PHARMACOLOGICAL EVALUATION OF TINOSPORA CORDIFOLIA ON NICOTINE DEPENDENCE IN MICE

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Abstract

The aim of the present study was to investigate the effects of a hydroalcoholic extract of Tinospora cordifolia on the prevention of the development of nicotine dependence and for the reduction of abstinence suffering following nicotine cessation in mice. Nicotine dependence was induced in mice by subcutaneous injections of nicotine (2mg/kg, s.c., 4 times/day) for seven days. Spontaneous abstinence syndrome was evaluated on 8th day, after the last nicotine administration followed by mecamylamine (3 mg kg−1, i.p.), by analysis of withdrawal signs. The hydroalcoholic extract of Tinospora cordifolia (100 and 400 mg/kg, p.o) was administered during nicotine treatment. Results showed that somatic signs are abolished by administration of Tinospora cordifolia hydroalcoholic extract in a dose-dependent fashion. In conclusion, our data encourage additional studies to define the use of Tinospora cordifolia extract as a therapeutic approach in the treatment of smoking cessation.

Keywords: Giloy; Tinospora cordifolia; Nicotine; Abstinence; Withdrawal; Somatic signs; Dependence.

Introduction

Nicotine addiction is the second-leading cause of death worldwide. The estimated numbers of individuals smoking ranges in developing countries with a percentage of 50 % men and 9 % women. Whereas, the developed countries includes the individuals with 35% of men and 22% of women addicted to smoking (Preedy, 2019; Pacek et al., 2019). Smoking is one the most common addiction causing deaths around 480,000 deaths in the United States by increasing risk factor of causing certain diseases like cancer, cardiovascular disease, and pulmonary disease, infections etc (Preedy, 2019; Perez-Rubio et al., 2019; Pratt et al., 2019). Despite of the advances in allopathic medicine, there is no available effective medicine encouraging the development of herbal extracts that have shown to have protective effect in nicotine addiction (Ozarowski et al., 2013; Tran et al., 2019; Saleh et al., 2019). The current available drug therapies only aid smoking cessation and ameliorating the signs and symptoms of acute nicotine withdrawal (Murthy, 1993; Sohn et al., 2003; Kozlowski et al., 2007; Ozarowski et al., 2013; Kathuria et al., 2018). Additionally, it is well established that the extensive and repetitive use of the psychoactive substances like nicotine, alcohol etc (Macedo et al., 2019; Prasad et al., 2019; Malinska et al., 2019; Kangiser et al., 2019). Therefore, this study serves as a preliminary screening of antioxidant constituent in Tinospora cordifolia which, in the future, may be included as a regimen for nicotine addiction and its withdrawal symptoms. Studies have validated Tinospora cordifolia extract as effective possible antioxidant treatment for other disease like parkinson; cancer and hepatotoxicity (Rashmi et al., 2019; Birla et al., 2019; Karamalakova et al., 2019). In this experiment, we have assessed the neuroprotective activity of hydroalcoholic extract of Tinospora cordifolia in nicotinictreated albino mice by measuring various behavioural parameters and abolishment of nicotine dependence induced withdrawal symptoms. The current study also attempted to propose a possible mechanism of activity by studying the various behavioral symptoms induced by withdrawal of the nicotine.

Materials and Methods

Preparatory actions

Authentication of plant material Preparatory actions

Tinospora cordifolia (Menispermaceae) was collected from Andhra Pradesh. The plant material has been identified and authenticated by Dr. K. Madhava Chetty; Assistant Professor & Assistant Dean in Sri Venkateswara University, Tirupati, India. The reference number of specimen was drug Authentication SVU/SC/22/47/18-19.

Extraction of Tinospora cordifolia

The stem of the Tinospora cordifolia was collected washed, dried at room temperature and grinded into powder. The powder was extracted in 70:30 ratio of hydroalcoholic
extract using a Soxhlet apparatus for 48 hours and concentrated in a rotary evaporator to form syrup like consistency. The obtained extract was stored in the refrigerator at 4°C. The percentage yield for the extract was 18.5%.

Animals used

Swiss albino mice weighing 25±2g obtained from Central Research Institute, Kasauli, India, maintained on standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and having free access to tap water were employed in the present study. They were housed in the departmental animal house and were exposed to a regular 12 hr cycle of light and dark. The experiments were conducted in a semi-sound proof laboratory. The observer was blind to the treatment group assignment. The experimental protocol was approved by the institutional animal ethical committee and care of the animals was done as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. 1181/PO/Ebi/08/CPCSEA).

