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EVALUATION OF EFFECT OF *ANETHUM GRAVEOLENS (SOWA)* IN ALCOHOL WITHDRAWAL SYNDROME IN RATS

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ABSTRACT

Objective: To evaluate the effect of *Anethum graveolense (Dill sowa)* on: anxiety, analgesic activity and muscles strength in Alcohol Withdrawal Syndrome in rats. **Methods:** Liquid diet with ethanol was administered to the rats for 21 d. The rats in the control group were given sucrose as a liquid diet that provided the same number of calories. After alcohol withdrawal, rats were examined at 6th and 24th hour for major withdrawal signs that included anxiety, analgesic and muscles strength activity. Ethanol withdrawan anxiety was tested using elevated plus maze, light and dark apparatus, analgesic activity tested using tail flick apparatus and muscles strength was tested using rota rod apparatus. *Anethum graveolens* sowa extract (150 and 300 mg/kg, oral) and diazepam (2 mg/kg, i.p) were administered to the treatment group animals 30 min before alcohol withdrawal estimation. Medication was administered 30 minutes prior to the 6 and 24 hr after Alcohol withdrwal. **Results**: Findings from the present study revealed that *Anethum graveolens* sowa extract treatment at doses 150 and 300 mg/kg, oral had a significant protective effect on signs and symptoms of ethanol withdrawal in alcohol-dependent rats. **Conclusions:** *Anethum graveolens* seems to be an active drug for the treatment of alcohol withdrawal syndrome.

KEYWORDS: *Anethum graveolens*, Alcohol withdrawal syndrome, Diazepam, Anxiety, Analgesic, Muscles strength, Elevated plus maze, Light and dark,Tail flick, Rota rod apparatus.

1) INTRODUCTION

Alcoholism and alcohol abuse are serious public health issues that continue to place a heavy burden on the healthcare sector and cause a variety of social issues, such as legal issues (like crime), financial issues (like property damage or loss, decreased productivity at work), and, most tragically, collateral harm to interpersonal and family relationships. Long-term excessive alcohol use can result in dependence, even though many people abuse alcohol without developing a substance dependence.

The development of symptoms linked to the alcohol withdrawal syndrome is a severe effect of dependence. A typical withdrawal symptom occurs when the dependent person's drinking is abruptly stopped or significantly decreased. Increased autonomic nervous system activation, symptoms of central nervous system (CNS) hyperexcitability, and, in its most extreme form, delirium tremens and hallucinations are clinical characteristics of alcohol withdrawal.[1,2] The withdrawal syndrome is characterized by a number of symptoms that contribute to psychological discomfort and bad affect in addition to the physical indicators of withdrawal.[3,4] While many withdrawal symptoms go away in 5–7 days, other symptoms (mostly those associated with mood and emotional disorders) have been known to persist for a long time[5]. The unpleasant affective or emotional aspects of withdrawal, despite their subtler nature, may be important motivators for relapsing into alcohol-seeking behavior. The recurring character of alcoholism is highlighted by the high recidivism rate. Alcohol-dependent people hence frequently go through several withdrawal episodes, with abstinence attempts ultimately failing and people returning to excessive, unhealthy drinking patterns.[6,7]

The development of alcohol dependency is a complicated and dynamic process that eventually reflects a maladaptive neurophysiological state. The development of alcohol dependence is believed to reflect an allostatic state fueled by progressive dysregulation of neurophysiologic systems beyond normal homeostatic limits.[8] Perturbations in a wide range of neurochemical systems produced by the chronic presence of alcohol contribute to significant changes in neural activity (neuroadaptations) that ultimately compromise the brain's functional integrity.[9,10] The formation and expression of many alcohol withdrawal symptoms are, in fact, caused by indications of an allostatic state, which

also contribute to a prolonged sensitivity to relapse. Alcohol's capacity to ease the discomfort of withdrawal-related stress and dysphoria may also be a strong motivator that encourages increasing alcohol use to even greater levels while simultaneously increasing the risk of relapse. This chapter outlines the salient characteristics of alcohol withdrawal syndrome and discusses neuroadaptations in a variety of neurotransmitter and neuromodulator systems in relation to the manifestation of different alcohol withdrawal symptoms and signs, as well as their connection to the serious clinical issue of relapse and uncontrolled, risky drinking.[10]

The consumption of ethanol causes changes in neurobiology and behavior, which are influenced by the N-methyl-D-aspartic acid (NMDA) excitatory and γ-aminobutyric acid (GABA)A inhibitory receptor systems. Dependence on ethanol results in a reduction of the GABAA receptor response and an increase in NMDA receptors, affecting reinforcement for ethanol drinking, drug discrimination, tolerance, dependence, and withdrawal. The GABAergic and NMDA/glutamatergic systems may serve as key targets for treatments aimed at achieving long-term abstinence from alcohol. Currently, there are very few treatments available for ethanol withdrawal syndrome, including benzodiazepines, acamprosate, naltrexone, and disulfiram; however, these medications primarily mitigate cravings for ethanol and do not address withdrawal symptoms. Among all benzodiazepines, this is the sole medication used for managing alcohol withdrawal syndrome. However, it is linked to side effects that resemble the symptoms of alcohol withdrawal, including anxiety, emotional distress, shaking, excessive sweating, headaches, sleep problems, irritability, nausea, weight loss, and muscle pain. There is a need for new medication options and effective treatments for ethanol withdrawal syndrome.[11]

Medicinal plants are safe, effective, and inexpensive resources that are screened by different communities for the isolation of bioactive compounds and new drug inventions. *Anethum graveolens* (dill) grows mainly in the southwest of Asia and southeast of Europe, typically grown in India, and traditionally has been used as a sedative and an appetizer. Various species of dill comprise different kinds of steroids and phytoestrogen, which could have anti-inflammatory and tranquilizing effects. *Anethum graveolens* sowa extract contained flavonoid, essential oil, steroids which shows the properties for the management of anxiety, hyperalgesia and muscles strength. Moreover, various components, such as monoterpenes (carvone, limonene, linalool, etc.) have been isolated from *Anethum graveolens* according to phytochemical screening. In the present report, we studied the anxiolytic effect, analgesic effect and muscles strength properties of dill extract on the behavior of male rats.[12]

2) MATERIALS AND METHOD

2.1 MATERIALS

2.1.1-Animals - Adult male Wistar rats weight: 200-250gm were utilized. Throughout the animal study, the animals were kept in wire mesh-topped polyethylene cages with a husk breeding system that was kept under controlled conditions of light (12 hours of light and 12 hours of darkness), temperature (25±2ºC), and humidity (60±5%). They were also fed a normal pellet diet and were given access to water without restriction.The CPCSEA and IAEC guidelines were followed for the housing and care of the rats. Under the research project number 650/PO/Re/S/2002/2024/CPCSEA/13, dated-8/1/2025 the Institutional Animal Ethics Committee (IAEC) approved the protocol for all animal studies.

