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Original Article

Assessing the Pharmacological Potential of Thymoquinone for Managing Alcohol Craving and Withdrawal Syndrome in Mice

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ABSTRACT

Aim/Background: The aim is to evaluate THMQ's impact on alcohol withdrawal and alcohol craving using an animal model. Mice were exposed to increasing ethanol doses to simulate dependency. Researchers assessed anxiety levels during withdrawal using behavioural and biochemical tests. THMQ was administered orally at 20 mg/kg and 40 mg/kg, compared to diazepam (1 mg/kg). **Materials and Methods:** Model Creation Mice received escalating ethanol doses (5% to 35% v/v) over specific days. Regular water was given during withdrawal periods. Assessment Tools Elevated Plus Maze (EPM), Elevated Zero Maze (EZM), Open Field Test (OFT), and Western Blot analysis. Treatment Intervention: THMQ (20 mg/kg and 40 mg/kg) vs. diazepam (1 mg/kg). **Results and Conclusion:** THMQ-treated group showed reduced anxiety and alcohol desire. Alcohol consumption decreased in THMQ-treated mice. No changes in GluA1 and sk2 protein levels. THMQ holds promise as a remedy for alcohol cravings and withdrawal symptoms

Keywords: Alcohol Craving, Alcohol Withdrawal, Thymoquinone, Western blot, Open Field Test.

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INTRODUCTION

Alcohol is considered the most widely abused substances globally due to its propensity for inducing tolerance and dependency with extended usage, observed in both animals and humans. For the context herein, "alcohol" pertains specifically to ethanol (C_2H_5OH), as it is the predominant type employed in the concoction of alcoholic beverages.[1,2] Alcohols emit an aroma often delineated as "pungent" and "persistent" in the nasal cavity.[3]

Alcohol dependence denotes a state of substance misuse wherein an individual develops a tolerance to alcohol and persists in consumption despite adverse outcomes. For a diagnosis of alcohol dependence, a minimum of three out of seven specified symptoms must manifest consistently over a duration of at least one year.[4] These symptoms encompass consuming more alcohol than intended, experiencing prolonged intoxication beyond anticipated durations, attempting unsuccessfully to curtail consumption, dedicating significant time to obtaining alcohol or recovering from its effects, and diminishing engagement in social, professional, and leisure pursuits as a consequence of excessive alcohol usage.[5]

The alcohol withdrawal syndrome encompasses a constellation of distressing physical and psychological manifestations that may arise when an individual abruptly discontinues alcohol consumption. Symptoms can vary from subtle tremors to severe manifestations like hallucinations and seizures. Repeated episodes of withdrawal can contribute to the development of alcoholism and lead to adverse health ramifications. A deeper understanding of the genetic and physiological underpinnings of alcoholism holds promise for the advancement of efficacious treatments targeting withdrawal and other facets of the disorder.[6,7] Withdrawal, a pivotal stage in the neural modulation cycle induced by alcohol, is a tangible phenomenon. Evidence suggests that acute alcohol intake can perturb the release of neurotransmitters and disrupt the functioning of neuronal membrane proteins, including receptor proteins binding neurotransmitters and ion channels facilitating ion influx (e.g., sodium or calcium.[8] Over time, the brain acclimates to alcohol, thereby diminishing its once-disruptive effects a phenomenon known as tolerance. Prolonged heavy drinking may potentially alter the structure and function of brain neurons to the extent that they require alcohol to maintain normal function, a condition termed physical dependence. Abrupt cessation of alcohol intake among heavy drinkers may precipitate rebound hyperexcitability, colloquially referred to as

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withdrawal syndrome, as the adaptive mechanisms previously deployed to counter alcohol's inhibitory effects are no longer operational.[6,9] To better comprehend and quantify the experience of withdrawal, researchers have employed diverse methodologies to induce physical dependency in animals. Common techniques involve intravenous alcohol administration, oral ingestion via tube insertion, or inclusion of alcohol in a balanced liquid diet (intubation).[10] Each method presents its advantages and limitations, all aimed at maintaining an individual's Blood Alcohol Content (BAC) within a safe threshold.[11] Withdrawal symptoms observed in animals may encompass tremors, motor dysfunction, and heightened autonomic activity. Seizures, a well-documented medical phenomenon, offer valuable insights. Their occurrence may be spontaneous or triggered by external stimuli, contingent upon the severity of withdrawal.[12]

The article delves into the symptoms associated with alcohol withdrawal, elucidating the utilization of animal models as a means to glean insights into the fundamental mechanisms of the disorder, including the roles played by the test drug in alleviating these symptoms.[13]

MATERIALS AND METHODS

Various methods exist to induce physiological dependence on alcohol in animals, with a balanced diet being one approach. In the present study, alcohol was administered by gavage. The direct effects of alcohol consumption were closely monitored and documented.[1]

Animals

Mice, aged between 6 to 8 weeks, were housed in a controlled environment with a temperature maintained between 21 to 23°C and a 12-hr light/dark cycle. Except for the duration of the trials, during which they were removed from their cages, the mice had unrestricted access to food and water. All animal procedures were conducted in accordance with the guidelines outlined by the Institutional Animal Ethics Committee (IAEC) and adhered to the regulations set forth by the Committee for Control and Supervision of Experiments on Animals (CCSEA) regarding the use of experimental animals in scientific research.[14]

Treatment

Experimental study consists of 8 distinct groups, each composed of 6 animals. The experimental groups were organized as follows:

1st Group (Vehicle control): Received 0.2 mL of water orally (i.g) from days 1 to 5, followed by access to normal drinking water in a water bottle until day 28.

2nd Group (Standard *per se*): Similar to the 1st group, but treated with diazepam at a dose of 1 mg/kg intraperitoneally (i.p).

3rd Group (Test drug *per se*): Similar to the 2nd group, but treated with thymoquinone at a dose of 40 mg/kg orally (p.o).

4th Group (Negative control): Administered 5% v/v ethanol in the water bottle along with 0.2 mL of 5% v/v ethanol orally (i.g) from days 1 to 5, followed by 10% v/v ethanol in the water bottle from days 8 to 12, 20% v/v ethanol from days 15 to 19, and 35% v/v ethanol from days 22 to 26. On days 6, 7, 13, 14, 20, 21, 27, and 28, regular drinking water was provided.

5th Group (Positive control): Underwent alcohol administration and withdrawal as described above for 4th group and administered with diazepam at a dose of 1 mg/kg i.p.

6th Group (Test drug- Low dose): Underwent alcohol administration and withdrawal as described above for 4th group and administered with thymoquinone at a dose of 20 mg/kg p.o.

7th Group (Test drug- High dose): Underwent alcohol administration and withdrawal as described above for 4th group and administered with thymoquinone at a dose of 40 mg/kg p.o.

8th Group: Underwent alcohol administration and withdrawal as described above for 4th group and administered with thymoquinone at a dose of 20 mg/kg p.o and diazepam at a dose of 1 mg/kg i.p.

Procurements of chemicals and drug

We obtained absolute alcohol from Sigma Aldrich, thymoquinone and diazepam from Carbanio.

