Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [7] June 2023: 127-132

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Chronic Treatment of *Boerhaavia diffusa* Prevents Alcohol withdrawal Induced anxiety and convulsions in mice

Manisha A.Tayde[1]*, Rupali A. Patil[2], Suvarna A. Katti [3], Shubhangi H. Pawar [4]Veena S. Kasture[5]
1-Department of Pharm. Chemistry, SPES Institute of Pharmacy College, Panchavati, Nashik, Maharashtra ,India
2-Department of Pharmacology,K.K.Wagh College of Pharmacy, Nasik, Maharashtra, India.
3- Department of Chemistry, MGV's Pharmacy College, Panchavati,Nasik, Maharashtra, India.
4- Department of Pharmacology, MGV's Pharmacy College, Panchavati,Nasik, Maharashtra, India.
5-Pinnaclebiomedicalresearchand Institute,Bhopal, Madhya Pradesh, India
*-Corresponding author's Email: mtyade82@gmail.com

ABSTRACT
In India's ancient medical system, the plant Boerhaavia diffusa is frequently employed as a nervine tonic. Animal models of anxiety, locomotion, and convulsions were used in the current work to examine the effects of acute and chronic administration of a methanolic extract of Boerhaavia diffusa. Mice were treated with Boerhaavia diffusa extract (30 mg/kg or 100 mg/kg) and ethanol (2 g/kg of 10 % ethanol) orally for 6 days and the tests were carried out on 7th day. In the elevated plus maze test, mice given extract and ethanol displayed an increase in occupancy in the open arm, but acute administration of extract to mice abstaining from ethanol (2g/kg of 10% ethanol for 6 days) exhibited a decrease in occupancy in the open arm. In open field apparatus, simultaneous administration with extract and alcohol (in the same doses) decreased movement, whereas extract administered after alcohol withdrawal boosted locomotion. While acute treatment of extract following alcohol withdrawal did not offer considerable protection against PTZ-induced convulsions, concomitant treatment of extract and alcohol protected mice from pentylenetetrazole-induced convulsions. The findings imply that the B. When administered after alcohol withdrawal, diffusa extract was ineffective in preventing the development of anxiety, hyperactivity, and convulsions caused by alcohol withdrawal.
***Keywords*:** *Elevated plusmaze,open field apparatus, pentylenetetrazole, Locomotion, GABA*

Received 28.03.2023 Revised 15.05.2023 Accepted 17.06.2023

INTRODUCTION

Alcohol is the most popular inebriant and its use is increasing alarmingly. A person willing to quit alcohol experiences various symptoms of alcohol withdrawal such as tremors, irritability, depression, anxiety, agitation and sometimes convulsions. Symptoms of alcohol withdrawal may develop within 6–24 h after the abrupt discontinuation or decrease of alcohol consumption [1]. These symptoms may be so severe that person is compelled to consume alcohol. Similar symptoms are observed in mice treated repeatedly with intoxicating doses of alcohol and then abruptly withdrawing alcohol. Such alcohol withdrawn mice exhibit a kindling-like state due to reduced inhibition [2].

The withdrawal syndrome that is felt during ethanol abstinence may be a factor in a person's decision to relapse into alcohol abuse [3].Rodent studies have lead to better understanding of complex relationship between alcohol intake, alcohol withdrawal, anxiety and relapse [4,5].In almost all studies involving alcohol withdrawal the effect of drugs is observed on two important behaviours, namely anxiety and convulsion. Serotonergic and GABAergic mechanisms are extensively studied in alcohol withdrawal. The inhibitory neurotransmitter in the central nervous system (CNS) is GABA, which by activating GABAA receptors and opening chloride channels lowers neuronal excitability [6].Pentylenetetrazole (PTZ),a GABAA antagonist, in subconvulsive dose(60mg/kgs.c.) precipitates convulsions in alcohol withdrawn mice[4].Alcohol withdrawal and its acute effects have been linked to GABAB receptors [7]. According to reports alcohol withdrawal has been associated with increased N methyl-D-aspartate (NMDA) function and decreased levels of dopaminergic function. [8,9,10].Anxiety-like behaviours following chronic ethanol exposure has been reviewed[11].Reports have shown that the anticonvulsant, gabapentin, protects against alcohol withdrawal-induced convulsions and anxiety [12]. A GABAergic anxiolytic compound etifoxine reduced anxiety in ethanol withdrawn mice [13]. Studies showed involvement of

GABAA in the anxiogenic effect in alcohol with drawn animals and involvement of 5-HT2 receptors in alcohol withdrawal-induced anxiety [6,14].

