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Research Article
Efficacy of Ashwagandha and Brahmi Extract on Alcohol Withdrawal Syndrome in Laboratory Rats

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Abstract

Background and Objective: Ashwagandha and Brahmi have shown GAB Anergic activity and possess neuroprotective, antianxiety and antioxidant properties which could be of use in combating alcohol withdrawal syndrome. The aim of the study was to find the effect of Ashwagandha and Brahmi extract on alcohol withdrawal syndrome in rats. **Materials and Methods:** Thirty Sprague Dawley rats of either sex were divided into 5 groups. Control rats were par-fed with the iso-caloric liquid diet. Modified Liquid Diet with ethanol at increasing dosage was given for 21 days to other groups. Ashwagandha (3.75 mg of withanolide glycosides per kg bodyweight) and Brahmi (10 mg of bacosides per kg bodyweight) were administered orally while Diazepam (2 mg kg^{-1}) was injected intraperitoneally 45 min before ethanol withdrawal to respective groups. After 6 h of the withdrawal period, seizure threshold and withdrawal signs of rats were studied. **Results:** Ashwagandha and Brahmi treated groups were significantly effective in controlling seizure and agitation when compared with the alcohol group. Brahmi and diazepam treated groups were effective in suppressing stereotypic behaviour when compared with alcohol groups and found similar to the control group. No significant difference was found in locomotor activity among all the groups. **Conclusion:** These herbs may be developed into a suitable formulation for the control of alcohol withdrawal symptoms.

Key words: Ashwagandha, brahmi, diazepam, alcohol withdrawal syndrome, seizures, stereotype behavior, agitation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chronic alcoholism leads to the development of physical dependence, resulting in withdrawal reactions after cessation of alcohol. Alcohol has a sedating effect or depressant effect on the brain and after the withdrawal of alcohol, sympathetic excitation ensues as a loss of inhibitory effect of alcohol on CNS mediated by GAB anergic activity. The alcohol withdrawal symptoms can range from mild anxiety to seizures and includes symptoms such as tremors, irritability, agitation, restlessness, nausea, depression etc[1]. Alcohol modulates the effect of GABA on the $GABA_A$ receptor causing a depressant effect on overall brain excitability. Tolerance develops to the effect of alcohol on chronic exposure due to a compensatory decrease of $GABA_A$ receptor response to GABA. Studies have shown that alcohol has got an inhibitory effect on NMDA receptor and chronic alcohol exposure results in the up-regulation of these receptors[2]. As a result, cessation of alcohol results in brain hyperexcitability, because NMDA receptors are no longer receptors inhibited by alcohol[2,3].

Around 2 billion people consume alcohol and 76.3 million are diagnosed to have alcohol use disorders. In India, prevalence rate of alcoholism is 21.4%[4]. If left untreated, 6% of alcohol-dependent patients develop clinically relevant symptoms of withdrawal, with up to 10% of those experiencing delirium tremens[5].

Benzodiazepines are the recommended drugs for the treatment of alcohol withdrawal symptoms. They have a slower onset of action and therefore are less likely to lead to abuse. Diazepam or Chlordiazepoxide is indicated as a treatment for the management of acute alcohol withdrawal syndrome. A reducing dose of diazepam over 5-7 days is commonly used[6]. Diazepam is a GABA agonist, causes opening of Cl^- channels leading to hyperpolarisation which is responsible for the inhibitory effect on CNS excitation and prevents seizures[7].

Withania somnifera, known as ashwagandha belongs to family Solanaceae and is considered as good as Chinese ginseng. In Ayurveda, it is enlisted as Rasayana (rejuvenation) and which is associated with promotion of physical and mental health along with rejuvenation of the body in debilitated conditions. It is also associated with increased longevity. Studies have shown that active principles of ashwagandha, i.e., sitoindosides VII-X and withaferin-A, to possess significant anti-stress activity in animal models of experimental stress. In acute toxicity studies the LD50 of Withania somnifera was found to be 1750 mg (p.o.) in albino mice[8]. Ashwagandha has been used as an adaptogen, antioxidant, anxiolytic, antidepressant, memory enhancer and antiulcerogenic agents. Experimental studies in animals have extensively demonstrated a GABA-mediated action of Ashwagandha[9].