2.1.2-Collection and authentication of plant material of *Anethum graveolens* (Dill sowa) extract : *Anethum graveolens* were collected in the month of January from the local area of Yavatmal, Maharashtra, (India). The plant material was identified and authenticated by Dr. Punjabrao Deshmukh Krishi Vidyapeeth Akola Vasantrao Naik College of Agricultural Biotechnology, Yavatmal. (Ref.No.VNCABT/Ytl/Hort/1440/2024 dated-29/10/2024).

2.2 METHODOLOGY

2.2.1- Extraction of dried powder of *Anethum graveolens* sowa with ethanol by maceration process : The *Anethum graveolens* sowa was collected, dried in shade and coarsely powdered plant material was stored in an airtight container and further defatted with petroleum ether. The air dried defatted plant material was extracted using maceration with ethanol for 3 days and, subsequently, the mixture was filtered and concentrated under reduced pressure by a rota evaporator at 40 degree Celsius.

2.2.2-Phytochemical screening.

The phytochemical screening tests of the ethanolic extract of *Anethum graveolens* were performed for the detection of phytoconstituents like alkaloids (Mayer test), carbohydrates (Fehling's test), saponins (Foam test), flavonoids (Alkaline reagent test), proteins (Millons test), glycosides (Modified Borntrager's test), steroids (Salkowski test), terpenoids (Salkowski test), anthraquinones (Borntrager's test), tannins (Braymers test).[13,14]

2.2.3- Experimental Design

- Animal groups

Rats were divided in the five groups, six rates in each group for this study.

Group I (Vehicle Control): Rats received saline solution only.

Group II (Negative Control): Treatment of alcohol in rats for 21 days and then withdrawal.

Group III (AG-150mg/kg): Treatment of alcohol in rats for 21 days and then withdrawal + AG (150mg/kg) of *Anethum graveolens* extract.

Group IV (AG-300mg/kg): Treatment of alcohol in rats for 21 days and then withdrawal + AG (300mg/kg) of *Anethum graveolens* extract.
Group V (Standard): Treatment of alcohol in rats for 21 days and then withdrawal + Diazepam (2mg/kg).

2.2.4- Study design
Rats were allocated into five groups [vehicle control group, negative control group, two treatment groups, and a standard diazepam group], with each group containing six rats (n=6). The rats were kept individually, and ethanol was administered through a modified liquid diet. No additional food or water was provided. A liquid diet was prepared that included 925 ml of cow milk, 25-75 ml of ethanol, 17 g of sucrose, and 5,000 IU of vitamin A. This mixture provides 1000.7 kcal/L.

For the initial 7 days of the study rats received a liquid diet without any ethanol, after that period, a 2.4% ethanol solution was added to the liquid diet for 3 days. Subsequently, the ethanol concentration was raised to 4.8% for the next 4 days, and then increased to 7.2% for a span of 14 days. Each day, a fresh liquid diet was prepared and provided at the same time. Rats in the control group were given an isocaloric liquid diet that used sucrose as a caloric substitute to ethanol. Ethanol was eliminated from the liquid diet after a total of 21 days. A dose of *Anethum graveolens* sowa extract (150 and 300 mg/kg, administered orally), saline, and diazepam (2 mg/kg, injected intraperitoneally) were administered to the rats 30 minutes prior to ethanol withdrawal assessment in their respective group.

Assessment of AWS
Anxiety, analgesic activity and muscles strength were assessed by the treatment given to the animals. After 21 days of AW rats at 6 hours and 24 hrs of anxiety was assessed with an Elevated plus maze (EPZ), Dark and Light apparatus, analgesic activity was assessed with an Tail flick apparatus and muscles strength was assessed with an Rota rod apparatus.[15]

2.2.5-Assessment of Alcohol Withdrawal Syndrome in rats
All rats in each group were assessed for the Alcohol Withdrawal Syndrome by using following apparatus.
1) Elevated plus maze apparatus
2) Light and dark box apparatus
3) Tail flick apparatus
4) Rota rod apparatus

1) Elevated plus maze apparatus
The elevated plus maze apparatus comprises two open arms and two closed arms positioned opposite each other, along with a central platform elevated to a height of 50 cm. Each animal was placed alone on the central platform, facing the open arm. An observer recorded the number of entries into the open arms, the total time spent there, as well as the number of entries into the closed arms and the total time spent in them, all within a 5-minute time frame. These observations were conducted under low red lighting.[11]

2) Light and dark box apparatus
The model features two sections, one illuminated and one shaded, housed within a rectangular enclosure connected by an opening measuring 7.5 cm by 7.5 cm in the dividing wall. Each animal was individually positioned in the center of the light section, facing the passage leading to the dark section. The observer documented the number of entries into both the light and dark sections, as well as the duration spent in each area, over a period of 5 minutes.[11]

3) Tail flick apparatus
The rat under test is held in a cylindrical holder of perforated zinc, clamped horizontally; its tail lies along the channel and over the hole, which must be not more than $1^{1/2}$in. from its tip. The circuit is closed when the rat has stopped moving in this posture. After an interval the rat withdraw its tail from the channel with a rapid and unique flick. This period of time, known as the reaction time, is measured using a stopwatch.
Rats weighing between 200-250 g. with clean and healthy tail were chosen for experiment.[16]

4) Rota rod apparatus
A horizontally oriented, revolving cylinder (rod) positioned above a cage floor—low enough to prevent injury but high enough to encourage fall avoidance—is used to test a mouse. In order to prevent falling to the ground, rodents instinctively attempt to remain on the revolving cylinder, or rota rod. The amount of time an animal spends on this revolving rod is a gauge of its motor planning, balance, coordination, and physical health. The rota rod's mechanically driven speed can be increased or maintained at a steady level.

Rats that could exercise on the rota rod and be trained to increase their speed were chosen. We conducted test trials with trained rats that revolved at 25 rpm and stopped at 60 minutes, timing how long they could stay on the rod. Because the rota rod methodology incorporates training, selection, and a 60-minute trial, it provides a sensitive and accurate method of assessing motor performance.[17]

Statistical Analysis
All data were expressed as the mean ± standard deviation (SD). For statistical analysis, group means were compared using one-way analysis of (ANOVA) followed by Dunnett test. A *p*-value of and it 0.01 was considered statistically significant.

3) RESULTS
3.1: Phytochemical Screening of the plant extract
Phytochemical detection tests of the *Anethum graveolens* sowa extract revealed the presence of chemical constituents like alkaloids, carbohydrates, flavonoids, saponins, tannins, proteins, steroids.

3.2: Elevated Plus Maze Test Results

Table No.3.2.1: Evaluation of Effect of *Anethum Graveolens* extract using Elevated Plus Maze Test (Close arm Entries).