Alcohol consumption

This structured approach allowed the researchers to observe the effects of progressively increasing ethanol concentrations on the animals, with intermittent breaks to assess any changes in behaviour or physiology during periods without ethanol exposure. Throughout the investigation, the animals were exposed to a progressive escalation in ethanol consumption. Both oral and water-based delivery of ethanol were conducted alcohol administration its withdrawal and the test schedule are depicted in Figure 1. Below is a comprehensive analysis of the ethanol administration timetable: The animals were administered a 5% v/v ethanol solution during the first five days.[1] Every animal was provided with this solution in their water bottles for uninterrupted availability, and they were also administered 0.2 mL of the same 5% v/v ethanol solution orally. Days 6 and 7: These days provided a little respite from the use of ethanol. The animals were given access to regular drinking water, at which time they consumed no ethanol. Days 8 to 12: Following the first exposure and a brief pause, the ethanol concentration was increased twice to 10% v/v. The augmented solution was present in their water bottles during the whole 5-day duration. Days 13 and 14: A two-day hiatus was introduced, during which the animals were provided with regular drinking water containing no ethanol. During days 15 to 19, the ethanol concentration increased again, reaching a level of 20% v/v. The elevated content was maintained in their water bottles over five days. On days 20 and 21, the animals were

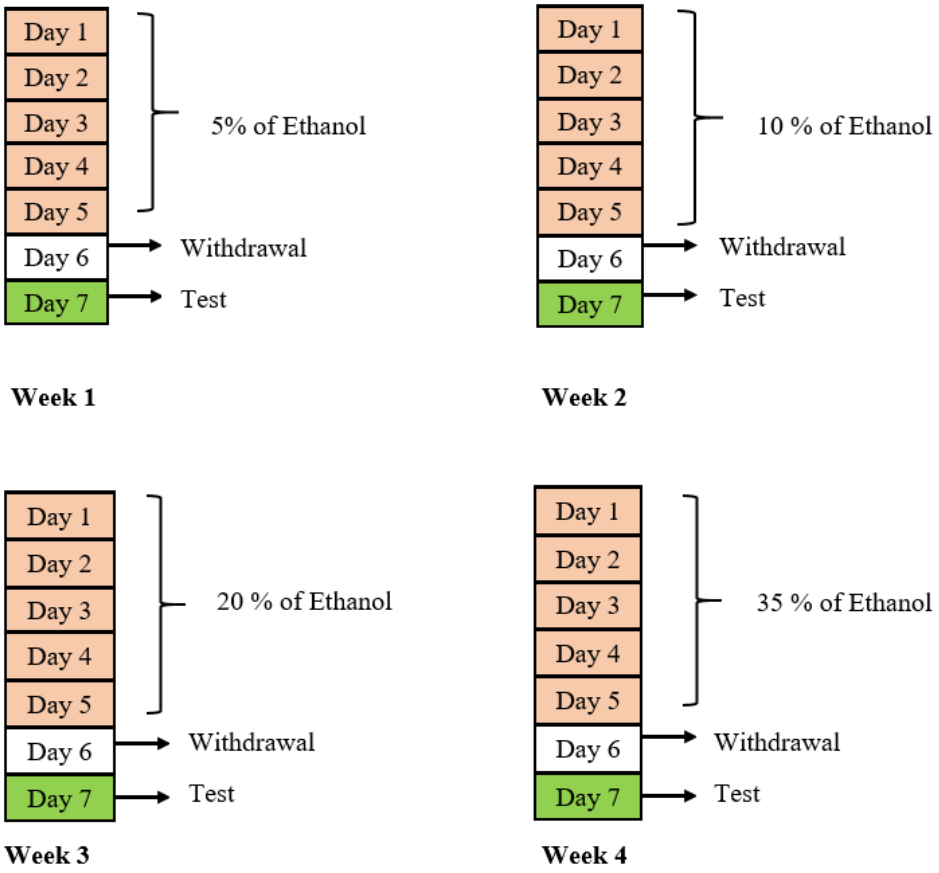

Figure 1: Alcohol administration, its withdrawal and test schedule.

given another period of days without ethanol and were supplied with regular drinking water once again. Days 22 to 26: During the last stage of ethanol administration, the concentration was raised to the maximum level of 35% v/v. The concentration remained constant in their water bottles throughout the five days. Days 27 and 28: The research was completed by providing participants with regular drinking water for two days, which indicated the completion of the time of administering ethanol. Using a systematic methodology, the researchers could closely monitor the impact of gradually escalating ethanol levels on the animals. They also took periodic intervals to evaluate any behaviour or physiology alterations when the animals were not exposed to ethanol.[1]

Evaluation parameters

Elevated Plus Maze (EPM)

A testing setup consists of a plus-shaped maze with both open and closed arms, featuring open roofs and varying heights from 40 to 70 cm above the ground. This paradigm is founded on research indicating that mice exhibit fear responses in expansive environments.[15] EPM was performed to evaluate anxiety related behaviours, after drug treatment, individual mice was placed at the centre of the maze, head facing an open arm. During the 5 min (300 sec) test period, the number of entries and time spent on the open arm were recorded automatically.[16]

Elevated Zero Mazes (EZM)

The EZM comprises a circular apparatus with elevated arms enclosing one half of the circumference while leaving the other half exposed. Within the closed segment of the elevated zero mazes (featuring walls standing at 20.5 cm, an outer perimeter measuring 119.4 cm, a platform width of 10.2 cm, and a distance from the floor to the contraption at 63.5 cm), the mice were introduced (wall height 20.5 cm, outer diameter 119.4 cm, platform width 10.2 cm, floor to apparatus 63.5 cm). The room lamps were disabled, and the maze was lighted from an above source. The duration of time spent in both the closed and open quadrants was recorded automatically for a period of 5 min (300 sec). Increased time spent in the closed quadrants is linked to behavior resembling anxiety.[16,17]

Open Field Test (OFT)

The Open Field Test (OFT) is a commonly used method for assessment of generalized locomotor activity. Rodents were introduced into a vacant rectangular enclosure and given 5 min to investigate. The movement was tracked using a beam break system and analysed using software to determine the

total distance travelled, number of movement and rest episodes, number of rearing events, amount of time spent in the centre, and level of stereotypic activity. Anxiety-like behaviour induced by alcohol withdrawal is characterised by excessive mobility and an increased amount of time spent in the centre area.[16,17]

Western Blot analysis

Dorsal (dHC) or Ventral (vHC) hippocampi of Swiss albino mice were harvested for protein extraction. The harvested tissue was homogenized using a buffer containing 50 mM Tris-Base (pH 7.35), 20 mM HEPES (pH 7.4), 5 mM EDTA (pH 8.0), and a protease and phosphatase inhibitor cocktail (HaltTM, Thermo Fisher). Synapto neurosomes were prepared following the same protocol. For western blotting, the samples were dissolved in Sodium Dodecyl Sulphate (SDS) sample buffer and run on a 4-20% gradient SDS polyacrylamide gel (Bio-Rad). Subsequently, they were transferred to nitrocellulose membranes. Fluorescent secondary antibodies and mouse anti-actin primary antibodies were used for the western blot analysis. The blots were then photographed using the infrared LICOR Odyssey CLx equipment.[17]

Statistical analysis

The results were expressed as mean S.E.M (n=6). The statistical analysis involving different groups was performed by Sigma Stat 4.5. One way ANOVA followed by Tukey test was performed. p value at < 0.05 was considered as statistically significant.

