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ORIGINAL ARTICLE

Effect of *Valeriana wallichii* on Alcohol Addiction in Mice

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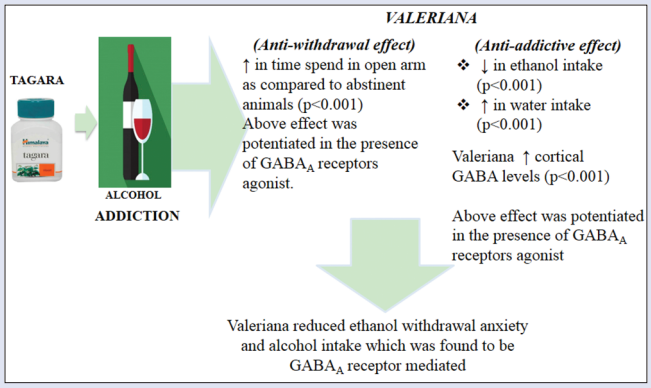
ABSTRACT

Background: Alcoholism is the most common form of addiction and a worldwide problem. Alcohol addiction primarily leads to alcohol dependence and on abstinence leads to withdrawal effects. Common pharmacological properties of compounds used against alcohol addiction include anti-anxiety, anticonvulsant, antidepressant, and nootropic actions. *valeriana wallichii* known ethnopharmacologically for its sleep-inducing effects have all the above-pharmacological properties. **Aim:** Here, we screen valeriana for possible anti-addictive potential. **Materials and Methods:** Effect of valeriana was measured on ethanol withdrawal anxiety using elevated plus maze. The role of valeriana on chronic ethanol consumption (21 days) was measured using two bottle choice method of voluntary drinking. We also measured the effect of the above herb on cortico-hippocampal gamma-aminobutyric acid (GABA) levels. **Results:** *Valeriana* was found to reduce ethanol withdrawal anxiety in a dose-dependent manner. The herb also decreased ethanol intake and increased water intake significantly ($P < 0.001$) after 4 days of administration. Both these effects were potentiated ($P < 0.001$) by $GABA_A$ agonist zolpidem, and not affected by N-Methyl D-aspartic acid antagonist memantine, suggesting the role of $GABA_A$ receptor. Chronic administration of valeriana (10 days) also significantly ($P < 0.01$) increased cortico-hippocampal GABA levels in mice. **Conclusion:** Thus, valeriana reduced both ethanol dependence and withdrawal in a $GABA_A$-dependent manner showing promising anti-addictive potential.

Key words: Addiction, alcohol, gamma-aminobutyric acid, *Valeriana*

SUMMARY

- *Valeriana* reduced ethanol withdrawal anxiety and showed anti-addictive property in a $GABA_A$ dependent manner.



Abbreviations Used: GABA: Gamma-aminobutyric acid; CNS: Central nervous system; CPP: Conditioned place preference; EPM: Elevated plus maze; NMDA: N-Methyl D-aspartic acid; HIV: Human Immunodeficiency virus.

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INTRODUCTION

Alcoholism is the most common form of addiction and a worldwide problem. Alcoholism is the fifth leading risk factor for premature death and disability. Alcoholism may result in numerous disorders and injury-related medical problem. This includes cirrhosis of liver, liver cancers, Human Immunodeficiency Virus transmission, and physical injuries primarily traffic accidents and falls.[1,2] Alcohol dependence is a multifactorial process that involves neurological, psychological and environmental factors.[3] An individual usually gets addicted to alcohol consumption primarily due to alcohol's rewarding effects including euphoria and anti-anxiety potential and its noxious withdrawal effects including anxiety, muscle cramp, and depression. Relapses are primarily blamed toward withdrawal-anxiety, which reflects neurological changes because of continued alcohol exposure. Association of the above effects of alcohol with environmental clues may primarily determine alcohol intake.[4] Alcohol withdrawal is associated with various unwanted effects such as agitation, sleep disturbances, anxiety, and increased sensitivity to pain, etc.[5] Disulfiram, naltrexone, and acamprosate are US Food and Drug Administration (US-FDA) approved medications for the treatment of alcohol dependence. While Ibogaine is a drug with anti-addictive potential in animals is undergoing clinical trials.[6] Ibogaine has shown serous cardiotoxicity; the other FDA approved compounds showed various adverse drug reactions including hepatotoxicity, psychosis, restlessness, constipation, optic neuritis, and are contraindicated in individuals with hepatic and cardiovascular complications. Thus, the commonly used drugs against alcohol addiction substantially affect the quality of life of the individual under anti-addictive therapy.