Group	Number of entries (%)(in Close arm) on Day 0	Number of entries (%) (in Close arm) on Day 21 after 6 hr	Number of entries (%) (in Close arm) on Day 21 after 24hr
Control	62.68 ± 2.35	63.08 ± 3.22	63.16 ± 2.30
Negative control	62.93 ± 1.51^{ns}	78.49 ± 3.63$^{@@}$	80.79 ± 7.03$^{@@}$
AG (150 mg/kg)	64.02± 3.46^{ns}	60.39 ± 2.97**	59.30 ± 4.72**
AG (300 mg/kg)	63.63 ± 5.74^{ns}	58.02 ± 4.60**	56.28 ± 3.82**
Diazepam (2mg/kg)	64.84 ± 4.69^{ns}	52.46 ± 4.87**	51.09 ± 2.34**

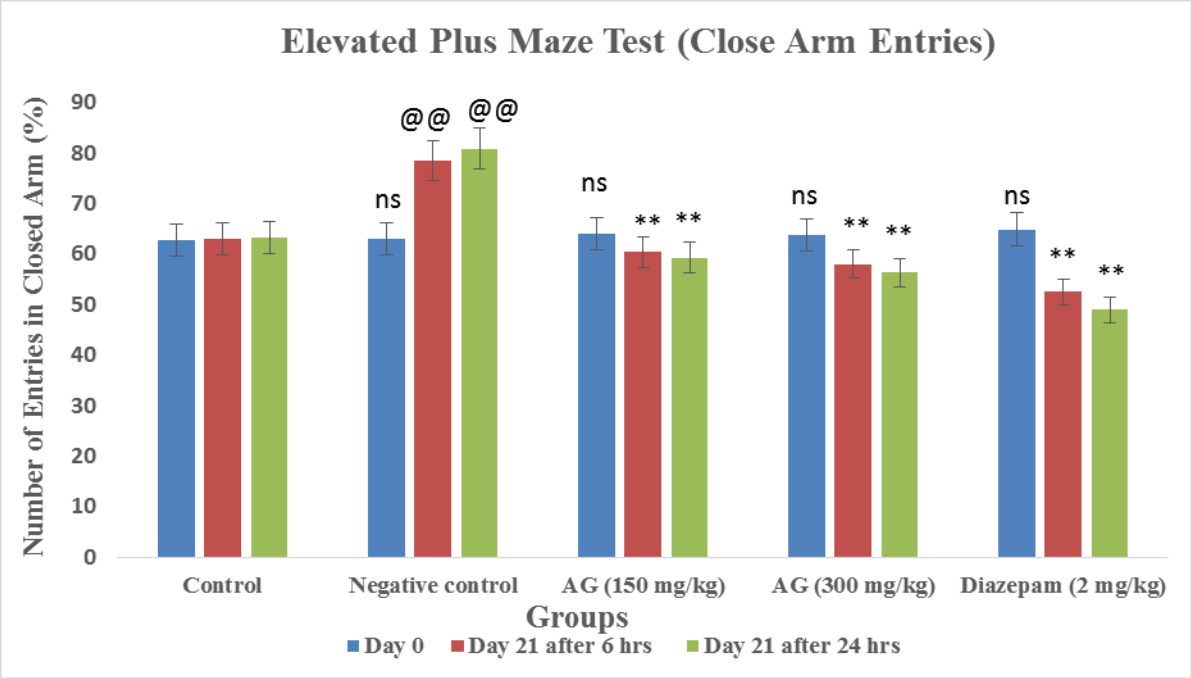

Figure 3.2.1:-Number of entries in Close arm.

All Values are expressed in Mean±SD (n=6): ns (not significant) $p>0.05$, $^{@@}p<0.01$ when compared to normal control group.

ns (not significant) $p>0.05$, $^{**}p<0.01$ when compared to negative control group.

Table 3.2.1 and Figure 3.2.1 shows that, there was a significant ($p<0.01$) increase in the close arm entries of negative control group as compare to normal control group. There was significant ($p<0.01$) decrease in the close arm entries in AG (150mg/kg), AG (300mg/kg), Diazepam (2mg/kg) group as compare to negative control group**.**

Table No.3.2.2: Evaluation of Effect of *Anethum Graveolens* extract using Elevated Plus Maze Test (Open arm Entries).

Group	Number of entries (%) (in Open arm) on Day 0	Number of entries % (in Open arm) on Day 21 after 6hr	Number of entries (%) (in Open arm) on Day 21 after 24hr
Control	37.32 ± 3.54	36.92 ± 2.34	36.84 ± 2.15
Negative control	37.07 ± 2 ^{ns}	21.51±1.61$^{@@}$	19.21 ± 1.40$^{@@}$
AG (150 mg/kg)	35.98 ± 1.96 ^{ns}	39.61± 3.22**	40.70 ± 2.66**
AG (300 mg/kg)	36.37 ± 1.58 ^{ns}	41.98 ± 2.10**	43.72 ± 3.47**
Diazepam(2 mg/kg)	35.16 ± 3.47 ^{ns}	47.54 ± 2.52**	48.91 ± 2.34**

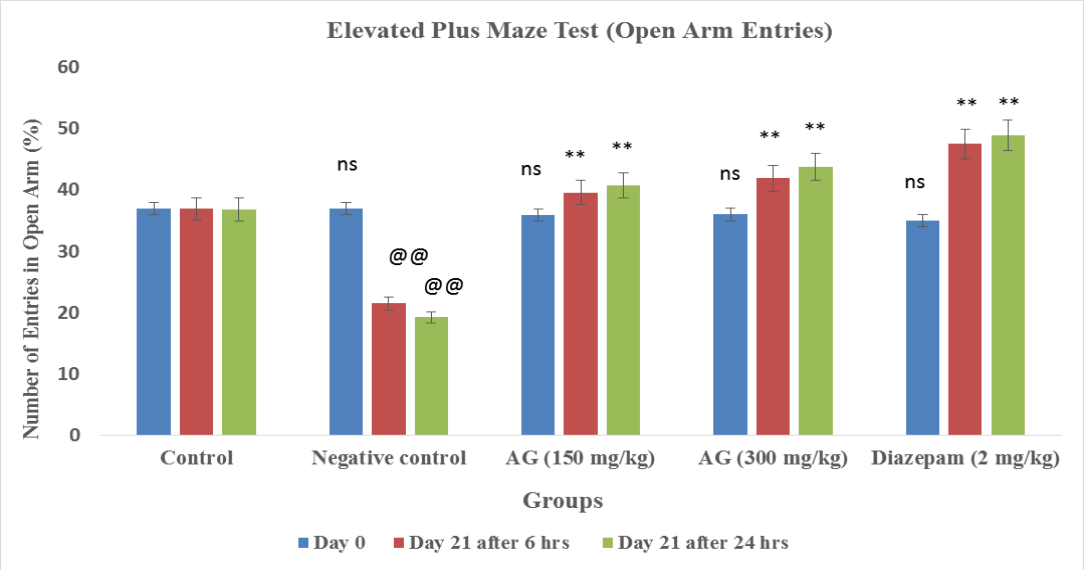

Figure 3.2.2:-Number of entries in open arm.

