AJBMS AFGHANISTAN Journal of Basic Medical Sciences
ISSN: 3005-6632

Afghanistan Journal of Basic Medical Sciences

2025 Jan 2(2): 158-167.

ajbms.knu.edu.af

Therapeutic Potential of Afghan *Hibiscus sabdariffa L.* Aqueous Extract: Antidepressant and Anxiolytic Effects in Male Rats

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ARTICLE INFO

Type: Original Article
Received: 30 April 2025
Accepted: 20 June, 2025

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To cite this article: Shojae MT, Hossaini D, Mustafa Ansari M. Therapeutic Potential of Afghan *Hibiscus sabdariffa L.* Aqueous Extract: Antidepressant and Anxiolytic Effects in Male Rats Afghanistan Journal of Basic Medical Sciences. 2025 Jan 2(2):158-167.
https://doi.org/10.62134/khatamuni.89

ABSTRACT

Background: We explored the antidepressant and anxiolytic effects of Afghan *Hibiscus sabdariffa*aqueous extract in male rats. With a growing interest in natural remedies for mental health disorders, we aimed to assess the efficacy of *H. sabdariffa* at various doses (50, 100, and 150 mg/kg) using established behavioral models.
Methods: The rats were randomly assigned to four groups: a control group receiving saline, a group exposed to alcohol (at doses of 5%, 10%, 15%, and 20%), and three experimental groups treated with *H. sabdariffa* extract alongside alcohol exposure. The treatment lasted for 14 days, after which the animals underwent behavioral assessments, including the Forced Swim Test (FST) to evaluate depressive behaviors and the Elevated Plus Maze (EPM) to assess anxiety-like behaviors.
Results: The findings revealed a significant (P<0.001) reduction in immobility time in the FST for the extract-treated groups compared to the alcohol-only group, indicating an antidepressant effect, with the 100 mg/kg dose showing the most pronounced impact. Additionally, EPM results demonstrated increased time spent in the open arms and a higher number of entries, suggesting reduced anxiety levels.
Conclusion: The aqueous extract of Afghan *H. sabdariffa* exhibits promising antidepressant and anxiolytic effects in male rats, particularly at the 100 mg/kg dosage, even in the context of alcohol exposure. These findings warrant further investigation into the therapeutic potential of *H. sabdariffa* for treating mood disorders in clinical settings, highlighting its role as a natural alternative in mental health management.

Keywords: Alcohol, Anxiety, Depression, *Hibiscus sabdariffa*, Rats

Introduction

Adolescence marks a critical period of brain development, involving profound structural and functional changes in neural circuits (1, 2). This stage is also notable for increased experimentation with substances, especially alcohol (3). Drinking alcohol during adolescence is associated with a heightened risk of developing alcohol use disorder (4) later in life, primarily due to its disruptive effects on the cortical and subcortical reward systems that are still maturing during this time (5). Both short-term and long-term ethanol (Eth) exposure can trigger structural, physiological, and functional alterations in the central nervous system (CNS), potentially leading to neurodegeneration, neuronal loss, and behavioral disturbances such as anxiety and depression (6, 7). Adolescent alcohol

exposure (AAE), in particular, has been linked to long-lasting behavioral outcomes that extend into adulthood, including elevated levels of anxiety and depressive symptoms (8-11). Beyond its direct effects, alcohol use during adolescence may also sensitize the brain to stress, thereby indirectly facilitating the development of mood-related disorders (12).

Emerging research has highlighted several key cellular pathways that may drive these associations, including oxidative stress (4, 13). Alcohol-induced oxidative damage can overwhelm antioxidant defences, resulting in excessive free radical production and lipid peroxidation, processes that significantly contribute to alcohol's harmful impact on the brain (14, 15).

Although harmful alcohol use continues to be a major public health issue, pharmacological treatments to support psycho-social therapies are still limited in both availability and use. The three most commonly used medications—disulfiram, acamprosate, and naltrexone—are often scarce in low-resource settings and are associated with significant adverse effects, including hepatotoxicity, renal dysfunction, and peripheral neuropathy. As a result, investigating complementary and alternative treatments, particularly herbal medicines with fewer side effects and potential health, social, and economic advantages, represents a promising research direction. The use of plant-based therapies for managing alcohol-related disorders is well-documented (16).