Bacopa monnieri (Brahmi), a nootropic plant from family Scrophulariaceae, has been studied widely for its cognitive enhancing, antidepressant, antihypertensive, anti-asthmatic, antiulcer, analgesic, neuroprotective, hepatoprotective and nephroprotective properties. Hepatoprotective, antioxidant as well neuromodulatory and nootropic effect of Brahmi is due to Bacoside-A which is the major chemical constituent present in it[10]. Bacopa monnieri exhibited a no-observed adverse effect level (NOAEL) of 500 mg kg^{-1} and a median lethal dose (LD_{50})[11] of 2400 mg kg^{-1}.

During epilepsy, cerebral cortex neurons get overstimulated and there occurs a decrease in GABA mediated inhibition. CREB gene expression, GABA and $GABA_A$ receptor binding get altered. Bacopa monnieri could reverse the changes occurred and can be used therapeutically in the management of epilepsy[12].

Diazepam being a GABA agonist is indicated in the treatment of alcohol withdrawal syndrome. Ashwagandha and Brahmi, do possess GABA facilitatory activity as seen in few preclinical studies. Hence, we undertook this study to explore the potential of herbal drugs (Ashwagandha and Brahmi) in the treatment of alcohol withdrawal symptoms.

MATERIALS AND METHODS

Study area: This study was conducted in Department of Pharmacology, Maulana Azad Medical College, Delhi from June, 2018-May, 2019 after getting approval of Institutional Animal Ethical Committee (IAEC: 01/2017). The study was conducted within framework of CPCSEA (Committee for purpose of control and supervision of experiments on animals) guidelines.

Method: Sprague Dawley (SD) rats of either sex, weighing 180-250 g were used in the present study. The animals were housed in a quiet room at 22±3°C temperature, 60±5% relative humidity and 12 h light/dark cycle. The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) were followed strictly throughout the experiment. The animals were provided with Modified Liquid Diet (MLD) in place of regular pellet diet *ad libitum*[13]. A total of healthy 30 SD rats were included in the study.

The animals were divided into 5 groups comprising of 6 rats in each group. Group 1 was kept as control group and rats were provided with Modified Liquid Diet (MLD) (cow milk

