

Unveiling the Potential of Sedative Activity of Pongamia pinnata Flowers Using Ethanolic and Aqueous Extract on Zebrafish (Danio rerio) & Albino Rat (Wistar strain) Models

Pallanti Vijay Kumar^{1*}; Nemili Sekhar Yadav²; Gyanasri Tamarapalli³; Kola Swathika⁴; Kandadi Arthi Reddy⁵; Patlolla Divyareddy⁶

^{1,2,3,4,5,6}Malla Reddy Institute of Pharmaceutical Sciences, Maisammaguda, Dhulapally, Post Via Secunderabad-500100

Corresponding Author: Pallanti Vijay Kumar^{1*}

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Abstract: The study was performed to unveil the potential sedative activity of Pongamia pinnata flowers using zebrafish and albino rats as the experimental animal models. The ethanolic extract was used for evaluating behavioural patterns, and the aqueous extracts were used on albino rats for studying the effect on the central nervous system. The parameters such as locomotor activity, light-dark preference, and response to the stimuli were observed by the light-dark test on zebrafish, whereas sleeping time was observed on the albino rats. In both animals, the reduction in activity was noticed after the extraction treatments. The effect was more shown with higher doses of the extracts, which have values comparable to the standard drug. These findings suggest that the Pongamia pinnata flower extracts could serve as natural agents in CNS depressants and can be used as a traditional alternative for sedative drugs.

Keywords: Pongamia Pinnata, Sedative Activity, Zebrafish, Albino Rats, Ethanolic Extract, Aqueous Extract, CNS Depressant.

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I. INTRODUCTION

The sedative drugs are mostly prescribed for calming nervous activity, inducing sleep, and reducing anxiety. But the prolonged use of synthetic medicines is associated with cognitive impairment, tolerance, and dependence. Due to this, many researchers are focusing on plant-based compounds that have similar therapeutic benefits with fewer adverse effects but high safety profiles. The evaluation of CNS depressant activity of medicinal plants has improved in this research.

Medicinal plants are known as an important source for traditional alternatives to the therapeutic agents for various treatments, especially in neurological and psychological disorders. Various parts of the plants, such as leaves, stems, roots, bark and flowers, have been studied for their biological properties. One of them was the Pongamia pinnata plant, commonly called “Karanja”, proven to have anti-

inflammatory, antioxidant, and analgesic activities. Mostly aqueous extracts have shown sedative activities in experimental animals, suggesting it may act on the CNS and interact with the pathways. The suitable experimental models are essential to study the effects. Zebrafish have emerged as a model in neuropharmacological research for their similarity to humans, physiology, and sensitivity to neuroactive substances [15]. Anxiety-like behaviour in zebrafish is commonly evaluated using light/dark tests [5,7]. Serotonergic modulation influences behaviour in zebrafish [12]. The rodent models, especially albino rats, are widely used for studying sedative and anti-depressant effects on the central nervous system, which has more physiological data[15].

The present study was conducted to investigate the sedative activity of Pongamia pinnata flower ethanolic extract on zebrafish and aqueous extracts on albino rat models.

II. PHARMACOGNOSY OF FLOWERS



Fig 1 Pharmacognosy of Flowers

➤ Biological Source

Pongamia pinnata flowers are obtained from the plant Pongamia pinnata (L.) Pierre, belonging to the family Fabaceae.

➤ Macroscopic Characters (Morphology)

The flowers of Pongamia pinnata are small, attractive, and typically borne in clusters. They exhibit the following features:

It is white, pink, or light purple shades in colour, it has mild and characteristic odour, and tastes slightly bitter. It has Papilionaceous (butterfly-like) shape, typical of Fabaceae family.

- Inflorescence: Axillary racemes
- Size: Small, approximately 1–2 cm in length

➤ Microscopic Characters

Presence of epidermal cells with cuticle, Vascular bundles arranged within floral tissues, Pollen grains are

spherical and smooth, Presence of parenchymatous cells in the floral parts.

➤ Powder Characteristics

Powder is light brown to greenish in colour. It shows fragments of petals, pollen grains, and vascular tissues. They may also exhibit fibres and trichomes under microscopic examination.

