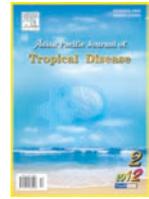


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## Evaluation of Ashwagandha in alcohol withdrawal syndrome

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## ABSTRACT

**Objective:** To evaluate the effect of Ashwagandha (ASW) in attenuation of alcohol withdrawal in ethanol withdrawal mice model. **Methods:** Alcohol dependence was induced in mice by the oral, once-daily administration of 10% v/v ethanol (2 g/kg) for one week. Once the animals were withdrawn from alcohol, the efficacy of ASW (200mg/kg and 500mg/kg) in comparison with diazepam (1 mg/kg) in the attenuation of withdrawal was studied using, pentylentetrazole (PTZ) kindling test for seizure threshold, forced swim test (FST) for depression and locomotor activity (LCA) in open field test (OFT). 6 hours after the last ethanol administration, seizure threshold was measured in all the groups by administering the convulsant drug, PTZ with a subconvulsive dose of 30 mg/kg i.p). In FST, mice were forced to swim and the total duration of immobility (seconds) was measured during the last 4 min of a single 6-min test session. In OFT, number of crossings of the lines marked on the floor was recorded for a period of 5 min. **Results:** Compared to ethanol group, ASW (500 mg/Kg) has suppressed the PTZ kindling seizures in ethanol withdrawal animals [0% convulsion], FST has shown decreased immobility time and OFT has exhibited increase in the number of line crossing activity by mice which may be the consequence of anxiolytic activity of ASW similar to that of diazepam. **Conclusions:** The present study provides satisfactory evidence to use ASW as a safe and reliable alternative to diazepam in alcohol withdrawal conditions.

## 1. Introduction

One of the problems of chronic alcohol intake in humans is the development of physical dependence, resulting in physical withdrawal reactions after cessation of alcohol. Alcohol withdrawal is the change that the body goes through when a person suddenly stops drinking after a chronic alcohol use. Alcohol has a slowing effect (also called a sedating effect or depressant effect) on the brain and over time, the brain adjusts its own chemistry to compensate for the effect of the alcohol. The alcohol withdrawal includes symptoms such as neural excitation (seizures), disturbed sleep, anxiety and depression etc[1]. A number of reviews state that benzodiazepines (BZDs) class of drugs viz. diazepam make an excellent choice for the treatment of alcohol withdrawal because they ameliorate both the anxiety and the seizures but, research indicates that diazepam poses the potential hazard of dependence[2].

*Withania somnifera* (WS), popularly known as

Ashwagandha (ASW) is widely considered as the Indian ginseng belonging to family Solanaceae [3,4]. It is classified as a rasayana (rejuvenation) in ayurveda and expected to promote physical and mental health, rejuvenate the body in debilitated conditions and increase longevity[5]. Various studies in animal models have shown that ASW has anticonvulsant[6], antidepressant[7–9], antianxiety[10], hepatoprotective[11] effects etc. The  $\gamma$ -amino butyric acid (GABA)ergic activity of ASW may explain its ability to attenuate motor seizures resulting from pentylentetrazol (PTZ) kindling in rodents[4].

ASW also exhibited an antidepressant effect comparable with that induced by imipramine, in the forced swim induced model of behavioral despair and helplessness[9]. The glycowithanolides present in ASW has anxiolytic effect and this finding was obtained in the elevated plus maze as well as in the test of social interaction and feeding latency in an unfamiliar environment[10]. It has also been reported that long term treatment with ASW resulted in the reversal of chemical induced damage to the liver functions in rats[11].

In Ayurveda, ASW have been found to have potential therapeutic role in almost every central nervous system related disorders. ASW's anticonvulsant, antidepressant,

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anxiolytic and hepatoprotective potential will help us to explore its usefulness in treating alcohol withdrawal as it does not produce any dependence and is non-sedating too whereas benzodiazepines are. Hence the present study has been planned to carry out to evaluate the said useful effect of ASW as an alternative to benzodiazepine class of drugs.