Among medical plants, *H. sabdariffa*, belonging to the Malvaceae family, is well known for its wide range of health-promoting effects. It is rich in bioactive constituents such as flavonoids, alkaloids, and phenolic acids, which are largely responsible for its therapeutic properties (17). We aimed to investigate the anti-anxiety and anti-depressant effects of Afghan *H. sabdariffa* in male rats.

Materials and Methods

Animals

This study involved the random selection of 30 male Sprague Dawley rats, aged 21 postnatal days (PND 21), from the Research and Technology Center at Khatam Al-Nabieen University. The rats were housed in standard Plexiglass cages with unrestricted access to food and water. They were maintained under controlled conditions, with a room temperature of 23±2 °C and a 12-hour light/dark cycle.

Plant Extraction

Calyces of *H. sabdariffa* were gathered from the Shigal and Sheltan districts in Kunar Province, Afghanistan. The plant material was dried in a laboratory setting and subsequently ground into a fine powder at the Research and Technology Center, Khatam Al-Nabieen University. To create the extract, 30 grams of *H. sabdariffa* calyces were immersed in 200 ml of boiling water for 30 minutes, facilitating the release of the plant's active compounds into the infusion. Following this, the mixture was filtered to remove solid residues, resulting in a clear liquid extract. The filtrate was concentrated through evaporation to remove water, yielding a dark red powder, which was stored at 4 °C (17).

Drugs and Experimental Procedure

Ethanol (Eth) with a purity of 99.5% (v/v) was obtained from Merck KGaA, Germany. Working Eth solutions were prepared by diluting the stock solution with drinking water to achieve final concentrations of 5%, 10%, 15%, and 20% (v/v). These graded Eth solutions were administered as the sole drinking source for the experimental animals to progressively increase ethanol exposure. An escalating Eth exposure protocol was employed to simulate binge-drinking patterns

typically observed during adolescence. From postnatal days (PND) 24–25, rats assigned to the Eth groups received 5% Eth in their drinking water. Once fluid consumption matched that of the control group, the Eth concentration was raised to 10% on days 26–27, followed by 15% on days 28–29. Upon adaptation to the 20% Eth solution, rats continued at this concentration from PND 30 to PND 60 (18, 19). Animals were randomly assigned into five experimental groups: Group 1 (vehicle control) received regular drinking water and daily intraperitoneal (i.p.) injections of normal saline; Group 2 (Eth withdrawal) was given increasing concentrations of Eth in drinking water along with daily i.p. saline injections; Groups 3–5 (Eth + HS) were exposed to the same Eth regimen as Group 2 but also received daily i.p. injections of *Hibiscus sabdariffa*extract at doses of 50, 100, and 150 mg/kg, respectively.

Behavioral evaluations

Open Field Test

The open field test (OFT) is frequently utilized to investigate the neurobiological mechanisms of anxiety and to identify potential anxiolytic compounds (20). The test was conducted in a square arena measuring 100 × 100 × 40 cm, made from opaque material and divided into 25 equal sections of 20 × 20 cm each. Each rat was individually placed in the center of the arena and allowed to explore freely for 5 minutes. Movements, including the time spent in central versus peripheral areas and the distance traveled, were recorded and analyzed using video tracking software (21). To eliminate contamination or scent-related biases, the apparatus was thoroughly cleaned with a 10% (w/v) alcohol solution between trials.

Elevated plus maze

The elevated plus maze (EPM) is a well-established method for assessing anxiety-like behaviors and general locomotor activity in rodents (22). The EPM was conducted one week after alcohol withdrawal to evaluate potential long-term anxiety outcomes. While previous research indicates that anxiety levels usually peak within 24 to 48 hours following withdrawal, a one-week interval was chosen to investigate more sustained behavioral effects (7). The EPM apparatus consisted of a plus-shaped platform elevated 50 cm above the ground, featuring two open arms and two enclosed arms, each measuring 50 cm long and 10 cm wide, with 40-cm-high walls on the closed arms. Rats were placed in the central junction facing an open arm and were allowed to explore the maze for 5 minutes. Behavioral metrics, such as the time spent in open versus closed arms, were recorded as indicators of anxiety-like responses.

