Sonawane, R., et al., (2024). *J Basic Appl Pharm Sci, 2*(1): 106
https://doi.org/10.33790/jbaps1100106

Journal of Basic and Applied Pharmaceutical Science

Preliminary Screening of Anxiolytic and Anti-depressant Potential of QintroTM a Polyherbal Formulation on Alcohol Withdrawal Syndrome in Experimental Mice

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Article Details
Article Type: Research Article
Received date: 23rd January, 2024
Accepted date: 20th March, 2024
Published date: 22nd March, 2024

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Citation: Sonawane, R., Kasar, G., Chavan, D., Mahajan, M., Upaganlawar, A., & Upasani, C., (2024). Preliminary Screening of Anxiolytic and Anti-depressant Potential of QintroTM a Polyherbal Formulation on Alcohol Withdrawal Syndrome in Experimental Mice. *J Basic Appl Pharm Sci, 2*(1): 106. doi: https://doi.org/10.33790/jbaps1100106

Abstract

Background: Chronic alcohol consumption is a major cause of mortality, morbidity, and economic consequences globally. Prolonged alcohol use and its sudden withdrawal are considered the triggers for anxiogenic effects in rodents and humans. Also, alcohol withdrawal (AW) is involved in depressive disorders. This study investigates the impact of Qintro™ on AW mice who were given anxiety and depression.

Methods: There were seven groups of mice (n = 6). 10% (v/v) ethanol was administered twice daily at a 2000 mg/kg dose, intragastrically on day 1 and once a day up to day 6. Other groups were given a 10% ethanol treatment (2000 mg/kg p.o.). with test formulation at different doses (mg/kg) designated Q-100, Q-200, Q-400, p.o. They were withdrawn from ethanol on the 7th day, and behavior was analyzed using the tests, which include the tail suspension test (TST), marble burying test (MBT), hole board test (HBT), elevated plus maze (EPM), and light-dark test (LDT).

Results: This investigation showed the beneficial effects of Qintro™ on current conditions. The concentration of Qintro™ used in the current research revealed significant differences ($p<0.05$) in time devoted (sec) in the open arm of EPM, time devoted in the bright region in LDT, and head droops in HBT. Additionally, when comparing the TST group to the AW group, there were substantial ($p<0.05$) decreases in MBT and reductions in immobility time.

In summary: The results of the study showed that Qintro™ has both antidepressant and anxiolytic effects in mice who are ethanol-deprived.

Keywords: Alcohol withdrawal (AW), Anxiety, Depression, Qintro™, polyherbal formulation, behavioral study.

Introduction

The most prevalent psychiatric disorders worldwide are anxiety and depression, which negatively impact the quality of life [1, 2]. Nearly 10% of the world's population is affected by anxiety and depressive disorders annually, which are widespread and debilitating psychiatric diseases [3]. The symptoms of anxiety and depression can coexist, and vice versa [4].

Ethanol is the most common alcohol among chronic consumers who may experience the adverse effects of excessive alcohol use [5]. It is now well known that chronic alcohol intake may lead to behavioral and biochemical alterations in humans and animals. Moreover, regular consumers are prone to developing AW syndrome, which is caused by the cessation of chronic intake. Alcohol withdrawal syndrome (AWS) is characterized mainly by anxiety, along with depression, agitation, hypertension, nausea, vomiting, hallucinations, insomnia, delirium, sweating, tachycardia, and tremors [6, 7]. AWS is a commonly occurring state that progresses after severe or regular alcohol consumption is immediately stopped, intentionally or unintentionally [8, 9].

Alcohol-induced imbalances in the neurotransmitters cause higher neuronal activity if the alcohol is revoked [10]. In humans, withdrawal significantly lowers brain GABA concentrations [11]. Alcohol increases the effect of GABA on GABAA receptors, which reduces overall brain excitability. Activation of the amygdala and hypothalamus is considered to play a leading role in treating AW anxiety-like behaviors in various limbic regions [6]. The central neurons that make serotonin (5HT) and dopamine (DA) may also experience neuroadaptive alterations brought on by alcohol. When alcohol is consumed, the dopaminergic and serotonergic pathways undergo the opposite adaptive changes as when alcohol is stopped [12].