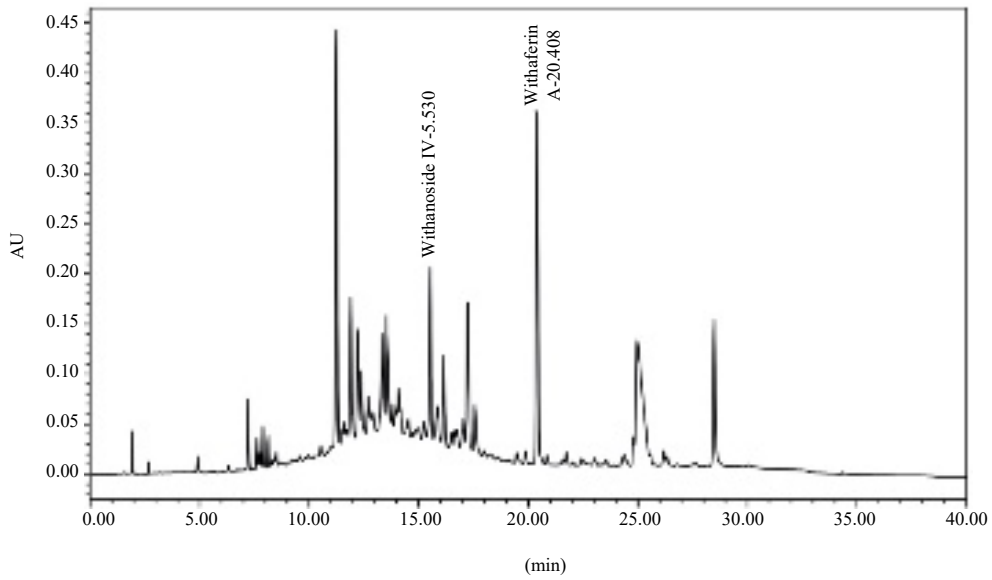

Fig. 1: HPLC Chromatogram of Ashwagandha extract

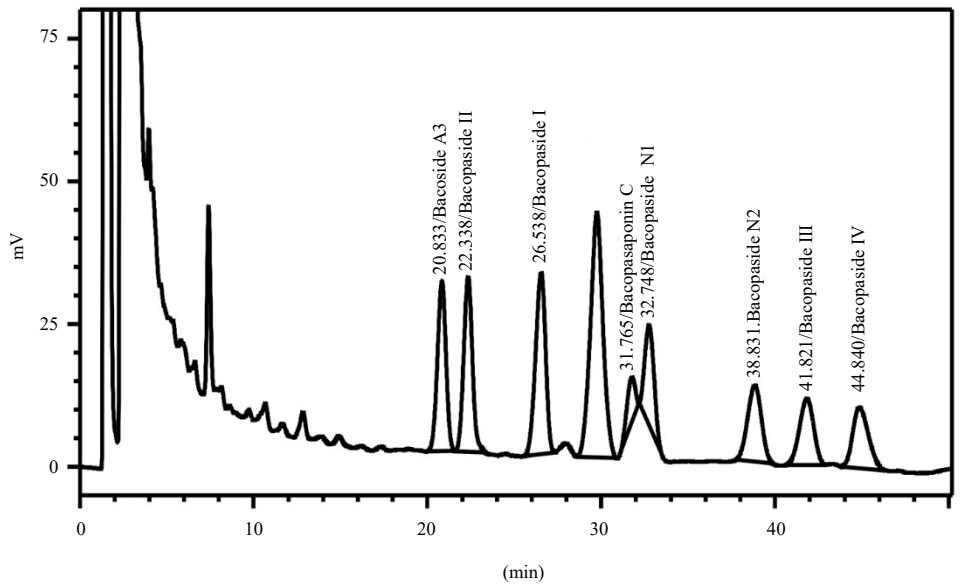

Fig. 2: HPLC chromatogram of Brahmi extract

925 mL, 17 g sucrose and 5000 IU Vitamin A) in the feeding bottle[13]. The animals of Group 2-5 were made alcohol dependent by providing ethanol along with MLD for 21 days. Animals of Group 3 and Group 4 were administered by oral gavage with test extracts ashwagandha (3.75 mg of withanolide glycosides per kg bodyweight) and Brahmi (10 mg of bacosides per kg bodyweight), respectively after 21 days of establishing alcohol dependence. Animals of Group 5 were injected with Diazepam (2 mg kg^{-1}) intraperitoneally after 21 days of establishing alcohol dependence.

Plant extraction: Ashwagandha and Brahmi extracts were used as the test products. Normal saline was used to dissolve the extracts. Diazepam used was purchased from Sigma, MO, USA whereas ethanol was procured from Jebsen and Jessen (GmbH and Co.) KG, Germany. Supplied extracts were made from plant materials of Withania somnifera (Ashwagandha) and Bacopa monnieri (Brahmi) which were identified by a qualified botanist. Roots and leaves of Ashwagandha were dried and pulverized for hydroalcoholic extraction. The extraction solvent used was ethanol: water at a proportion of 60: 40. The solution obtained is treated and concentrated

under controlled conditions and dried to pale brown powder. Ashwagandha extract obtained contains 35% withanolide glycosides confirmed by HPLC analysis method based on US Pharmacopoeia (Fig. 1). Hydroalcoholic extractions of dried and powdered Brahmi leaves were done. The extraction solvent used was ethanol: water at a proportion of 80:20. The solution obtained is treated and concentrated under controlled condition to brown to deep brown powder. Brahmi extract obtained contains 21.4% total bacoside analyzed by HPLC method (Fig. 2)[14].

Alcohol dependence was produced by a validated animal model described by Uzbay *et al.*[13] In brief, the rats were housed individually and ethanol was added in the MLD. The MLD contained about 1000.7 kcal L^{-1}. The composition of the MLD with ethanol contained 925 mL cow milk, 25-75 mL ethanol, 5000 IU vitamin A and 17 g sucrose. Fresh cow milk was procured daily.

Rats were provided with MLD without ethanol for 7 days. Then, liquid diet with 2.4% v/v ethanol was added in MLD for 3 days. The ethanol concentration was increased to 4.8% v/v for the following 4 days and finally to 7.2% v/v for 14 days. Liquid diet was freshly prepared daily. Control rats were par fed an isocaloric liquid diet containing sucrose as a caloric substitute to ethanol.

At the end of exposure to 7.2% v/v ethanol-containing diet, ethanol was withdrawn from the diet. Ashwagandha (3.75 mg of withanolide glycosides per kg bodyweight, orally), Brahmi (10 mg of bacosides per kg body weight, orally) and diazepam (2 mg kg^{-1}, intraperitoneally) were administered to the rats 45 min before ethanol withdrawal in respective groups. The rats were observed for 5 min at 6th h of withdrawal period for locomotor activity, agitation, stereotypic behavior and seizures.

