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Berberine Alleviates Anxiety, Depression, and Motor Impairment Associated with Alcohol Withdrawal in Mice: A Preclinical Study

Jayesh Bagul , Manojkumar Mahajan , Aman Upaganlawar , Chandrashekhar Upasani

Department of Pharmacology, SNJB's Shriman Sureshdada Jain College of Pharmacy, Neminagar, Chandwad 423101, India

Correspondence
Manojkumar S. Mahajan
Email: mahajan.mscop@snjb.org

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Muhammad Torequl Islam, PhD
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Abstract: Alcohol withdrawal is a serious condition that often leads to heightened anxiety, depressive symptoms, and problems with motor coordination, all of which can significantly affect quality of life. These effects are largely tied to chemical imbalances in the brain, particularly involving GABA and glutamate systems, as well as oxidative stress. Berberine, a natural plant alkaloid long recognized for its therapeutic potential, is known to have calming, mood-stabilizing, and neuroprotective properties. In this study, mice were exposed to repeated alcohol intake (10% v/v, 2 g/kg, p.o) followed by withdrawal, which produced marked behavioral changes resembling anxiety, depression, and poor motor performance. When treated with berberine (10 or 30 mg/kg, p.o.) , however, these animals showed a clear reversal of withdrawal-induced problems. At both lower and higher doses, berberine reduced anxiety-like behavior, improved exploratory activity, lessened depressive signs, and improved motor coordination, with the higher dose offering stronger benefits. Importantly, berberine by itself did not cause any harmful effects, suggesting that it is safe in this context. These findings indicate that berberine may offer meaningful protection against the emotional and motor disturbances caused by alcohol withdrawal. Its ability to ease anxiety and depression while supporting brain function makes it a promising candidate for managing alcohol withdrawal syndrome.

Keywords: Berberine, Alcohol withdrawal, Anxiety, Depression, Motor coordination,

1. Introduction

Alcohol has been an integral part of human society for centuries, with patterns of consumption steadily rising over time. Historical records show that in 1790 the average annual intake was 5.8 gallons of pure alcohol, which increased to 7.1 gallons by 1830. Today, alcohol remains one of the most widely consumed psychoactive substances, and excessive use has become a global health challenge. Alcohol use disorder (AUD) is frequently linked to a range of psychiatric illnesses including drug use disorders, major depression, bipolar I disorder, and antisocial personality disorder as well as serious medical conditions such as alcohol withdrawal, liver disease, pancreatitis, and cancers of the head, neck, liver, colon, and rectum. On a societal level, it contributes to accidents, aggression, violence, and suicide. Between 2015 and 2019, alcohol-related causes were responsible for an estimated 140,557 deaths annually (Kranzler, 2023).

The neurobiological basis of alcohol dependence and withdrawal is complex. Alcohol enhances the release of opioid peptides and dopamine (DA), reinforcing craving and dependence. Abrupt cessation disrupts the balance between inhibitory GABAergic and excitatory NMDA activity, producing classical withdrawal symptoms such as nausea, tremors, hypertension, hallucinations, and seizures (Goldfine et al., 2023). Ethanol also alters neurotransmitter systems involving DA, GABA, glutamate, serotonin (5-HT), and norepinephrine (NE), while directly affecting cellular membranes, receptor activity, and ion channels (Celada et al., 2013; Esel et al., 2021). The result is increased central nervous system excitability a compensatory adaptation that becomes unmasked when alcohol is removed.

Chronic exposure to alcohol strengthens excitatory signalling and weakens inhibitory control, primarily through GABA receptor downregulation and glutamate receptor upregulation (Goldfine et al., 2023). Withdrawal from this state triggers hyperexcitability,

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Journal Home Page: Journal of Phytochemical Insights

seizures, and delirium tremens, while excessive glutamate activity further contributes to oxidative stress through calcium overload and mitochondrial dysfunction (Koob & Le, 2001, Mayo-Smith et al., 2004). At the same time, reduced dopamine leads to dysphoria and depression, major drivers of relapse. Meanwhile, norepinephrine rebounds during withdrawal, fueling autonomic hyperactivity and stress responses, which in turn raise cortisol levels and amplify inflammatory and oxidative damage (Ngui et al., 2022).