Compounds derived from natural sources have provided us with new lead molecules with high-therapeutic efficacy and low toxicity thus playing a major role in drug development.[6] *Valeriana wallichii*[7] is native to India, Nepal and China. This plant includes its rhizome part from which the active constituents are extracted. It has been traditionally used against insomnia, obesity, Central nervous system (CNS) disorders and also skin diseases. *V. wallichii* is widely known for its actions on CNS against anxiety, insomnia, epilepsy, antidepressant, and hysteria. Valerian contains valerianic oil (volatile oil) containing valerenic acid, isovaleric acid and terpineol. Valerinic acid, decreases the breakdown

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of gamma-aminobutyric acid (GABA) in the brain and act as $GABA_A$ receptor substrate resulting in its sedative and sleep-enhancing properties. Previous work suggests, CNS depressant action of *Valeriana* is due to the flavonoid glycosides such as hesperidin and linarin thus causing a general inhibition of neuronal activity. Flavone derivatives have shown $GABA_A$ receptor binding with benzodiazepine binding site which may be responsible for their depressant actions in mice. Valerian may inhibit α-ketoglutarate dehydrogenase and GABA transaminase thus preventing the breakdown of GABA and increasing its levels. It may also enhance the response of $GABA_A$ receptor and thus acts as $GABA_A$ agonist.[8,9] Here, we report that acute administration of *Valeriana* may prevent ethanol withdrawal anxiety, whereas it's long-term administration may reduce ethanol intake in mice. Chronic administration of *Valeriana* extract led to change in cortico-hippocampal GABA levels, whereas $GABA_A$ agonist potentiated its anti-addictive properties. The, above results suggest that *V. wallichii* may prevent ethanol addiction in a $GABA_A$-dependent manner.

MATERIALS AND METHODS

Animals

Swiss albino mice (20–30 g) were used in this study. Animals were issued from the Institutional Animal House of Birla Institute of Technology, Mesra. All animals were kept in polyacrylic cages and maintained under standard conditions (room temperature 24°C–27°C and humidity 60%–65% with 12:12 light: dark cycles). The food was provided in the form of dry pellets and water *ad libitum*. The animals were allowed to get acclimatized to the laboratory conditions for 7 days before the commencement of the experiment. All experiments involving animals complied with the ethical standards of animal handling and approved by the Institutional Animal Ethics Committee (BIT/PH/IAEC/11/2016).

Estimation of blood alcohol levels

Blood was collected by retro-orbital bleeding with animals in light ether anesthesia after 20 min of ethanol administration. Ethanol levels were measured using ultraviolet (UV) assay kit (DRI ethyl alcohol assay) for alcohol estimation based on manufacturer's protocol (Thermo Fisher Scientific [India] Pvt. Ltd.).

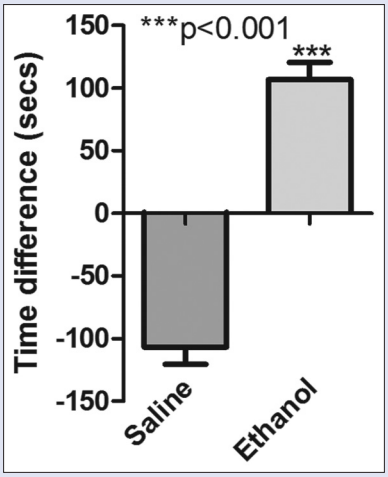

Figure 1: Condition place preference on ethanol administration. Time spent in alcohol and saline administration chamber on day 11 by control and ethanol-treated group. Ethanol-treated animals showed significant increase in ($P < 0.001$) time spent in ethanol-paired alcohol compared with saline-paired chamber. Values expressed as mean ± standard error of the mean $n = 6$

Development of conditioned place preference model

Apparatus

The conditioned place preference (CPP) apparatus contain three compartments. The two compartments (30.5 cm × 26.5 cm × 37 cm) were connected by a central corridor (12.75 cm × 23 cm × 15.25 cm). The compartment on the left had black walls with a perforated stainless steel floor with round holes on staggered centers. The central corridor was transparent with a smooth floor, and the right compartment had white walls with a stainless-steel mesh floor.