Evaluation of ethanol withdrawal syndrome

Agitation and stereotypic behavior: Six h after alcohol withdrawal, rats were observed for agitation and stereotyped behavior. The parameters were scored and graded as described in Table 1. Grooming, sniffing, head weaving, gnawing and chewing were observed as major stereotyped behaviors during the ethanol withdrawal in the study. Subjective ratings for agitation and stereotypic behavior were done by an independent observer who was unaware of treatment groups[15].

Pentylenetetrazole kindling: Six h after ethanol withdrawal, seizure threshold was measured in all groups by administering the convulsant drug, Pentylenetetrazole (PTZ) at sub-convulsive dose of 30 mg kg^{-1} intraperitoneally. Animals entering into convulsions were shown convulsive waves axially through body, myoclonic jerks and rearing, clonic forelimb convulsions, generalized tonic-clonic seizure. Behavior of animals was noted for a period of 30 min after administering PTZ and the number of animals showing convulsions were recorded in each group[16].

Table 1: Rating scale for agitation and stereotyped behavior signs induced by ethanol withdrawal in rats

Signs	Scoring	Characteristics observed
Agitation	0	No irritability or aggressive behavior
	1	Rats showing mild or moderate irritability
	2	Very irritable
	3	Handling vocalization and moderately aggressive
	4	Handling vocalization and very aggressive
	5	spontaneous vocalization and very aggressive
Stereotyped behavior	0	No stereotyped behavior
	1	Rats showing only one stereotyped behavior
	2	Two stereotyped behavior
	3	Three stereotyped behavior
	4	Four stereotyped behavior
	5	All of stereotyped behavior

Locomotor activity: Open field test was used for recording the locomotor activity. The animals were 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 60×60 cm wooden maze with 5×5 crossings. Number of lines crossed by the animals from various treatment groups was recorded[17].

Statistical analysis: The results were expressed as Mean±Standard Deviation (SD). The statistical analysis involving different groups was performed by chi-square with four degrees of freedom for parameter 'seizures'. Analysis of variance at 0.2 level followed by Dunnett's test was applied for parameter 'agitation'. Tukey-Kramer test was applied as post hoc test for the parameter 'stereotype'. F-test was applied for locomotor activity.

RESULTS

Bodyweight of the rats and ethanol consumption: There was no significant difference between the groups with regard to alcohol consumption. Bodyweight changes were observed in all groups, barring the control group, mean weight of rats significantly increased in all groups with regard to their respective baseline values (Table 2). The ethanol

Table 2: Changes in weight gain of the rats

	Bodyweight (g) (Mean±SD)	
Groups (n = 6)	Day 0	Day 21
Control	237.50±18.4	249.67±16.6
MLD+Alcohol	203.16±7.8	224.67±13.7*
MLD+Alcohol+Ashwagandha	181.83±4.0	194.16±7.5*
MLD+Alcohol+Brahmi	197.84±8.7	214.67±11.9*
MLD+Alcohol+Diazepam	209.84±11.0	229.84±29.5*

*p<0.05 in comparison to day 0 readings in respective groups (paired t test)

Table 3: Effect of test drugs on seizure activity

Groups (n = 6)	No. of rats with seizures (%)*
Control	0 (0)[b]
MLD+Alcohol	6 (100)[a]
MLD+Alcohol+Ashwagandha	3 (50)[b]
MLD+Alcohol+Brahmi	0 (0)[b]
MLD+Alcohol+Diazepam	0 (0)[b]

*2 sided Pearson's Chi-square was applied. χ^2 (4, n = 30) = 22.8571, p = 0.0001, [a]Significantly different from control, [b]Significantly different from MLD+Alcohol

consumption of the rats in all the groups ranged from 10.53±0.18-16.40±0.90 g kg^{-1} daily during the exposure to ethanol (7.2%).

PTZ kindled seizures in rats: In the control group, sub convulsive dose of PTZ (30 mg kg^{-1}, I.P) administered after 21 days of MLD did not produce seizures in any of rats whereas all the rats (100%) treated with MLD+Alcohol developed seizures after PTZ administration. The computed chi-square with 4 degrees of freedom, χ^2 (4, n = 30) = 22.8571, exceeds the critical value $\chi^{24,0.05}$ = 9.49. Hence, the null hypothesis is rejected. Control group and alcohol (untreated control) group were found to be statistically significant. Ashwagandha, Brahmi and diazepam treated groups were statistically not different with control group but significantly different with alcohol group (Table 3).