Given this neurochemical and behavioral consequences, there is an urgent need for effective therapeutic strategies that not only reduce withdrawal symptoms but also protect against long-term neurobehavioral disturbances.

Berberine shows strong neuroprotective activity that supports its usefulness in alcohol-withdrawal–induced anxiety and depression. It reduces neuroinflammation by inhibiting the NLRP3 inflammasome and lowering pro-inflammatory cytokines, thereby helping restore synaptic plasticity and neuronal health (Chandler et al., 1998). Berberine also enhances the PI3K/Akt/CREB/BDNF pathway, which is essential for mood regulation, neurogenesis, and stress resilience (Zhu et al., 2022, Liu et al., 2017). Its antioxidant and anti-apoptotic actions further protect neurons from oxidative stress and excitotoxicity, major contributors to withdrawal-related emotional disturbances (Kumar et al., 2023). Recent preclinical analyses also show that berberine consistently improves depressive -like behavior by reducing inflammation and increasing BDNF expression (Li et al., 2025). Together, these mechanisms make berberine a biologically plausible candidate for alleviating alcohol-withdrawal–related anxiety and depression.

This study addresses this gap by exploring the potential of berberine, a natural plant-derived alkaloid with established neuroprotective, anxiolytic, and antidepressant properties, as a candidate for managing alcohol withdrawal and its associated complications.

2. Materials & Methods

2.1. Drugs and Chemicals

Berberine hydrochloride was procured from Yucca Enterprises, Navi Mumbai, and analytical grade ethanol (99.9%) from Bio Liqua Research Pvt. Ltd. All other chemicals used were of analytical grade.

2.2. Experimental Animals

Adult male albino mice (30–40 g) were obtained from LACSMI Biofarms, Alephata, Pune, and randomly allocated into six groups (n=6 per group). Animals were housed individually in polypropylene cages with paddy husk bedding under standard laboratory conditions (23 ± 2°C, 55 ± 10% relative humidity, 12 h light/dark cycle) with free access to water and standard pellet diet (Nutrivet Lab, Pune, India). All experimental procedures were conducted in accordance with CPCSEA guidelines and approved by the Institutional Animal Ethics Committee (IAEC), SSDJ College of Pharmacy, Neminagar, Chandwad (Approval No.: SSDJ/IAEC/24-25/01).

2.3. Experimental Design

Adult male Swiss albino mice (30–40 g) were randomly divided into six groups (n=6 per group) as follows:

Group I (Control, CON): Received distilled water.

Group II (Ethanol Withdrawal, EW): Received 10% v/v ethanol at 2 g/kg p.o. for six consecutive days, followed by withdrawal on day 7 (Joshi et al., 2005).

Group III (EW + Berberine 10, EW-B10): Received ethanol as in Group II, along with berberine hydrochloride at 10 mg/kg p.o., followed by withdrawal on day 7.

Group IV (EW + Berberine 30, EW-B30): Received ethanol as in Group II, along with berberine hydrochloride at 30 mg/kg p.o., followed by withdrawal on day 7.

Group V (Berberine 30, B30): Received berberine hydrochloride at 30 mg/kg p.o. only followed by withdrawal on day 7.

Ethanol (10% v/v) was freshly prepared, and doses were calculated based on the animal's body weight. Berberine hydrochloride doses (10 and 30 mg/kg) were prepared and administered orally. All treatments were given once daily between 10:00 a.m. and 4:00 p.m. from day 1 to 6. On day 7, 24 hours after the last ethanol dose, animals were evaluated for behavioral, exploratory, compulsive, depressive, and motor coordination parameters.

Behavioral assessments included the elevated plus maze (EPM), and light-dark test (LDT) for anxiety; hole board test (HBT) for exploratory activity; marble burying test (MBT) for compulsive behavior; tail suspension test (TST) for depressive-like behavior; and stumbling test for motor coordination.

2.4. Behavioral Assessments

2.4.1. Assessment of Anxiolytic Activity Using Elevated Plus Maze

Male albino mice (30–40 g) were used for the study. Each mouse received its respective treatment 30 minutes prior to testing and was then placed individually at the center of the Elevated Plus Maze. The animals were observed for 5 minutes, and the following parameters were recorded: the number of entries into the open and closed arms, and the total time spent in each arm (Pellow et al., 1985).