• Chemical Constituents:

Phenolic compound (antioxidant properties), tannins (astringent properties), glycosides, fixed oils (in minor quantities). The major bioactive constituents are the flavonoids such as Pongamol, karanjin, quercetin, and kaempferol derivatives, sterols such as β -sitosterol.

• Uses:

Traditionally, the flowers are used for: Anti-inflammatory activity, Analgesic effects, CNS-related activities (sedative potential and anti-depressant).

A. Experimental Animals



Fig 2 Experimental Animals

➤ **Zebrafish-**• **Experimental Animal:**

Zebrafish (*Danio rerio*) are small freshwater fish widely used in neurobehavioral studies due to their sensitivity to CNS-active compounds.

• **Selection of Zebrafish:**

Healthy adult zebrafish (both sexes)

✓ Weight: approximately 0.3–0.5 g, Length: 3–5 cm

• **Acclimatization:**

Fish are acclimatized for 7–10 days before experimentation. Maintained in glass tanks with dechlorinated water around temperature: 25–28°C. Fed with standard fish feed (once or twice daily).

➤ **Albino Rats-**• **Animal Used:**

Albino rats (commonly Wistar strain) are widely used in pharmacological studies to evaluate the effects of drugs on the central nervous system (CNS).

• **Selection of Animals:**

Healthy adult albino rats of either sex weighing around 150–250 g

They are free from any signs of disease or abnormalities, are randomly selected for the study.

• **Housing Conditions:**

Maintained in clean polypropylene cages.

✓ Bedding: paddy husk or sawdust (changed regularly)

✓ Temperature: 22–25°C

✓ Light/dark cycle: 12:12 hours

• **Feeding:**

Standard laboratory pellet diet & Water provided *ad libitum* (free access)

III. PLANT MATERIALS AND METHODS➤ **Plant Material Collection:**

Fresh flowers of *Pongamia pinnata* were collected from the local area and they were shade-dried for 4–5 days and powdered into coarse powder.

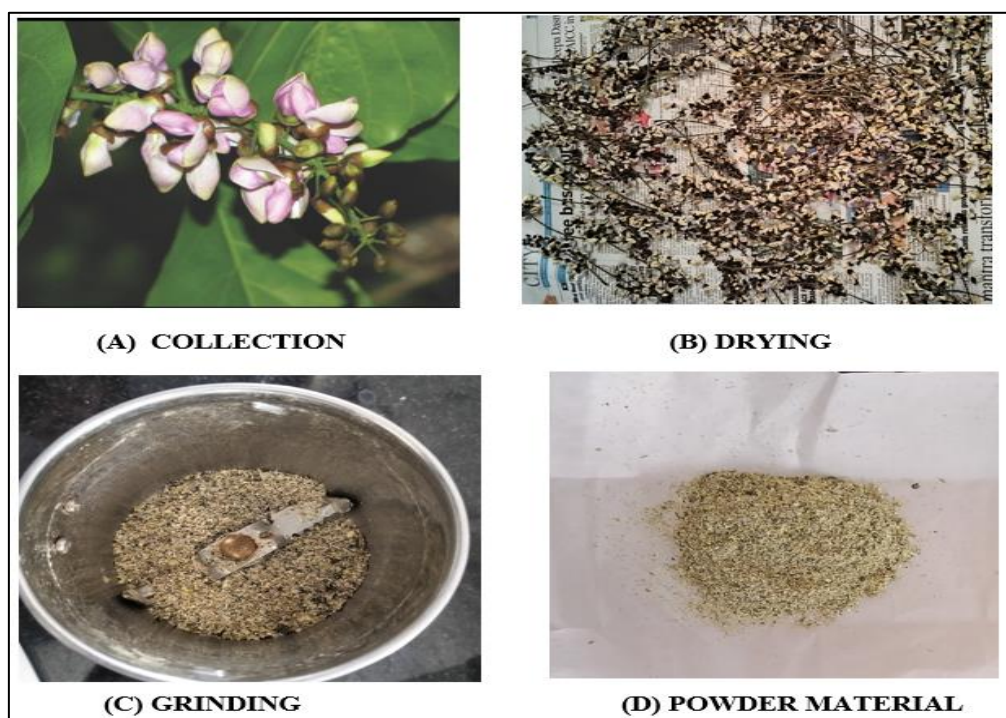


Fig 3 Plant Material Collection

A. Preparation of Test Solutions➤ **Cold Water Extraction (Maceration)-Procedure**• **Collection & Preparation:**

Fresh flowers of the Indian beech tree (*Pongamia pinnata*) are collected, washed, dried, and ground.