Agitation: Mean scoring for parameters of agitation was found to be 0.33±0.52 in the control group whereas in the group administered with MLD+Alcohol, mean scoring was higher and noted as 2.33±1.2. In the groups treated with ashwagandha, Brahmi and diazepam, mean scores were 0.00±0.0, 0.17±0.41 and 0.33±0.52, respectively. Analysis of variance at 0.2 level was significant (p = 0.00846) and was unequal. Welch test was done and found that there is a significant difference between treatments (p = 0.0343). On further analysis with Dunnett's test, it was observed that there was a significant difference between the control group and MLD+Alcohol group (p = 0.000019) whereas, there is no significant difference between the control group compared with ashwagandha, Brahmi and diazepam treated groups

Table 4: Statistical analysis for agitation, stereotype behaviour and locomotor activity

	Groups					
Symptoms	Control	MLD+Alcohol	MLD+Alcohol+Ashwagandha	MLD+Alcohol+Brahmi	MLD+Alcohol+Diazepam	p-value
Agitation						
B/w treatments						0.03433***
Mean (SD)	0.33 (0.52)	2.33 (1.2)	0 (0)	0.17 (0.41)	0.33 (0.52)	
Control vs each group*		0.000019	0.388806	0.664769	1	
MLD+Alcohol vs each group*	0.000019		0.000002	0.000006	0.000019	
Stereotype						
B/w treatments						0.01201****
Mean (SD)	0.67 (0.52)	2 (0.89)	1.5 (0.55)	1 (0.63)	1.17 (0.41)	
Control vs each group**		0.00103	0.0287	0.362	0.1759	
MLD+Alcohol vs each group**	0.001029		0.175969	0.010047	0.028721	
Locomotor						
B/w treatments						0.11805****
Mean (SD)	58.67 (23.65)	41.83 (24.51)	40.67 (34.23)	73.5 (33.14)	36.33 (10.54)	

*Dunnett's test, **Tukey Kramer test, ***Welch's test, ****F-test, MLD: Modified liquid diet, SD: Standard deviation

(p>0.05). Mean agitation in MLD+Alcohol+Ashwagandha was nill when compared with MLD+Alcohol+Brahmi group (Table 4).

Stereotype behavior: Stereotypic behavior in rats was observed after 6 h of alcohol deprivation. The mean score in the control group was 0.67±0.52 whereas, in the alcohol treated group it was 2.00±0.89. In the group treated with Ashwagandha, mean score was found to be 1.50±0.55. Rats treated with Brahmi and diazepam had mean score 1.00±0.63 and 1.17±0.41, respectively.

Analysis of Variance at 0.2 level was not significant among groups (p = 0.36909) and can be considered as equal. F-test was done and found that there is a significant difference between control group and treatment groups (p = 0.01201). On further analysis with Tukey-Kramer test, it was found that there is a significant difference between the control group and alcohol group (untreated control) (p = 0.001029) whereas, there is no significant difference between the control group compared with Brahmi and diazepam treated groups. All the statistical tests are summarized in Table 4.

Locomotor activity: The results for locomotor activity are presented in Table 4. Locomotor activity was observed in open field apparatus and number of crossings was found to be 58.67±23.65 in the control group. The locomotor activity in alcohol dependent group (untreated control) was less as the number of crossings was recorded as 41.83±24.51 only. In the group administered with Ashwagandha extract, locomotor activity was found to be 40.67±34.23. Analysis of variance at 0.2 level was not significant (p = 0.2775) and the groups were equal. F-test was done and found that there is no significant difference between treatments (p = 0.11805).

DISCUSSION

In our study, we observed that Ashwagandha and Brahmi were effective in controlling seizure and agitation when compared to untreated control rats, which is indicative of withdrawal anxiety. The animal model opted for alcohol dependence in this study is well documented and reliable. Alcohol administration with a multiple intermittent paradigms in animals has been found to produce more persistent signs of withdrawal[18]. Ashwagandha and Brahmi known to possess GABA and serotonergic activity may explain its ability of possible usefulness in the treatment of alcohol withdrawal, reduction in the risk of withdrawal seizures and prevention of alcohol relapse.

