in line with the results of prior research that has shown that polyphenolic compounds exert a suppressive effect on morphine withdrawal signs **(16)**. Thus, it may be expected that the flavonoids, the main component of D. stramonium, are effective in decreasing the severity of the withdrawal signs in morphine-dependent animals.

It is possible to speculate that the effect of D. stramonium extracts in attenuating morphine withdrawal behavior is relevant to its antidepressant proprieties **(17)**. It has been described that treatment with extract of Datura fastuosa elicits a robust antidepressant profile in low doses and acts as an antidepressant agent **(18)**. In this regard, it is believed that the D. stramonium extracts could enhance bioavailability and increase neurotransmission of serotonergic, noradrenergic, and monoaminergic systems in the brain. In line with this, activation of these systems has been implicated in limiting the severity of morphine withdrawal **(19)**. Besides, other mechanisms may also be involved in reducing withdrawal syndrome. D. stramonium via scopolamine, a constituent in the plant, acts as an antagonist of muscarinic cholinergic receptors **(20)**. For example, Large and colleagues have mentioned that brain levels of ACh are elevated during withdrawal **(21)**. Thus, the extract may modulate withdrawal syndrome by this mechanism. Furthermore, in an experimental study, evidence showed that injections of morphine for several days to mice led to induction of oxidative stress in brain tissues **(21)**. In addition, administration of naloxone in morphine-dependent animals produced a significant reduction in the activity of brain intracellular antioxidant enzymes and notable elevations of malondialdehyde (MDA) concentration in the brain **(21)**. In addition, it has been reported that the D. stramonium leaf extracts showed an effective antioxidant activity **(22)**. In support of this hypothesis, it will probably be considered that the D. stramonium extract attenuated morphine withdrawal by an antioxidant mechanism.

Conclusion

In summary, the present study for the first time demonstrated that D. stramonium extract could prevent some major signs of morphine withdrawal in animal models of addiction in mice. However, the exact mechanism by which D. stramonium extract promotes its beneficial effects is not completely clear and further clinical and biochemical studies are needed to verify its precise mechanism of action.

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Conflict of Interest

The authors declare no potential conflicts of interest.

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