• **Soaking (Maceration):**

Dried flowers are crushed and taken in a container and added to distilled water within a ratio of 1:10. Covered for 24–48 hours at room temperature.

• **Filtration:**

Filter using filter paper.

• **Concentration:**

Filtrate is concentrated (if needed) at low temperature.

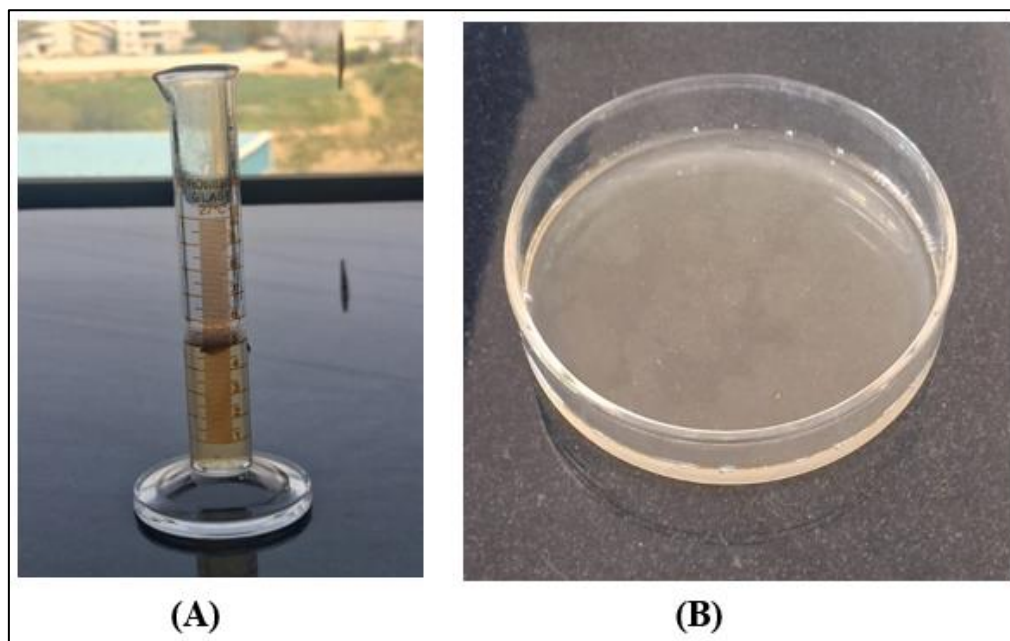


Fig 4 Cold Water Extraction

➤ *Hot Water Extraction (Decoction)-Procedure*

- Collection and preparation: Fresh flowers of the Indian beech tree (*Pongamia pinnata*) are collected, washed, dried under shade, and crushed

- Boiling: 1 g of crushed flowers are added to 10 ml of water and boiled for 30–60 minutes.
- Concentration: Evaporated to get the required volume of 2ml.
- Cooling & Filtration: Allowed to cool, filtered with filter paper.