All Values are expressed in Mean±SD (n=6).
ns (not significant) $p>0.05$, $^{@@}p<0.01$ when compared to normal control group.
ns (not significant) $p>0.05$, $^{**}p<0.01$ when compared to negative control group.
Table 3.2.2 and Figure 3.2.2 shows that, there was a significant ($p<0.01$) decrease in the open arm entries of negative control group as compare to normal control group. There was significant ($p<0.01$) increase in the open arm entries in AG (150mg/kg), AG (300mg/kg), Diazepam (2mg/kg) group as compare to negative control group.

Table No.3.2.3: Evaluation of Effect of *Anethum Graveolens* extract using Elevated Plus Maze Test (Time spent in close arm).

Group	Time Spent (in Sec in Close arm) on Day 0	Time spent (in Sec in Close arm) on Day 21 after 6hr	Time spent (in Sec in Close arm) on Day 21 after 24hr
Control	94.90 ± 2.4	96.11 ± 6.7	95 ± 2.2
Negative control	94.80 ± 2.02^{ns}	123.33 ± 2.3$^{@@}$	136.67 ± 4.5$^{@@}$
AG (150 mg/kg)	95.30 ± 2.6^{ns}	92. ± 4.8**	89.2 ± 1.1**
AG (300 mg/kg)	94.30 ± 2.1^{ns}	88.33 ± 8.6**	86.1 ± 8.5**
Diazepam (2 mg/kg)	93.70± 2.3^{ns}	87.10 ± 5**	84 ± 1.9**

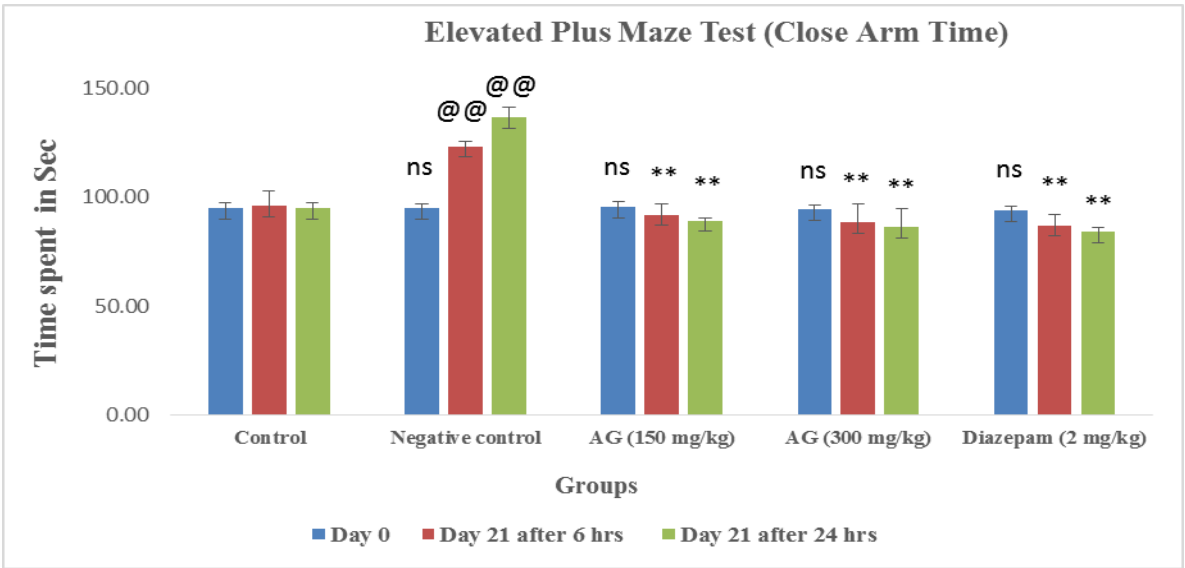

Figure 3.2.3:- Time spent in Close arm.

All Values are expressed in Mean±SD (n=6):
ns (not significant) $p>0.05$, $^{@@}p<0.01$ when compared to normal control group.
ns (not significant) $p>0.05$, $^{**}p<0.01$ when compared to negative control group.
Table 3.2.3 and Figure 3.2.3 shows that, there was a significant ($p<0.01$) increase in the time spent in close arm of negative control as compare to normal control. There was significant ($p<0.01$) decrease in the time spent in close arm in AG (150mg/kg), AG (300mg/kg), Diazepam (2mg/kg) group as compare to negative control group.

Table No.3.2.4: Evaluation of Effect of *Anethum Graveolens* extract using Elevated Plus Maze Test (Time spent in open arm).

Group	Time Spent (in Sec in Open arm) on Day 0	Time spent (in Sec Open arm) on Day 21 after 6hr	Time spent (in Sec in Open arm) on Day 21 after 24hr
Control	82.70 ± 2.77	83.00 ± 4.13	81.1 ± 2.5
Negative control	83.20 ± 3.2^{ns}	71.80 ± 4.2$^{@@}$	69.48 ± 4.7$^{@@}$
AG (150 mg/kg)	82.10 ± 4.1^{ns}	82.70 ± 3.1**	84.3 ± 5.7**
AG (300 mg/kg)	83.30 ± 2^{ns}	86.27 ± 2.17**	88.4 ± 4.4**
Diazepam (2 mg/kg)	83.20 ± 3.1^{ns}	89.10 ± 4**	93.00 ± 7.1**

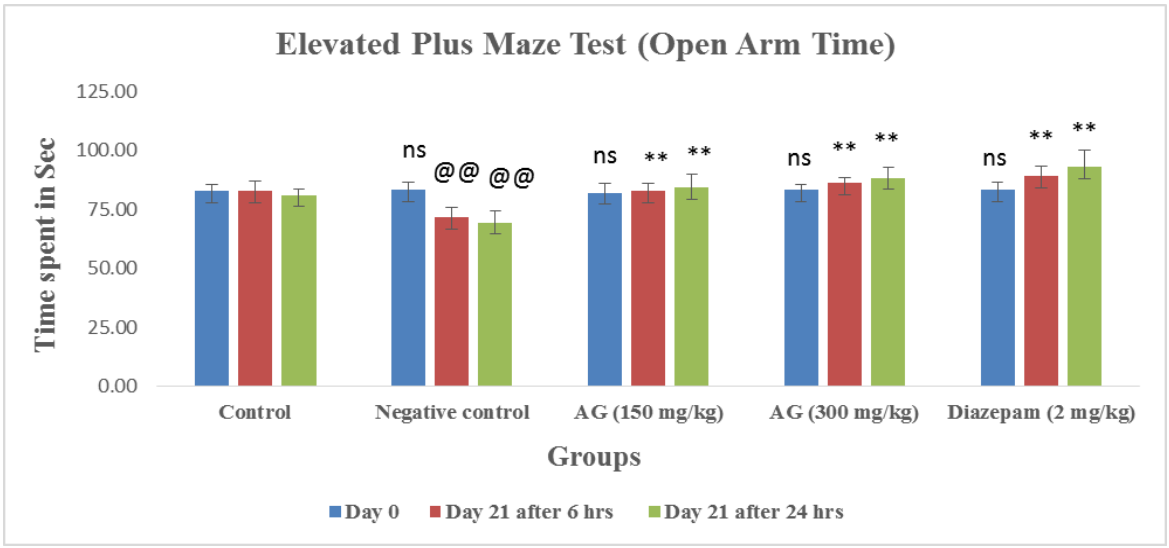

Figure 3.2.4:-Time spent in Open arm.