RESULTS

Alcohol intake

Day wise alcohol intake is depicted in Figure 2, On the 7th day, no significant difference was observed among different groups. On the 14th day, thymoquinone and diazepam administered groups exhibited significant decrease in alcohol intake, as compared to the positive control group (p<0.05). The combination of thymoquinone (20 mg/kg) with diazepam showed the highest level of significance (p< 0.001). On the 21st and 28th days, similar results were observed and the thymoquinone treatment, especially when combined with diazepam, exhibited best effect, compared to the positive control group.

Effect of thymoquinone on alcohol intake. Data represented as mean ± SD (n = 6).*,**,*** represents p<0.05, p<0.01, p<0.001 statistical significance, respectively, when compared to negative control group; $^{\#,\#\#,\#\#\#}$ represents p<0.05, p<0.01, p<0.001 statistical significance, respectively, when compared to positive control group.

Elevated Plus maze

Over the course of the study, significant differences were observed on days 7, 14, 21, and 28. The findings are summarized as follows: The depicted Figure 3 show that there were consistently significant differences (p<0.001) between the vehicle control group and the alcohol-treated disease control group. Furthermore, there were notable distinctions between the alcohol-treated disease control group and the group administered with diazepam (1 mg/kg, i.p) (p<0.01). Additionally, the group receiving alcohol and thymoquinone (20 mg/kg, oral) alone did not vary significantly from the group receiving alcohol, thymoquinone (20 mg/kg, oral), and diazepam (1 mg/kg, intraperitoneal) (p<0.001). The groups treated with alcohol and thymoquinone (20 mg/kg, oral) with diazepam (1 mg/kg, i.p) and the group treated with alcohol and thymoquinone (20 mg/kg, oral) alone did not, however, differ significantly. These results demonstrate the consistent effects of alcohol plus diazepam and alcohol plus thymoquinone on the observed outcomes.

Effect of thymoquinone on time spent in open arm. Data represented as mean ± SD (n = 6). $^{!,!!,!!!,}$ represents p<0.05, p<0.01, p<0.001 statistical significance, respectively, when compared to negative control group; $^{\#,\#\#,\#\#\#}$ represents p<0.05, p<0.01, p<0.001 statistical significance, respectively, when compared to combination of THMQ+Diazepam group; *,**,*** represents p<0.05, p<0.01, p<0.001 statistical significance, when compared to positive control group $^{\$,\$}$.

Elevated Zero Maze

As depicted in Figure 4, significant differences were continuously noted at every research time point. On Days 7, 14, 21, and 28, there was a significant difference (p < 0.001) between the vehicle control group and the alcohol-treated disease control group. When compared to groups treated with alcohol and diazepam (1 mg/kg, i.p.) and alcohol and thymoquinone (20 mg/kg and 40 mg/kg, p.o.), the alcohol-treated disease control group demonstrated substantial differences (p-values ranging from < 0.05 to < 0.001). Furthermore, significant differences (p < 0.001) were seen on all days between the alcohol and diazepam (1 mg/kg, i.p.) group and the alcohol and thymoquinone (20 mg/kg, p.o.) group. Additionally, on Days 21 and 28, there was a significant difference between the alcohol and thymoquinone (40 mg/kg, p.o.) group and the alcohol and diazepam (1 mg/kg, i.p.) group (p < 0.01 and p < 0.001, respectively). According to these findings, the vehicle control group provided a stable baseline, and the addition of alcohol along with either thymoquinone or diazepam demonstrated distinct therapeutic effects, with the combined addition of these two medications producing significantly different outcomes than their individual administration.

Effect of thymoquinone on alcohol withdrawal syndrome induced anxiety. Data represented as mean ± SD (n = 6). $^{!,!!,!!!,}$ represents p<0.05, p<0.01, p<0.001 statistical significance, respectively, when compared to negative control group; $^{\#,\#\#,\#\#\#}$ represents p<0.05, p<0.01, p<0.001 statistical significance, respectively, when compared to combination of THMQ+Diazepam group;

*,**,*** represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, when compared to positive control group $,$$.

Number of Open Entries

As depicted in Figure 5, significant difference were found between the groups at every stage of the investigation. When compared to other treatment groups, the Vehicle Control group demonstrated significant changes on Days 7, 14, 21, and 28 ($p < 0.001$). With p-values ranging from < 0.05 to < 0.001, the Alcohol Disease Control group differed substantially from all other treatment groups. Significant differences ($p < 0.001$) were continuously seen when comparing the Alcohol + Diazepam 1mg/kg i.p. group to other treatment groups, such as the Alcohol + Thymoquinone 20 mg/kg p.o. group and the Alcohol + Thymoquinone 20 mg/kg p.o. + Diazepam 1mg/kg i.p. group. Additionally, there was a significant difference ($p < 0.05$ on Day 7 and $p < 0.001$ on Days 14, 21, and 28) between the Alcohol + Thymoquinone 20mg/kg p.o. group and the Alcohol + Thymoquinone 20mg/kg p.o. + Diazepam 1mg/kg i.p. group. These findings demonstrate the unique therapeutic benefits of alcohol in conjunction with thymoquinone or diazepam and emphasise the findings' continuous statistical significance over the course of the investigation.

Effect of thymoquinone on alcohol withdrawal syndrome induced anxiety. Data represented as mean ± SD ($n = 6$). !,!!,!!! represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, respectively, when compared to negative control group; #,##,### represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, respectively, when compared to combination of THMQ+Diazepam group; *,**,*** represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, when compared to positive control group $,$$.

Open Field Test

Centre Ambulation

As depicted in Figure 6, throughout the period of the observation, the study continuously showed significance differences between the various treatment groups. Significant differences were seen between the "Alcohol Treated Disease Control" group and the Vehicle Control group as well as other treatments, such as "Alcohol + Diazepam (1 mg/kg i.p.)" and "Thymoquinone (20 mg/kg p.o. and 40 mg/kg p.o.)" ($p < 0.001$). There was a significant difference between all other treatments and the Alcohol Treated Disease Control group ($p < 0.01$ to $p < 0.001$). Further significant differences were observed between the "Alcohol + Thymoquinone (20 mg/kg p.o. and 40mg/kg p.o.)" group and the combination treatment of "Alcohol + Thymoquinone (20 mg/kg p.o.) + Diazepam (1 mg/kg i.p.)" ($p < 0.001$). These differences also applied to the "Alcohol + Thymoquinone (20 mg/kg p.o.)" group and the "Alcohol + Thymoquinone (20 mg/kg p.o.)" group.