Qintro™, a polyherbal formulation manufactured by Rudraksha Ayurveda-Pharma, Mumbai, India, is indicated for insomnia, anxiety, and sleep disturbance. The components used in this formulation are popular in Indian and Chinese medicine to treat a variety of neurological disorders. Sarpagandha (*Rauwolfia serpentina*) is commonly used to treat psychotic illnesses, including anxiety and depression [13, 14]. Guduchi (*Tinospora cordifolia*) has anxiolytic and anti-depressant properties [15, 16]. Vacha (*Acorus calamus*)

possesses anxiolytic and antihypertensive properties [17, 18]. Bramhi (*Bacopa monnieri*) has anti-depressant and anti-anxiety activity [19, 20]. Tagar (*Valeriana wallichii*) effectively treats insomnia, anxiety, and depression [21]. Jatamansi (*Nardostachys jatamansi*) treats mild to moderate sleep difficulties, anxiety, and depressive behavior [22, 23], and Shankhpushpi (*Convolvulus prostratus*) has been shown to reduce anxiety associated with AW and alcohol addiction [24, 25].

The purpose of this study is to conduct a preliminary screening to evaluate the anxiolytic and antidepressant potential of Qintro™, a polyherbal formulation, in alleviating symptoms of alcohol withdrawal syndrome in experimental mice.

Materials and Methods

Animals

Male mice (albino, 20–25 g) for the study were procured from Lacsmi Biofarms, Pune, India. Mice were housed in polypropylene cages and kept in conditions (room temperature 25±2°C, humidity 45–55%, cycle of light and dark 12:12 hrs). Allow animals to be acclimated to the laboratory for 7 days before starting the experiment. Mice were fed on food pellets, and water was given *ad libitum*. Food but not water was withheld two hours before the administration of alcohol and the test formulation. All experiments were performed following the CCSEA Laboratory Animal Use and Care Guidelines. The protocol for this investigation was acknowledged by the IAEC (*SSDJ/IAEC/2022-23/02*).

Drugs and chemicals

QintroTM was purchased from Rudraksha Ayurveda-Pharma, Mumbai, India. The supplier of absolute ethanol was Changshu Hong Sheng Fine Chemical Co. Ltd. in China. The supplier of diazepam was Neon Laboratory Limited, in Thane, India. Imipramine was purchased from Modern Pharmaceutical, Mumbai, India.

Study Design

Six groups of mice were created, with six mice in each group.

Group I: saline (control); **Group II:** ethanol withdrawal (2000 mg/kg, p.o. 10% v/v) till day 6; and saline on day 7 (ethanol) [26]. **Group III:** Ethanol (2000 mg/kg, p.o. 10% v/v) till day 6, followed by Q-100 (p.o.) on day 7 (ETH + Q100); **Group IV:** Ethanol (2000 mg/kg, p.o. 10% v/v) till day 6, followed by Q-200 (p.o.) on day 7 (ETH + Q200); **Group V:** Ethanol (2000 mg/kg, p.o. 10% v/v) till day 6, followed by Q-400 (p.o.) on day 7 (ETH + Q400); **Group VI:** Mice treated with ethanol till day 6, followed by diazepam (1 mg/kg, i.p.) or imipramine (10 mg/kg, i.p.) considered as a standard (STD) on day 7 [27].

All the treatments were given from 10.00 to 11.00 a.m. in the morning and for 30 minutes, before carrying out behavioral tests.

Induction of alcohol withdrawal

Mice were given two doses of 10% v/v ethanol, 2000 mg/kg, intragastrically on the first day and once a day up to day 6. Mice were examined for withdrawal symptoms on the seventh day. On the 7th day, the treatment was switched, and the chronic ethanol treatment group received only saline on the 7th day [26].

Assessment of Qintro™ for anti-anxiety and anti-depression effects

Elevated plus maze: The EPM is an extensively employed behavioral test to examine anxiogenic and anxiolytic behaviors in rodents [28]. The EPM has four arms arranged in the form of '+'. Two arms (open arms: 25×5 cm) don't have side or end walls. The remaining two arms (closed arms: 25×5×15 cm) are open at the top but have side and end walls. At the intersection of the four arms, there is a square platform measuring 5×5 cm. The EPM apparatus was placed 50 cm off the ground [29]. The mice were put in the center, facing an open arm, at the start of each test. The mice were given 5 minutes to explore the EPM to record the duration of stay in each arm and the entries [30].