All Values are expressed in Mean±SD (n=6):
ns (not significant) $p>0.05$, $^{@@}p<0.01$ when compared to normal control group.
ns (not significant) $p>0.05$, $^{**}p<0.01$ when compared to negative control group.
Table 3.2.4 and Figure 3.2.4 shows that, there was a significant ($p<0.01$) decrease in the time spent in open arm of negative control as compare to normal control. There was significant ($p<0.01$) increase in the time spent in open arm in AG (150mg/kg), AG (300mg/kg), Diazepam (2mg/kg) group as compare to negative control group.

3.3: Evaluation of Effect of *Anethum Graveolens* extract using Light and Dark Box Apparatus Results.
Table No. 3.3.1: Effect of *Anethum Graveolens* extract using Light and Dark box test in (Dark box Entries).

Group	Number of entries (%) (in Dark box) on Day 0	Number of entries (%) (in Dark box) on Day 21 after 6hr	Number of entries (%) (in Dark box) on Day 21 after 24hr
Control	56.66 ± 3.89	59.98 ± 4.05	58.1 ± 6.26
Negative control	56.32 ± 2.59^{ns}	72.36 ± 3.70$^{@@}$	83.6 ± 4.04$^{@@}$
AG (150 mg/kg)	57.99 ± 2.96 ^{ns}	57.00 ± 2.90**	56 ± 2.61**
AG (300 mg/kg)	58.94 ± 4.01 ^{ns}	56.32 ± 2.45**	51.04 ± 5.19 **
Diazepam (2 mg/kg)	57.56 ± 3.85 ^{ns}	50.62 ± 7.31 **	45.45± 1.36**

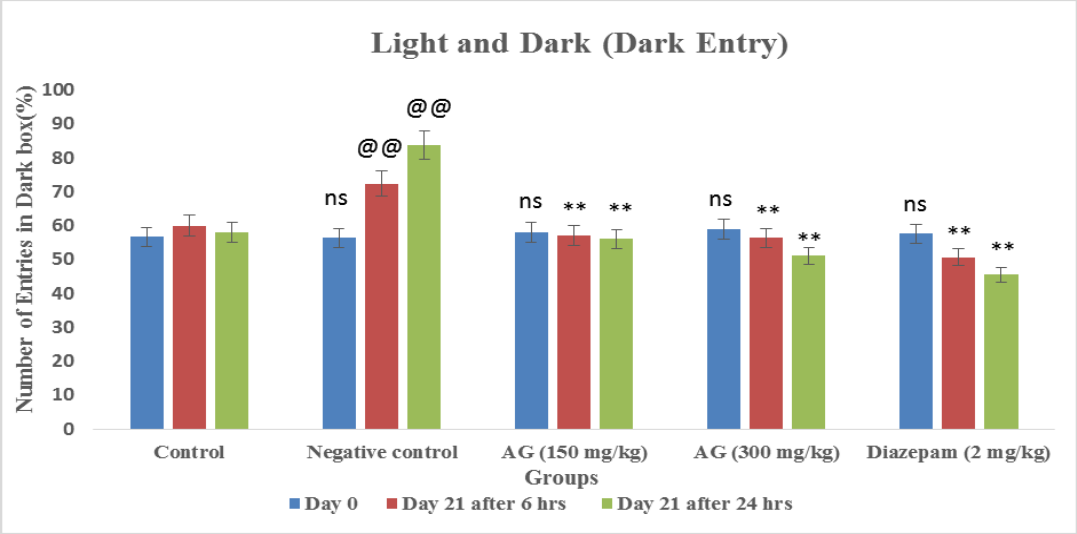

Figure 3.3.1:- Light and Dark box test (Dark box entries).

All Values are expressed in Mean±SD (n=6):
ns (not significant) $p>0.05$, $^{@@}p<0.01$ when compared to normal control group.
ns (not significant) $p>0.05$, $^{**}p<0.01$ when compared to negative control group.
Table 3.3.1 and Figure 3.3.1 shows that, there was a significant ($p<0.01$) increase in the dark box entries of negative control group as compare to normal control group. There was a significant ($p<0.01$) decrease in the dark box entries in AG (150mg/kg), AG (300mg/kg), Diazepam (2mg/kg) group as compare to negative control group.

Table No. 3.3.2: Effect of *Anethum Graveolens* extract using Light and Dark box test in (Light box Entries).

Group	Number of entries (%) (in Light box) on Day 0	Number of entries (%) (in Light box) on Day 21 after 6hr	Number of entries (%) (in Light box) on Day 21 after 24hr
Control	15.95 ± 0.59	16.68 ± 1.44	16.45 ± 1.17
Negative control	16.02 ± 1.92 ^{ns}	10.07 ± 0.51$^{@@}$	8.35 ± 0.72$^{@@}$
AG (150 mg/kg)	16.01 ± 1.06^{ns}	16.08 ± 0.46**	17 ± 1.49**
AG (300 mg/kg)	15.02 ± 1.03^{ns}	17.05 ± 0.79**	18.02 ± 0.66**
Diazepam (2mg/kg)	15.07 ± 1.16^{ns}	17.07 ± 1**	18.5 ± 1.17**