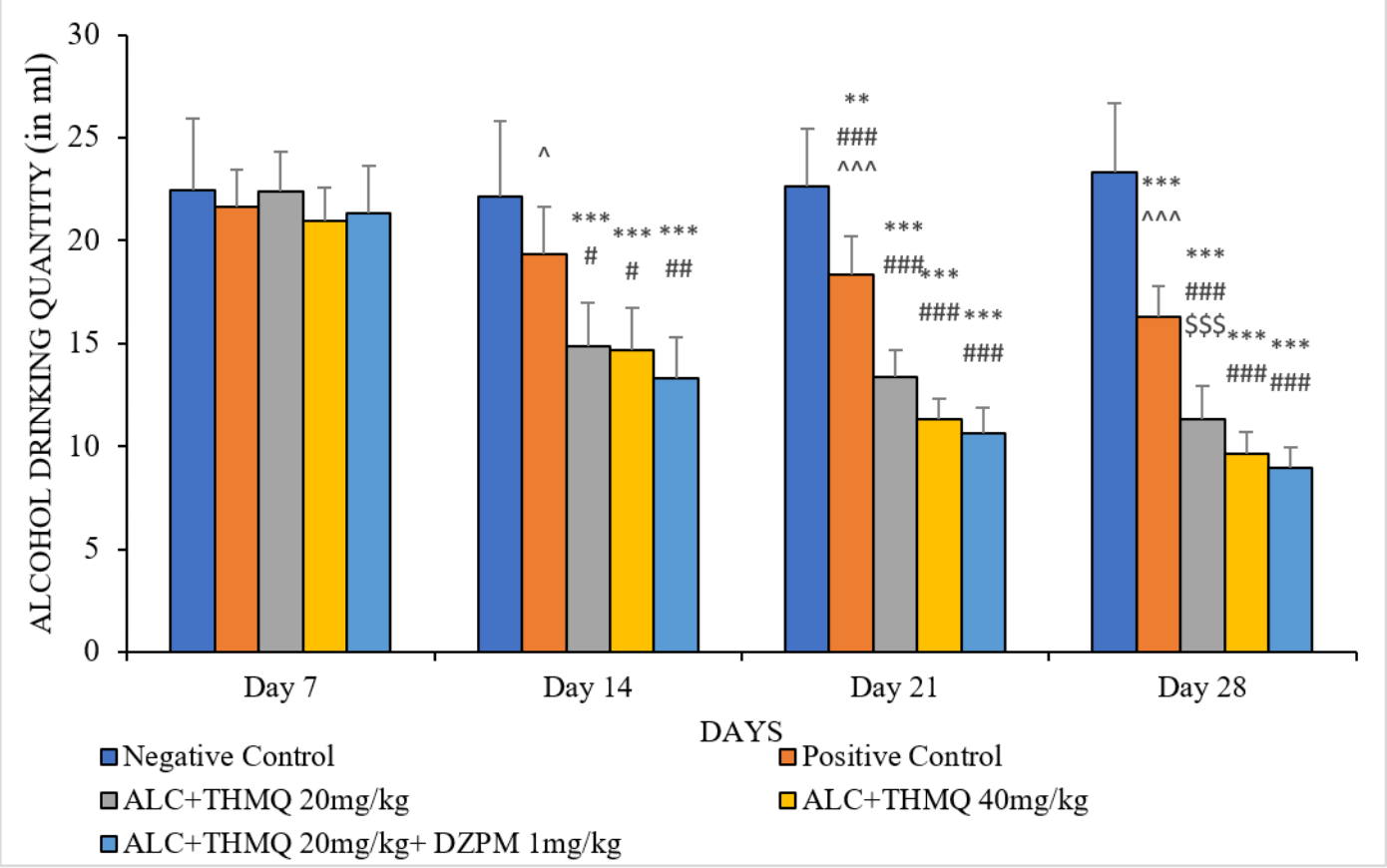

Figure 2: Alcohol intake day wise.

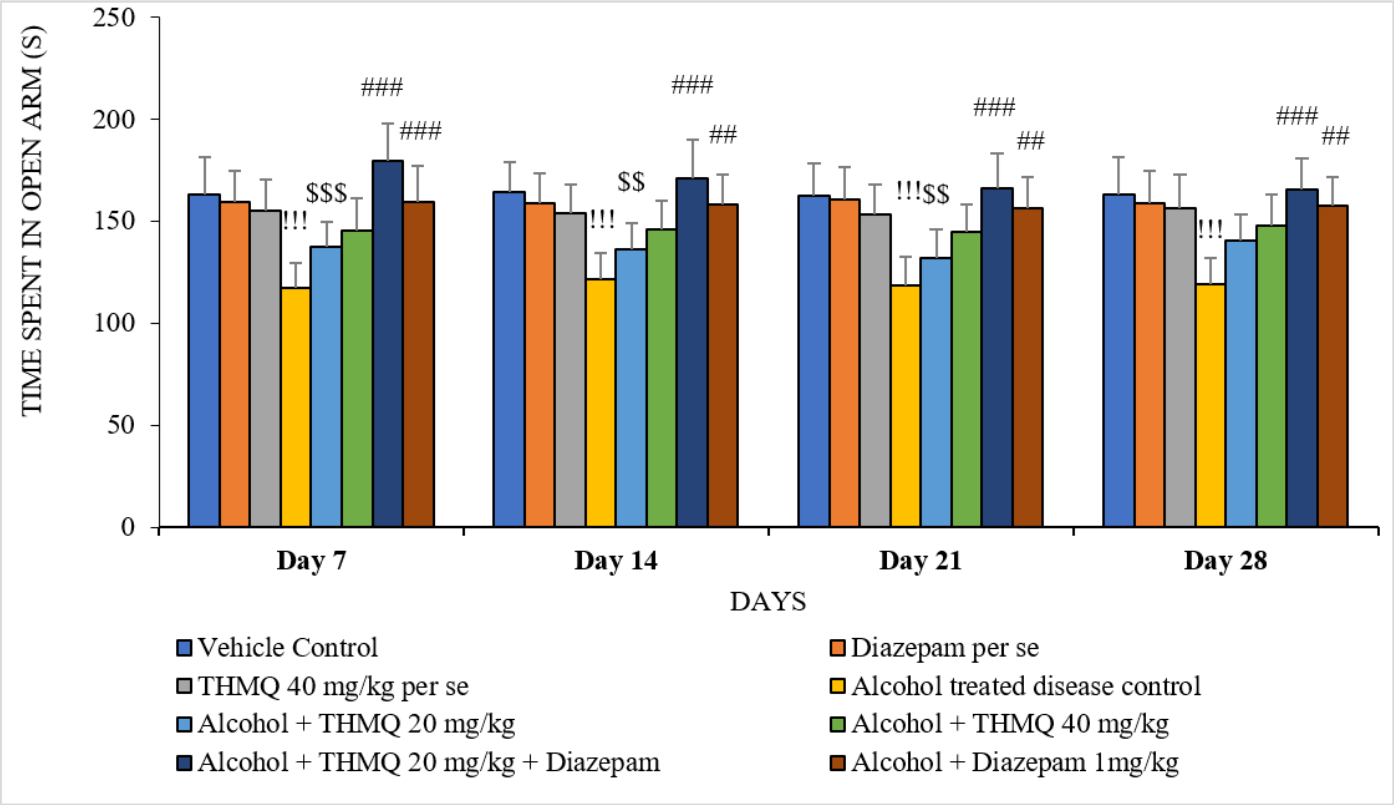

Figure 3: Elevated Plus Maze.

