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# 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 8$^{th}$ 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 arounds 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 psychostimulant drugs, including cocaine, nicotine have been found to exert potent neurotoxic effects by releasing the neurotransmitters in the Nucleus accumbens and Ventral tegmental areas of limbic system like Glutamate, Acetylcholine, GABA as well as the serotonin and dopamine levels (Hendrickson *et al.*, 2013; Zoli *et al.*, 2015; Maurer *et al.*, 2019; Bechard, 2019; Zarrindast *et al.*, 2019; Solinas *et al.*, 2019; Wilar *et al.*, 2019; Zulkifli *et al.*, 2019). The involvement of dopaminergic neurotransmission in pleasure feeling by activating reward like pathway causing repetitive and compulsive need of use these psychoactive substances and further leading to increase the ability to increase the formation of reactive oxygen and nitrogen species (ROS and RNS) as well as intensification of lipids peroxidation processes (Solinas *et al.*, 2019; Wilar *et al.*, 2019; Macedo *et al.*, 2019; Wilar *et al.*, 2019). However, the nicotine research area is lacking the investigation of involvement of oxidative stress mechanisms and the production of the ROS and RNS may be the cause of desensitization of the receptors in the brain cause due to 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 constitute 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 hepatoxicity (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 nicotinic-treated 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 4ºC. 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 (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).

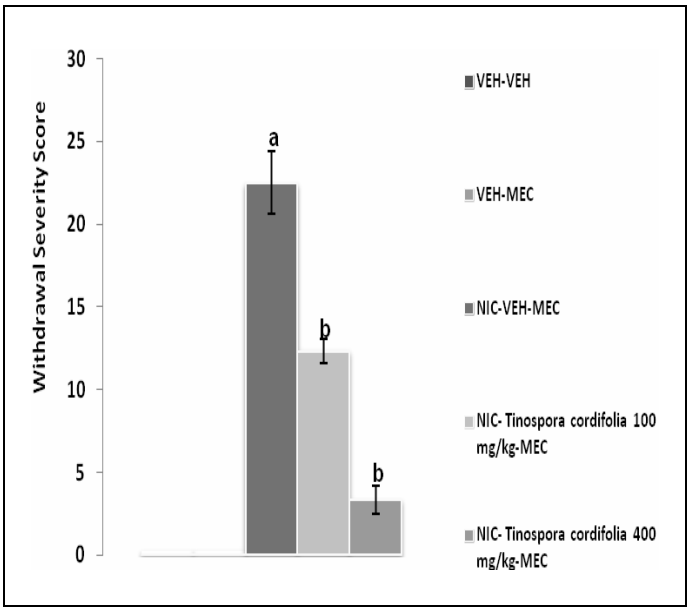


**Fig. 1:** Effect of treatment(s) on mecamylamine induced withdrawal severity score in mice.

[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* 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).

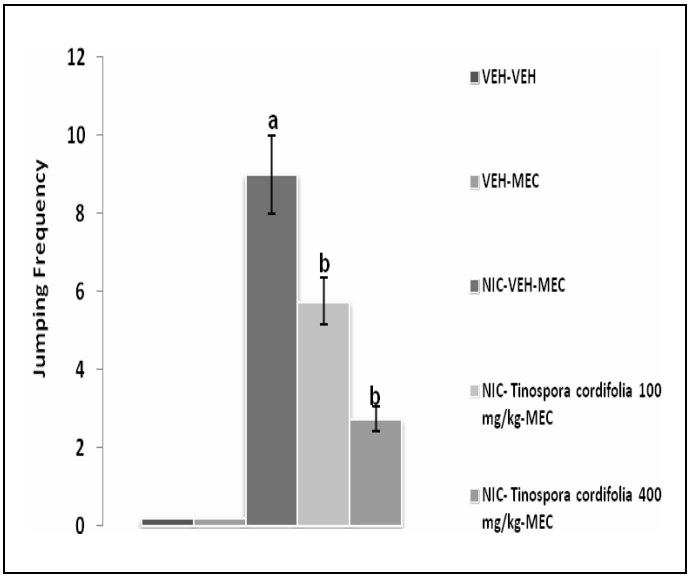


**Fig. 2:** Effect of treatment(s) on mecamylamine induced Jumping Frequency in mice.

[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).

