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Withania somnifera attenuates nicotine induced locomotor sensitization and withdrawal symptoms in mice

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ABSTRACT

Objective: To investigate the effect of *withania somnifera* extract (WSE) on nicotine mediated reinforcement effect and withdrawal symptoms which attributed for the addiction liabilities of nicotine. **Methods**: In Swiss albino mice nicotine mediated locomotor sensitization and anxiogenic effects of chronic and acute nicotine treatment respectively was tested *per se* or in combination with WSE. In addition, nicotine withdrawal induced anxiety-like behavior was also studied. Locomotor sensitization was tested by employing open field test (OFT), while symptoms of anxiety were evaluated by subjecting mice to elevated plus maze (EPM). **Results:** Daily treatment with nicotine (subcutaneous) for 7 days showed gradual increase in the locomotor activity in OFT as compared to saline group indicating the development of locomotor sensitization. Following 3 days of drug free period, nicotine challenge on day 11 also showed rise in locomotor activity depicting expression of sensitization. WSE pretreatment inhibited the nicotine induced development and expression of locomotor sensitization. WSE+nicotine treated group showed decreased ambulations as compared to per se nicotine group on day 1-7 and day 8 ($P<0.05$). In EPM, acute nicotine treated mice spend more time in open arms as compared to saline indicating the anxiolytic behavior. WSE pretreatment reversed this anxiolytic effect. Nicotine withdrawal mice showed significant increase in the number of entries in arm and total time spend in closed arm indicating anxiety-like behavior. WSE treatment in nicotine withdrawal mice inhibited the nicotine withdrawal induced increased number of entries and time spend in closed arms. **Conclusion:** These results indicated that WSE may serve an effective herbal medicine in arresting nicotine mediated reinforcement and withdrawal signs.

Keywords: *Withania Somnifera*, Nicotine, Locomotor sensitization, Anxiety.

INTRODUCTION

Chronic tobacco use has become a major health problem worldwide. Nicotine is the major psychoactive constituent of tobacco and its dependence has been accepted as a major cause underlying tobacco addiction [1]. Similar to many other drugs of abuse, nicotine addiction also initiates with behavioral phenomena termed as locomotor sensitization. It is widely supposed that sensitization may have relevance to the initiation, maintenance and escalation of drug use that is characteristic of the transition from casual experimentation with drugs to drug craving and abuse in humans. Sensitization may also contribute to the reinstatement of drug taking in individuals after prolonged abstinence [2]. Sensitization is also suggested as a potential adaptation that impacts the pursuit and self-administration of nicotine [2]. These evidences indicate that modulation of nicotine-induced locomotor sensitization may help in the control of development of addiction.

There are number of approved therapeutics for the management of nicotine dependence. However, most of the studies were focusing on the later stage of addiction such as physical/mental dependent, reinstatement, relapse and withdrawal symptoms. Early target at the development of locomotor sensitization may help to prevent the addiction. *Withania somnifera* or its psychotropic preparation is known to play a critical role in morphine, alcohol and benzodiazepines addiction. *Withania somnifera* Dunal (commonly known as Ashwagandha or Indian ginseng) is used in many indigenous systems of medicine, primarily Ayurveda in India [3, 4] for the treatment of a number of diseases. This herb is found to be effective in the treatment of carbohydrate and lipid metabolism dysregulation [5], anxiety [6], enhancement of immune modulation [7], prevention of neurodegenerative diseases [8] and cancer [9]. WSE prevents the acquisition and expression of morphine-elicited conditioned place preference, possibly through a GABAB receptor-mediated mechanism [10]. In addition, WSE impairs motivation for drinking ethanol [11]. Pretreatment with WSE protects from the neuronal changes induced by morphine withdrawal [12] and inhibit withdrawal anxiety of alcohol [13].