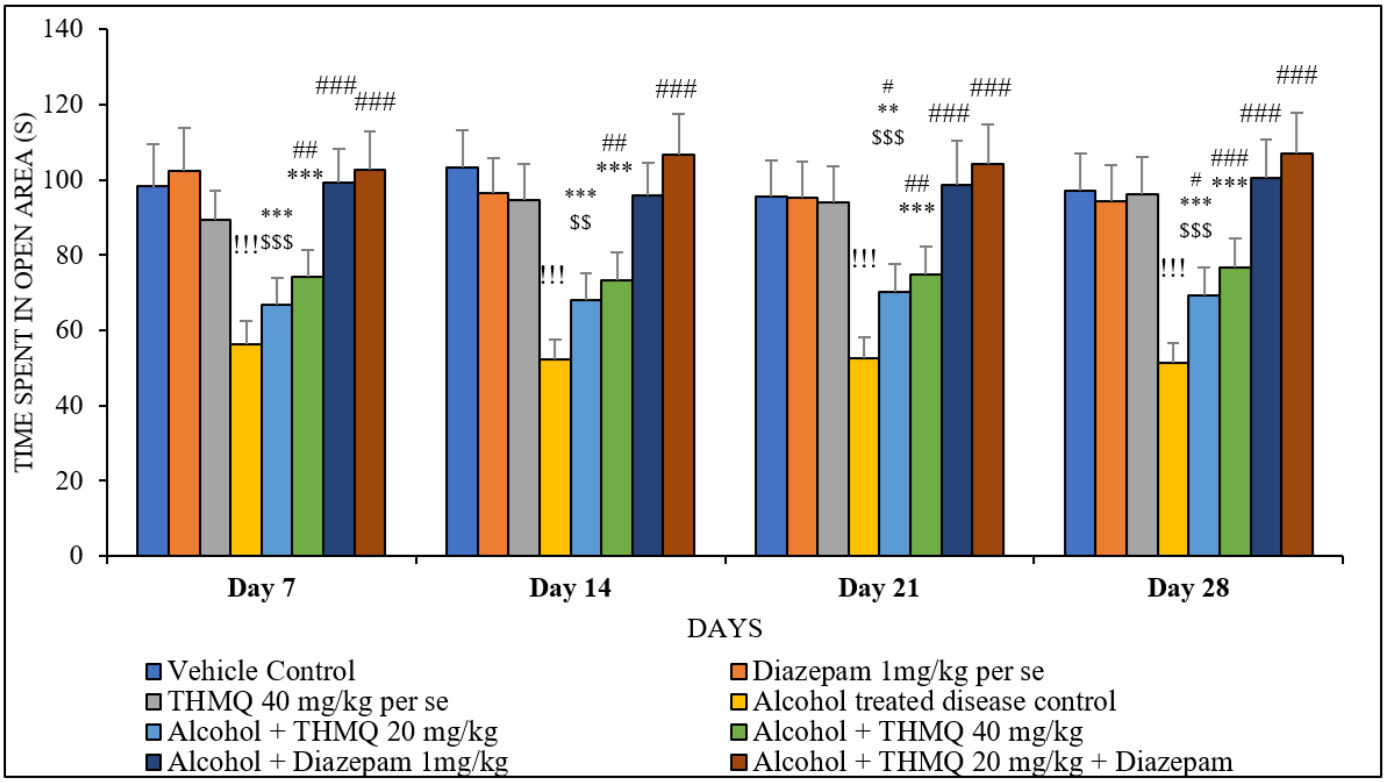

Figure 4: Time spent in Open Area.

These findings highlight the unique therapeutic benefits of alcohol in conjunction with either thymoquinone or diazepam, with combination treatments demonstrating markedly different results from their separate equivalents.

Effect of thymoquinone on alcohol withdrawal syndrome induced anxiety. Data represented as mean ± SD (n = 6). $^{!,!!,!!!}$ represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, respectively, when compared to negative control group; $^{\#,\#\#,\#\#\#}$ represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, respectively, when compared to combination of THMQ+Diazepam group; *,**,*** represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, when compared to positive control group $^{\$,\$\$}$.

Open Field Test

Time Spent In center

Time spent in center is depicted in Figure 7, significant differences ($p < 0.001$) were noted between the Vehicle Control and Alcohol Treated Disease Control groups throughout the investigation. Significant differences were seen on all days ($p < 0.01$) between the groups that received alcohol plus diazepam (1 mg/kg i.p)

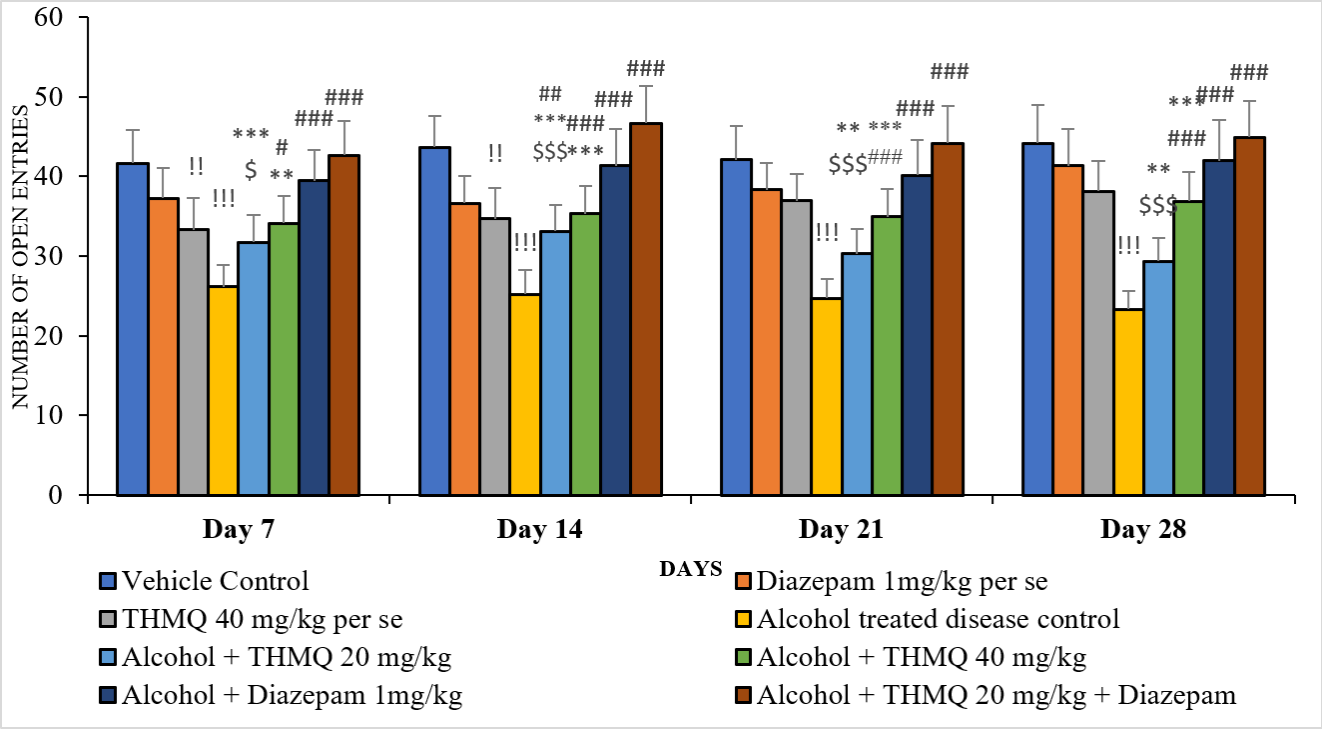

Figure 5: Number of open entries.