Light and Dark Test:

The LDT is a widely used rodent test for investigating unconditioned anxiety-like behavior based on a conflict between an accession or evasion urge to explore different regions and an aversion to brilliantly lit, open spaces [31]. There are two compartments on the LDT device. Two-thirds of the box comprises the open, well-lit illumination section. A covered, dark compartment makes up one-third of the entire box. The two compartments are joined by a 7-cm door [32]. The mice were first put in the center of the light [33]. The transitions and the time spent in the darkness to the brightness compartment were captured for a duration of five minutes [34].

Hole board test:

Measurements of a mouse's propensity to poke its nose or snout through holes have been performed using hole boards or hole boxes. One of the noteworthy behaviors is hole-poking, also known as head-dipping, which has been proven to be extremely sensitive to drug effects [35]. An HBT apparatus (35 cm x 35 cm x 15 cm) was used to observe the mice's anxiogenic effects. The arena floor is made of black wooden plate with 16 holes divided into 16 equal parts, and the walls are made of wooden sheets (3.5 cm in diameter). The apparatus was lifted 56 cm off the ground. Each mouse was located in the center of the apparatus, and the number of heads dipping was counted for 5 minutes [36].

Marble Burying Test:

The MBT is a widely used experiment examining repeated behavior and a phenotype resembling anxiety [37]. A test for marble burying was performed utilizing a propylene cage and 12 marbles made of clean glass that were spaced evenly, or 4 cm apart, on the husk. QintroTM at varied doses was given to mice 30 minutes before the test. After that, mice were put in the cage with the marbles. After 30 minutes, the number of buried marbles was counted.

Tail Suspension Test (TST):

The TST is a behavioral test for desperation in which mice are positioned 50 cm off the ground by the distal end of their tails. Blinded observers timed their total immobility for six minutes. The immobility time in this test can be decreased by anti-depressants [38].

Statistical Analysis:

The mean and SEM for each group's data is shown. A one-way ANOVA was used for statistical analysis, followed by Dunnette's test utilizing Graph-Pad Prism version 5.0 (USA). Statistics were found to be significant at p values < 0.05. The graphs showed $^{a}p< 0.05$ in contrast to group I and $^{b}p< 0.05$ contrasted with group II.

Results

Impact of Qintro™ on the exploratory behavior of ethanol withdrawal mice in EPM

As shown in **Figure 1**, alcohol withdrawal animals exhibited the anxiogenic effects after chronic ethanol consumption, as indicated by significantly ($f_{(5,30)} = 40.67$, $p < 0.05$) more time invested in the close arm. Contrarily, administration of Qintro™ post-withdrawal was observed to reduce anxiety, as evidenced by a considerably ($f_{(5,30)} = 50.25$, $p < 0.05$) longer open arm time compared to ethanol withdrawal group II.

Effect of Qintro™ on the behavior of ethanol withdrawal mice in LDT

Figure 2 illustrates the result of Qintro™ on the tested settings in the LDT apparatus. The mice in both the control group (which received saline according to methods) and those experiencing ethanol withdrawal exhibited a significant increase in the amount of time they spent in the dark chamber compared to the light compartment ($f_{(5,30)} = 16.78$, $p< 0.05$), suggesting elevated anxiety levels. However, it's important to note that while the withdrawal group experienced anxiety related to alcohol cessation, the control

group did not undergo alcohol withdrawal. The anxiolytic efficacy of the polyherbal formulation was indicated by a significant ($f_{(5,30)} = 29.18$, $p < 0.05$) increase in the amount of time mice treated with Qintro™ spent in the light chamber. Compared to the vehicle-treated and ethanol withdrawal groups, group V, which got Q-400, exhibited a more notable effect.