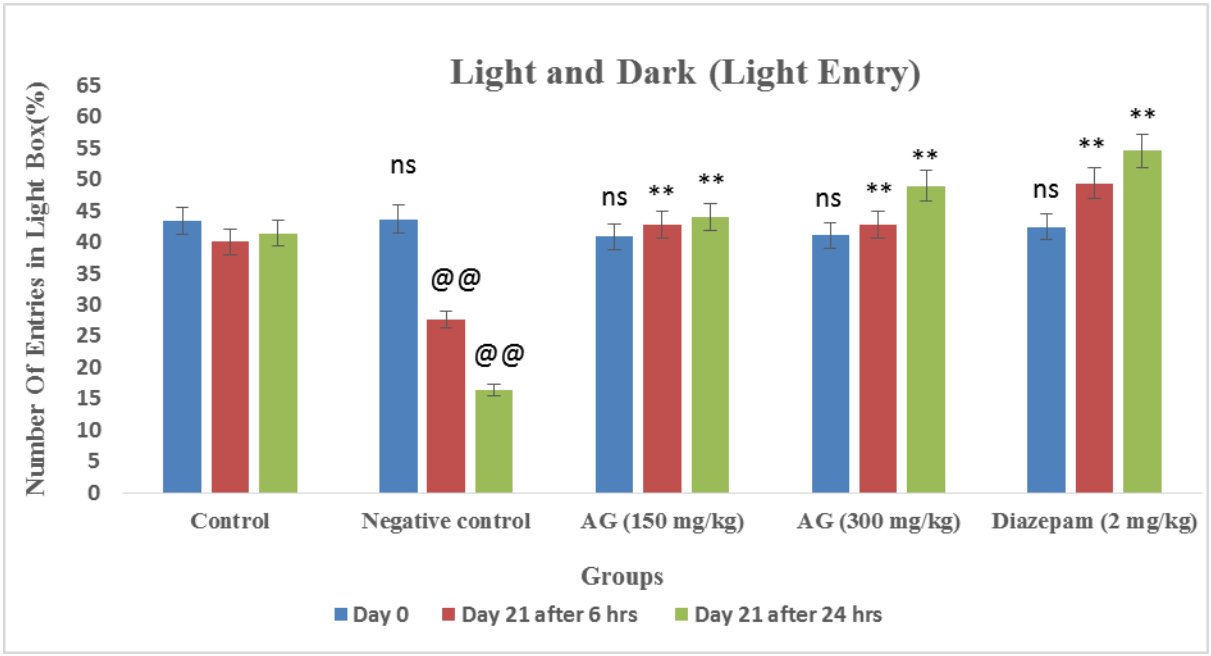

Figure 3.3.2:- Light and Dark box test (Light box entries).

All Values are expressed in Mean±SD (n=6):
ns (not significant) $p>0.05$, $^{@@}p<0.01$ when compared to normal control group.
ns (not significant) $p>0.05$, $^{**}p<0.01$ when compared to negative control group.
Table 3.3.2 and Figure 3.3.2 shows that, there was a significant ($p<0.01$) decrease in the light box entries of negative control group as compare to normal control group. There was a significant ($p<0.01$) increase in the light box entries in AG (150mg/kg), AG (300mg/kg), Diazepam (2mg/kg) group as compare to negative control group.

Table No 3.3.3: Effect of *Anethum Graveolens* extract using Light and Dark box test (Time spent in Dark box).

Group	Time Spent (in Sec in Dark box) on Day 0	Time spent (in Sec in Dark box) on Day 21 after 6hr	Time spent (in Sec in Dark box) on Day 21 after 24hr
Control	42.66± 1.13	39.24 ± 2.03	41.01 ± 2.04
Negative control	43.09± 4.02^{ns}	51.86 ± 2.04$^{@@}$	54.63± 3.02$^{@@}$
AG (150 mg/kg)	41.22 ± 2.09^{ns}	39.4 ± 3.00**	36.49 ± 3.00**
AG (300 mg/kg)	42.08± 3.06^{ns}	35.94 ± 1.05**	32.61 ± 2.08**
Diazepam (2 mg/kg)	40.22± 3.13^{ns}	36.38 ± 1.05**	31.18 ± 1.09**

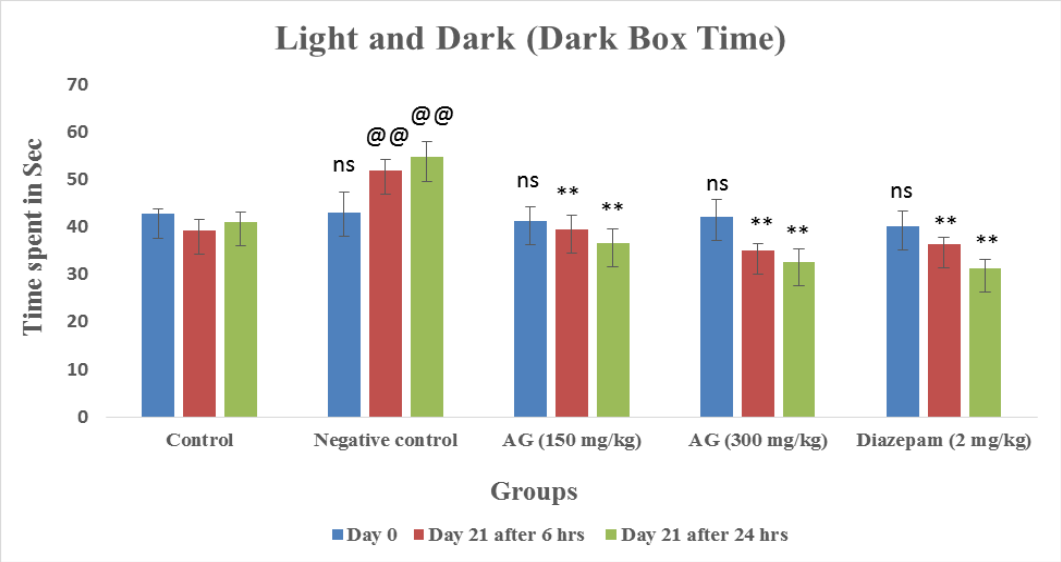

Figure 3.3.3:-Time spent in Dark box.

All Values are expressed in Mean±SD (n=6):
ns (not significant) $p>0.05$, $^{@@}p<0.01$ when compared to normal control group.
ns (not significant) $p>0.05$, $^{**}p<0.01$ when compared to negative control group.
Table 3.3.3 and Figure 3.3.3 shows that, there was a significant ($p<0.01$) increase in the time spent in dark box of negative control group as compare to normal control group. There was a significant ($p<0.01$) decrease in the time spent in the dark box in AG (150mg/kg), AG (300mg/kg), Diazepam (2mg/kg) group as compare to negative control group.