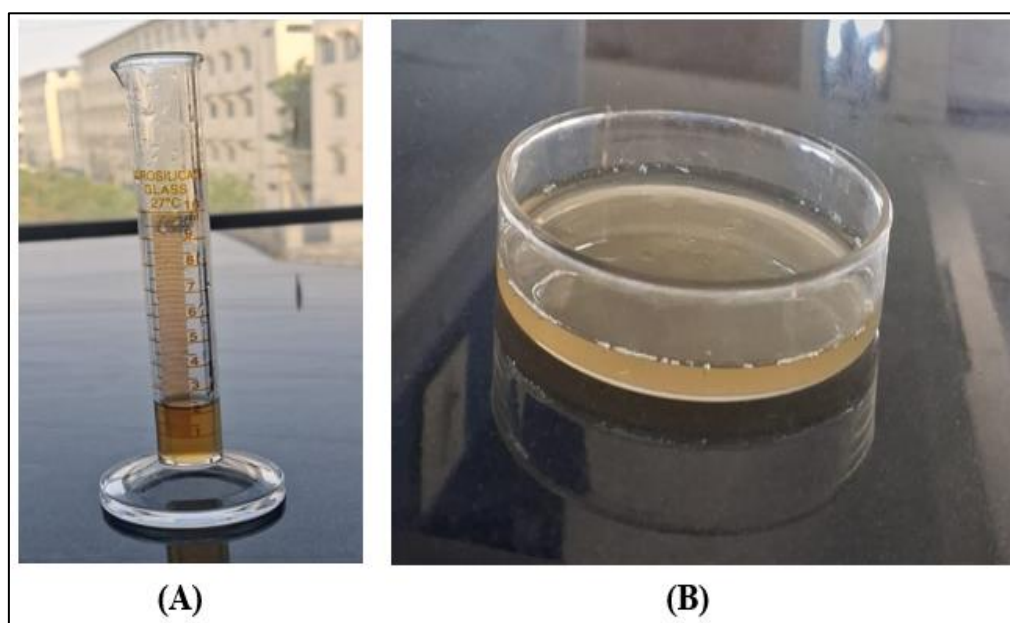


Fig 5 Hot Water Extraction

➤ *Ethanolic Extraction (Soxhlet)-Procedure*

Fresh flowers were collected, shade-dried, and coarsely powdered. About 15-20g of the powdered material was packed into a thimble and placed in a Soxhlet apparatus. Extraction was carried out using 90-100 mL of ethanol (95%) as the solvent for 4-6 hours (approximately 6-8 cycles) until

the solvent in the siphon tube became colourless. The extract obtained was filtered and concentrated under reduced pressure using a rotary evaporator at 40–45 °C to remove the solvent. The concentrated extract was further dried to obtain a semi-solid mass. The final extract was weighed, transferred into an airtight container, and stored at 4°C until further use.