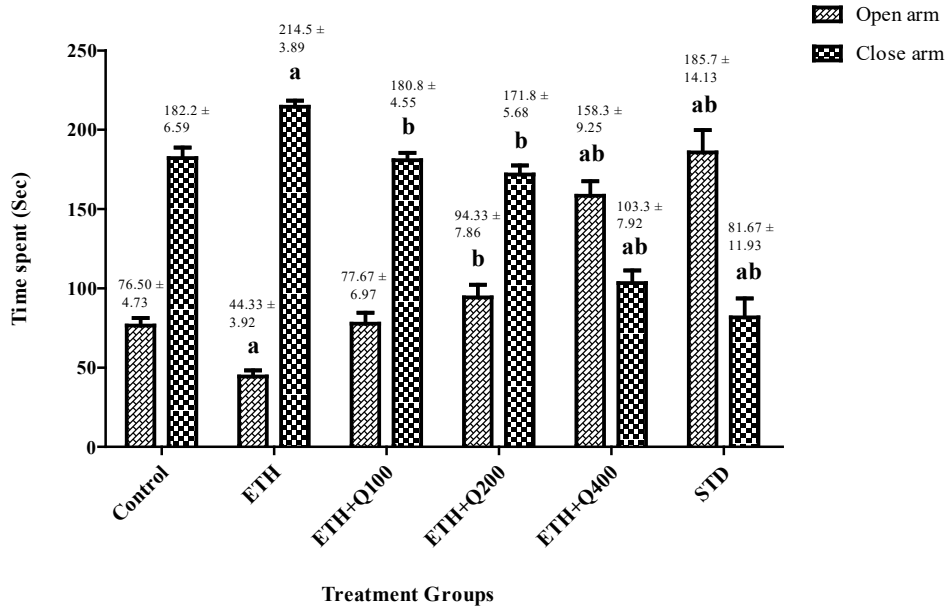

Figure 1: Impact of Qintro™ on the behavior of ethanol withdrawal mice in the EPM test. The graph showed a comparison between the open and closed arms, and the bar represented the mean (n = 6) of time spent (sec) in each arm.

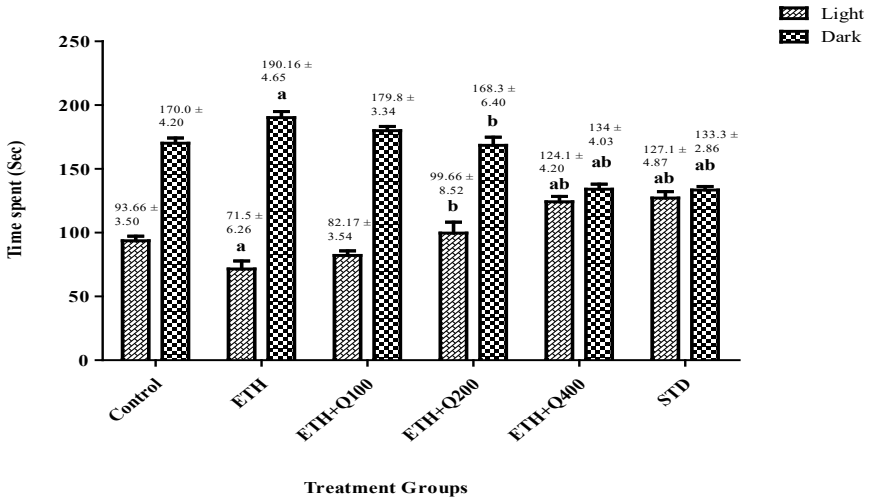

Figure 2: Impact of Qintro™ on behavior of ethanol withdrawal mice in LDT. The graph showed a comparison between the light and dark compartments, and the bar represented the mean (n = 6) of time spent (sec) in each compartment.

Effect of Qintro™ on the behavior of ethanol withdrawal mice in HBT

Figure 3 illustrates the results on specifications tested in the HBT apparatus. The control and ethanol withdrawal mice exhibited a significant decrease in head dips ($f_{(5,30) = 20.63}$, $p < 0.05$), indicative of anxious behavior stemming from abstinence. However, it is noteworthy that while the withdrawal group experienced anxiety related to alcohol cessation, the control group did not undergo alcohol withdrawal. Mice treated with Qintro™ revealed a significant ($p < 0.05$) increase in head dips in mice, demonstrating the polyherbal formulations anxiolytic activity. A stronger impact was observed in group V Q-400 as opposed to vehicle-treated groups and groups that disengage from ethanol.