In Sanskrit, *Boerhaavia diffusa* Linn (Nyctaginaceae) is referred to as Punarnava, and the name denotes the plant's capacity to aid in tissue regeneration. It has a long history of use as a rejuvenator to speed up healing [15]. The roots of *Boerhaavia diffusa* (*B. diffusa*) have long been used to ease gastrointestinal pain, treat jaundice, and stimulate digestion [16]. Pharmacological research has established the diuretic, hepatoprotective, anticonvulsant, and anti-inflammatory activities of *B. diffusa*[17, 18, 19, 20]. From the roots of *B. diffusa*, a substance with calcium channel blocking action has been identified [21]. According to studies, *B. diffusa* anticonvulsant efficacy is caused by its calcium channel antagonist action [22]. Additionally, it has been claimed that *B. diffusa* roots have anti-stress, adaptogenic, and immunological [23]. Since the roots are endowed with anticonvulsant activity, in the present study the effect of methanolic extract of *B.diffusa* (BDE) on alcohol withdrawal-induced anxiety and convulsions in mice has been assessed.

MATERIAL AND METHODS

Extract preparation

B. diffusa roots and rhizomes were bought from Ayurved Seva Sangh-Aushadhi Bhavan in Nasik in June 2017, and Prof. S. C. Pal, Head of the Pharmacognosy Department at MVP's Pharmacy College in Nasik, authenticated them. The roots and rhizomes (1.0 kg) were coarsely ground, defatted with petroleum ether, and macerated in methanol for seven days. The extract was dried in the air after being concentrated under reduced pressure (yield- 4.4 g). Just before use, the extract was placed in the refrigerator and suspended in 0.5 percent sodium CMC. Doses of 30 and 100 mg/kg were then given orally.

Experimental Animals and drugs

Male Albino mice were obtained from Agro-Biological Farms in Karjat, Mumbai, weighing 20–25 g. Five animals were kept in each cage at a conventional laboratory temperature of (25 2oC), with access to food and water, relative humidity levels of (45–55%), and a 12-hour light/dark cycle. All studies were conducted in the early morning hours. and noon, twelve. Water but not food was withheld from 12 hours before doses until the experiment's conclusion that day. The protocol for this investigation was approved by the institutional animal ethics committee (IAEC).(MGV/PC/CPCSEA/XXXVI/01/2018/18.

Dissolution of Pentylenetetrazole (PTZ, Sigma, USA) was performed in distilled water. Ethanol (2g/kg of 10% v/v) was administered orally, while subcutaneous injection of PTZ (60mg/kg) was given. Extract was administered in a constant volume of 1ml/100g of body weight of animals.

Phytochemical analysis

Phytosterols (Liebermann's test), alkaloids (Dragendorff's test), flavonoids (Shinoda test), phenolics and tannins (5 percent ferric chloride and diluted potassium permanganate), and proteins (Millon's test) were all identified through qualitative phytochemical analysis of the BDE(methanol)[23].

Treatment schedule

The treatment schedule was essentially the same as used previously[4].The elevated plus-maze and open field apparatus were used to evaluate the withdrawal reaction, and a subconvulsive dosage of pentylenetetrazole was used to measure convulsive action.

Acutetoxicity

A dose of (2000 mg/kg p.o.) of BDE(methanol) was given, and after 24 hours, the % mortality was observed.

Measurement of anxiety using Elevated plus-maze

The raised plus maze was used to measure the anxiolytic action (EPM). The EPM employed in this investigation had two opposed 20 x 5 cm wide open arms and two opposite 48 x 5 cm wide closed arms (20 x 5 x 22 cm). The EPM was raised to a 25 cm height. The test animals were initially placed one by one in the plus-center, maze's facing an open arm. The following metrics were recorded during the five-minute test session: (1) the number of entries the animal made in open and closed arms; and (2) the total amount of time spent in each arm [25].

Assessment of Locomotor activity

The withdrawal-induced changes in the locomotor behaviour of mice were evaluated using an open field setup. The open field gadget had wooden construction for its proportions (56 x 56 x 40 cm). The ground was divided into 16 identical, equal-sized squares. The device was set up with each animal carefully placed in a corner, and after five minutes the number of squares crossed and the number of rearing were counted[26].

