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# EVALUATION OF ANTI ANXIETY ACTIVITY OF ETHANOLIC EXTRACT OF *Allium porrum* IN EXPERIMENTAL MICE

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***Abstract:*** This study assessed the anxiolytic effects of an ethanolic extract of *Allium porrum* leaves (APLE) in mice. Using two standard anxiety tests, the elevated plus-maze (EPM) and the light-dark box, researchers administered APLE at doses of 200mg/Kg and 400mg/Kg. Mice given APLE displayed increased time spent and entries in the open arms of the EPM and spent more time in the illuminated side of the light-dark box, indicating reduced anxiety behaviors. These findings suggest that APLE may have significant anti-anxiety properties. The extract's potential efficacy is attributed to its flavonoid, alkaloid, and mineral content. Overall, the study concluded that APLE could be a natural and effective anxiolytic agent based on its positive results in animal models.

***Keywords - Allium Porum*, Anxiety, Elevated plus maze, Light and dark transition model.**

## I. INTRODUCTION

Anxiety is a natural response to stress that becomes problematic when excessive or triggered by imagined threats. [1,2] People with anxiety face higher healthcare costs, and globally, mental health disorders represent a growing burden, with many lacking accesses to treatment.[3,4] Current treatments include therapy, medication, and behavioral strategies, but side effects from drugs like benzodiazepines have led to the exploration of safer alternatives. Research on the 5-HT1A receptor has driven interest in drugs like azapirones, which reduce anxiety without severe side effects. [5,6] Additionally, traditional medicines, such as Ayurveda, offer potential in developing herbal anxiolytics, like *Allium porrum* (leek), although its efficacy remains unproven. [7,8] The need for new treatments with fewer side effects and improved patient compliance persists, especially in exploring plant-based compounds.[9,10,11]

## II. RESEARCH METHODOLOGY

### 2.1 Collection and Authentication of The Leaf

The leaves of *Allium porrum* were collected from Mangalore, Karnataka. The taxonomy was authenticated by Dr. Siddaraju M N (Assistant professor and Research guide, Department of Botany, University College Mangalore).

### 2.2 Preparation of Methanolic Extract of *Allium porrum*

*Allium porrum* leaves were cleaned, shade-dried, and ground into powder, then stored in a freezer. For extraction, 60g of powder was processed with 80% ethanol in a Soxhlet extractor at 37°C for two days. The extract was filtered, dried under vacuum at 40°C, and stored at 4°C until needed for experiments. [12,13]

**2.3 Dose Fixation**[12]
Dose of 200mg /kg and 400mg/kg body weight was chosen as per previous works.

**2.4 Experimental Animals**
Healthy Swizz albino mice (18 to 25gms) of both sexes were sourced from the animal house of Srinivas college of pharmacy, Mangalore, for the experiment. The mice were maintained under controlled conditions, with a temperature of 22 ± 2°C, relative humidity of 60 ± 5%, and a 12-hour light/ dark cycle. They were provided with unrestricted access to standard pellet diet and water. The animal was housed in sanitized polypropylene cages with sterile paddy husk bedding. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (Approval no.**SCP/IAEC/F150/P215/2023**), in compliance with guidelines from the Committee for Control and Supervision of Experiments on Animals (CCSEA). All procedures adhered to the "Guide for the Care and use of Laboratory Animals" by the National Academy of sciences, as published by the National Institute of Health. The mice were acclimatized for at least one week prior to the experiment.

**2.5 Experimental Methods**

**2.5.1 Elevated plus maze**[14]

**Introduction:**
This rodent model of anxiety has been widely utilized to assess novel anxiolytic agents and explore the psychological and neurochemical mechanisms underlying anxiety. It has been designed for the selective identification of both anxiety – reducing and anxiety-inducing drugs. Anxiolytic compounds, by lowering anxiety, lead to increased exploration of the open arms, while anxiogenic compounds produce the opposite effect.
The key indicators are the ratio of entries into the open arms and the duration spent in the open arms, expressed are a percentage of the total time spent in both open and closed arms.