Drugs and chemicals:

The chemicals used were of analytical grade and all drug solutions were freshly prepared before use.

Laboratory procedures:

Induction of nicotine withdrawal syndrome in mice:

Sub-acute administration of nicotine followed by a single injection of mecamylamine was used to induce nicotine withdrawal in mice (Damaj et al., 2003; Biala and Weglinska, 2005; Singh et al., 2013 a, b; Rehni et al., 2012). Nicotine dependence was induced by repeated s.c. injections, four times daily, at an interval of 4 h starting for 7 days (2.5 mg/kg, s.c).

Assessment of withdrawal severity score (WSS):

A set of withdrawal severity score was employed to quantitate the magnitude of withdrawal syndrome in mice (Rehni et al., 2012; Singh et al., 2013 a, b).

Assessment of nicotine withdrawal syndrome in terms of jumping frequency:

Jumping frequency observed in a period of 30 min was used as a quantitative symptom of Nicotine withdrawal immediately after mecamylamine administration (Biala and Weglinska, 2005; Singh et al., 2013 a, b).

Assessment of nicotine withdrawal syndrome in terms of piloerection frequency:

Piloerection frequency observations were made for a period of 30 min after mecamylamine administration (Singh et al., 2013 a, b).

Assessment of nicotine withdrawal syndrome in terms of body tremor frequency:

Body tremor frequency observations were made for a period of 30 min after mecamylamine administration (Singh et al., 2013 a, b).

Experimental Design:

The current study protocol is of 7 days and was employed with five groups comprising of 06 animals in each group.

Group I (Vehicle-vehicle control):

Vehicle (Saline, 10 ml/kg, i.p.) for Nicotine was administered four times daily for a period of 7 days. Vehicle (Saline, 10 ml/kg, i.p.) for Tinospora cordifolia was simultaneously injected once daily for the same period of 7 days. Vehicle (10 ml/kg, i.p.) for Mecamylamine was then injected on the morning of day 8, 1 hr after administering vehicle (Saline, 10 ml/kg, i.p.) for nicotine.

Group II (Vehicle-mecamylamine control):

Vehicle (Saline, 10 ml/kg, i.p.) for Nicotine was administered four times daily for a period of 7 days. Vehicle (Saline, 10 ml/kg, i.p.) for Tinospora cordifolia was simultaneously injected once daily for the same period of 7 days. Mecamylamine (3 mg/kg, i.p.) was then injected on the morning of day 8, 1 hr after administering Vehicle (Saline, 10 ml/kg, i.p.) for nicotine.

Group III (Nicotine–Mecamylamine control):

Nicotine (2.5 mg/ kg, s.c.) was administered four times daily for a period of 7 days. Vehicle (Saline, 10 ml/kg, i.p.) for Tinospora cordifolia was simultaneously injected once daily before 1 hour of the nicotine for 7 days. Mecamylamine (3 mg/kg, i.p.) was then injected on the morning of day 8, 1 hr after administering Nicotine (2.5 mg/ kg, s.c.).

Group IV (Tinospora cordifolia 100 mg/kg+ nicotine-mecamylamine control):

Vehicle (Saline, 10 ml/kg, i.p.) for nicotine was administered four times daily for a period of 7 days. Tinospora cordifolia (100 mg/kg, p.o.) was administered once daily before 1 hour of the nicotine for 7 days. Mecamylamine (8 mg/kg, i.p.) was then injected on the morning of day 8, 1 hr after administering Vehicle (Saline, 10 ml/kg, i.p.) for nicotine.

Group V (Tinospora cordifolia 400 mg/kg+ nicotine-mecamylamine):

Nicotine (2.5 mg/ kg, s.c.) was administered four times daily for a period of 7 days. Tinospora cordifolia (400 mg/kg, p.o.) was administered once daily before 1 hour of the nicotine for 7 days. Mecamylamine (3 mg/kg, i.p.) was then injected on the morning of day 8, 1 hr after administering Nicotine (2.5 mg/ kg, s.c.).

Statistical analysis

All the results were expressed as mean ± standard error of mean (S.E.M.). Data of the results was analyzed using ANOVA followed by post-hoc comparison using Sheffe’s multiple range test. A value of P<0.05 was considered to be statistically significant. The statistical analysis was done using Sigma Stat 6.0 software.