CPP was performed as described Thanos *et al.* and Ledesma *et al.*[10,11] with slight modifications. It mainly consists of three phases:

1. Preconditioning phase: (1st and 2nd day). The animals were placed in the middle chamber and allowed to explore both the chambers for 30 min
2. Conditioning phase: (3rd–10th day). Each mouse was treated for eight consecutive sessions with the alternate oral administration of ethanol and saline. On days 3, 5, 7, and 9, the animals were administered ethanol (2 g/kg body weight; i.p. 10% [v/v]) and placed in one compartment for 30 min. Besides, on days 4, 6, 8, and 10, the animals were administered saline and placed in opposite compartment
3. Postconditioning phase: (11th–12th day) Mice were placed in the middle chamber and allowed free access to both chambers for 30 min. Time spent in ethanol and saline-paired chamber was measured
4. Treatment protocol: After the development of withdrawal symptoms (15th day after 3 days of abstinence from ethanol), the following treatment schedule was followed:

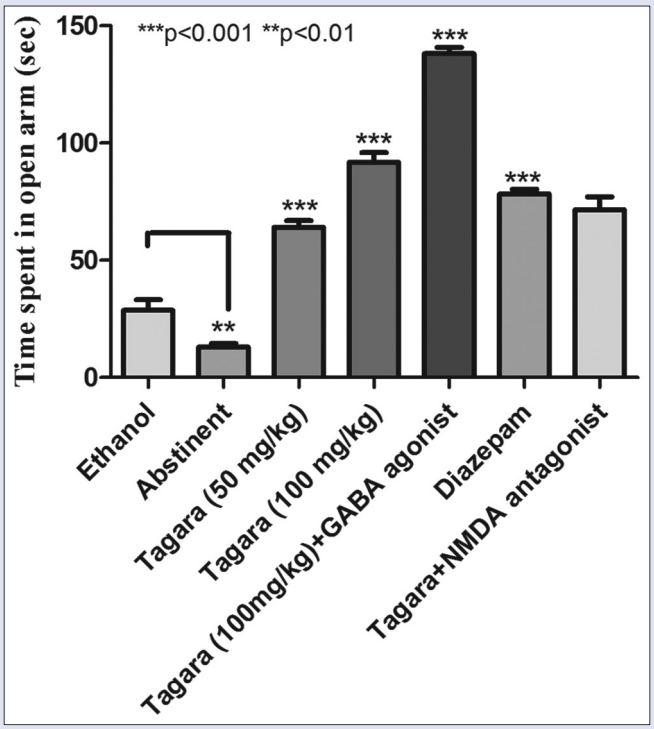

Figure 2: Effects of *Valeriana* on ethanol-withdrawal anxiety using elevated plus maze test. Ethanol abstinence significantly decreased ($P < 0.01$) time spend in open arm compared with ethanol-treated animals. *Valeriana* treatment to abstinent animals significantly (50 mg/kg, $P < 0.001$ and 100 mg/kg, $P < 0.001$) increased time spend in open arm compared with abstinent animals. Diazepam also significantly increased time spend in open arm over abstinent and ethanol-treated groups ($P < 0.001$). Animals treated with *Valeriana* in the presence of $GABA_A$ agonist spent significantly less time in the open arm ($P < 0.001$) compared with abstinent animals. Values expressed as mean ± standard error of the mean $n = 6$

- Group 1: Saline
- Group 2: Ethanol
- Group 3: Ethanol abstinent + Tagara (Himalaya Drug Company; 50 mg/kg)
- Group 4: Ethanol abstinent + Tagara (100 mg/kg)
- Group 5: Ethanol abstinent + Tagara (100 mg/kg) + $GABA_A$ agonist
- Group 6: Ethanol abstinent + Diazepam (1 mg/kg)
- Group 7: Ethanol abstinent + Tagara (100 mg/kg) + N-Methyl D-aspartic acid (NMDA) antagonist.