Forced Swimming Test

The forced swim test (FST) is utilized to evaluate depressive-like behaviors in rodents by placing them in a confined water-filled environment and observing their active and passive responses (24). Rats were placed in glass cylinders filled with water at a temperature of 24 ± 2 °C and a depth of 30 cm, ensuring they could not reach the bottom. The FST protocol consisted of a 15-minute pretest session, followed by a 5-min test session conducted 24 hours later. During both sessions, the rats' behaviors, including swimming, climbing, and immobility, were recorded. Increased time spent immobile was interpreted as a sign of behavioral despair. After each session, the animals were dried with paper towels, placed in heated cages for 30 minutes, and then returned to their home cages. The 24-hour interval between the pretest and test sessions allowed for the assessment of the stability of depressive-like behaviors (25).

Statistical Analyses

The data were analyzed using GraphPad Prism software (version 8), utilizing one-way ANOVA followed by Tukey's post hoc tests for statistical assessment. Results are presented as mean ± standard error of the mean (SEM), with a significance level set at $\alpha = 0.05$, corresponding to a 95% confidence interval for evaluating statistical significance.

Results

H. sabdariffa Mitigates Ethanol Withdrawal-Induced Anxiety-Like Behavior

Results from the OFT indicated that animals exposed to Eth exhibited heightened anxiety-like behavior, evidenced by a significant reduction in time spent exploring the central area of the open field and an increase in time spent in the peripheral area, compared to the vehicle group (P<0.001, Fig. 1 A and B). Notably, higher doses of *H. sabdariffa* (100 and 150 mg/kg) significantly increased the time spent in the central area (P<0.05 and P<0.001, Fig. 1 A) and decreased the time spent in the peripheral area (P<0.01 and P<0.001, Fig. 1 B) relative to the Eth group.

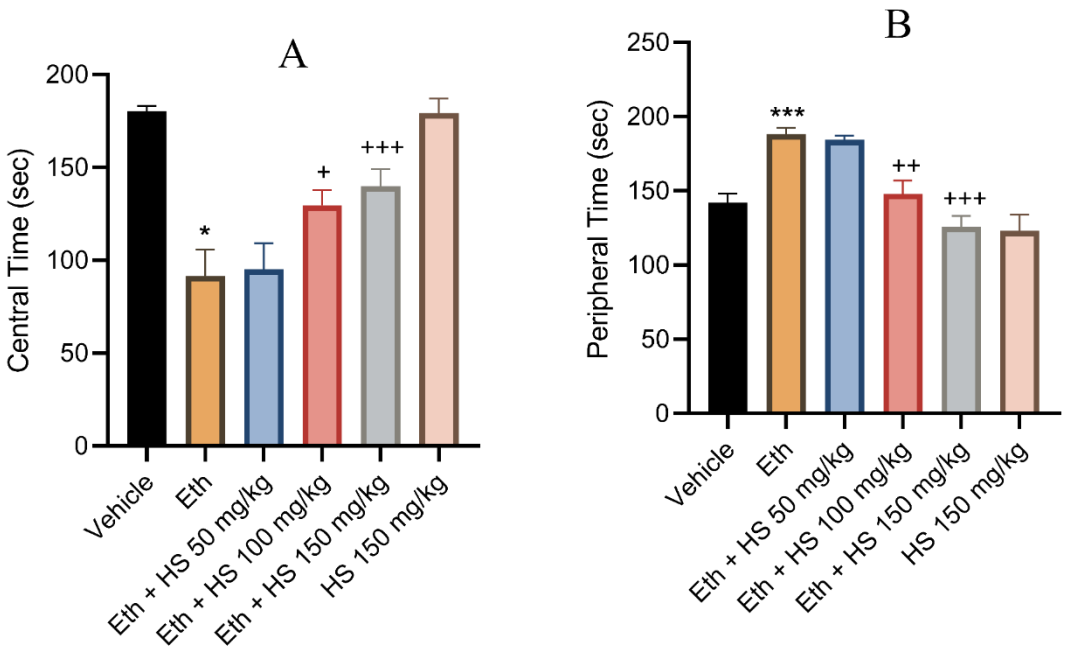

Fig. 1: Evaluation of anxiety-related behaviours in the OFT. A) the time spent in the central area of the open field box; and B) the duration of animal exploration in the peripheral sections of the experimental groups. The data are presented as means ± SEM. Significant differences were observed with P<0.001*** compared to the control group, and P<0.05^{+}, P<0.01^{++}, and P<0.001^{+++} compared to the Eth group