Assessment of anticonvulsant activity

Following administration of a subconvulsive dose of PTZ (60 mg/kg; s.c.) in vehicle-treated and ethanol-

withdrawn animals, the beginning of Straub's tail, myoclonic spasms, clonic convulsions, and tonic convulsions followed by death were noted. Following PTZ therapy, every animal was watched individually for up to 30 minutes [4].

Statistical analysis

The data was expressed as Mean±SEM and analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test.$P<0.05$ was considered significant.

Results and discussion

Acute toxicity

After 24 hours, the animals who received a dose of 2000 mg/kg, p.o., survived.

Phytochemical investigations

The BDE in methanol indicated presence of proteins, alkaloids, phenolics and tannins and flavonoids. It revealed presence of proteins, phenolics and tannins, saponins, alkaloids and flavonoids.

Impact of BDE on alcohol withdrawal induced anxiety

When mice were given an acute oral dose of 10% ethanol (2 g/kg), the duration of occupancy in open arms was significantly longer (94.8 5.75 s) than it was in mice given a vehicle treatment (62.4 9.10 s). Following acute ethanol treatment, mice showed less preference for closed arm entry compared to the vehicle-treated group. In contrast to mice treated with vehicles (62.4 9.10 s), mice removed from chronic ethanol treatment preferred closed arm entry more (80%) and spent much less time in open arms (28.4 6.0 s). Mice that were regularly given ethanol treatments for six days, followed by a challenge with extract (100 mg/kg) on day seven, exhibited anxiogenic behaviour. In comparison to the ethanol withdrawal group's duration of stay (28.4 6.0 s), the time spent in open arms was much less (9.1 1.64 s). In mice that had been regularly given ethanol for six days prior to being challenged with extract (30 mg/kg) on the seventh day, the quantity of time spent in open arms did not significantly increase (20.0 4.77 s). Mice receiving prolonged treatment with extract (30 and 100 mg/kg) followed by ethanol did not display any withdrawal-induced anxiety, and the time spent in open arms considerably increased compared to the ethanol-withdrawn group (Figure 1, Figure 2).

Effect of BDE extract on ethanol withdrawal induced locomotor activity

Comparatively to the vehicle-treated group, mice who were taken off a chronic ethanol administration displayed more square crossings (hyper locomotion). The ethanol withdrawal-induced hyper locomotor activity was significantly increased in mice that had been regularly given ethanol for six days before being challenged with extract on day seven. The mice treated with the vehicle travelled through 136.6 9.02 squares, but the mice treated with the ethanol travelled through 86.8 8.06 squares, a considerably (p 0.05) smaller number. In mice treated abruptly with BDE (methanol)as well as mice with ethanol withdrawal, locomotor activity increased, and the effect of the extract was dose dependent. Lower doses of the extract and ethanol combination in mice did not result in any discernible change, whereas larger doses (100 mg/kg) significantly reduced locomotor activity (p 0.05). In none of the groups, the number of rearing significantly changed (Figure3).

Effect of BDE on ethanol withdrawal induced Pentylenetetrazole convulsion

Following treatment of PTZ (60 mg/kg; s.c.), acute administration of ethanol showed delayed onset of convulsions and 20% mortality. Following PTZ injection, ethanol-withdrawn mice experienced severe tonic-clonic convulsions and 80% death. Mice repeatedly given ethanol for six days, then challenged with extract (100 mg/kg) on the seventh day, then given PTZ, experienced severe tonic-clonic convulsions, with a 40% fatality rate. Group of mice given extract (30 and 100 mg/kg) plus ethanol concurrently for 6 days demonstrated delayed start and no mortality after receiving PTZ on day 7, in contrast to the mice group given ethanol withdrawal (Table1).

The results emanated in the present study indicate that simultaneous treatment of the BDE(methanol)along with ethanol exhibited anxiolytic activity in mice whereas acute administration of the extract to ethanol withdrawn mice showed anxiogenic effect. The chronic treatment with the extract in the ethanol- withdrawn mice also inhibited PTZ- induced convulsions. The BDE(methanol)did not produce any mortality in dose sun to 2000mg/kg; p.o. indicating its wide margin of safety.

Several previous reports based on EPM test suggests that withdrawal of ethanol following chronic treatment gene rates anxiety[27, 28, 29].