The behavioral test (elevated plus maze test) was performed 60 min after oral drug administration and 30 min after i. p. administration.

Behavioral studies to measure ethanol withdrawal anxiety

Elevated plus maze

Elevated plus maze (EPM) was performed as described by Kokare *et al.*[12] After drug treatment, individual mice were placed at the center of the maze, head facing an open arm. During the 5 min test period, the number of entries and time spent on the open arm were recorded automatically (Medicraft Electromedical, Lucknow, Uttar Pradesh, India).

Chronic-treatment study to measure ethanol intake

Two bottle choice ethanol drinking

We used the standard two-bottle choice protocol, which is a widely used animal model to capture aspects of voluntary ethanol consumption in humans.[13] Following 7 days of acclimatization, animals were subjected to an ethanol drinking acquisition regimen. The animals remained in their home cages at all times throughout the study but had their water bottles removed during a 4 h and ethanol presentation period. During this time, animals were exposed to a free choice between ethanol (15% v/v) and water for 20 days but with no drug pretreatment.

After 20 days of ethanol administration, animals were divided into different groups for 10 days of treatment. Each day, the bottles were weighed before and after 4 h of limited access period and the differences were used to calculate the water and ethanol intake. The mean intake was expressed as g/kg body weight/day of water and g/kg body weight/day of ethanol intake. All animals were given unrestricted food access. Every 2 days, the bottles were switched to eliminate place preference.[14] After 20 days of pretreatment with ethanol (15% v/v), the animals were divided into different treatment groups ($n = 7$ per group) as follows:

- Group 1: (control) received saline 30 days
- Group 2: received free choice ethanol (15% v/v)/water 30 days
- Group 3: received free choice ethanol (15% v/v)/water and Tagara (100 mg/kg) 21st–30th day
- Group 4: received free choice ethanol (15% v/v)/water and diazepam 21st–30th day
- Group 5: received free choice ethanol (15% v/v)/water, $GABA_A$ agonist and Tagara (100 mg/kg) 21st–30th day.

After the above experimental protocol of 30 days, five animals per group were sacrificed under ether anesthesia by cervical dislocation for biochemical estimation.

Estimation of gamma-aminobutyric acid levels in brain tissue

Brain tissue was homogenized in 5 mL of 0.01 M HCl. In this homogenate, 8 mL of ice cold ethanol was added and kept for 1 h at 0°C. The contents were centrifuged for 10 min at 16,000 rpm, and supernatant was collected in a petri dish. The precipitate was washed three times with 5 mL of 75% ethanol. The washes were combined with supernatant and evaporated to dryness. To the dry mass, 1 mL water and 2 mL chloroform were added and centrifuged at 2000 rpm. Upper phase containing GABA was separated, and 10 µL of it was applied as spot on Whatman filter paper. The mobile phase consisted of n-butanol, acetic acid, and water in 4:1:5 ratios. The chamber was saturated for half an hour with mobile phase. The paper chromatogram was developed with ascending technique. The paper was dried in a hot air oven and

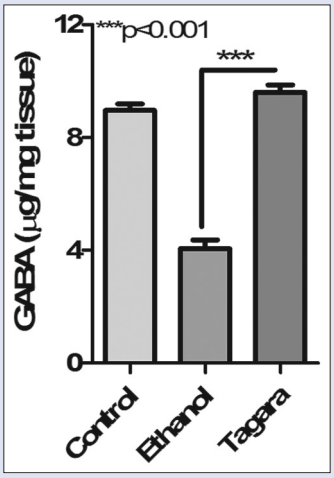

Figure 3: Effect of *Valeriana* on cortical GABA levels. Treatment with *Valeriana* significantly increased gamma-aminobutyric acid levels ($P < 0.001$). Values expressed as mean ± standard error of the mean $n = 6$