The EPM test further corroborated these findings, showing that animals in the Eth group displayed increased anxiety-like behavior, with a significant decrease in time spent in the open arms and an increase in time spent in the closed arms compared to the vehicle group (P<0.001, Fig. 2 A and B). Interestingly, administration of *H. sabdariffa* (150 mg/kg) significantly increased the time spent in the open arms (P<0.001, Fig. 2 A), while both doses of *H. sabdariffa* (100 mg/kg and 150 mg/kg) significantly reduced the

time spent in the closed arms compared to the Eth group (P<0.05 and P<0.001, Fig. 2 B).

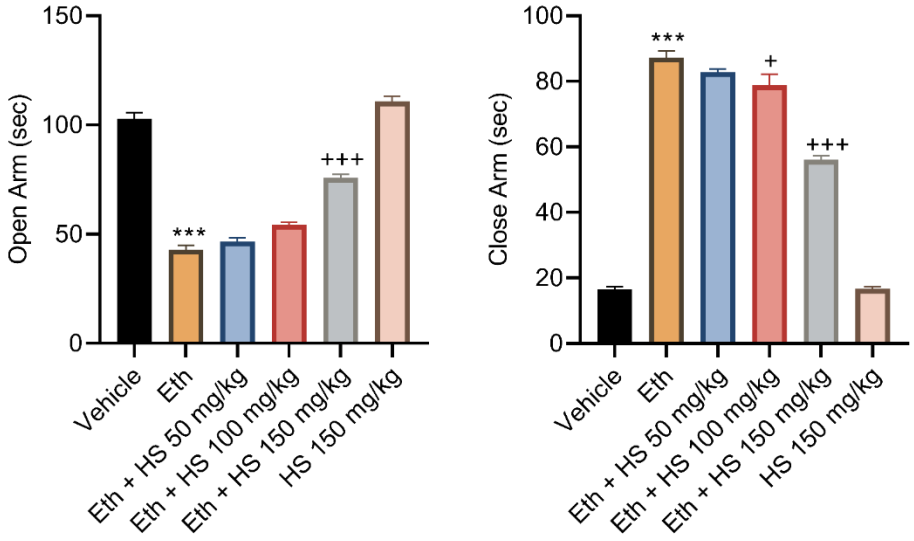

Fig. 2: Evaluation of anxiety-related behaviours in the EPM. A) The duration of time spent in the open arms of the platform and B) the duration of animal exploration within the enclosed areas were documented in various experimental groups. The data are presented as the mean ± SEM. The statistical analysis revealed significant differences with a P<0.001*** when compared to the control group and P<0.05^{+} and P<0.001^{+++} when compared to the Eth group

H. sabdariffa Mitigates Ethanol Withdrawal-Induced Depression-Like Behaviour

Results from the FST revealed that animals in the Eth group displayed increased depressive behaviours when compared to the vehicle group. This was indicated by a significant decrease in struggling time, an increase in immobility, and a reduction in swimming time (P<0.001, Fig. 3 A-C). Importantly, administering a higher dose of *H. sabdariffa* (150 mg/kg) significantly increased both struggling and swimming times while decreasing immobility relative to the Eth group (P<0.05 and P<0.01, Fig. 3 A-C). Furthermore, administration of *H. sabdariffa* (150 mg/kg) alone also enhanced struggling and swimming times compared to the vehicle group (P<0.05, Fig. 3 A and C).