Table No. 3.3.4 Effect of *Anethum Graveolens* extract using Light and Dark box test (Time spent in Light box)

Group	Time Spent in (Sec in Light box) on Day 0	Response (in Sec in Light box) on Day 21 after 6hr	Response (in Sec in Light box) on Day 21 after 24hr
Control	26.68 ± 1.09	27.42 ± 1.9	28.55 ± 1.2
Negative control	27.17± 2.08^{ns}	21.83± 1.6$^{@@}$	17.52± 0.55$^{@@}$
AG (150 mg/kg)	28.54 ± 1.05^{ns}	29.93 ± 1.6**	30.06 ± 1.4**
AG (300 mg/kg)	28.45 ± 1^{ns}	31.87 ± 2.5**	33.33 ± 2.22**
Diazepam (2 mg/kg)	27.27 ± 1.06^{ns}	33.74 ± 2.5**	39.11 ± 2**

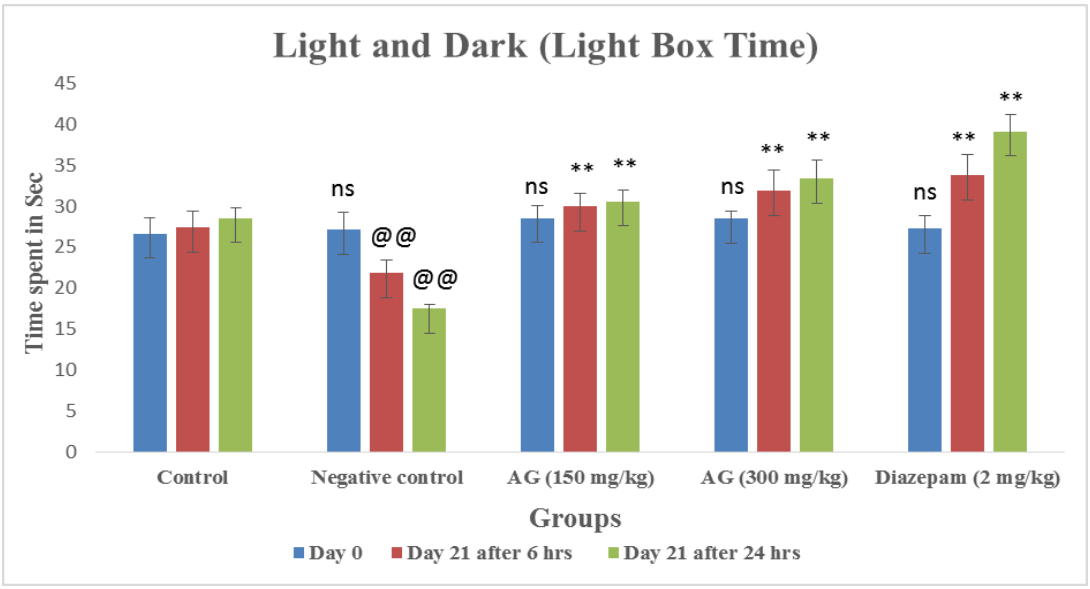

Figure 3.3.4:-Time spent in Light box.

All Values are expressed in Mean±SD (n=6):
ns (not significant) $p>0.05$, $^{@@}p<0.01$ when compared to normal control group.
ns (not significant) $p>0.05$, $^{**}p<0.01$ when compared to negative control group.
Table 3.3.4 and Figure 3.3.4 shows that, there was a significant ($p<0.01$) decrease in the time spent in light box of negative control group as compare to normal control group. There was a significant ($p<0.01$) increase in the time spent in the light box in AG (150mg/kg), AG (300mg/kg), Diazepam (2mg/kg) group as compare to negative control group.

3.4: Tail flick Response Result

Table No.3.4.: Evaluation of Effect of *Anethum Graveolens* extract using Tail-flick Apparatus.

Group	Tail- Flick Response (in Sec) on Day 0	Tail- Flick Response (in Sec) on Day 21 After 6hrs	Tail- Flick Response (in Sec) on Day 24 After 6hrs
Control	5.5± 0.19	5.8± 0.4	5.6 ± 0.36
Negative control	5.3± 0.43^{ns}	3.6± 0.16$^{@@}$	3 ± 0.25$^{@@}$
AG (150 mg/kg)	5.4± 0.36 ^{ns}	5.5 ± 0.19**	5.6 ± 0.33**
AG (300 mg/kg)	5.3± 0.39 ^{ns}	5.6 ± 0.29**	5.7 ± 0.35**
Diazepam (2mg/kg)	5.6 ± 0.32 ^{ns}	5.8± 0.44**	6 ± 0.46**

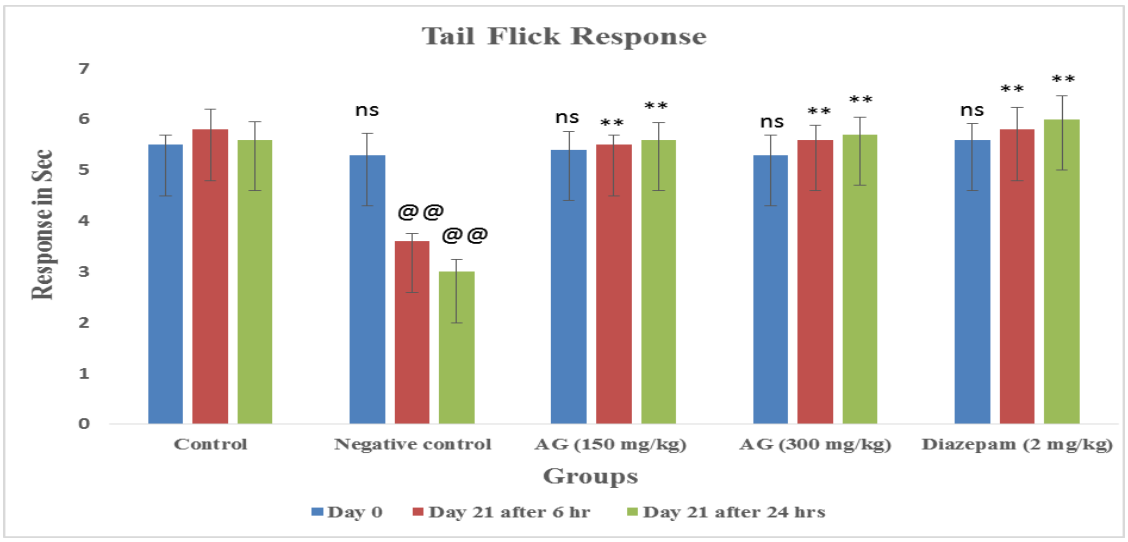

Figure 3.4:- Tail Flick Response.

All Values are expressed in Mean±SD (n=6)
ns (not significant) $p>0.05$, $^{@@}p<0.01$ when compared to normal control group.
ns (not significant) $p>0.05$, $^{**}p<0.01$ when compared to negative control group.
Table 3.4 and Figure 3.4 shows that, there was a significant ($p<0.01$) decrease the in the analgesic response in tail flick test of negative control group as compare to normal control group. This decrease in analgesic effect confirm the hyperalgesia in rats.There was a significant ($p<0.01$) increase in the analgesic response in tail flick test in AG (150mg/kg), AG (300mg/kg), Diazepam (2mg/kg) group as compare to negative control group.

3.5: Rota Rod Response Result

Table No.3.5: Evaluation of Effect of *Anethum Graveolens* extract using Rota-Rod Apparatus.