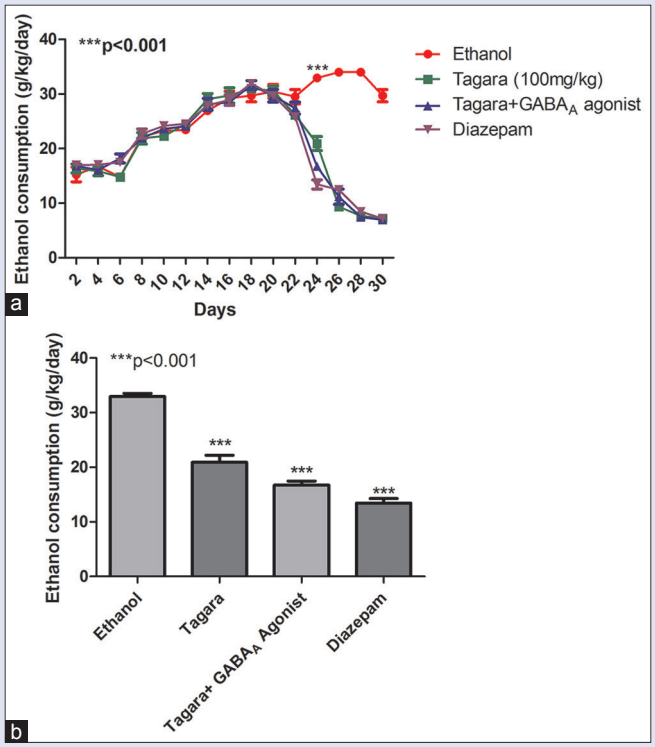

Figure 4: Effect of *Valeriana* on ethanol consumption (a) Ethanol consumption patterns from day 2–30 (b) Ethanol consumption on day 24. There was a significant decrease in ethanol intake ($P < 0.001$) after treatment with *Valeriana* alone or in combination with GABAA agonist and on diazepam treatment. Values expressed as mean ± standard error of the mean $n = 6$

then sprayed with 0.5% ninhydrin solution in 95% ethanol. The paper was dried. Blue spot developed on paper, which was cut and heated with 2 mL ninhydrin solution on a water bath at 60°C–65°C. Water was added to the solution and kept for 1 h and supernatant was used. Absorbance was measured at 570 nm on a UV-visible spectrophotometer.

RESULTS

Blood alcohol levels

Blood alcohol levels were measured by DRI Ethyl Alcohol Assay. This method is based on the high specificity of Alcohol Dehydrogenase for ethyl alcohol. The alcohol concentration in blood was found to be 40 mg/dl.

Conditioned place preference

The CPP test, consists of three phases-precondition, condition and postcondition. In precondition phase, the preference of animals was observed. Whereas, in condition phase, the animals were given the alternate dose of ethanol or saline in ethanol chamber and saline chamber respectively and finally postcondition phase was done on 11th day, where the animals were allowed to explore both the chambers freely and to make choice to spend more time in either of the environments provided to them. This environment provides a direct measure of the conditioned reinforcing effect of a drug. Animals were found to prefer the ethanol-paired chamber over the saline-paired chamber.

Ethanol CPP is illustrated in Figure 1 on 11th day as total time spent in each chamber. There was a significant increase in time spent in the ethanol-paired chamber as compared to the saline-paired chamber as compared to the saline-paired chamber ($P < 0.001$) suggesting that the animals were addicted to ethanol.