2.4.2. Assessment of Anxiolytic Activity Using Light and Dark Apparatus

Anxiety-like behavior was assessed using a Light-Dark apparatus consisting of two connected boxes (25 × 25 × 25 cm). One box was darkened by covering its top, while the other was left open to natural light. Male albino mice (30–40 g) received their respective treatments 30 minutes prior to testing. Each mouse was placed in the light compartment facing the doorway and allowed to move freely between the two boxes for 5 minutes. The time spent in the light and dark compartments, along with the number of transitions between compartments, was recorded as measures of anxiety-like behavior (Campos-Cardoso et al., 2023).

2.4.3. Assessment of Exploratory Behavior in Hole Board Apparatus

Exploratory behavior was evaluated using a Hole Board apparatus consisting of a 40 × 40 cm floor with 16 evenly spaced holes (1.5 cm apart) elevated 20 cm above the base (File & Wardill, 1975). Male albino mice (30–40 g) received their respective treatments 30 minutes prior to testing and were individually placed in one corner of the apparatus. Each mouse was observed for 5 minutes, and the number of head dips was recorded as a measure of exploratory activity (Pisula et al., 2021; Takeda et al., 1998)

2.4.4. Assessment of Compulsive-like Behaviour Using the Marble Burying Test

Compulsive-like behavior was evaluated using a standard mouse cage or transparent plastic box (approximately 40 × 30 × 15 cm) with 4–5 cm of bedding spread evenly across the floor. Twenty-one clean glass marbles were placed equidistantly (≈4 cm apart) on the bedding. Male albino mice (30–40 g) received their respective treatments prior to testing and were then placed individually in the cage. The mice were allowed to explore for a set period, and the number of marbles buried to at least two-thirds of their surface area was recorded as a measure of compulsive-like behavior

(Witkin and Smith, 2023; Njung'e & Handley, 1991).

2.4.5. Assessment of Motor Coordination Using Stumbling Test

Motor coordination and anxiety-like behavior were evaluated using an apparatus measuring 28 × 28 × 20 cm with a floor containing 36 holes arranged in a 6 × 6 grid (each hole 2 cm apart and 1 cm deep). Male albino mice (30–40 g) received their respective treatments 30 minutes prior to testing. Each mouse was observed for the number of stumbles and foot slips, which were recorded as indicators of impaired motor coordination and anxiety-like behavior (Lepicard et al., 2003).

2.4.6. Assessment of Antidepressant Activity Using the Tail Suspension Test

Antidepressant-like activity was evaluated using the Tail Suspension Test. Male albino mice (30–40 g) were divided into six groups and received their respective treatments 30 minutes prior to testing. Each mouse was suspended by the tail using adhesive tape placed approximately 1 cm from the tip of the tail, at a height of 50 cm above the floor. Immobility time was recorded over a 5-minute observation period, with immobility defined as the absence of body movement while the mouse hung passively (Pinto Brod et al., 2016; Porsolt et al., 1977).

2.5. Statistical Analysis

All data are presented as mean ± standard error of the mean (SEM). Statistical comparisons among multiple groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. GraphPad Prism version 8.0 was used for all analyses and generation of graphs.

3. Results

3.1. Evaluation of Berberine Hydrochloride on Anxiety-like Behaviour in Ethanol Withdrawal Mice Using EPM

Chronic ethanol exposure significantly altered anxiety-like behavior in mice. Ethanol-withdrawn mice spent considerably less time in the open arms (11.64 ± 4.73 s) of the EPM (**Fig. 1A**) compared to control animals (open arm: 35.36 ± 6.81 s). Treatment with berberine hydrochloride at 10 mg/kg (EW-B10) partially improved this behavior, with mice spending 46.23 ± 5.51 s in the open arms, which was significantly higher than the ethanol-withdrawal group ($p < 0.05$). A higher dose of berberine (30 mg/kg, EW-B30) produced an even more pronounced anxiolytic effect, increasing open-arm time to 74.19 ± 7.14 s ($p < 0.05$ vs. ethanol-withdrawal). Mice receiving berberine alone at 30 mg/kg (B30) also showed a significant increase in open-arm time (84.51 ± 8.12 s) compared to the ethanol-withdrawal group. In addition, mice treated with ethanol plus berberine (B10 and B30) displayed a significantly higher number of entries into both arms than the ethanol-withdrawal group, further indicating reduced anxiety-like behaviour (**Fig. 1B**).