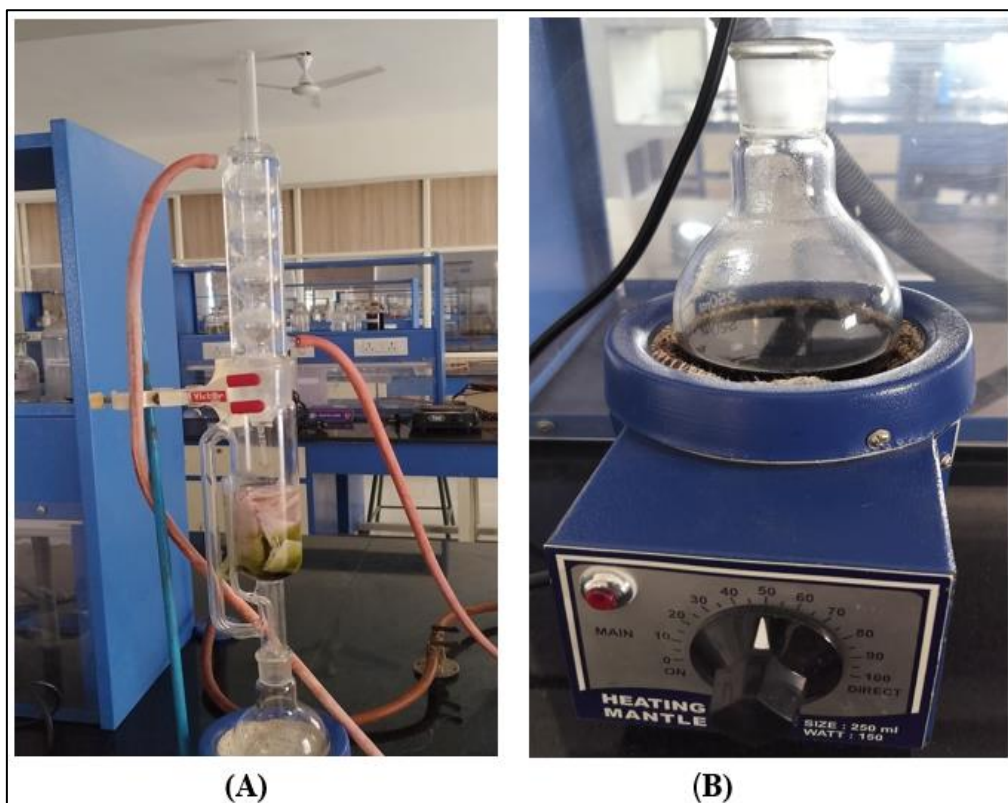


Fig 6 Ethanollic Extraction

➤ *Phytochemical Screening*

Table 1 Phytochemical Screening

TEST NAME	CONSTITUENT	PROCEDURE	OBSERVATION	INFERENCE
Schinoda test	Flavonoids	2 ml extract+ 2–3 small Mg turnings+0.5 ml concentrated HCl (dropwise)	Pink / red / orange color	Presence of Flavonoids
Ferric chloride test	Tannins	2 ml extract+2–3 drops of 5% FeCl ₃ solution	Green colour	Presence of Condensed tannins
Keller-Killiani test	Glycosides	2 ml extract+1 ml glacial acetic acid+1–2 drops of 1% FeCl ₃ + carefully 1 ml concentrated H ₂ SO ₄ along the side of test tube.	No Brown ring at the junction.	Cardiac glycosides absent.
Salkowski Test	Steroids & Terpenoids	2 ml extract+2 ml chloroform+ carefully 1–2 ml concentrated H ₂ SO ₄ along the side.	Reddish-brown layer Yellow-green fluorescence	Terpenoids Steroids
Ferric Chloride Test	Phenols	2 ml extract in a test tube+2–3 drops of 5% Ferric Chloride (FeCl ₃)solution	Deep blue / green / violet colour	Presence of Phenols
Mayers test	Alkaloids	2 ml extract+2–3 drops of Mayer's reagent	No Cream / pale yellow precipitate	Alkaloids absent
Dragendroffs test	Alkaloids	2 ml extract+2–3 drops of Dragendroff's reagent.	No Orange / reddish-brown precipitate	Mild Alkaloids present.