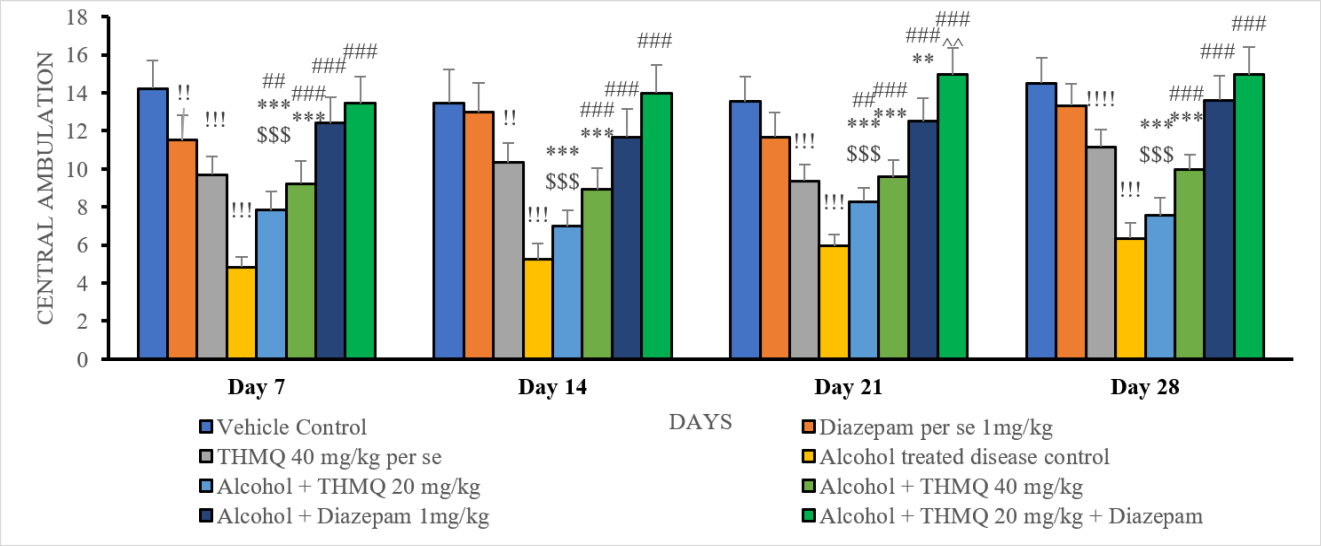

Figure 6: Centre Ambulation.

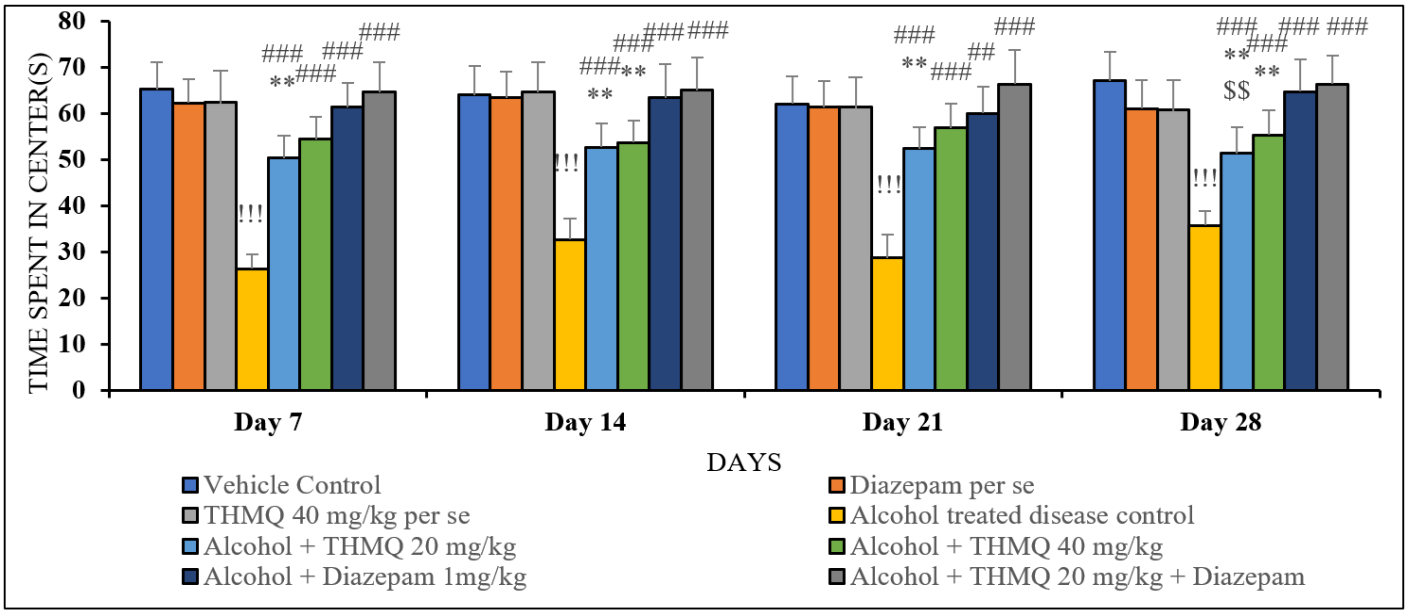

Figure 7: Time Spent in center.

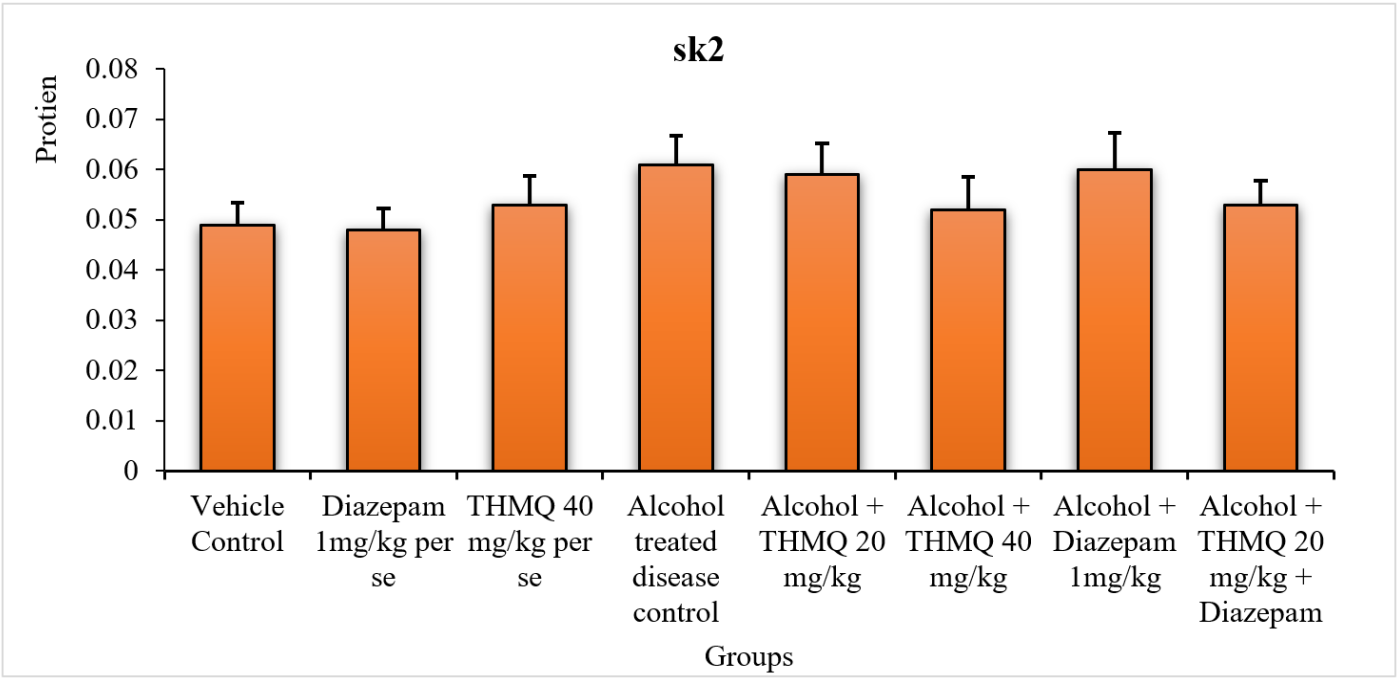

Figure 8: Western Blot sk2 Protein.