Hence, in light of the above evidence, in the present study we investigated the role of WSE in the regulation of nicotine-induced sensitization and nicotine withdrawal. Swiss-albino mice were injected daily with nicotine and immediately subjected for the OFT to assess the locomotor activity. Effect of WSE was tested by giving its pre-treatment along with nicotine. Assessment of WSE for its effect on withdrawal symptoms induced after chronic nicotine administration through evaluation of anxiety (EPM), locomotor activity (actophotometer) and somatic signs 24 h., following last nicotine administration was done.

MATERIALS AND METHODS

Animals

Swiss albino mice (22-30 g) were used in the study. Animals were obtained from the animal house of the National Institute of Nutrition, Hyderabad, India and kept in the animal house facility of SKB college of Pharmacy, Kamptee, Nagpur. Animals were housed in group of 10 mice per cage) in acrylic cages (24×17×12 cm) under a constant room temperature (25±2 °C), relative humidity (50±5%), and maintained under a controlled 12:12-h light-dark cycle (lights on at 07:00-h). Rodent chow food pellets and tap water were offered to the animals *ad libitum*. All the experimental procedures were approved and carried out under strict compliance with Institutional Animal Ethics Committee, constituted for the purpose of control and supervision of experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India (853/PO/Re/S/04/CPCSEA). Experiments were performed during the light cycle between 09:00 to 14:00-h to avoid circadian variations. Each experimental group had a separate set of animals and the care was taken to ensure that animals used for one experiment were not employed elsewhere. Animals were handled 5 days prior to actual starting of experiment and brought to experimental room at least 1-h before the test to minimize the non-specific stress induced behavioral alteration, if any.

Chemicals and reagents

Nicotine (Sigma-Aldrich, India), *Withania somnifera* extract (Green heaven laboratory, Nagpur)

Development of sensitization to nicotine and effect of WSE

The protocol outlined by Shim *et al*, 2002 [14] was used for induction of nicotine sensitization. Mice were divided into three groups. I. Saline II. Nicotine hydrogen tartarate (0.2 mg/kg, twice daily, 6 h apart) and III. Nicotine +WSE. These drug treatments were given daily for 7 days (development phase). Locomotor activity was measured once daily at 09.00 h for 20 min immediately after every first injection of saline or nicotine using the OFT. Animals were kept drug free on days 8, 9 and 10 of the experiment (withdrawal phase). Mice previously treated with nicotine were challenged with the same dose of nicotine (sc) on day 11 (expression phase) and locomotor activity was assessed.

Development of nicotine withdrawal and effect of WSE

Nicotine dependence was induced by repeated sc administration of nicotine four times daily, at an interval of 6 h starting at 09:00 h, for 10 days. The control animals received saline employing same injection schedule. Twenty-four hours of last injection, mice were evaluated for anxiety-like behavior by subjecting to EPM and OFT. In EPM, time spends in closed arm and numbers of entries were measured, while in OFT, rearing, ambulations and grooming was counted. In third group of animals, on d 11, mice were given concomitant treatment of nicotine and WSE and subjected to EPM and OFT [15, 23].

Influence of WSE on acute nicotine anxiolysis.

Anxiolytic activity increases time spent in open arms or in number of open arms entries in EPM, anxiogenic effects was characterized by decreases in these measures.

Separate group of animals received nicotine or saline as single injection and were tested 30 min after the last injection.

Another set of experiment was designed to investigate whether WSE have any influence on anxiety related effect of nicotine. For this purpose, distinct groups of mice were injected with WSE before nicotine injection [16].

Statistical analysis

Effect of nicotine on development of locomotor sensitization and withdrawal was analyzed using repeated measures two-way ANOVA, with group (saline vs nicotine) as the independent factor and the days of the tests as the repeated measures factor. Effect of challenge dose was compared between control and sensitized mice by using one-way ANOVA.

RESULTS

Effect of WSE on sensitization to nicotine

Administration of nicotine hydrogen tartarate (0.2 mg/kg, ip) gradually increases locomotor activity in mice. Ambulations in the nicotine treated mice were significantly more as compared to saline group ($p<0.05$) on day 1-7. Challenged with the same dose of nicotine on day 11 (expression phase) also increased ambulations as compared to saline ($p<0.05$). In contrast, concomitant treatment with WSE (100 mg/kg ip) prior to nicotine blocks the nicotine mediated rise in ambulations ($p<0.05$) on day 1-7. Similarly, on day 11, WSE pretreatment prior to nicotine challenge attenuated the increase in ambulations ($p<0.05$). This indicated the attenuation of nicotine induced development and expression of locomotor sensitization.