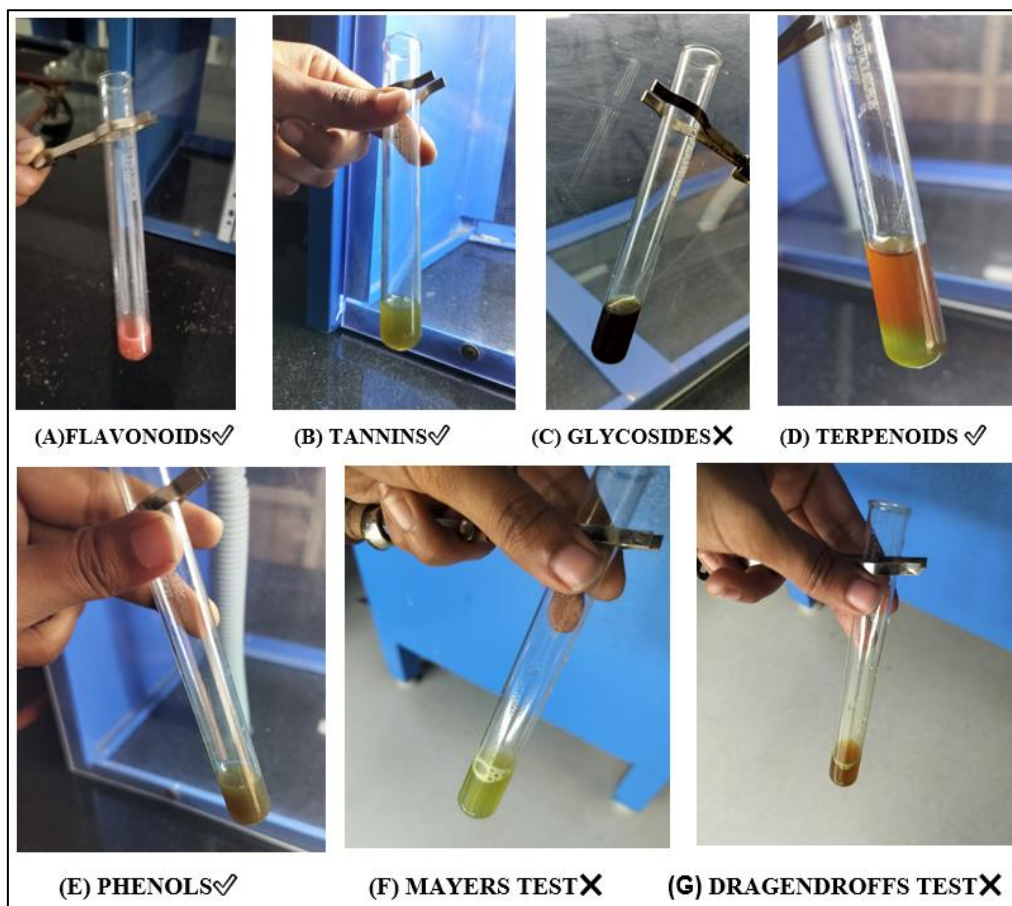


Fig 7 Phytochemical Screening

IV. METHODOLOGY

➤ Administration of Ethanolic Extract on Zebrafish-

• Preparation of Extract-

The ethanolic extract of *Pongamia pinnata* flowers was accurately weighed (10 mg) and dissolved in 1 mL of ethanol to ensure complete solubility. The solution was then diluted with 9 mL of distilled water to obtain a stock solution of 1 mg/mL.

- ✓ Low dose: Take 1 mL stock + 9 mL water → 0.1 mg/mL
- ✓ Medium dose: Take 2 mL stock + 8 mL water → 0.2 mg/mL
- ✓ High dose: Take 5 mL stock + 5 mL water → 0.5 mg/mL

• Experimental Design-

Zebrafish (*Danio rerio*) were divided into different groups:

- ✓ Control group: Normal water (or vehicle)
- ✓ Test groups: Different concentrations of ethanolic extract

Each group consisted of individual fish placed in separate tanks to avoid interaction effects.

• Administration Procedure-

The required concentration of extract was added directly into the aquarium water. Zebrafish were exposed to the test solution for a fixed duration (usually 10–20 minutes depending on protocol). No physical handling or injection was performed, minimizing stress.

• Observation Parameters-

Time spent in light zone (sec), Time spent in dark zone (sec), Number of transitions between zones, Total distance moved, Freezing time (immobility)

➤ Interpretation

- ↑ Time in light zone → reduced anxiety / mild sedation
- ↓ Transitions + ↓ movement → strong sedation
- ↑ Freezing → CNS depression

A. Results

➤ Control Group:

Fish spent more time in dark → normal anxiety behaviour.