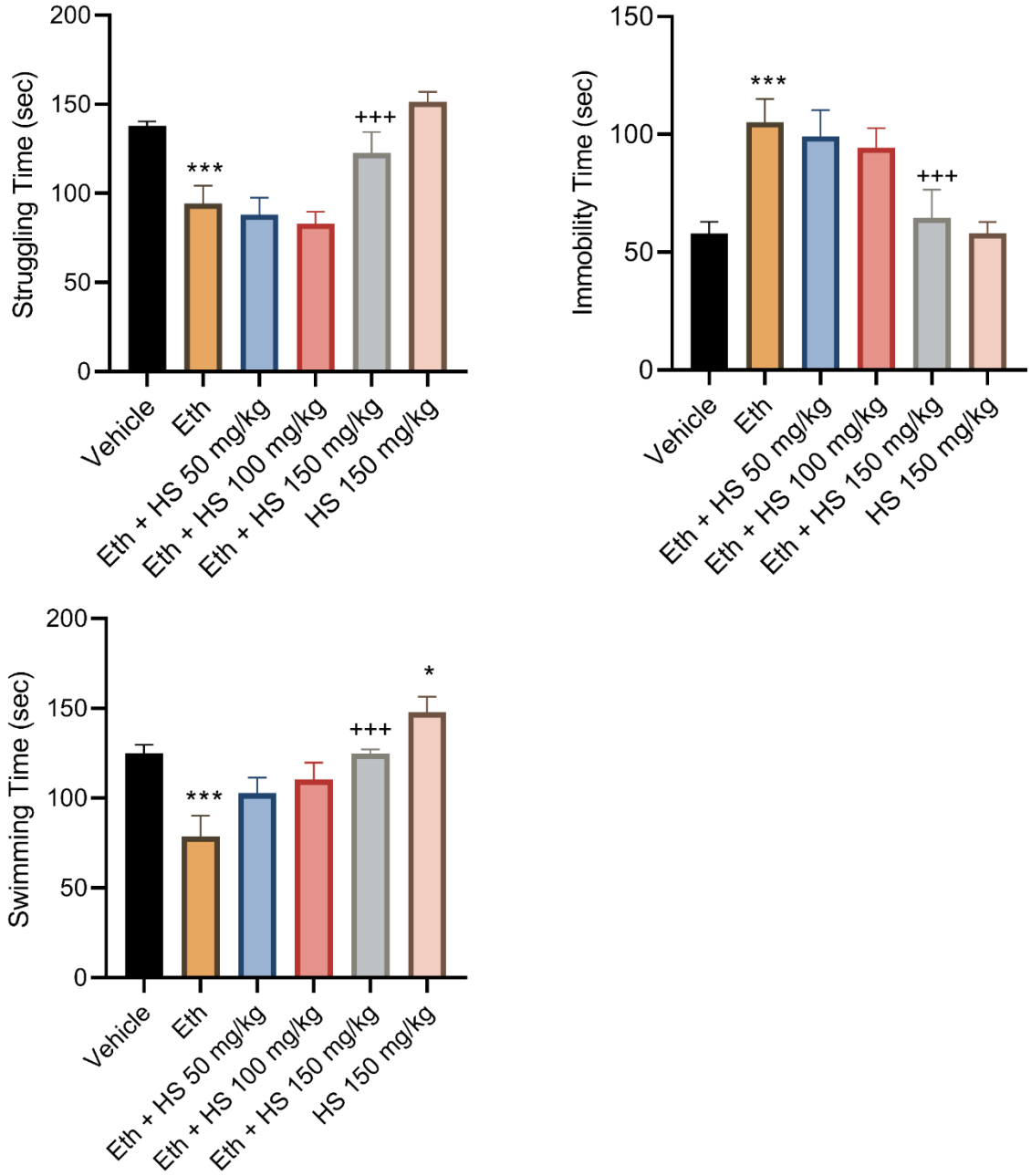

Fig. 3: Evaluation of depressive-like behaviours in the FST. A) the time of the animal's struggle for survival in water; B) the duration of immobility, which refers to the time when the animal does not actively swim to save itself; and C) the duration of normal swimming in various experimental groups. The data are presented as the mean ± SEM. Significant differences were observed with $P<0.05$*, $P<0.01$** $P<0.001$*** compared to the control group and $P<0.001^{+++}$ compared to the Eth group

Discussion

The present study provides robust evidence that *H. sabdariffa* administration significantly attenuates both anxiety-like and depression-like behaviors induced by ethanol (Eth) withdrawal in rodent models. These findings are particularly important given the well-documented neurobehavioral disturbances associated with Eth withdrawal, which include heightened anxiety, dysphoria, and depressive symptoms. The therapeutic potential of *H. sabdariffa* in this context is underscored by its consistent anxiolytic and antidepressant-like effects across multiple behavioural assays.