**Design Of Instrument**
The plus maze features two open arms measuring 30 x 5 cm and two enclosed arms measuring 30 x 5 x 20 cm, all extending from a central platform of 5 x 5 cm. The maze is dark brown, mounted on a wooden base, and elevated 30 cm above the floor in a dimly lit room. A 40W light is positioned above the maze for illumination.

**Treatment:**
The Swiss albino mice (18- 25gms) of either sex was selected. The mice were divided into following groups (n=6) as follows:
Group I: Received 1ml control (p.o.)
Group II: Received 1 mg/Kg Diazepam (i.p.)
Group III: Received 200mg/Kg of *Allium porrum* leaves extract (p.o.)
Group IV: Received 400mg/Kg of *Allium porrum* leaves extract (p.o.)
The treatment was given through oral route. All animals were pretreated for 20 days except diazepam treated animals. On 21st day, animals were treated, 30 min before the evaluation.

**Procedure**
Prior to starting the experiment, the mice were handled daily to reduce stress. Two hours after the oral administration of the test drugs and 30 min after the intraperitoneal administration of diazepam, the animal was placed in the center of the maze, facing one of the enclosed arms. Thereafter, the number of entries and time spent in the open and closed arms were recorded during the next 5 min. An arm entry being defined when all four paws are in the arm.
Following parameters measured

1. Number of open and closed arm entries.
2. Percentage time spent in open and closed arm.

At the end of each trial the apparatus was wiped clean in order to eliminate any olfactory clues, which might modify the behavior of next animal.
The procedure was conducted preferably in a sound attenuated room, with observations made from an adjacent room via web camera (CyberPix S-300) attached to the computer system.

**Evaluation**
In this model, the change in the latency to move from the open arm to the closed arm of the Elevated Plus Maze serves as an indicator of anti-anxiety activity. The treatment was given through oral route. All animals were pretreated for 20 days except diazepam treated animals. On 21st day, animals were treated, 30 min before the evaluation.
APLE at 200 and 400 mg/Kg made the animals spend way more time in and go into the open arms, while they spent less time and went into the closed arms. This shows it really has some anxiety-relieving effects.

**2.5.2 Light and Dark model**[15]

**Principle**
The light-dark box model is a straightforward behavioral test in mice used to identify compounds with anxiolytic properties. Mice naturally explore new environments but will avoid the aversive nature of a brightly lit open area in favor of the dark, enclosed section.

After treatment with anxiolytic agents, mice exhibit increased movement between the two chambers, along with heightened overall locomotor activity. The number of transitions between the light and dark compartments is measured as a key indicator.

**Treatment**

The Swiss albino mice (18- 25gms) of either sex was selected. the mice were divided into following groups (n=6) as follows:
Group I: Received 1ml Control (p.o.)
Group II: Received 1 mg/Kg Diazepam (i.p).
Group III: Received 200mg/Kg of *Allium porrum* leaves extract (p.o.)
Group IV: Received 400mg/Kg of *Allium porrum* leaves extract (p.o.)
The treatment was given through oral route. All animals were pretreated for 20 days except diazepam treated animals. On 21st day, animals were treated, 30 min before the evaluation.

**Procedure**

The testing apparatus consisted of a light and dark chamber separated by a photocell-equipped zone. A polypropylene cage measuring 44 x 21 x 21 cm had a dark section with black spray covering one-third of the cage, while the remaining two-thirds remained brightly lit. The cage was placed on an Animex® activity monitor to count total locomotor activity. A small partition with a 13 cm long x 5 cm high opening separated the dark chamber from the light chamber.
In this two-chamber system, where animals can freely move between a brightly lit open field and a darker corner, they exhibit more crossings between chambers and increased locomotor activity following anxiolytic treatment. The number of crossings between the light and dark sections was recorded, along with movements through the partition and time spent in each chamber. Male mice were introduced into the cage for testing.
The animals were treated 30 min before the experiment with test drugs or vehicle intraperitoneally and then observed for 10 min, groups of 6 animals are used for each dose. The following behavioral were measured:

1) The number of entries in dark and light chamber.
2) Time spent in minutes in dark and light chambers.