## 2. Materials and methods

### 2.1 Drugs

*Withania somnifera* (ASW) root extract from Himalaya Drugs Co. Bangalore, India was suspended in 0.5% w/v carboxy methyl cellulose (CMC) in distilled water and administered via oral route. Absolute ethanol 99.8% (Merck), Diazepam tablets (Calmose– Ranbaxy Research Laboratories) and PTZ (Sigma, MO, USA) were purchased and used in the study.

### 2.2 Animals

In-house laboratory bred healthy Albino mice of either sex weighing (25 – 35 g) used in the study were drawn at random for test and control groups. The experimental protocol was approved by the Institutional Animal Ethics Committee. Animal ethical guidelines and good laboratory practice guidelines were followed throughout the experimental period. In addition, all the precautions were taken to minimize pain and discomfort to the animals.

### 2.3 Experimental Design

The rats (n=6 per group) were divided into 5 groups.

1. Group I – Control group treated with water
2. Group II – Ethanol alone administered animals (Drug control)
3. Group III – Animals administered Ethanol + diazepam (1 mg/kg)<sup>[12]</sup>
4. Group IV – Animals administered Ethanol + ASW (200 mg/Kg/day)<sup>[12]</sup>
5. Group V – Animals administered Ethanol + ASW (500 mg/Kg/day) <sup>[12]</sup>

### 2.4 Chronic intermittent ethanol procedure <sup>[13]</sup>

Chronic intermittent ethanol (CIE) exposure regimen consists of repeated episodes of ethanol intoxication and withdrawal. Albino mice housed in the vivarium under a 12:12 h light/dark cycle with free access to food and water, were given 2 g/kg ethanol as 10% (v/v) solution in distilled water by oral intubation on an everyday regimen for one week and then the animals were withdrawn. Ethanol withdrawal behaviors were hyperexcitability (seizures) and this hyperexcitability was behaviorally present in terms of super sensitivity to sub convulsive doses of PTZ a

convulsant. Together with these physical reactions, alcohol withdrawal resulted in a reduced exploratory behaviour which was confirmed by locomotor activity (LCA) in open field, reflective of anxiety-like behavior and immobility in forced swim test (FST).

### 2.5 PTZ Kindling

6 hours after the last ethanol administration, seizure threshold was measured in all the groups even the control by administering the convulsant drug, PTZ with a subconvulsive dose of 30 mg/kg i.p). After each administration the behavior of the mice was noted for a period of 30 min. Animals entering into convulsions have shown convulsive waves axially through body, Myoclonic jerks and rearing, Clonic forelimb convulsions, Generalized tonic-clonic seizure. (No of animals shown convulsions and percentage of animals convulsed were calculated<sup>[14,15]</sup>).

### 2.6 FST

The test was carried out according to the method described by Porsolt et al<sup>[16]</sup> with modifications. Briefly, mice were forced to swim in a transparent glass vessel (8 inch height, 13.5 inch length and 8.5 inch width) filled with 6 inch water at 24–26°C. The total duration of immobility (seconds) was measured during the last 4 min of a single 6–min test session. Mice were considered immobile when they made no attempts to escape except the movements necessary to keep their heads above the water. Immediately after swimming session, the mice were removed from the cylinder, patted dry and then returned to home cage. After testing of each animal, water of glass tank was replaced with the fresh water. Mean immobility time in second were calculated<sup>[9,17]</sup>.

### 2.7 Open field test (OFT)

Hall originally described the open field test for the study of emotionality in rats<sup>[18]</sup>. The procedure consists of subjecting an animal, usually a rodent, to an unknown environment from which escape is prevented by surrounding walls. The procedure generally usually involves forced confrontation of a rodent with the situation. The animal is placed close to the walls of the apparatus and number of crossings of the lines marked on the floor was recorded for a period of 5 min. The dimensions of the box were 14.2 inch length and breadth which is divided equally into 4 squares. An increase in central locomotion or number of crossings of the lines marked can be interpreted as an anxiolytic-like effect while the contrary, that is a decrease of these variables, is associated with anxiogenic effects. No of lines crossed by the animals from various treatment groups were recorded<sup>[19]</sup>.