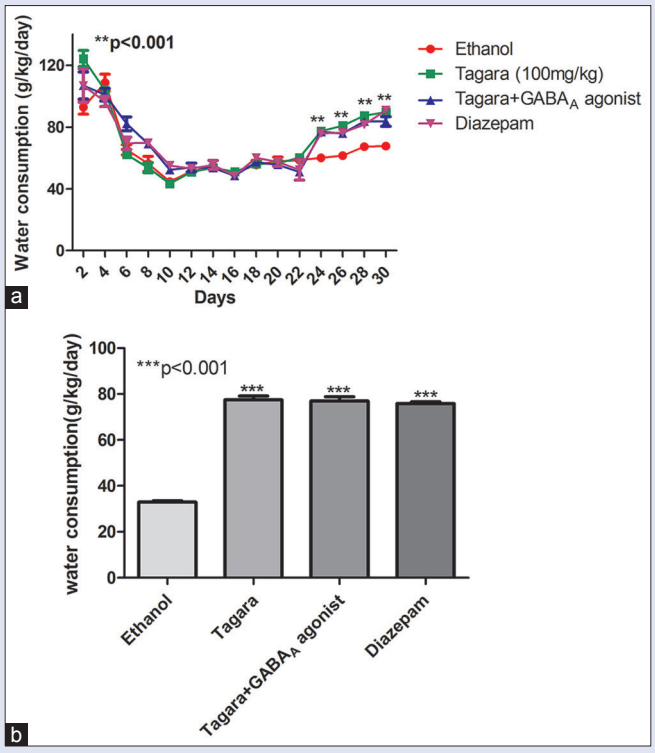

Figure 5: Effect of *Valeriana* on water consumption (a) ethanol consumption patterns from day 2–30 (b) Ethanol consumption on day 24. There is a significant increase in water intake ($P < 0.001$) for *Valeriana*, *Valeriana* in combination with GABA$_A$ agonist and on diazepam treatment. Values expressed as mean ± standard error of the mean $n = 6$

Effect of valeriana on withdrawal anxiety

Ethanol withdrawal anxiety was measured by EPM test after 8 days of conditioning phase in which alternate dose of ethanol and saline was given for 10 days, followed by 5 days of abstinence. After 5 days of abstinence from ethanol (2 mg/kg b. w; i. p. 10% v/v, 8 days), animals showed significant decrease in time spent in the open arm of the EPM as compared to the control suggesting withdrawal anxiety. Administration of Valeriana (50 and 100 mg/kg) led to decrease in withdrawal anxiety as evidenced by the significant increase in time spent in the open arm of EPM. The above effect was found to be dose-dependent [Figures 2 and 5b]. Diazepam was used as a standard anxiolytic. The combination of valeriana + GABA$_A$ agonist, also showed significant ($P < 0.001$) anxiolytic action thus suggesting that anti-anxiety effect of valeriana may be mediated by GABA$_A$ receptors. However, NMDA antagonist memantine did not alter the effect of Valeriana alone [Figure 2].

Effect of valeriana on GABA levels

Brain GABA level was estimated in cortical homogenates. The level of GABA in the cortical region significantly reduced after ethanol administration for 20 days. However, 10 days of treatment with Valeriana significantly increases the GABA levels in the cortical region and go it back to levels seen in normal animals [Figure 3].

Effect of valeriana on Ethanol intake

For the chronic study, two bottle choice ethanol drinking was used, and the study was conducted for 30 days. For the first 20 days, choices were between ethanol and water which was given freely to the animals i.e., 4 h of ethanol and rest of the time water was given. After that for the next 10 days treatment was given with *Valeriana*, *Valeriana* and GABA agonist (zolpidem) combination. Ethanol intake was found to increase till 20th day. From 24th day, there was a significant decrease in ethanol intake ($P < 0.001$) for animals treated with *Valeriana*, *Valeriana* in combination with GABA$_A$ agonist and diazepam groups when compared to ethanol-treated animals. The above results suggest that *Valeriana* prevented chronic ethanol intake while this effect may be mediated by GABA$_A$ receptors [Figure 4a and b]. From 24th day water intake was also found to significantly increase ($P < 0.001$) in *Valeriana*, *Valeriana* in combination with GABA$_A$ agonist and diazepam treated animals [Figure 5a and b]. Here, diazepam was used as standard drug. The above results confirm that valeriana may prevent chronic ethanol intake while this effect may be mediated by GABA$_A$ receptors.