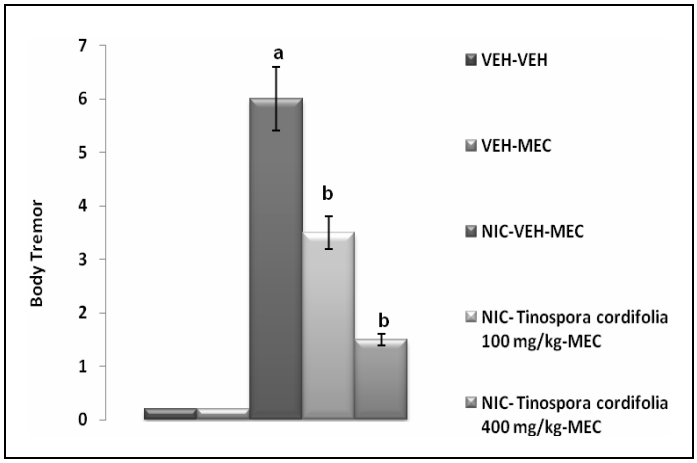


**Fig. 3**: Effect of treatment(s) on mecamylamine induced Body tremor in mice.

[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).

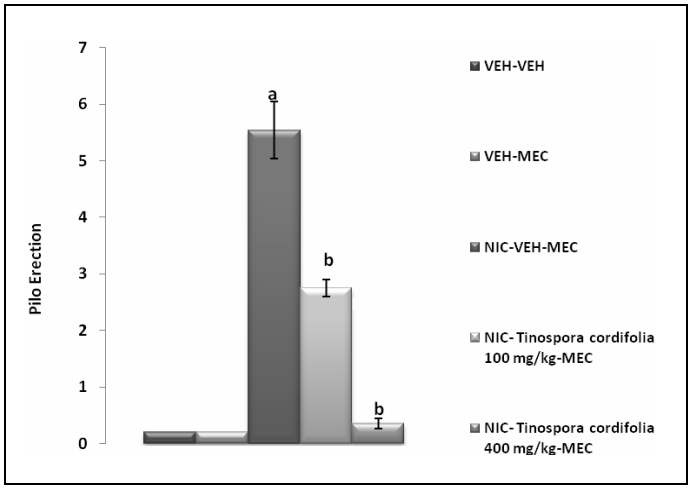


**Fig. 4:** Effect of treatment(s) on mecamylamine induced Piloerection in mice.

[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* profoundly attenuating both developed nicotine dependence and the withdrawal signs associated with nicotine cessation. The aim of the present study is to investigate the protective effects of *Tinospora Cordifolia* hydroalcoholic extract in preventing the development of nicotine dependence and in reducing the abstinence suffering following nicotine cessation in mice. The primary hypothesis of this study was that *Tinospora cordifolia* hydroalcoholic extract would antagonize the mecamylamine precipitated nicotine withdrawal syndrome in mice. Chronic exposure to nicotine produced alternation in the level of excitatory and inhibitory neurotransmitters in rodents. The challenge dose of mecamylamine on the 8$^{th}$ day caused an increase in withdrawal severity score. Hydroalcoholic extract of *Tinospora cordifolia* blocks the mecamylamine induced withdrawal syndrome in mice, it indicates antagonism of nicotine addiction. *Tinospora cordifolia* is widely used herbal plant in Ayurvedic system for anti-inflammatory anti-spasmodic, anti-arthritic, anti-allergic, anti-lepritic, antioxidant, anti-cancer, immune stimulating, nerve cell protecting and anti-diabetic properties (Kattupalli *et al.*, 2019; Reddi, 2019; Antul *et al.*, 2019). In various research studies *Tinospora cordifolia* tends to possess the stress-attenuating activity. In addition, the *Tinospora cordifolia* has several other therapeutic properties generally associated with adaptogenic antioxidant properties. It is also known to possess beneficial effects on learning, anxiety, stress and memory (Fan *et al.*, 2019; Sumran, 2019; Patankar, 2019; Balamithra *et al.*, 2019). *Tinospora cordifolia* may modulate the GABAergic system and assist to overcome the anxiety in rodents (Mishra *et al.*, 2016). Due to its anxiolytic potential and neurotransmitter modulation properties *Tinospora cordifolia* played a prominent role in attenuation of nicotine dependence in mice. Hydroalcoholic extract of *Tinospora cordifolia* ameliorating withdrawal signs of nicotine dependence in line with previous studies (Rehni *et al.*, 2012; Singh *et al.*, 2013). In the present study, the *Tinosporia cordifolia* extract was given together with the nicotine during the development of addiction for eight days. The results of this study concluded that neurotransmitter modulation played a prominent role in nicotine addiction and the *Tinosporia cordifolia* extract treatment modulating these factors making this research directly relevant to the treatment of human addiction. In routine case, the current available therapy for nicotine addiction is targeting the anxiety, depression like withdrawal symptoms or nicotine replacement therapies. The current research evaluated the use of hydroalcoholic extract of *Tinosporia cordifolia* attenuating nicotine addiction and withdrawal symptoms and providing a future direction for *Tinosporia 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 neuroprotective effective in abolishing the nicotine dependence and its withdrawal symptoms induced in mice. Hence, the active constitutes of *Tinosporia cordifolia* tends to be future direction for nicotine addiction therapy or may be included as a regimen for nicotine addiction and its withdrawal symptoms.

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