### 2.8 Statistical Analysis

The results were expressed as mean±S.E.M ( $n=6$ ). The statistical analysis involving different groups was performed by means ANOVA followed by Dunnett test.  $p$  value at  $< 0.05$  was considered as statistically significant. Data were processed with graph pad prism version 5.00 software.

### 3. Results

#### 3.1 PTZ Kindled seizures in Ethanol withdrawal mice model

In normal mice a subconvulsive dose of PTZ (30 mg/kg i.p) has not shown any convulsions (0% convulsion). Whereas PTZ administration after 7 days of Ethanol treatment in mice has shown convulsion in the Ethanol treated animals (100% convulsion). Ethanol + Diazepam (1mg/Kg) and Ethanol + Ashwagandha ASW (500mg/Kg) showed a significant increase in seizure threshold (0% convulsion), however Ethanol + ASW (200 mg/Kg) was not that effective in minimizing convulsions (29% convulsions) [Figure 1].

#### 3.2 FST in Ethanol withdrawal mice model

Compared to control, Ethanol + Diazepam (1mg/Kg) ( $P<0.001$ ) and Ethanol + ASW (500 mg/kg) ( $P<0.01$ ) have produced decrease in immobility time in the FST. But compared to Ethanol group, all the other groups (Ethanol + Diazepam (1mg/Kg), Ethanol + ASW (200 mg/kg) and Ethanol + ASW (500mg/kg) have decreased the immobility time ( $P<0.001$ ) [Figure 2].

#### 3.3 OFT in Ethanol withdrawal mice model

Compared to control, Ethanol + Diazepam (1mg/Kg) ( $P<0.001$ ) and Ethanol + ASW (500mg/kg) ( $P<0.01$ ) have produced an increase in the number of lines crossed during open field test. Similarly, compared to Ethanol group, Ethanol + Diazepam (1mg/Kg) ( $P<0.001$ ) and Ethanol + ASW 500mg/kg ( $P<0.01$ ) have produced an increase in the number of lines crossed during open field test [Figure 3].

### 4. Discussion

Alcohol withdrawal is the change that the body goes through when a person suddenly stops drinking after a chronic alcohol use. CIE exposure regimen, a different model of long-term ethanol treatment, consists of repeated episodes of ethanol intoxication and withdrawal. In humans, long-term alcohol consumption typically follows an intermittent or repeat pattern characterized by regular bouts of intoxication interspersed with multiple periods of withdrawal from ethanol. Alcohol administration with a multiple intermittent paradigm in animals has been found to produce more persistent signs of withdrawal[20].

ASW is known to possess GABA and serotonergic activity thereby suggests possible usefulness in the treatment of alcohol withdrawal, reduction in the risk of withdrawal seizures and prevention of alcohol relapse. The aim of the study was to evaluate the potential of ASW in attenuation of alcohol withdrawal and also to check whether ASW can replace the untoward effects of benzodiazepines in alcohol withdrawal in mice. The criteria for evaluation were anti-depressant activities and increase in the seizure thresholds during alcohol withdrawal.

One of the problems associated with alcohol withdrawal is neural excitation (seizures)[21]. Kokka et al[15] showed that rats on a CIE regimen showed a persistent reduction in seizure threshold to the convulsant drug PTZ. The gradual increase in seizure susceptibility produced by the chronic intermittent administration of ethanol and the persistence of that effect suggests that a kindling-like process may be involved in the development of ethanol withdrawal. In the present study compared to ethanol group, ASW (500 mg/Kg) has suppressed the PTZ kindling seizures in ethanol withdrawal animals similar to that of standard drug Diazepam (1 mg/Kg) [both have shown 0% convulsion]. Whereas, ASW 200 mg/kg (lower dose) has not suppressed the seizures to the same level (shown 29 % convulsion). In the earlier studies Kulkarni et al [22] found that a methanolic extract of ASW attenuated PTZ-induced seizures in rats in a dose-dependent fashion. The extract also inhibited GABA and t-butylbicyclophosphorothionate binding to rat brain cerebral cortical membranes in vitro. Finally, the extract showed additive effects with pentobarbitone in protecting against PTZ seizures. These findings also suggested that ASW has GABAergic actions. Biochemical studies by Mehta et al[23] suggested that the WS extract contains an ingredient which has a GABA-mimetic activity. WS offered a significant protection against PTZ-induced kindling, possibly via a GABAergic mechanism.