Alcohol withdrawal is associated with neurogenic excitation leading to seizures[19]. In published literature, it was observed that rats on chronic exposure of alcohol showed a significant reduction in seizure threshold to the convulsant drug, PTZ. PTZ kindling as a stimulant has been proposed to be responsible for causing withdrawal seizures. In the earlier studies, it was established that methanolic extract of the Ashwagandha attenuates PTZ induced seizures in rats in a dose-dependent fashion[20]. In another study, Ashwagandha was found to have antioxidant property and protected the glial and neuronal cells from oxidative as well as glutamate insult which may be responsible for onset of seizures[21]. In our study, we found similar observations with Ashwagandha and Brahmi in inhibiting or preventing seizure activity after alcohol withdrawal.

In our study, we did not observe any significant change in the locomotor activity in control and test groups. In the alcohol treated group, numbers of crossings were decreased in open field test which signifies low exploratory activity. This might be due to prolonged sedative effect of alcohol and reflects non-achievement of withdrawal stage. This finding was non-consistent with observations in previous studies where an increase in locomotor activity was observed which may be due to anxiety and restlessness during alcohol withdrawal[22].

Agitation is prominent during alcohol withdrawal period and is one of the important parameter along with seizure prevention for screening potential activity of drugs against withdrawal symptoms[22]. We observed that Ashwagandha and Brahmi suppressed agitation in alcohol-treated rats which was comparable to diazepam treated rats. This finding is consistent with earlier study where Ashwagandha and Brahmi have shown antianxiety and antiepileptic potential. Agitation is also related with increase in sympathetic activity which is responsible for symptoms in alcohol withdrawal. Ashwagandha, Brahmi and diazepam because of facilitation of GABA activity suppress sympathetic activity[20,23,24].

Brahmi acts as a neuroprotective agent and its mechanism has been studied. It has shown various mechanisms, viz. anti-oxidant activity, reduction in β-amyloid, potentiation and modulation of monoamine, inhibition of acetylcholinesterase, activation of choline acetyltransferase activation and increase in cerebral blood flow[11].

Neuroprotective and anti-epileptic of Brahmi has been widely studied. There is paucity of literature for its anti-anxiety and GABA modulating activity. This drug has the potential for cognitive functions and behavior. In a placebo-controlled, double-blind phase I clinical trial by using single-dose (20 mg up to 300 mg), in 31 healthy male adults with

bacosides A and B, no adverse effects were reported. Another multiple dosage trial (100-200 mg oral per day) was conducted for 4 weeks with Brahmi. In this study, clinical, hematological and biochemical assays revealed no abnormalities[25]. These studies indicate that Brahmi is safe for human consumption as a drug.

The GABA-mimetic activity of Ashwagandha root extract has lent support to the Behavioral experiments. It has GABA mimetic effect and was observed to promote formation of dendrites. It has anxiolytic effect and improves energy levels and mitochondrial health[8]. A study was conducted on rodents with induced chronic stress by electric shock method which led to various adverse effects like gastric ulceration, glucose intolerance, mental depression, immunosuppression and sexual dysfunction. It was found that glycowithanolides isolated from Ashwagandha has shown significant adaptogenic and anti-stress effect[26]. Another study states that GABA (Gamma Amino-butyric acid) which is an inhibitory neurotransmitter function by attenuating the neuron activity and GABA-like activity of Ashwagandha is responsible for its anti-anxiety effect[27]. Glycowithanolides (Sitoindosides VII to X) and Withaferin A are the active constituents of Ashwagandha and are responsible for its antioxidative and adaptogenic or stress response modulating activity[28]. Limitations of our study were mechanism of drug action was not studied and only one dose of test drugs was studied which could have jeopardized some results. Other limitations were sample size was small and potential sex difference were not accounted for in the study.

CONCLUSION

In conclusion, we observed the beneficial effects of Ashwagandha and Brahmi in controlling of alcohol withdrawal symptoms in rats. The standard drug diazepam was also effective in the same manner as observed with Ashwagandha and Brahmi extracts. Further studies at different doses are needed to explore their potential in neurobehavioral animal models for drug addiction and rehabilitation.

SIGNIFICANCE STATEMENT

This study discover the beneficial effect of Ashwagandha (Withania somnifera) and Brahmi (Bacopa monnieri) extracts in controlling alcohol withdrawal symptoms in rats and were comparable to the standard drug diazepam. This study will help the researcher to uncover the critical areas of alcohol withdrawal syndrome that many researchers were not able to explore. Thus, these herbs can be used for developing suitable formulation for the control of alcohol withdrawal symptoms.

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