DISCUSSION

In the present work, we studied the effect of *Valeriana* on withdrawal anxiety and chronic ethanol consumption in mice. The CPP results suggested that when given a free choice of environment after acute administration of ethanol (2.0 mg/kg b. w.; i. p., 10% v/v) on 11th day the animals preferred the ethanol-paired chamber to that of saline/water paired chamber thus showing ethanol addiction. The anxiolytic potential of *Valeriana* and diazepam was studied on acute ethanol withdrawal anxiety using EPM as a behavioral model of anxiety. Chronic ethanol consumption followed by withdrawal, results in abstinence syndrome.[15,16] Alcohol withdrawal commonly results in anxiety disorders, which may be primarily responsible for reverting back to alcohol use.[17] Ethanol may also be responsible for inter-receptor communication contributing to development of tolerance, dependence and withdrawal symptoms.[18] During ethanol withdrawal, upregulation of NMDA receptors[19] and a downregulation of GABA$_A$ receptors have been observed.[20] In our study, valeriana reversed the withdrawal anxiety after ethanol abstinence in a dose-dependent manner as reveled by treated animals spending increased time in the open arm of the EPM compared to untreated

ethanol-withdrawn animals.[9] Valeric acid, from *V. wallichii* with GABA like property may also acts as NMDA-receptor antagonist.[21] However, in our work, NMDA antagonist mamentine was unable to potentiate the effect of *V. wallichii* against ethanol withdrawal anxiety. The above result suggests the lack of involvement of NMDA receptors in anxiolytic effect of valeriana against ethanol withdrawal anxiety. However, zolpidem a $GABA_A$ agonist, potentiated the effect of valeriana thus suggesting the role $GABA_A$ receptors in valeriana mediated reduction in withdrawal anxiety. Valeric acid may also ameliorate various neuropsychiatric disorders.[22] Valeric acid may inactivate α-ketoglutarate dehydrogenase resulting in increased GABA shunt activity which eventually increases GABA levels.[23] It may also prevent the GABA transaminase function[24] or act as $GABA_A$ receptor agonist.[25] Downregulation of $GABA_A$ receptor and/or decrease in the GABAergic transmission may be responsible for ethanol withdrawal. The findings from the present investigation suggest that *V. wallichii* may reduce ethanol withdrawal anxiety in a $GABA_A$-dependent manner. Long-term ethanol exposure may lead to altered neurotransmitter functions resulting in the development of alcohol dependence. Alcohol may inhibit NMDA receptors while prolonged ethanol intake may result in compensatory "upregulation" of this receptor functions. Previous studies have reported that ethanol alters the function of a number neurotransmitter receptors including $GABA_A$.[26] This imbalance between excitatory and inhibitory processes is primarily responsible for alcohol withdrawal symptoms. The upregulation of NMDA receptor functions after chronic ethanol intake is to sustain ethanol exposure. In our study after 30 days of chronic ethanol intake animals showed decreased cortical level of GABA. Evidences showed a decrease in GABAergic function after chronic administration of ethanol in experimental animals may be due to decrease in the $GABA_A$ receptor levels or altered receptor function. *Valeriana* treatment for 10 days after 20 days of ethanol treatment increased the GABA levels in cortex back to normal. In two bottle, choice model of voluntary drinking *Valeriana* treated animals showed a significant decrease in ethanol intake and a significant increase in water consumption as compared to control group from 24th day or after 4 days of therapy. The results were comparable with diazepam treated animals. Hence, *Valeriana* showed decreased withdrawal anxiety as well as decrease chronic ethanol intake, signifying its anti-addictive potential. The reduction in the chronic ethanol intake in *Valeriana* and *Valeriana* in combination with zolpidem-treated groups may also be a function of GABAergic system. Essential oil from *V. wallichii* may increase the levels of norepinephrine and serotonin responsible for its antidepressant actions. The study showed the involvement of the L-arginine-NO-cGMP pathway in the antidepressant-like effect of *V. wallichii*.[27] It has also been shown to have analgesic property.[28] While roots and rhizomes of *V. wallichii* contains valepotriates which may also facilitate GABA noradrenergic and/or dopaminergic transmission thus acting as an antidepressant.[29] The above compounds with their respective physiological properties may also be responsible for anti-addictive potential of *V. wallichii*. Future studies should characterize specific phytoconstituents and define their molecular signaling responsible for the anti-addictive properties of *Valeriana*.

CONCLUSION

In the present study, we report anti-addictive activity of *Valeriana* against ethanol addiction in mice; it showed significant reduction in chronic ethanol intake as well as efficiently reduced withdrawal effects of ethanol in mice. The primarily mechanism responsible for both anti-addictive and anti-withdrawal effect of *Valeriana* was found to be GABA mediated.

Acknowledgement

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Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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