Eth withdrawal is known to induce a hyperexcitable state in the central nervous system due to abrupt cessation of chronic alcohol exposure, leading to behavioural manifestations such as anxiety and depression. In the (OFT, a reduction in central area exploration is considered indicative of heightened anxiety, while in the EPM, decreased time in the open arms reflects similar anxiety-like behaviour. Consistent with previous findings (26, 27), animals exposed to Eth withdrawal in the current study exhibited both of these behavioural markers. Notably, administration of *H. sabdariffa* at doses of 100 mg/kg and 150 mg/kg significantly reversed these effects by increasing time spent in the central zone of the OFT and open arms of the EPM. These outcomes suggest that *H. sabdariffa* possesses anxiolytic properties that can effectively counteract the behavioural consequences of alcohol withdrawal.

The FST is a validated assay widely employed to evaluate depression-like states in rodent models. In this paradigm, increased immobility time is interpreted as a behavioural correlate of despair or anhedonia, key components of depression. As observed in this study, Eth-withdrawn animals showed significant increases in immobility time and reductions in active behaviours such as struggling and swimming. *H. sabdariffa* administration at a higher dose (150 mg/kg) significantly reversed these effects, decreasing immobility while enhancing both active components of the test. These findings suggest that *H. sabdariffa* exhibits potent antidepressant-like activity under conditions of Eth withdrawal, which aligns with reports that some natural and synthetic compounds can alleviate withdrawal-induced affective disturbances (28). Although the precise mechanisms underlying the therapeutic effects of *H. sabdariffa* remain to be elucidated, several plausible pathways are worth considering. Eth withdrawal is associated with pronounced neurochemical imbalances, particularly involving GABAergic and glutamatergic systems. Chronic alcohol consumption enhances GABAergic inhibition and suppresses glutamatergic excitation, but upon withdrawal, these systems rebound in opposite directions—GABAergic tone decreases while glutamatergic activity surges—resulting in neuronal hyperexcitability and increased anxiety (28). *H. sabdariffa* may exert anxiolytic effects by stabilizing the neurochemical imbalances associated with alcohol withdrawal, particularly by influencing the GABAergic and glutamatergic systems. GABAergic Signalling: *H. sabdariffa* has been shown to enhance GABAergic signalling, which is crucial for reducing anxiety. Extracts from *H. sabdariffa* can significantly increase the time spent in open arms in the elevated plus maze test, a common measure of anxiety in animal models, suggesting its potential to enhance GABAergic activity (29). *H. sabdariffa* may also inhibit excessive glutamatergic activity, which is often elevated during ethanol withdrawal. This modulation can help restore balance in neurotransmission, thereby reducing anxiety levels (30). By acting on

these systems, *H. sabdariffa* promotes stability in neurotransmission, which is essential for managing anxiety and improving emotional regulation during withdrawal (31).

Additionally, *H. sabdariffa* has been shown to influence the NMDA receptor-mediated nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) signalling pathway, which is vital for synaptic plasticity and emotional regulation (32). Disruptions in this pathway during ethanol withdrawal can lead to depression-like symptoms, suggesting that HS's ability to modulate this signalling may contribute to its antidepressant effects observed in behavioural tests like the FST (30, 33).

Taken together, the findings of this study suggest that *H. sabdariffa* might serve as a promising candidate for the development of pharmacotherapies aimed at mitigating the negative affective states associated with Eth withdrawal. Future studies should aim to delineate the specific molecular targets of *H. sabdariffa*, explore its effects in chronic administration models, and assess its translational potential in clinical settings.

Conclusion

H. sabdariffa effectively attenuates anxiety-like and depression-like behaviours associated with Eth withdrawal in rodents. These findings suggest that *H. sabdariffa* holds potential as a therapeutic agent for managing alcohol withdrawal symptoms. Further research is warranted to explore the underlying mechanisms and to assess the efficacy of *H. sabdariffa* in clinical settings.

Funding

None.

Conflict of Interest

Non-declared

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