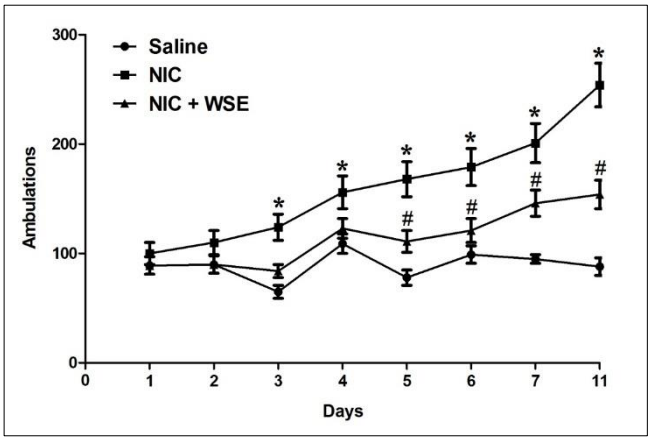

Figure 1: Represents the ambulations after treatment of saline, nic and nic + WSE in OFT. $p<0.05$ vs saline and $^{\#}p<0.05$ vs nicotine treatment.

Effect of WSE on acute nicotine induced anxiolysis

While acute nicotine treatment showed increased time spent by mice in open arm as compared to saline treated mice, WSE (100 mg/kg ip) before nicotine administration inhibits the anxiolytic effect of acute nicotine administration. WSE pretreatment increased the time spend by mice in open arm of EMP as compared to per se nicotine treated mice ($P<0.05$, Fig. 2A). Administration of Nicotine hydrogen tartarate (0.2 mg/kg, ip) shows significant ($p<0.05$) increase in time spent and number of entries in open arm EPM as compared to saline treated group representing the anxiolytic effect (Fig. 2B).

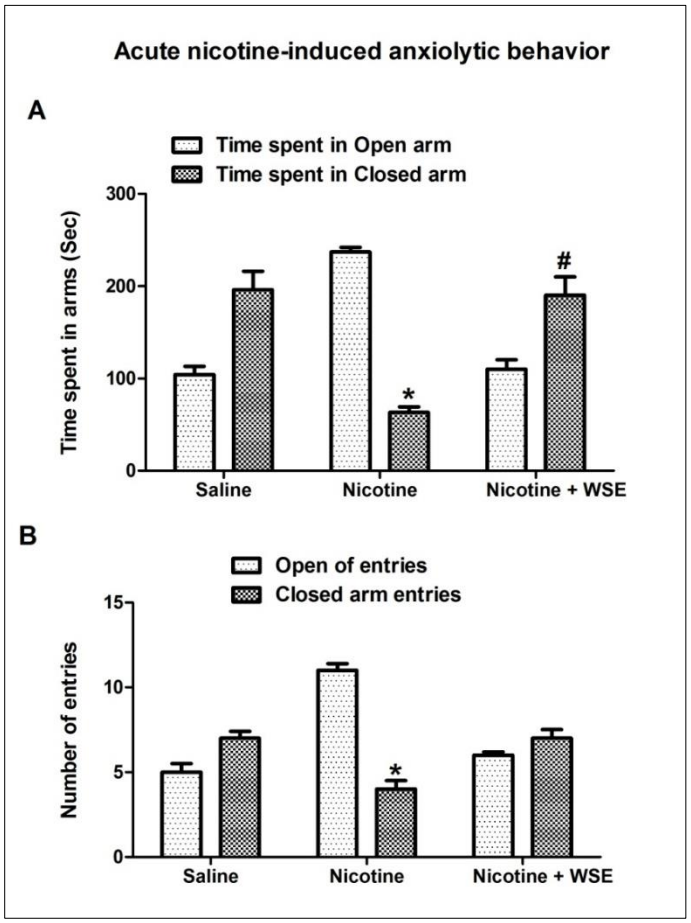

Figure 2: Represents time spent (A) and number of entries (B) in open and closed arm after treatment of saline, nicotine and nicotine + WSE in EPM. Where *p<0.05 vs saline and #p<0.05 vs nicotine treatment.