and alcohol plus thymoquinone (20 mg/kg p.o). On Days 7, 14, and 21, there were no appreciable variations between the groups receiving alcohol plus thymoquinone (20 mg/kg p.o) + diazepam (1 mg/kg i.p.) and alcohol plus diazepam (1 mg/kg i.p.). The "Alcohol + Thymoquinone (20 mg/kg p.o) + Diazepam (1 mg/kg i.p)" group and the "Alcohol + Thymoquinone (20 mg/kg p.o)" group showed a significant difference on Day 28 ($p < 0.01$). These results emphasise diverse therapeutic effects over time and show sustained significant differences across important treatment groups.

Effect of thymoquinone on alcohol withdrawal syndrome induced anxiety. Data represented as mean ± SD ($n = 6$). $^{!,!!,!!!}$ represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, respectively, when compared to negative control group; $^{\#,\#\#,\#\#\#}$ represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, respectively, when compared to combination of THMQ+Diazepam group; *,**,*** represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, when compared to positive control group $^{\$,\$}$.

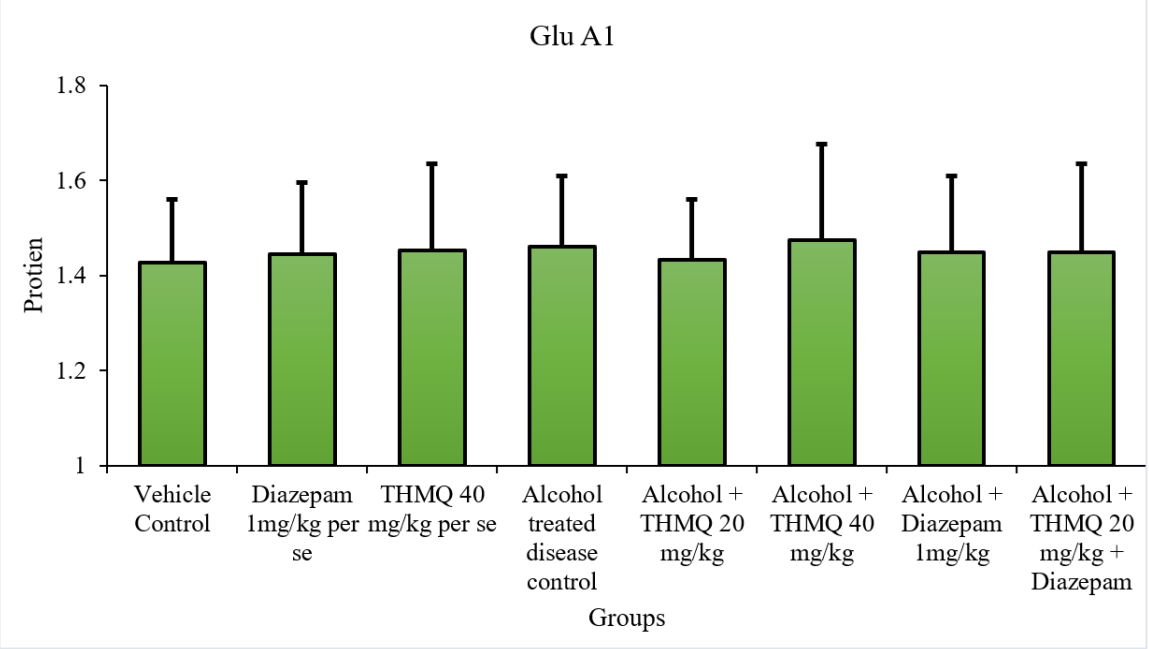

Figure 9: Western Blot Glu A1 Protein.

Western Blot

No significance difference was observed in sk2 and Glu A1 protein vs actin as depicted in Figures 8 and 9 respectively. The study used Western blot analysis as a primary method to examine the expression of SK2 protein in synaptosomes obtained from the Ventral Hippocampus (VHC) of Swiss albino mice. This analytical methodology allows identifying and measuring specific proteins in a given sample. SK2, a protein that activates potassium channels in response to calcium, is critical in regulating the excitability of neurons. The group of Swiss albino mice used for this study consisted of individuals with the same genetic makeup, which allowed for consistent and dependable findings the investigation aimed to evaluate any possible structural changes in GluAl and SK2 proteins under specified circumstances. GluAl most likely refers to glutamate receptors, perhaps AMPA receptors, which play a crucial role in synaptic transmission. There were no noticeable changes in the shape of these proteins throughout the range of GluAl and SK2 protein concentrations that were investigated. In addition, the research examined the subtle variations in GluAl and SK2 protein concentrations between the Ventral (VHC) and Dorsal Hippocampus (DHC). Examining these diverse hippocampus areas closely is essential since they often display varying functional properties. The findings showed no notable differences in GluAl or SK2 protein levels between the two situations. Within the confines of this investigation, it can be inferred that alcohol withdrawal did not significantly impact the manifestation of these proteins in the group of Swiss albino mice that were examined. The work offers unique insights into the dynamics of SK2 protein expression and its possible consequences in the hippocampus circuitry of Swiss albino mice. This is achieved by rigorous Western blot analysis and detailed comparative evaluations.

Effect of thymoquinone on alcohol withdrawal syndrome induced anxiety. Data represented as mean ± SD (n = 6). $^{!,!!,!!!,}$ represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, respectively, when compared to negative control group; $^{\#,\#\#,\#\#\#}$ represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, respectively, when compared to combination of THMQ+Diazepam group; *,**,*** represents $p<0.05$, $p<0.01$, $p<0.001$ statistical significance, when compared to positive control group $^{\$,\$}$.

DISCUSSION

The results demonstrate a significant decrease in alcohol consumption intensity from day 7 to day 28, among the treatment groups in case of negative control group. Day 7 can observe that all group drinking same quantity of alcohol on day 14 we observe that the groups were treated by Thymoquinone and Diazepam are slightly less when we compare them to negative control group. On day 21 the group were treated by Thymoquinone and Diazepam are drinking less as compare to day 7 and 14 respectively. On day 28 graphs show that clearly the groups of mice which is on Thymoquinone they have less urge to drink alcohol. As per the above graph its clearly indicating that groups of animals on Thymoquinone significantly decreases craving and urge for alcohol. Day 7 No significant change in alcohol consumption among different treatment groups. Day 14 to 28 No significant change in alcohol consumption for the negative control group. However, a significant decrease was observed in diazepam-treated, low and high dose Thymoquinone-treated, and Thymoquinone plus diazepam combination-treated groups. This

decrease indicates reduced alcohol craving due to the respective treatments.