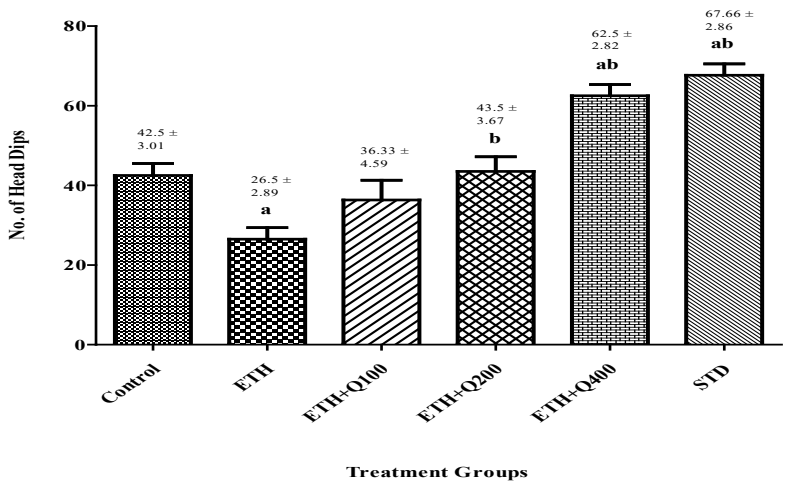

Figure 3: Impact of Qintro™ on behavior of ethanol withdrawal mice in HBT. The graph showed the comparison of group I to groups II, III, IV, V, VI and group II to group III, IV, V, VI and the bar represented the mean (n=6) of the numbers of head dips in each group.

Effect of Qintro™ on the behavior of ethanol withdrawal mice in MBT

In mice, continuous consumption of ethanol decreased marble burying in MBT in mice compared to group I. Mice administered with QintroTM (Groups III, IV, and V) and groups IV and V showed a significant ($f_{(5,30) = 15.51}$, $p< 0.05$) decrease in marble-burying compared to Group II. Mice treated with Q-400 had a more notable impact, suggesting that QintroTM has anti-anxiety properties in mice that remove ethanol (Figure 4).

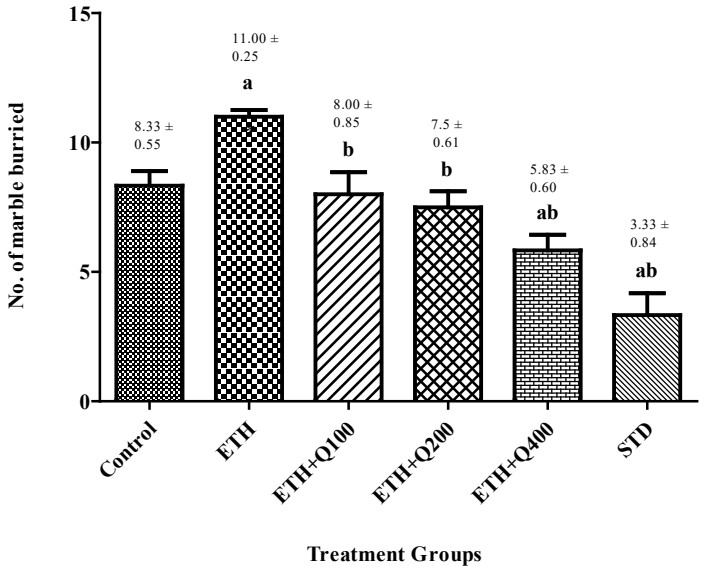

Figure 4: Effect of Qintro™ on behavior of ethanol withdrawal mice in MBT. The graph showed the comparison of group I to groups II, III, IV, V, VI and group II to group III, IV, V, VI, and the bar represented the mean (n=6) of the number of marble buried in each group.

Effect of Qintro™ on the behavior of ethanol withdrawal mice in TST

As shown in **Figure 5**, alcohol withdrawal animals exhibited anxiogenic effects after chronic ethanol consumption, as indicated by a significant ($f_{(5,30) = 13.13}$, $p< 0.05$) raised immobility moment in the TST. Contrarily, administration of QintroTM post-withdrawal was observed to reduce immobility, as evidenced by a considerable ($p< 0.05$) decreased inactivity duration in contrast to group II.