3.2. Evaluation of Berberine Hydrochloride on Anxiety-like Behaviour in Ethanol Withdrawal Mice Using LDT

Mice withdrawn from chronic 10% ethanol exposure exhibited a significant decrease in time spent in the light compartment (11.64 ± 8.84 s) compared to control animals (35.32 ± 6.81 s, $p < 0.05$), indicating heightened anxiety-like behavior. Treatment with berberine hydrochloride at 10 mg/kg (EW-B10) improved this behavior, with mice spending 46.23 ± 5.51 s in the light compartment. A higher dose of berberine (30 mg/kg, EW-B30) produced a more pronounced anxiolytic effect, increasing light-compartment time (**Fig. 2A**) to 74.19 ± 7.14 s ($p < 0.05$ vs. ethanol-withdrawal). Additionally, mice receiving berberine alone at 30 mg/kg (B30) also spent significantly more time in the light compartment (84.51 ± 8.12 s) compared to ethanol-withdrawn mice (**Fig. 2B**), confirming the anxiolytic potential of berberine in both withdrawal and normal conditions.

3.3. Evaluation of Berberine Hydrochloride on Exploratory Activity in Ethanol Withdrawal Mice Using HBT

Chronic administration of 10% ethanol significantly reduced exploratory behavior, as evidenced by a decrease in the number of head dips (8.55 ± 7.22) compared to the control group (23.15 ± 5.08, $p < 0.05$). Treatment with berberine hydrochloride along with ethanol at 10 mg/kg (EW-B10) and 30 mg/kg (EW-B30) significantly improved exploratory activity, with mice performing 29.50 ± 4.11 and 37.67 ± 5.02 head dips, respectively, compared to the ethanol-withdrawal group ($p < 0.05$). Furthermore, mice treated with berberine alone at 30 mg/kg (B30) displayed the highest exploratory activity, with 48.50 ± 4.78 head dips, significantly higher than ethanol-withdrawn mice, highlighting the strong pro-exploratory effect of berberine (**Fig. 3**).

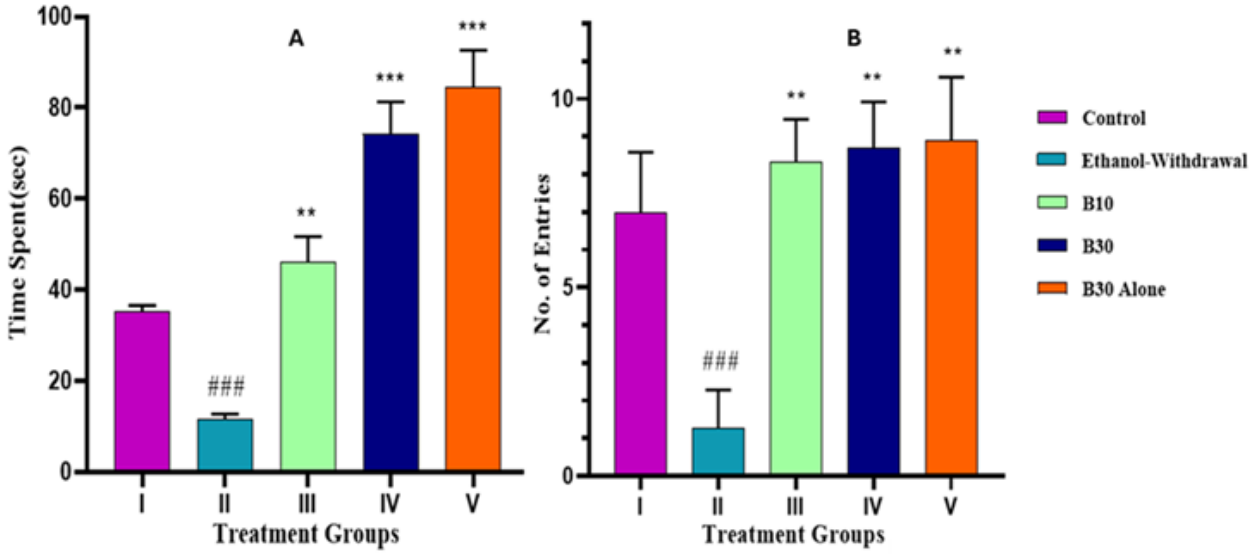

Fig. 1. Time spent & entries in open arm.Values are expressed as mean ± SEM (n=6), analysed by one-way ANOVA followed by Tukey's post hoc test. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.01$ as compared to control group and *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ as compared to Ethanol-withdrawal group.