Effect of WSE on nicotine withdrawal symptoms

Fig. 3 showed effect of nicotine withdrawal in mice. 24 hrs following last injection of nicotine animals were tested for sign of anxiety by subjecting to EPM (Fig. 3A-B) and OFT (Fig. 3C). In EPM, nicotine withdrawal showed significant increase in the number of entries in arm ($P<0.05$) and total time spend in closed arm ($P<0.05$). On the other hand, WSE inhibited the nicotine withdrawal induced increase no. of entries and time spend ($P<0.05$). OFT data suggest that nicotine withdrawal resulted into increased ambulations and rearing as compared to control group which re prevented by WSE treatment during withdrawal (Fig. 3C).

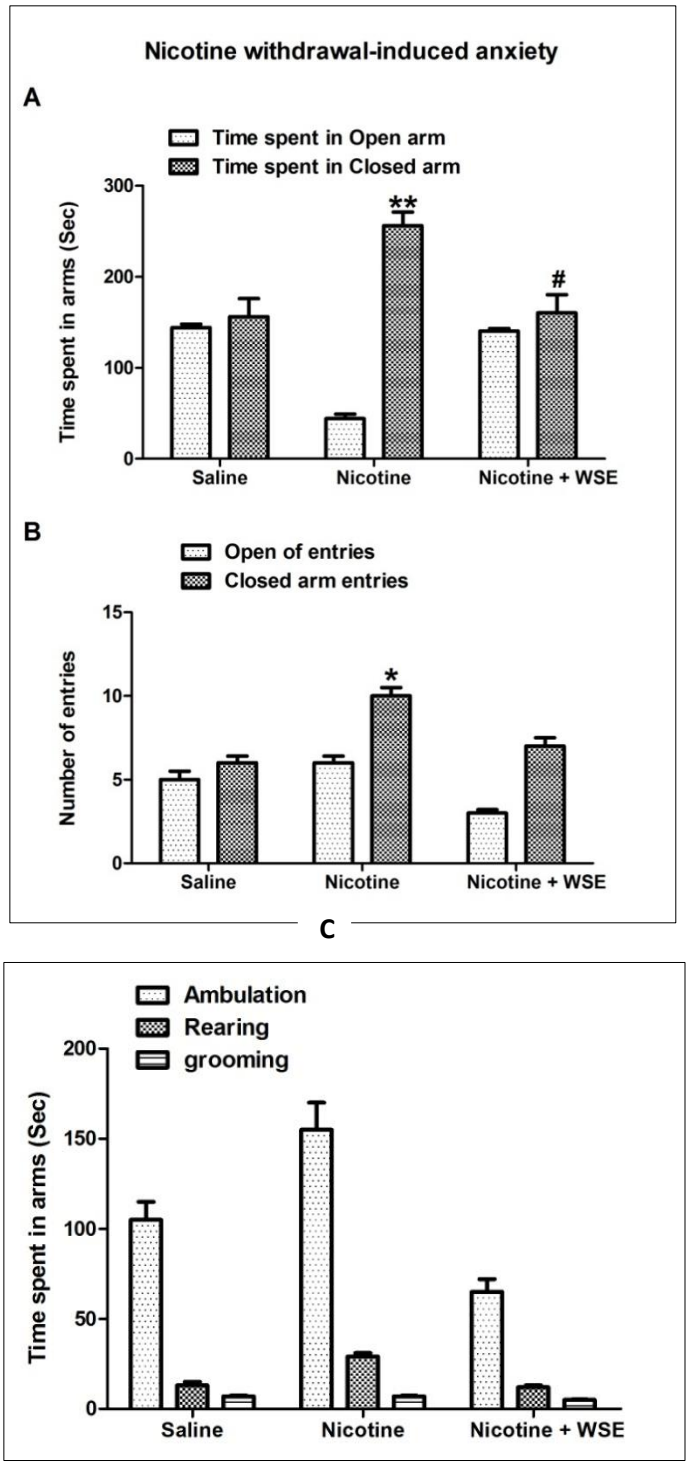

Figure 3: Represents time spent in closed and open arms (A) and number of entries (B) in mice treated with saline, nicotine or WSE+nicotine. (C) represents ambulation in OFT after same treatment as above. *p<0.05 vs saline and #p<0.05 vs nicotine treatment.

DISCUSSION

The results of present study demonstrate the anti addictive potential of WSE. It have shown to inhibit the three most important mechanism of nicotine addiction i.e. Sensitization, withdrawal symptoms and anxiolysis. Plants of withania somnifera have been widely

investigated for potential drug addiction activities. In context to drug addiction, withania somnifera inhibits morphine tolerance and dependence and also inhibited ethanol withdrawal induced anxiety. However, its role in nicotine addiction is not well documented.

Also in our earlier findings, we found WSE enhance the nicotine induced condition place preference in mice. In view of this, several studies have already established a role of withania somnifera in attenuation of ethanol and morphine withdrawal syndrome. It also decreases cocaine and fentanyl self-administration and in habited morphine CPP. These findings supported by available literature. Several studies have demonstrated the antistress, adaptogenic and immunomodulatory potential of Withania somnifera [17, 18, 19, 20]. Indian ginseng like to Hypericum perforatum and Panax ginseng shows 5-HT, GABA, acetylcholine and dopaminergic modulations [21, 22, 23, 24], accounting for its CNS effects.