The procedure was conducted preferably in a sound attenuated room, with observations made from an adjacent room via web camera (CyberPix S-300) attached to the computer system.

**Evaluation:**

The light-dark test is a simple behavioural model in mice used to identify compounds with anxiolytic properties. Mice and rats naturally explore new environments but prefer to avoid the discomfort of a brightly lit open space. In a two-chamber arrangement, where animals can move freely between a well-lit area and a darkened corner, they typically exhibit increased crossings between chambers and enhanced locomotor activity after treatment with anxiolytic agents.
The treatment was given through oral route. All animals were pretreated for 20 days except diazepam treated animals. On 21st day, animals were treated, 30 min before the evaluation. APLE at 200 and 400 mg/Kg made the animals spend way more time in and go into the light chamber, while they spent less time and went into the dark chamber. This shows it really has some anxiety-relieving effects.

## III. RESULTS

### 3.1 Elevated Plus Maze

In the EPM test (Table No. 1), animals treated with two doses of APLE (200 mg/kg and 400 mg/kg) demonstrated a dose-dependent increase in time spent in the open arms, which was significant compared to the control group.

**Table No. 01: Effect of APLE on EPM in mice:**

| Group No. | Drug Treatment | Dose (mg/Kg) | Number of entries (mean ± SEM) | | Time spent in sec (mean ± SEM) | |
|---|---|---|---|---|---|---|
| | | | Open arm | Closed arm | Open arm | Closed arm |
| I | Control | 1ml | 4.12±1.78 | 10.33±2 | 38.14±3.34 | 198.22±3.84 |
| II | Diazepam | 1 | 12.23±1.89*** | 3.24±1.67*** | 188.21±5.17*** | 56.12± 4.56*** |
| III | APLE | 200 | 7.28±1.41* | 6.22±2.28* | 64.333±1.33* | 159.167±2.57** |
| IV | APLE | 400 | 9.16±2.13** | 4.667±1.50*** | 100±2.76*** | 81.167±3.31*** |

Values are expressed as Mean ± SEM; (n=6). Data analysis was performed using Dunnett's test. Where * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control group animals.