3.4. Evaluation of Berberine Hydrochloride on Compulsive/ Anxiety-linked Behaviour in Ethanol Withdrawal Mice Using MBT

Chronic ethanol withdrawal significantly increased compulsive-like behavior, as indicated by a higher number of marbles buried (20.46 ± 2.30) compared to the control group (14.11 ± 1.57, $p < 0.05$). Treatment with berberine hydrochloride at 10 mg/kg (EW-B10) and 30 mg/kg (EW-B30) along with ethanol significantly reduced the number of marbles buried to 14.22 ± 2.79 and 14.17 ± 1.94, respectively, compared to the ethanol-withdrawal group ($p < 0.05$). Mice treated with berberine alone at 30 mg/kg (B30) exhibited the lowest marble burying activity (11.50 ± 2.56), highlighting the strong anti-compulsive and anxiolytic potential of berberine (**Fig. 4**).

3.5. Evaluation of Berberine Hydrochloride on Depressive-like Behaviour in Ethanol Withdrawal Mice Using TST

Chronic ethanol withdrawal significantly increased depressive-like behavior, as indicated by a prolonged duration of immobility (222 ± 7.16 s) compared to the control group (107.20 ± 2.77 s, $p < 0.05$). Treatment with berberine hydrochloride at 10 mg/kg (EW-B10) and 30 mg/kg (EW-B30) along with ethanol markedly reduced immobility times to 106.7 ± 4.05 s and 96.17 ± 2.54 s, respectively, compared to the ethanol-withdrawal group ($p < 0.05$). Mice treated with berberine alone at 30 mg/kg (B30) exhibited the shortest immobility duration (87.67 ± 2.20 s), further confirming the antidepressant-like effect of berberine in both ethanol-withdrawal and normal conditions (**Fig. 5**).

3.6. Evaluation of Berberine Hydrochloride on Motor Coordination in Ethanol withdrawal Mice Using the Stumbling Test

Chronic ethanol withdrawal significantly impaired motor coordination, as evidenced by an increased number of stumbles (51.78 ± 2.62) compared to control mice (13.17 ± 2.54, $p < 0.05$). Administration of berberine hydrochloride at 10 mg/kg (EW-B10) and 30 mg/kg (EW-B30) alongside ethanol significantly reduced the number of stumbles to 36.52 ± 2.93 and 30.17 ± 3.13, respectively ($p < 0.05$ vs. ethanol-withdrawal). Mice treated with berberine alone at 30 mg/kg (B30) exhibited the lowest number of stumbles (10.55 ± 1.82), demonstrating a strong protective effect of berberine on ethanol withdrawal-induced motor deficits (**Fig. 6**).