On day 7, no significant change in the alcohol consumption quantity was observed among different treatment groups. From day 14 to 28, no significant change in the alcohol consumption quantity was observed for negative control group. However, a significant decrease in the alcohol consumption quantity was observed among diazepam treated, thymoquinone low and high dose treated and thymoquinone plus diazepam combination treated groups from day 14 to day 28. This decrease in the alcohol consumption quantity indicates the reduced alcohol craving of animals due to respective treatment.[18]

The research used a progressive alcohol-inducing method to mimic the gradual development of dependency on alcohol precisely. This method was used extensively to examine the treatment capabilities of THMQ. The lack of variations in GluA1 and sk2 protein levels suggests that THMQ may exert its effects via pathways that differ from those influenced by standard treatments, which include Diazepam. This particularity might be beneficial in mitigating adverse reactions and enhancing patient results. Nevertheless, there are still some unresolved questions. The precise neurochemical routes via which THMQ functions are uncertain. Subsequent investigations should clarify these pathways, opening up possibilities for more precise treatments. In addition, while the study's results show promise, applying these findings from animal models to human patients is only sometimes direct. THMQ must undergo clinical studies to ascertain its effectiveness and safety in human subjects.[19] Furthermore, the study's emphasis on the serotonin system, while warranted by its established function in addiction, may neglect the participation of other neurotransmitter systems. The impact of alcohol on the brain is intricate and diverse, and a thorough comprehension of its addictive processes would probably need a more comprehensive approach. Ultimately, this study highlights the potential of THMQ as an innovative remedy for alcohol addiction. The notable aspect of its efficacy lies in its capacity to diminish cravings and ameliorate withdrawal symptoms while having no impact on GluA1 and sk2 protein levels. In the future, it will be essential to expand on these discoveries, investigating the whole spectrum of THMQ's impacts and the underlying neurochemical processes implicated. The transition from experimental research to practical use is arduous and filled with obstacles. However, the potential shown by THMQ in this investigation is a promising stride towards the development of more efficient therapies for those afflicted with alcohol addiction.[20,21]

The alcohol consumption data indicates that the animals treated with thymoquinone exhibited reduced craving for alcohol, demonstrating thymoquinone's ability to decrease alcohol craving. Additionally, the data from the EPM suggests that mice experienced anxiety due to alcohol withdrawal, but after treatment with thymoquinone, they showed a reduced anxiety which was comparable to the diazepam effect. Similar result was observed in the EZM test. Subsequent analysis in the OFT revealed that animals treated with thymoquinone displayed increased central ambulation and spent more time in the centre, suggesting an anti-anxiety effect of thymoquinone after alcohol withdrawal. Western blot analysis indicated no changes in GluA1 and Sk2 protein levels. This suggests the potential of thymoquinone in the management of alcohol withdrawal symptoms. Thymoquinone's protective effects associated to its antioxidant and anti-inflammatory properties. It potentially modulates neurotransmitter systems, reducing oxidative stress and inflammation in the brain, which are common during alcohol withdrawal. This modulation might help in restoring the balance of neurotransmitters like GABA and glutamate, thereby reducing anxiety and craving.

Elevated Plus Time spent in Open arm Significant differences were seen on the 7th, 14th, 21st, and 28th days between the "Vehicle control" and "Alcohol-treated disease control" groups, as well as between the "Alcohol-treated disease control" and "Alcohol + Diazepam 1mg/kg i.p" groups. The group that got the combination of "Alcohol + thymoquinone (20mg/kg p.o + Diazepam 1mg/kg i.p)" exhibited significant differences when compared to the group that received "Alcohol + thymoquinone (20 mg/kg p.o)". Elevated zero maze Time spent in open area on the 7th, 14th, 21st, and 28th days, there were significant differences between the "Vehicle control" group and the "Alcohol treated disease control", "thymoquinone 20 mg/kg p.o" and "Alcohol + Diazepam 1 mg/kg i.p" groups ($p < 0.001$). This implies that the likelihood of these discrepancies occurring due to random chance is less than 0.1%. The combination of Alcohol, thymoquinone (20 mg/kg p.o), and Diazepam (1 mg/kg i.p) showed a significant difference compared to the combination of Alcohol and thymoquinone (20 mg/kg p.o) alone ($p < 0.01$, $p < 0.001$). This indicates that the probability of these discrepancies occurring by random chance is less than 1% or 0.1%, respectively. Number of Open Arm Entries On the 7th day, a significant difference was seen between the group administered with thymoquinone (20 mg/kg p.o) and the control group receiving no treatment. Significant differences were seen between the disease control group treated with Alcohol and the groups treated with alcohol in combination with thymoquinone (40 mg/kg orally) and Diazepam (1 mg/kg i.p.). In every instance, the group that received a combination of Alcohol, thymoquinone (20 mg/kg p.o), and Diazepam (1 mg/kg i.p) exhibited a significant difference when compared to the group treated just with Alcohol and thymoquinone (20 mg/kg p.o). Open field test revealed that the "Vehicle control" group exhibited significant differences compared to the "Alcohol + Diazepam 1mg/kg i.p", "thymoquinone 20 mg/kg p.o", and "Alcohol treated disease control" groups on all four days (7th, 14th, 21st, and 28th). The administration of Alcohol, thymoquinone (20 mg/kg orally), and Diazepam (1 mg/kg i.p resulted in a significant Difference compared to only of Alcohol + thymoquinone (20 mg/kg p.o).

Overall, the concurrent delivery of Alcohol, thymoquinone, and Diazepam resulted in a substantial and discernible effect when compared to other therapies. The *p*-values suggest that these Significant differences are implying that they are improbable to have arisen randomly. A smaller *p*-value indicates a reduced probability that the outcome is a consequence of random chance. $p<0.001$ Marked three symbols, $p<0.01$ marked as two symbols and $p<0.05$ marked as one symbol.

CONCLUSION

This study investigated the potential of thymoquinone (THMQ) in reducing alcohol craving and withdrawal symptoms. The results demonstrate that THMQ significantly decreased alcohol consumption intensity and reduced anxiety-like behaviour in mice undergoing alcohol withdrawal. Notably, THMQ's effects were comparable to those of diazepam, a standard treatment for alcohol withdrawal. Western blot analysis revealed no changes in GluA1 and Sk2 protein levels, suggesting that THMQ's mechanisms may differ from those of traditional treatments. These findings suggest that THMQ may be a promising adjunctive therapy for managing alcohol withdrawal symptoms and cravings.

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CONFLICT OF INTEREST

The authors declare that there is no Conflict of Interest.

ETHICAL APPROVAL

Got the IAEC approval for the conduct of the study at JLS. No:1757/PO/RcBiBt/S/14/CPCSEA), Hyderabad. Jeeva Life Sciences Medchal -Malkajgiri Dist, Hyderabad.

ABBREVIATIONS

ALC: Alcohol; **THMQ:** Thymoquinone; **EPM:** Elevated Plus Maze; **EZM:** Elevated Zero Maze; **OFT:** Open Field Test; **i.p:** Intraperitoneal; **p.o:** Per Oral; **GluA1:** Glutamate Receptor Inonotrpic1; **Sk2:** Ca^{+} K^{+} activated channel; **AMPA:** α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; **DHC:** Dorsal Hippocampus; **VHC:** Ventral Hippocampus; **EDTA:** Ethylenediamine tetra acetic acid; **HEPES:** N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; **SDS:** Sodium dodecyl Sulphate; **V/V:** Volume by volume; **i.g:** Intragastric; **IAEC:** Institutional animal ethics committee; **BAC:** Blood alcohol content.

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