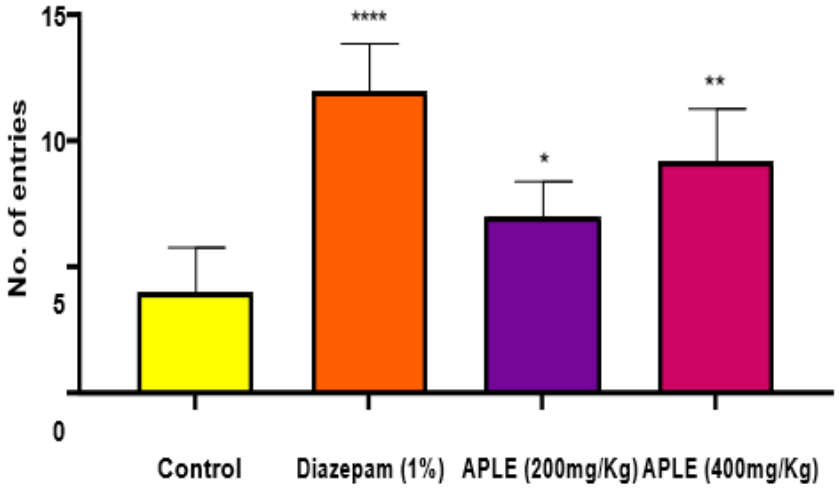



**Fig. No. 2: Anxiolytic effect of *Allium porrum* leaves extract using Elevated Plus Maze**

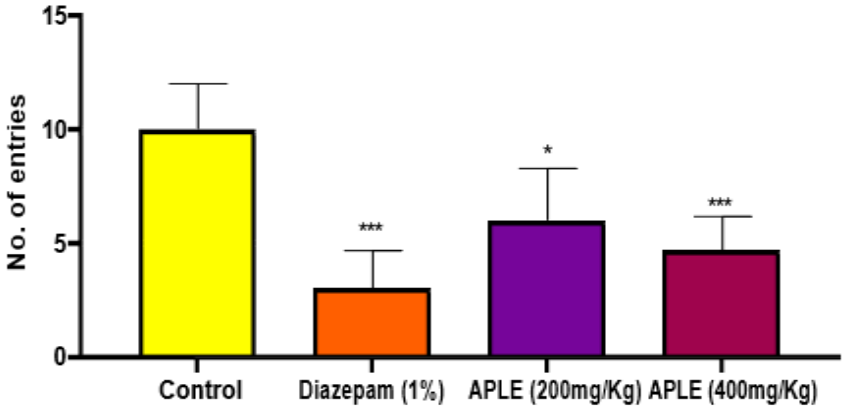
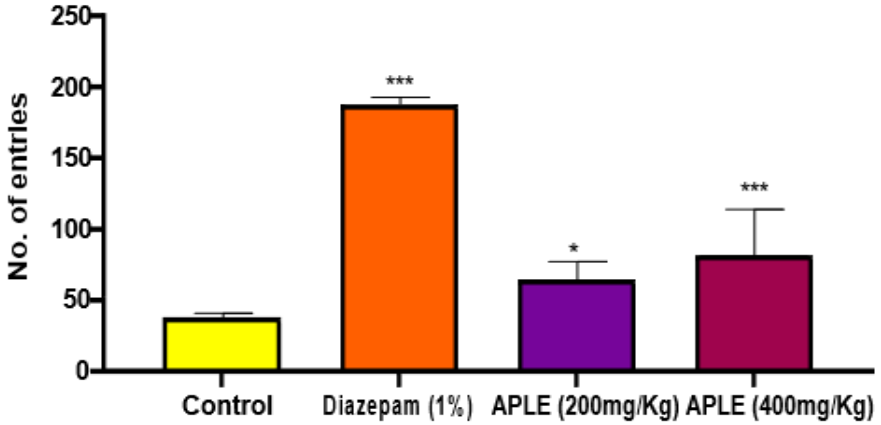
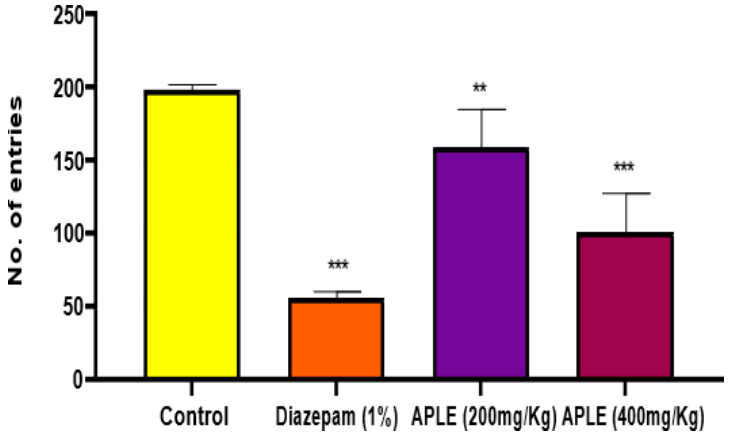



**Fig. No. 3: Anxiolytic effect of *Allium porrum* leaves extract using Elevated Plus Maze**






### 3.2 Light and Dark model

In the light-dark test (Table No. 2), animals treated with two doses of APLE (200 mg/kg and 400 mg/kg) displayed a dose-dependent decrease in time spent in the dark chamber, accompanied by an increase in time spent in the light chamber compared to the control group.

**Table No. 2: Effect of APLE on LDT in mice:**

| Group No. | Drug Treatment | Dose (mg/Kg) | No. of entries (Mean ±SEM) | | Time spent in min (Mean ±SEM) | |
|---|---|---|---|---|---|---|
| | | | Dark | Light | Dark | Light |
| I | Control | 1ml | 14.23±2.82 | 2.21±0.89 | 88.33 ±1.95 | 28.15±3.34 |
| II | Diazepam | 1 | 4.12±1.41*** | 13.23±2.13*** | 29.50 ±3.61*** | 88.23±2.27*** |
| III | APLE | 200 | 8.51±3.93** | 6.51±1.51** | 64.33 ±1.27** | 54.33±1.33** |
| IV | APLE | 400 | 5.33±1.86*** | 10.53±1.53*** | 44.66 ±1.37*** | 67.83±2.83*** |

Values are expressed as Mean ± SEM; (n=6). Data analysis was performed using Dunnett's test. Where * P< 0.05, ** P <0.01, *** P < 0.001 vs. control group animals.