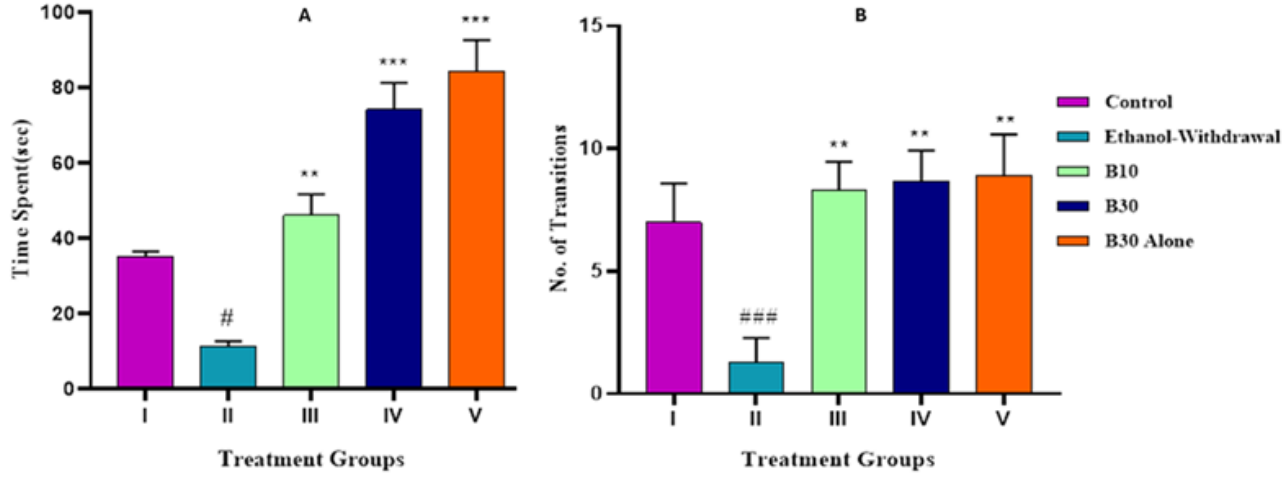

Fig. 2. Time spent & transitions in light compartment.

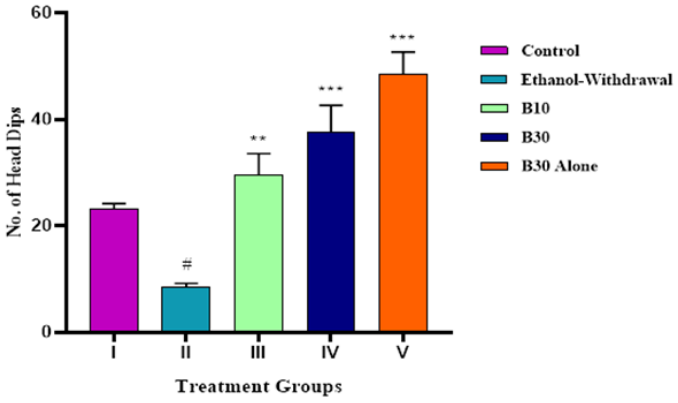

Fig. 3. No. of head dips.

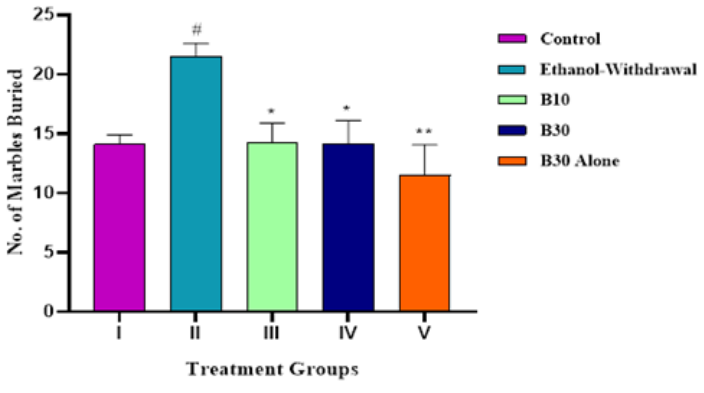

Fig. 4. No. of marbles buried.

[Values are expressed as mean ± SEM (n=6), analysed by one-way ANOVA followed by Tukey's post hoc test. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.01$ as compared to control group and *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ as compared to Ethanol-withdrawal group.]

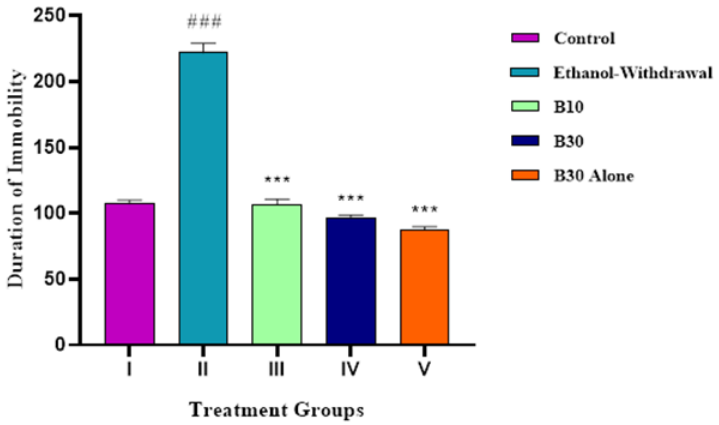

Fig. 5. Duration of immobility.