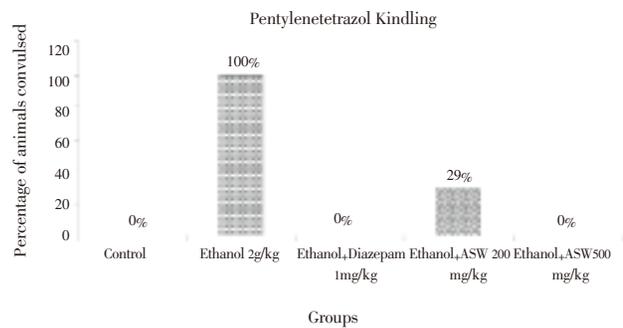
In the present study, the increase in immobility time in control group may be due to fear and anxiety which was manifested in the form of increased defecation. It has been reported that symptoms of alcohol withdrawal also includes anxiety and depression. This could be the reason behind a trend towards further increase in the immobility time in ethanol group in comparison with control group. Whereas the standard anxiolytic drug diazepam by virtue of its calming effect has decreased immobility time in FST. Similar effect was also observed with ASW, the immobility time was less at a higher dose of ASW (500mg/kg). Since catecholamine and 5-hydroxy tryptamine (HT) have been implicated in the etiology of depression, the positive effect of these drugs in FST seems to be due to increased availability of these neurotransmitters at the postsynaptic receptor sites following their re-uptake inhibition[8]. Serotonergic system has been shown to play an important role in the regulation of ethanol intake, preference, and dependence via central mechanisms[24–27]. Uzbay et al[28] indicated a significant

decrease in striatal serotonin levels of rats during early ethanol withdrawal and chronic ethanol consumption. These observations imply that there might be a correlation between decreased serotonergic activity and ethanol withdrawal. In earlier studies Bhattacharya et al<sup>[29]</sup> found that ASW exhibited an antidepressant effect, comparable with that induced by imipramine, in the forced swim-induced ‘behavioural despair’ and ‘learned helplessness’. Tripathi et al<sup>[30]</sup> demonstrated that chronic dosing with ASW downregulated 5-HT<sub>1</sub> and upregulates 5-HT<sub>2</sub> receptors in the rat brain; these changes were accompanied by a decrease in behavioral indices of anxiety and depression. ASW offered a significant reduction in depression, possibly via a serotonergic mechanism.

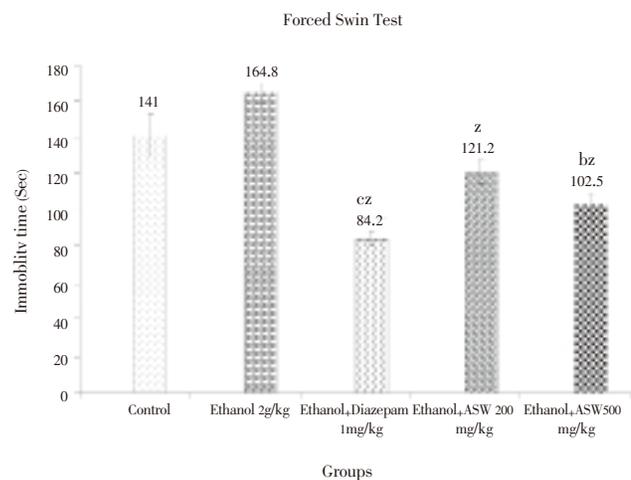
The open field is a very popular animal model of anxiety-like behavior<sup>[19]</sup>. Alcohol withdrawal resulted in a reduced exploratory behaviour which was confirmed by LCA in open field. The reduction in exploratory behaviour, often referred to as withdrawal anxiety, was present in terms of decrease in number of line crossing activity by mice which may be the consequence of withdrawal anxiety. The inhibition of exploration was present after 6 h of withdrawal and disappeared from 24 h onwards. Baldwin et al<sup>[31]</sup> reported similar reduction in exploration after alcohol withdrawal in rats in elevated plus maze and the social interaction test. This test has further witnessed the increase in number of line crossing activity by mice which may be the consequence of anxiolytic activity of ASW at higher dose (500 mg/kg) similar to that of diazepam. In the earlier studies Bhattacharya et al<sup>[29]</sup> showed that, in rats, ASW glycowithanolides had an anxiolytic effect comparable to that of lorazepam. This finding was obtained in the elevated plus-maze as well as in the test of social interaction and feeding latency in an unfamiliar environment. ASW and lorazepam both reduced rat brain levels of tribulin, an endocoid marker of clinical anxiety, as the levels were increased after administration of the anxiogenic agent PTZ. As demonstrated earlier by Mehta et al<sup>[23]</sup> and Kulkarni et al<sup>[22]</sup>, WS possessed GABA-mimetic properties. Since GABA agonism has been linked to anxiolysis<sup>[32]</sup> the extracts of WS may have beneficial effect in anxiety and related disorders.