WSE at dose of 100 mg/kg ip blocked the expression of locomotor sensitization as revealed by decreased ambulation and rearing activity in OFT. Thus it has shown to inhibit the positive reinforcement. Similarly the negative reinforcers like abstinence symptoms have been the most critical cause of relapse. Hence we have evaluated the potential of WSE in nicotine withdrawal. The results of this experiment clearly signifies the beneficial role of WSE in attenuating the symptoms like anxiety and hyper locomotion induced by nicotine withdrawal as indicated by decreased ambulation in OFT and increased time spent and number of entries in open arm of EPM.

Thus WSE was found effective in expression of sensitization as well as neuroadaptive changes in the form of withdrawal symptoms. Furthermore we were interested to explore the effect of WSE on acute effects of nicotine. To evaluate the same we administered WSE followed by nicotine and subjected animals to EPM. The outcome suggested that nicotine in acute dose shows profound anxiolysis, which was blocked by WSE. Investigations for the detail molecular mechanism underlying these effects of WSE are warranted. WSE have been tremendously explored phytomedicine by cumulative research work. As an adaptogen it decreases the hyperactivity of HPA. Its neurological benefits are attributed to its GABAergic and seotonergic activity. It is known anti-stress agent. All of these mechanisms are tooled here before for management of addiction. Hence it suggests for further biochemical and neurological investigations at molecular level to identify exact anti-addictive mechanism of WSE.

CONCLUSION

The results of the present investigation suggest that WSE blocked the expression of locomotor sensitization, prevented the acute nicotine mediated anxiolysis and withdrawal induced anxiety like behavior of nicotine. This suggests WSE may block the positive reinforcement effect of nicotine that lead to suppression of addiction liability of nicotine. In addition, consumption of WSE may be effective in reversing the development of nicotine withdrawal induced psychological deficits. This proposed the promising anti-addictive potential of WSE in the management of nicotine abuse.

Author Contribution

Nitin G. Dumore - Protocol design and animal experimentation.

Milind J. Umekar - Inspection of experimental protocol.

Manish M. Aglawe – Drafting of manuscript and statistical analysis of data.

Brijesh G. Taksande – Final editing of manuscript.

Conflict of Interest: None

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