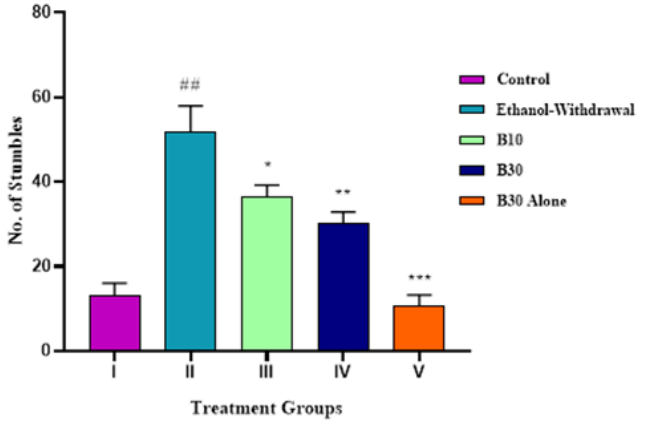

Fig. 6. No. of stumbles in different treatment groups.

[Values are expressed as mean ± SEM (n=6), analysed by one-way ANOVA followed by Tukey's post hoc test. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.01$ as compared to control group and *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ as compared to Ethanol-withdrawal group.]

4. Discussion

The present study provides compelling preclinical evidence for the therapeutic potential of berberine, a naturally occurring isoquinoline alkaloid, in mitigating behavioral, emotional, and motor deficits associated with alcohol withdrawal syndrome (AWS). Using a reproducible 7-day murine ethanol withdrawal model, we demonstrated that oral berberine administration at 10 and 30 mg/kg significantly reversed withdrawal-induced neurobehavioral impairments in a dose-dependent manner, highlighting its anxiolytic, antidepressant, and motor coordination-enhancing effects. These findings underscore berberine's multifaceted pharmacological actions and its potential as a natural, safe intervention for managing the complex symptomatology of AWS.

Chronic ethanol exposure followed by abrupt cessation is well-established to induce hyperexcitability of the central nervous system, resulting in a spectrum of behavioral and neurophysiological disturbances, including anxiety, depression, compulsive behaviors, and motor incoordination (Morisot & Ron, 2017; Poudel et al., 2020).

In the current model, mice received 10% ethanol (2 g/kg, p.o.) over six consecutive days, followed by 24 hours of withdrawal. This approach successfully induced a cluster of AWS-like behaviors, validating the translational relevance of the model and providing a reliable platform for testing therapeutic interventions.

Behavioral assessment using the EPM revealed that ethanol-withdrawn mice spent significantly less time and made fewer entries into the open arms, reflecting heightened anxiety and avoidance of unprotected spaces. This pattern is consistent with classical anxiogenic responses observed during alcohol withdrawal in rodents. Remarkably, berberine treatment restored these behaviors in a dose-dependent manner. This effect is likely mediated by modulation of central monoaminergic systems, including serotonergic and noradrenergic pathways, as well as GABAergic signaling and attenuation of neuroinflammatory processes (Fan et al., 2021). These findings indicate that berberine can effectively reduce the heightened anxiety observed in ethanol withdrawal, potentially lowering the risk of relapse driven by negative affective states.

Complementary evaluation using the light/dark test (LDT) assay corroborated these anxiolytic effects. Ethanol-withdrawn mice spent less time in the illuminated compartment and exhibited fewer transitions between compartments, indicative of reduced exploratory drive and increased aversion to novelty. Berberine administration reversed these behaviors, normalizing exploration and reducing light avoidance. These consistent observations across EPM and LDT paradigms strengthen the conclusion that berberine exerts a robust anxiolytic effect, effectively counteracting ethanol withdrawal-induced hyperarousal and fear-like behavior.

Exploratory behavior assessed using the HBT further supported these findings. Ethanol withdrawal significantly reduced the number of head dips, reflecting neophobia and diminished curiosity. Berberine treatment restored head-dipping activity in a dose-dependent manner, suggesting enhanced exploratory drive and reduced anxiety. Similarly, compulsive-like behavior, measured using the MBT, was elevated in ethanol-withdrawn mice, reflecting heightened stress and repetitive coping strategies. Berberine significantly reduced marble burying, highlighting its ability to mitigate compulsive tendencies, potentially through modulation of brain-derived neurotrophic factor (BDNF) and anti-inflammatory signaling (Deacon, 2006; Angoa-Perez et al., 2013).