In the present study, acute administration of ethanol produced a significant increase in the time spent in open arms of elevated plus maze suggesting an anxiolytic profile of ethanol in mice. Acute administration of extract on the 7thday to ethanol-withdrawn animals displayed considerable anxiogenic response, increased hyperlocomotor activity and also produced severe tonic-clonic convulsions. Similar observations have been reported earlier. [4,25] This suggests that acute treatment of extract is not effective in reversing symptoms of alcohol withdrawal. The animals treated chronically with extract

followed by ethanol for 6days displayed a significant inhibition of withdrawal- induced anxiety on the 7th day. Following administration of a subconvulsive dose of PTZ, chronic treatment of the extract at both dosages significantly reduced alcohol withdrawal-induced increased locomotor activity and exhibited delayed onset. These observations are in congruence with previous report [4].

Animals chronically treated with ethanol and then withdrawn are typically more prone to seizure activity than naïve controls. Several studies show that GABAA receptor subunit mRNA and protein level change during repeated ethanol exposure [6].

Both the acute and the chronic effects of ethanol on the central nervous system are primarily mediated through GABAA receptors [4]. Changes in GABAA receptor subunits expression are implicated in development of ethanol tolerance and in central hyper excitability associated with alcohol withdrawal [30,31] . The ability of BDE to prevent PTZ-induced convulsions in alcohol-free mice suggests that the extract works through the GABAergic system.

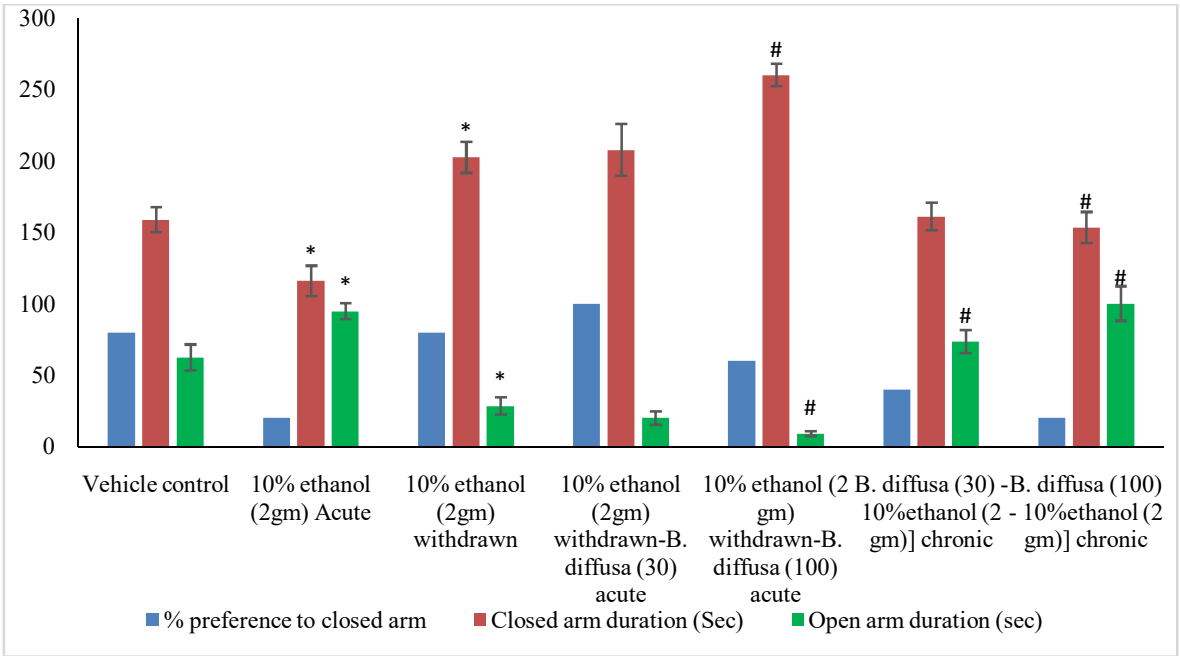

Fig. 1: Effect of methanolic extract of *B.diffusa* on alcohol withdrawal-induced anxiety in mice using elevated plus maze- % Preference and Closed and Open arm duration.