Group	Fall off time (in Sec) on Day 0	Fall off time (in Sec) on Day 21 after 6hr	Fall off time (in Sec) on Day 21 after 24hr
Control	61.3 ± 4.4	61.2± 2.9	60.03 ± 4.1
Negative control	62.5 ± 2.3 ^{ns}	33.9± 2.8$^{@@}$	33.02 ± 2.3$^{@@}$
AG (150 mg/kg)	60.1 ± 4.4 ^{ns}	61.3 ± 1.8**	62.7 ± 2.2**
AG (300 mg/kg)	61.4± 5.7 ^{ns}	65.1 ± 4.1**	66.2 ± 6**
Diazepam(2mg/kg)	62.7 ± 2.5 ^{ns}	70.2 ± 2.8**	71.2 ± 7**

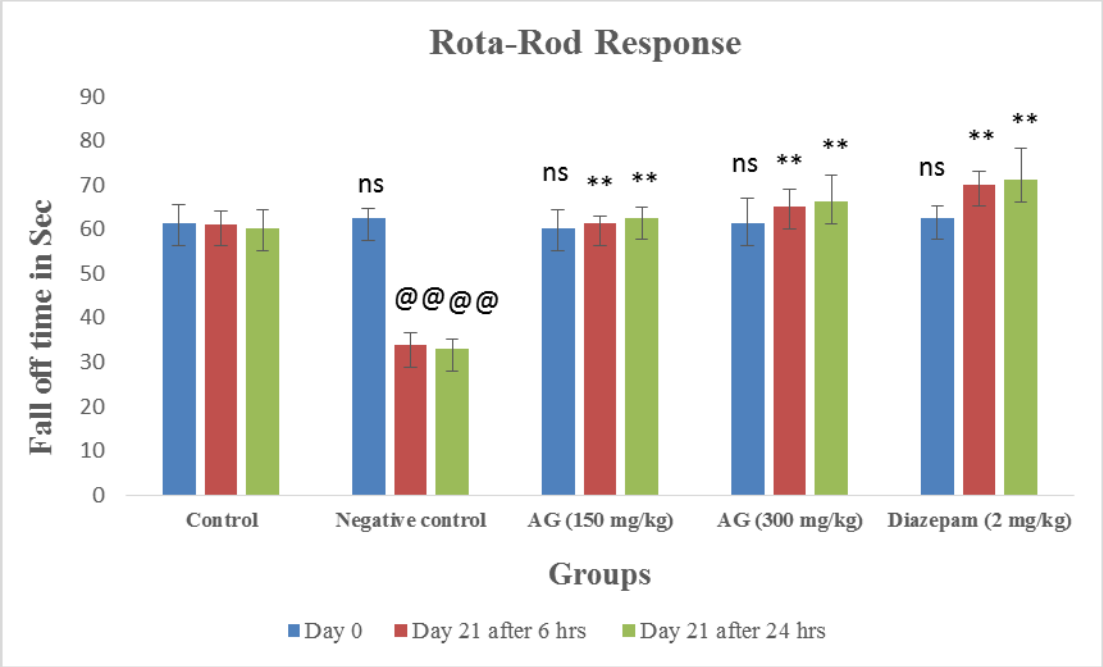

Figure 3.5:- Rota Rod Response.

All Values are expressed in Mean±SD (n=6)
ns (not significant) $p>0.05$, $^{@@}p<0.01$ when compared to normal control group.
ns (not significant) $p>0.05$, $^{**}p<0.01$ when compared to negative control group.
Table 3.5 and Figure 3.5 shows that, there was a significant ($p<0.01$) decrease in the muscular activity in rota rod test of negative control group as compare to normal control group. There was a significant ($p<0.01$) increase in the muscular activity in rota rod test in AG (150mg/kg), AG (300mg/kg), Diazepam (2mg/kg) group as compare to negative control group.

4) DISCUSSION

The findings from this study revealed the inhibitory effects of the *Anethum garveolens* sowa extract on ethanol withdrawal symptoms in alcohol-dependent rats. Ethanol administration with liquid diet is a relevant and well-established model for ethanol consumption in rats[18,19]. In rats, daily ingestion of more than 9 g/kg of ethanol for 15 consecutive days causes dependence, and cessation results in a variety of symptoms.[20]

In this study, it was observed major signs of alcohol withdrawal such as withdrawal anxiety, analgesic and weak muscles strength. The incidence of alcohol withdrawal anxiety observed in the alcohol-fed rats at 21 days after 6th hour and 24th hour of abstinence.[22-24] The present study demonstrates the significant effect of the *Anethum graveolens* in reducing anxiety at 150mg/kg and 300 mg/kg which is similar to some of the previous findings.[25,26] The earlier studies reported the anti-anxiety effects of the *Anethum graveolens* extract in normal rats while the current study revealed the anti-anxiety effects of *Anethum graveolens* in alcohol abstinence animals for the first time. *Anethum*

graveolens extract at 150 mg/kg and 300 mg/kg showed a significant anti-anxiety effect by using Elevated plus maze apparatus and Light and Dark apparatus in ethanol-withdrawn rats as compared to negative group. Alcohol withdrawal induced hyperalgesia upon thermal stimulation in rats by using tail flick apparatus.[27] A significant hyperalgesia was seen in the alcohol-dependent rats at 21 days after the 6th and 24th hour of the alcohol abstinence when compared to the normal group rats. *Anethum graveolens* extract at 150 and 300 mg/kg showed a significant analgesic effect in ethanol-withdrawn rats as compared to negative group. Diazepam (2 mg/kg) was used as a standard drug. When comparing the alcohol-dependent rats to the normal group rats at 21 days following the 6th and 24th hours of alcohol abstinence, weak muscle strength was seen.[28,29] *Anethum graveolens* extract at 150mg/kg and 300 mg/kg significantly normalized the muscles strength in the rota road apparatus test in the rats as compared to negative group.

NMDA receptors and the glutamatergic system are believed to have a critical role in the progress of the alcohol withdrawal signs.[30] The phytochemical study revealed that the flavonoids, tannins, steroids, and alkaloids were among the components found in ethanolic extract of *Anethum graveolens*. *Anethum graveolens* sowa extract contained flavonoids, and essential oil which shows the properties for the management of anxiety, hyperalgesia and weak muscles strength.[31,32] Hence, such phytoconstituents may have a protective role in the ethanol abstinence syndrome and these constituents may ameliorate the ethanol deprivation effects as *Anethum graveolens* is proved to have inhibitory effects on the reuptake of neurotransmitters such as noradrenaline, serotonin, dopamine and modifies neuronal excitability via GABAergic and glutamatergic.

5) CONCLUSION

Anethum graveolens sowa extract seems to be pharmacologically active by suppressing ethanol withdrawal signs and symptoms and may have therapeutic potential in treating ethanol dependence.

Thus, the present findings indicates that the ethanolic extract of *Anethum graveolens* sowa shows anxiolytic activity, analgesic activity and improve muscles strength at (150mg/kg) and (300mg/kg).

Thus, *Anethum graveolens* sowa is a promising herbal option in the pharmaceutical world for the management of of Alcohol Withdrawal Syndrome such as anxiety, hyperalgesia and to improve muscles strength.

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