The TST revealed that ethanol-withdrawn mice exhibited prolonged immobility, indicative of depressive-like behavior. Berberine significantly reduced immobility time, demonstrating antidepressant-like effects. These effects may be attributed to its capacity to enhance serotonergic and dopaminergic neurotransmission, promote hippocampal neurogenesis, and reduce neuroinflammatory processes, which are all implicated in the pathophysiology of withdrawal-associated depression (Peng et al., 2007).

Motor deficits are a prominent and clinically relevant feature of AWS, often resulting from ethanol-induced neurotoxicity in motor control regions such as the cerebellum and basal ganglia. The stumbling test revealed that ethanol withdrawal increased the number of stumbles, reflecting impaired coordination. Berberine treatment significantly improved motor performance, reducing stumbles and increasing fall time. These improvements are likely mediated by its antioxidant and neuroprotective properties, counteracting oxidative stress and preserving neuronal integrity in motor circuits (Klein, 2023; Meyer & Caston, 2005).

Overall, berberine demonstrated consistent efficacy across multiple behavioral paradigms, including the EPM, LDB, HBT, MBT, TST, stumbling tests, highlighting its multitarget pharmacological potential in AWS. Its polypharmacological actions modulation of

GABAergic, serotonergic, and glutamatergic systems, antioxidant activity, anti-inflammatory effects, and neurotrophic support likely contribute to the observed improvements across affective, cognitive, and motor domains.

Although the behavioral findings of the present study are robust, several limitations must be acknowledged. Only male mice were used, which restricts generalizability, as sex-specific differences in alcohol withdrawal severity and pharmacological responses are well established (Becker & Lopez, 2004). Moreover, the study did not investigate the underlying molecular mechanisms through which berberine exerts its beneficial effects. Future research should incorporate neurochemical and molecular evaluations, such as inflammatory cytokines, oxidative stress markers, BDNF–CREB signaling, glutamatergic and GABAergic balance, and NLRP3 inflammasome pathways to validate mechanistic hypotheses. Assessing these biomarkers in key brain regions including the amygdala, hippocampus, and prefrontal cortex, will help delineate the precise neurobiological actions of berberine. Additionally, future studies should include female cohorts and explore chronic administration or relapse-related models to better establish long-term efficacy and translational relevance (Cicero & Baggioni, 2016).

In summary, our findings show that berberine noticeably improves anxiety, depressive-like behavior, and motor coordination problems that arise during alcohol withdrawal in mice. These improvements suggest that berberine may help stabilize the key brain disturbances that occur when alcohol use is stopped, highlighting its promise as a supportive treatment option. At the same time, more work is needed to understand exactly how berberine produces these effects. Future studies should include molecular investigations, examine both male and female subjects, and explore longer treatment or relapse-related models. Overall, this study lays a strong foundation for advancing berberine as a potential therapeutic approach for managing the neurobehavioral challenges associated with alcohol withdrawal.

5. Conclusion

Overall, our study shows that berberine can genuinely help reduce the anxiety, low mood, and movement problems that arise during alcohol withdrawal in mice. These improvements suggest that berberine may offer real support during the challenging early phase of abstinence. Still, there is more to learn, especially about how berberine works in the brain, how it affects both males and females, and whether its benefits last over longer periods or during relapse-like situations. Even with these unanswered questions, our findings give a strong and encouraging starting point for exploring berberine as a helpful option for easing the difficulties of alcohol withdrawal.

Conflict of Interest

The author states that there is no conflict of interest.

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Author Contributions

Concept – M. M., J.B.; **Design** – M. M., A. U., J. B., C. U.; **Supervision** – M.M., A. U., C. U.; **Resources** –A. U., C. U.; **Materials** – J. B., M.M.; **Data Collection and/or Processing** – J.B. M.M., A. U.; **Analysis and/or Interpretation** – M. M., A. U., C. U.; **Literature Search** – J. B., M. M., **Writing** – J.B., M. M., A.U.; **Critical Reviews** –M. M., A. U., C. U.

Ethical Approval

This study was approved by the Institutional Animal Ethics Committee (IAEC), SSDJ College of Pharmacy, Neminagar, Chandwad (Approval No.: SSDJ/IAEC/24-25/01).

Informed Consent Statement

Not applicable.

Data Availability

All data supporting the findings of this study are included within the article

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