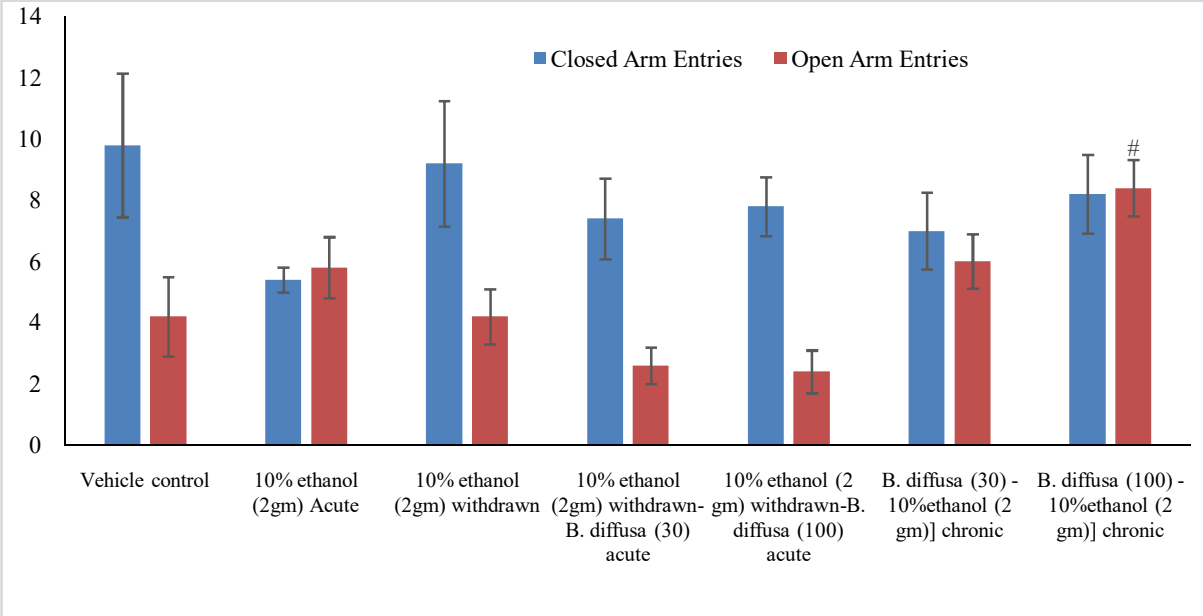

Fig. 2: Effect of methanolic extract of *B.diffusa* on alcohol withdrawal-induced anxiety in mice using elevated plus maze- Closed and Open arm entries.