**Fig. No. 5: Anxiolytic effect of *Allium porrum* leaves extract using light and dark model**

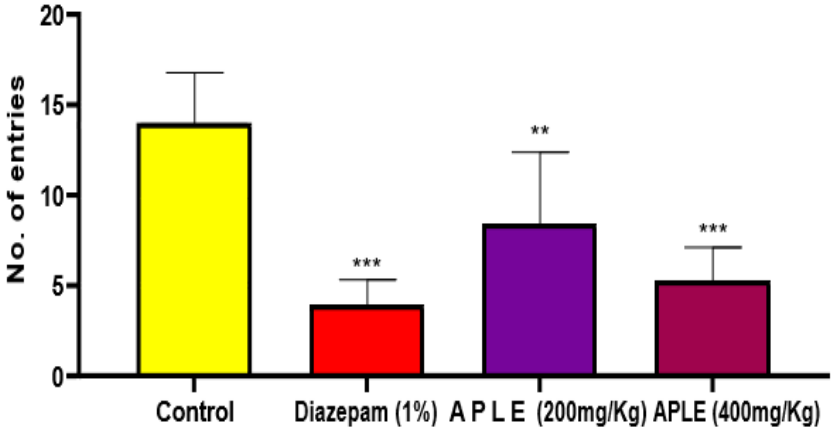


**Fig. No. 6: Anxiolytic effect of *Allium porrum* leaves extract using light and dark model**

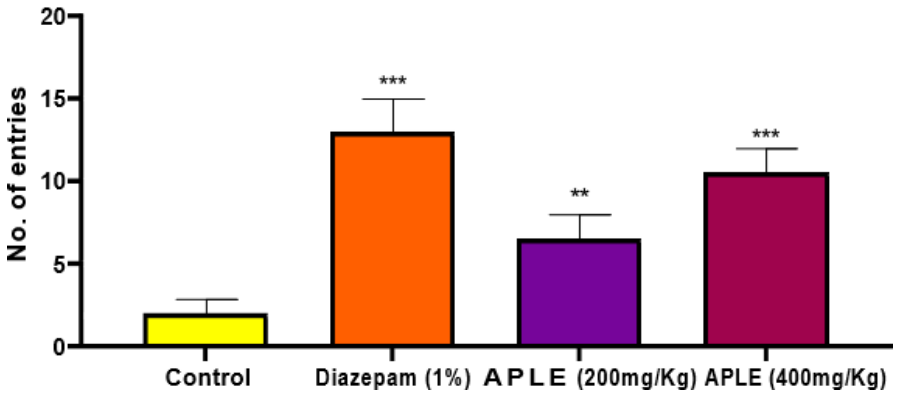



**Fig. No. 7: Anxiolytic effect of *Allium porrum* leaves extract using light and dark model**

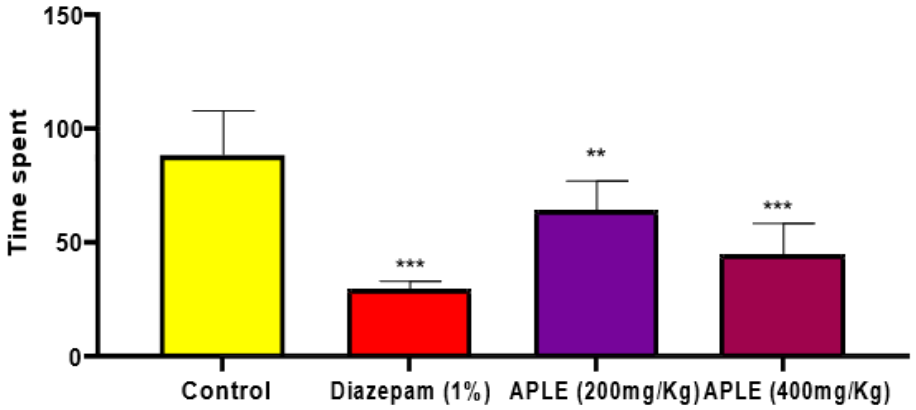
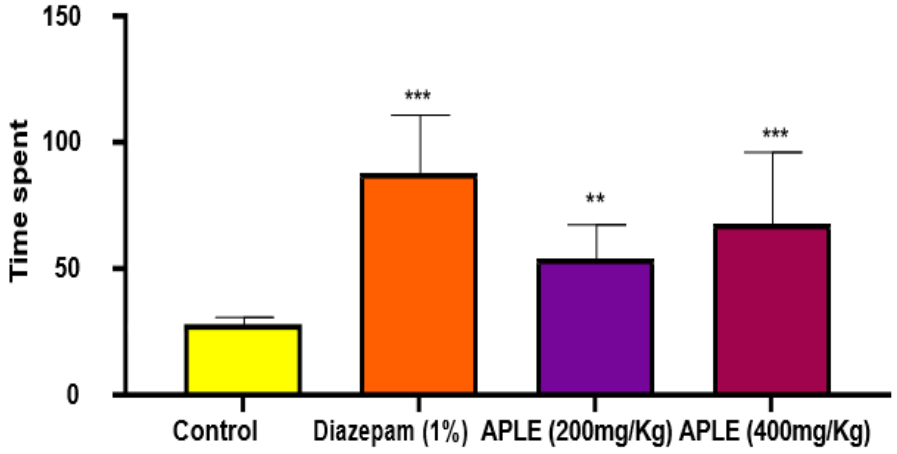



**Fig. No. 8: Anxiolytic effect of *Allium porrum* leaves extract using light and dark model**



## IV. DISCUSSION

Anxiety is a common emotion that many people experience. It's an uncomfortable feeling of worry or fear, often accompanied by physical symptoms like a racing heart or sweating. [16] This study evaluated the anxiolytic effects of an ethanolic extract of *Allium porrum* leaves (APLE) using animal anxiety models, specifically the elevated plus maze (EPM) and light-dark box. APLE was administered to mice at doses of 200 mg/kg and 400 mg/kg, and its effects were nearly comparable to those of diazepam, a standard anxiolytic. The treated mice displayed increased time in the open arms of the EPM and in the light compartment of the light-dark box, indicating reduced anxiety levels.

The anxiolytic effects may be linked to the extract's phytochemical composition, including flavonoids, alkaloids, and phenolic acids. Flavonoids, in particular, share structural similarities with diazepam, suggesting a possible mechanism of action. These compounds, commonly associated with therapeutic effects on the central nervous system, likely contribute to the observed anxiolytic effects. [17]

The study's findings support traditional uses of *Allium porrum* in folk medicine for managing anxiety-related symptoms. While APLE shows significant anxiolytic potential, further research is necessary to clarify its precise mechanisms and identify the specific active compounds responsible for its effects. These insights could enhance clinical applications and lead to the development of natural, plant-based treatments for anxiety.

## V. CONCLUSION

The present study was designed to expose the anxiolytic activity of ethanolic extract of *Allium porrum* leaf in Swiss albino mice using animal models of anxiety namely, Elevate plus maze, Light dark test.

The data obtained was both satisfactory and conclusive, successfully achieving our objectives. In conclusion, the present findings demonstrate that the administration of APLE to mice exhibits anxiolytic activity, supporting traditional claims of its anxiolytic properties. Preliminary phytochemical analysis of the ethanolic extract of *Allium porrum* leaves revealed the presence of alkaloids, carbohydrates, flavonoids, proteins, phenols, and amino acids.

The precise mechanism behind the anxiolytic effect remains unclear, but it appears to be linked to the active compounds present in APLE. Therefore, further research is needed to determine which specific components are responsible for the observed anxiolytic activity.

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