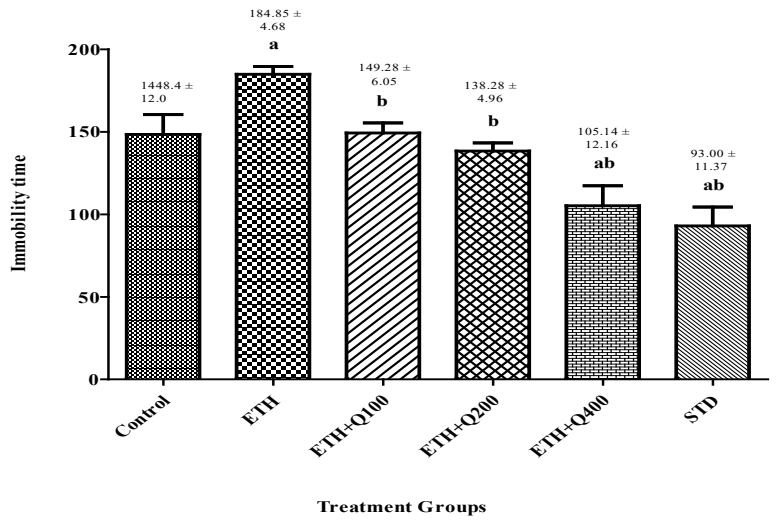

Figure 5: Effect of Qintro™ on behavior of ethanol withdrawal mice in TST. The graph showed the comparison of group I to groups II, III, IV, V, and VII and group II to group III, IV, V, and VI and the bar represented the mean (n=6) of immobility time in each group.

Discussion

In the current investigation, we investigated the effect of Qintro™ on the anxiety and depression induced by AW in mice. It has been observed that chronic ethanol treatment followed by withdrawal results in anxious-like behavior. AW is induced by an abrupt cessation of drinking after prolonged alcohol consumption. Thus, abstinence develops due to prolonged, excessive ethanol use and withdrawal. Several researchers have shown the link between AW and disruption of ion channel function and changes in neurotransmitter levels in the brain, leading to the development of anxiety and depression [39, 40, 41].

AW has been associated with decreased GABAergic transmission and down-regulated GABAA receptors, creating the hyper-glutamatergic state, which, when combined with decreased GABA function, results in excessive excitatory signaling and AW anxiety [42]. The characteristic of anti-addictive substances is that they inhibit withdrawal syndrome [43]. We also evaluated whether QintroTM can mitigate anxiety-like behavior associated with AW. According to data from the EPM, LDT, HBT, MBT, and TST, these are well-validated models to screen the agents used for relieving anxiety-like behavior. In the current investigation, Qintro™ significantly reversed the AW-induced anxiety in a dose-dependent manner in all the models [44]. It is a combination of seven components with a variety of pharmacological effects. It's possible that the sum of all these things helped to alleviate withdrawal symptoms.

The anxiolytic activity may be due to its components, including Sarpagandha (*Rauwolfia serpentina*) [13], Vacha (*Acorus calamus*) [18], Jatamansi (*Nardostachys jatamansi*) [23], and Shankhpushpi (*Convolvulus prostratus*). These components showed an anti-anxiety effect in ethanol withdrawal and alcohol-addicted mice [25]. Most of these herbs have previously been shown to modulate the GABAergic system and provide protection against anxiety and addiction [13,18,23].

In the present work, AW mice was evaluated by TST. AW mice exhibited more immobility time, indicating a depressive effect due to sudden alcohol cessation [45]. On the other hand, Qintro™ treatment decreases the duration of immobility, indicating the anti-depressant effect of this polyherbal formulation.

The components of Qintro™, like Guduchi (*Tinospora cordifolia*) [16], Bramhi (*Bacopa monnieri*) [20], and Tagar (*Valeriana wallichii*) [21], were previously shown to possess anti-depressant activity. Most of these herbs modulate the serotonin (5-HT) level and provide protection against depression [46].

Taken together, our study has shown the positive effect of Qintro™ in mice where alcohol abstinence caused anxiety and despair and might be useful as an anti-addiction remedy, especially in individuals with alcohol addiction.

Conclusion

Our results suggest that prolonged alcohol intake and sudden withdrawal are associated with the induction of anxiety and depression in laboratory mice. Whereas, administration of this polyherbal formulation may help prevent the development of tolerance to and dependence on ethanol. To sum up, these results provide the initial proof of the anti-addiction property of Qintro™, and it may be helpful against protection from AW syndrome and associated behavioral and biochemical changes.

Funding:

There was no external funding to conduct this study.

Acknowledgement

The authors express gratitude to the Management of *SNJB's College of Pharmacy* for providing the necessary facilities and Dr. *Gaurav Thakor (MD)*, for providing Qintro™ a polyherbal formulation and guidance for the experimental work.

Conflicts of Interest: According to the writers, there is no conflict of interest.

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