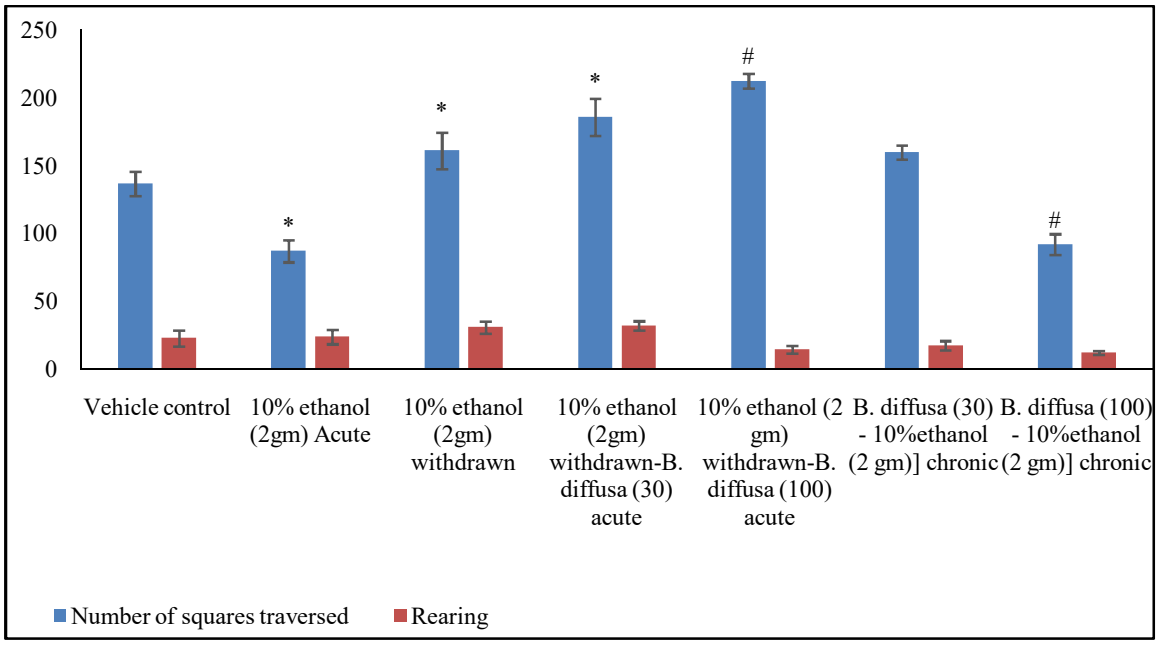

Fig.3: Effect of methanolic extract of *B.diffusa* on alcohol withdrawal-induced locomotor activity in mice using open field apparatus.

TABLE 1 :EFFECT OF BDE (METHANOL)ONETHANOLWITHDRAWAL-INDUCEDPTZ CONVULSION.

Treatment groups	Latency to Straub tail (sec)	Latency to Myoclonic jerk (sec)	Latency to clonic convulsion (sec)	Latency to tonic convulsion (sec)	% Mortality
Vehicle control	45.2 ± 2.9	123.0 ± 6.8	133.8±29.98	190.0 ±71.8	20
10%ethanol (2gm) Acute	208.4 ±7.84*	239.8± 54.97*	806.8±112.6*	903.0±95.21*	20
10%ethanol (2gm) W/D	38.2 ±6.45	46.0 ±4.03*	72.0 ± 17.15	89.0 ±28.4*	80
10% ethanol(2 gm) W/D +BDE(30)acute	19.6 ±8.9	48.2 ±2.2	62.2 ±19.21	90.0 ±13.6	60
10% ethanol(2gm)W/D+ BDE (100) acute	17.6 ±5..24	32.1 ±4..34	51.6 ±26.34	86.6 ±19.6	40
BDE (30)+10% ethanol(2gm)chronic	112.6±3.69#	248.0±16.83#	271.2±16.87#	404.7±83.7#	00
BDE (100mg)+ 10% ethanol(2gm)chronic	299.3 ±15.5#	436.8±33.33#	471.0 ±49.85#	748.2 ± 86.5#	00

CONCLUSION

From the results of study it can be said that BDE has anxiolytic and anticonvulsant properties, and its concurrent usage reduces anxiety, hyperactivity, and convulsions brought on by alcohol withdrawal. Once mice were taken off alcohol, the acute dose of extract was ineffective in reversing the impact. To identify the mechanism of action of *B. diffusa's* anxiolytic activity and to pinpoint the active component(s) responsible for the activity, more investigation is needed.

ACKNOWLEDGEMENT

Authors are very thankful to Dr.S.B.Kasture, Director, Pinnacle Biomedical Research Institute, Bhopal, Madhya Pradesh for his valuable support during the project work.

REFERENCES

1. Becker HC (2000). Animal models of alcohol withdrawal. *Alcohol Res. Health*, 24: 105-113.
2. Kliethermes CL,Cronise K,Crabbe JC(2004). Anxiety like behavior in mice in two apparatus during withdrawl from chronic ethanol vapours inhalation, Alcohol.*Clin Exp Res*,28:1012-1019.
3. Kulkarni SK, Verma(1993). Protective effect of Mentat (BR-16A) A Herbal Preparation, on alcohol abstinence induced anxiety and convulsions. *Indian J.Exp Biol*,31:435-439.
4. LovingerDM,CrabbeJC 2005).Laboratory models of alcoholism: Treatment target identification and insights into mechanisms. *NatNeurosci*, 8:1471-1480.