Various research articles have indicated that diazepam though used as an excellent choice for the treatment of alcohol withdrawal, it possess the potential hazard of dependence<sup>[2]</sup>. The present study provides satisfactory results of ASW on various parameters tested, which provides satisfactory evidence to use ASW as potential agent which can be used as a safe drug in alcohol withdrawal conditions as a reliable alternative to the commonly used diazepam.

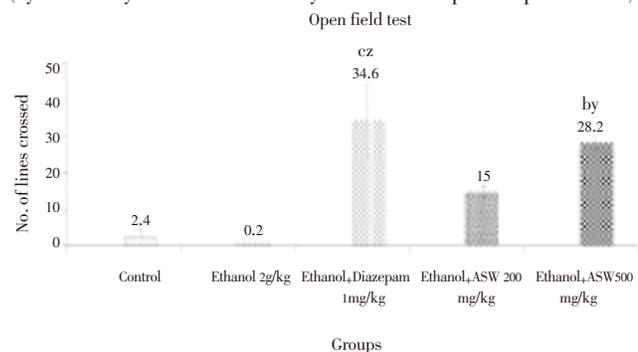
In conclusion, the result of the PTZ kindling, FST and OFT using alcohol withdrawal animals have shown similar response in diazepam 1mg/kg and ASW 500mg/kg. Therefore present study suggests that ASW can be used as a safe drug in alcohol withdrawal conditions as a reliable alternative to the commonly used BZD class of drugs.



**Figure 1.** Percentage of animals convulsed upon administration of pentylenetetrazol (30mg/kg) to Control, Ethanol (2g/Kg), Ethanol + Diazepam (1 mg /kg), Ethanol + Ashwagandha (ASW) 200 mg/kg and Ethanol + ASW 500 mg/kg treated groups in ethanol withdrawal mice model.



**Figure 2.** Effect of Control, Ethanol (2g/Kg), Ethanol + Diazepam (1 mg/kg), Ethanol+ Ashwagandha (ASW) 200 mg/kg and Ethanol + ASW 500 mg/kg during forced swim test in Ethanol withdrawal mice model. Bars represent mean±SEM (n=6). p values: b<0.05, c<0.001, as compared to control; z<0.001, as compared to ethanol treated group (by one – way ANOVA followed by Dunnett multiple comparison test).



**Figure 3.** Effect of Control, Ethanol (2g/Kg), Ethanol + Diazepam (1 mg/kg), Ethanol + Ashwagandha (ASW) 200 mg/kg and Ethanol + ASW 500 mg/kg using open field test in Ethanol withdrawal mice model. Bars represent mean±SEM (n=6). p values: b<0.05, c<0.001, as compared to control; y<0.05, z<0.001, as compared to ethanol treated group (by one – way ANOVA followed by Dunnett multiple comparison test).

## Conflict of interest statement

We declare that we have no conflict of interest.

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