Results and Discussion

Effects of hydroalcoholic extract of Tinospora cordifolia on withdrawal severity score in nicotine dependent mice

Administration of hydroalcoholic extract of Tinospora cordifolia (100 & 400 mg/kg, p.o.) significantly (p<0.05) and dose dependently attenuated nicotine-mecamylamine induced withdrawal syndrome in mice, when compared to that of the nicotine/ mecamylamine treated group (Figure 1).
Effects of hydroalcoholic extract of *Tinospora cordifolia* on jumping behavior in nicotine dependent mice:

Administration of hydroalcoholic extract of *Tinospora cordifolia* (100 & 400 mg/kg, p.o.) significantly (p<0.05) and dose dependently attenuated mecamylamine induced stereotyped jumping behavior in mice, when compared to that of the nicotine/ mecamylamine treated group (Figure 2).

![Figure 2: Effect of treatment(s) on mecamylamine induced jumping frequency in mice.](image1)

**Figure 2:** Effect of treatment(s) on mecamylamine induced jumping frequency in mice.  
[Values are mean ± S.E.M.]  
a = P<0.05 vs. VEH-VEH control;  
b = P<0.05 vs. NIC-VEH-MEC.  
VEH: Vehicle; NIC: Nicotine; MEC: Mecamylamine.

Effects of hydroalcoholic extract of *Tinospora cordifolia* on body tremor in nicotine dependent mice:

Administration of hydroalcoholic extract of *Tinospora cordifolia* (100 & 400 mg/kg, p.o.) significantly (p<0.05) and dose dependently attenuated mecamylamine induced body tremor behavior in nicotine dependent mice, when compared to that of the nicotine/ mecamylamine treated group (Figure 3).

![Figure 3: Effect of treatment(s) on mecamylamine induced body tremor in mice.](image2)

**Figure 3:** Effect of treatment(s) on mecamylamine induced body tremor in mice.  
[Values are mean ± S.E.M.]  
a = P<0.05 vs. VEH-VEH control;  
b = P<0.05 vs. NIC-VEH-MEC.  
VEH: Vehicle; NIC: Nicotine; MEC: Mecamylamine.

Effects of hydroalcoholic extract of *Tinospora cordifolia* on piloerection behavior in nicotine dependent mice:

Administration of hydroalcoholic extract of *Tinospora cordifolia* (100 & 400 mg/kg, p.o.) significantly (p<0.05) and dose dependently attenuated mecamylamine induced piloerection behavior in nicotine dependent mice, when compared to that of the nicotine/ mecamylamine treated group (Figure 4).

![Figure 4: Effect of treatment(s) on mecamylamine induced piloerection in mice.](image3)

**Figure 4:** Effect of treatment(s) on mecamylamine induced piloerection in mice.  
[Values are mean ± S.E.M.]  
a = P<0.05 vs. VEH-VEH control;  
b = P<0.05 vs. NIC-VEH-MEC.  
VEH: Vehicle; NIC: Nicotine; MEC: Mecamylamine.
Discussion

The present study findings have provided a validated results showing the ability of the hydroalcoholic extract of Tinospora cordifolia to attenuate nicotine withdrawal syndrome in mice. The hydroalcoholic extract blocks the mecamylamine-induced withdrawal syndrome in mice, indicating antagonism to the development of addiction for eight days. The results of this study concluded that neurotransmitter modulation played a prominent role in nicotine addiction and its withdrawal symptoms. Hydroalcoholic extract of Tinospora cordifolia blocks the mecamylamine-induced withdrawal syndrome in mice, indicating antagonism to the development of addiction for eight days. The results of this study concluded that neurotransmitter modulation played a prominent role in nicotine addiction and its withdrawal symptoms. The current study revealed the use of hydroalcoholic extract of Tinospora cordifolia attenuating nicotine addiction and withdrawal symptoms and providing a future direction for Tinospora cordifolia possessing active constituents might be included as a new regimen for nicotine addiction and its withdrawal symptoms.

Conclusions

Tinospora cordifolia hydroalcoholic extract at 400 mg/kg possesses an antidepressant effect in abolishing the nicotine dependence and its withdrawal symptoms induced in mice. Hence, the active constituents of Tinospora cordifolia tend to be future direction for nicotine addiction therapy or may be included as a regimen for nicotine addiction and its withdrawal symptoms.

References