5. Davies M (2003). The role of GABA $_A$ receptors in mediating the effects of alcohol in the CNS. *J. Psych Neurosci*, 28:263-274.
6. HumeniukRE, White JM, Ong J (1994). The effects of GABAB ligands on alcohol withdrawal in mice.*Pharmacol Biochem Behav*, 49:561-566.
7. Hu XJ,TickuMK(1997).Functional characterization of a kindling- like model of ethanol withdrawal in cortical cultured neurons after chronic intermittent ethanol exposure. *Brain Research,* 767:228–234.
8. Volkow ND,Fowler JS,Wang GJ (1999). Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. *Psychopharmacol*, 13:337-345.
9. Kliethermes CL (2005). Anxiety-like behaviors following chronic ethanol exposure*Neuroscience and Biobehavioral Reviews,*28:837–850
10. WatsonWP, Robinson E, Little HJ(1997). The novel anticonvulsant, gabapentin, protects against both convulsant and anxiogenic aspects of the ethanol withdrawal syndrome. *Neuropharmacology,* 36:1369–75.
11. Verleye M, Heulard I,Gillardin JM (2009).The anxiolytic etifoxine protects against convulsant and anxiogenic aspects of the alcohol withdrawal syndrome in mice.*Alcohol*, 43:197-206.
12. Lal H,Prather PL,Rezazadeh SM (1993). Potential role of 5HT1C and/or 5HT2 receptors in the mianserin-induced prevention of anxiogenic behaviors occurring during ethanol withdrawal. *AlcoholClin ExpRes*, 17:411-417.
13. DharML, DharMM, Dhawan BN, Mehrotra BN, Ray C(1968). Screening of Indian plants for biological activity,partI.*Indian JExpBiol* ,6:232–247.
14. Kirtikar KR, Basu BD(1956). Indian Medicinal Plants. Ed 2nd. (3). *Lalit MohanBasu*,Allahabad, 1956.
15. Gaitonde BB, Kulkarni HJ, Nabar SD(1974). Diuretic activity of punarnava (*Boerhaavia diffusa*).*Bul lHaffkine Institute*, 2:24-24.
16. Rawat AK ,Mehrotra S ,Tripathi SC,Shome U(1997). Hepatoprotective activity of *Boerhaaviadiffusa* L. Roots-a popular Indian ethnomedicine. *J. Ethnopharmacol*. 56:61–66.
17. Adesina SK(1979). Anti-convulsant properties of the roots of *Boerhaaviadiffusa.Q J. CrudeDrugRes*, 17:84–86.
18. Bhalla TN, Gupta MB, Bhargava KP(1971).Antiinflammatory activity of *Boerhaavia diffusa*L. *Ind J. MedRes*, 6:11-15.
19. Lami N, Kadota S, Ikuchi TK, Momose Y(1991). Constituents of the roots of *Boerhaavia diffusa*L.III. Identification of Ca channel antagonistic compound fromthemethanolextract.*ChemPharmBull,*39:1551–1555.
20. Kaur M, Goel RK (2011). Anti-Convulsant activity of *Boerhaavia diffusa*: Plausible role of calcium channel antagonism. *Evidence-Based Complementary AlternativeMed*, 2011.https://doi.org/10.1093/ecam/nep192.
21. Sumanth M,Mustafa SS (2007). Antistress, adoptogenic and immunopotentiating activity roots of *Boerhaavia diffusa* in mice,*IntJ. Pharmacol*, 3:416-420.
22. Khandelwal KR (2004).Practical Pharmacognosy: Technique and Experiments.E d . 12.Nirali Prakashan,Pune,p185-186.
23. Lister RG (1987). The use of a plus maze to measure anxiety in the mouse. *Psychopharmacology*,92:180-185.
24. Turner RA (1978). Screening Procedure in Pharmacology. Academic Press, New York,p87-99.
25. Pandey SC, Zhang D, Mittal N,Nayyar D (1999). Potential role of gene transcription factor cyclic –AMP responsive element binding protein in ethanol withdrawal related anxiety. *J. Pharmacol Exp Ther*, 288:866.
26. Gupta GL,Rana AC (2008). Effect of *Withania Somnifera* Dunalin ethanol induced anxiolysis and withdrawal induced anxiety in rats.*Ind J. Exp Biol,* 46:470-75.
27. Kokare DM,Chopade CT,Subhedar NK(2006).Participation alpha- melanocyte stimulating hormone in ethanol induced anxiolysis and withdrawal anxiety in rats. *Neuropharmacology*, 51:536.
28. Sanna E, Maria CM, Fabio B, Talani G. et al (2003). Changes in GABA$_A$ Receptors gene expression associated with selective alteration in receptor function and Pharmacology after alcohol withdrawal. *The journal of Neuroscience,* 23(37):11711-11724.

CITATION OF THIS ARTICLE

Manisha A.T, Rupali A. P, Suvarna A. K, Shubhangi H. P, Veena S. K. Chronic Treatment of *Boerhaavia diffusa* Prevents Alcohol withdrawal Induced anxiety and convulsions in mice. Bull. Env. Pharmacol. Life Sci., Vol 12[6] June 2023: 127-132.