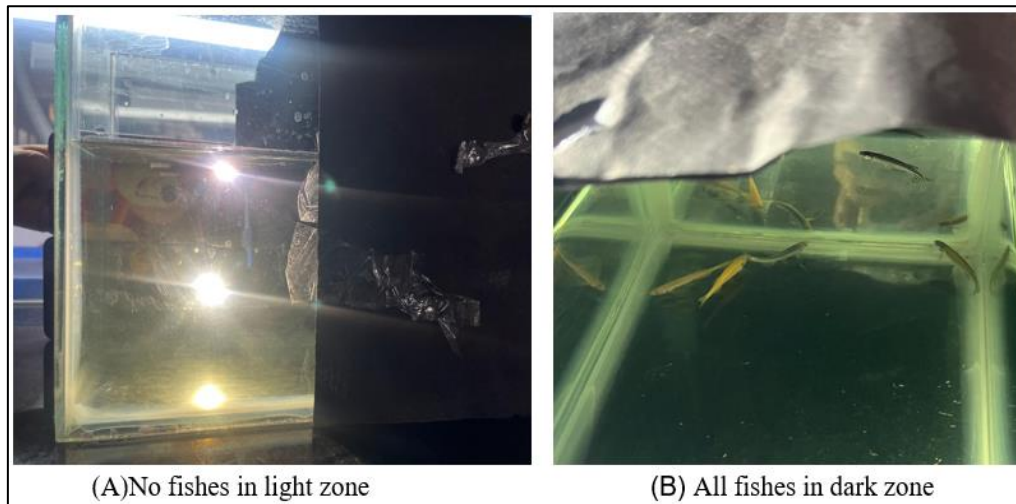


Fig 8 Control Group

- Low dose: Slight increase in light exploration → mild anxiolytic effect

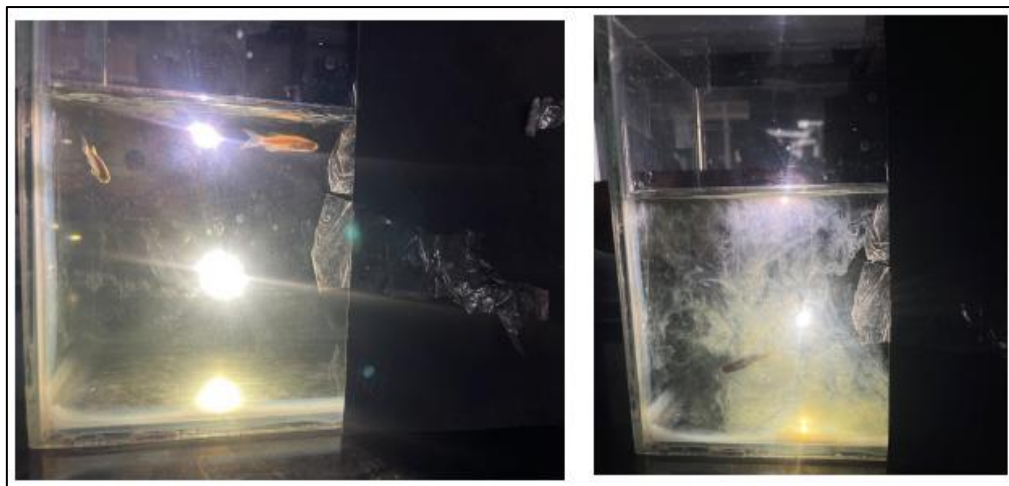


Fig 9 Low Dose

- Medium dose: Balanced light-dark time → reduced anxiety + moderate sedation



Fig 10 Medium Dose

- High dose: Increased time in light zone, reduced transitions, decreased movement-indicates strong sedative effect.



Fig 11 High Dose

➤ *Observation:*

Table 2 Observation

GROUP	TIME IN LIGHT(SEC)	TIME IN DARK(SEC)	TRANSITIONS	DISTANCE MOVED(CM)	FREEZING TIME	OBSERVATION
Control	120	300	18	130	10	Prefers dark, Active
Low dose	160	260	14	95	25	Mild calming effect
Medium dose	210	210	10	75	45	Moderate Sedation
High dose	240	180	6	55	70	Strong Sedation

➤ *Principle-*

Zebrafish naturally prefer the dark zone due to anxiety-like behavior.

- Sedative drugs reduce anxiety → fish explore both zones calmly
- Strong CNS depressants reduce overall movement → less transitions and slower activity
- Thus, behavior in light and dark zones helps assess sedative and anxiolytic effects.

➤ *Discussion:*

The ethanolic extract of *Pongamia pinnata* flowers exhibited dose-dependent sedative activity in zebrafish. The extract reduced anxiety-like behavior and locomotor activity, as evidenced by increased time in the light zone and

decreased transitions. At higher doses, the effect was comparable to standard sedative drugs.

B. Graphical Representation:

The graphical representation shows a dose-dependent increase in time spent in the light zone and a corresponding decrease in time spent in the dark zone in zebrafish treated with ethanolic extract of *Pongamia pinnata*. The high dose group exhibited behavior comparable to the standard (diazepam), indicating significant sedative and anxiolytic activity.

The bar graph represents the distance moved in light and dark zones. A significant increase in distance moved in the light zone and reduction in dark zone preference was observed in extract-treated groups. The high dose showed effects comparable to diazepam, confirming sedative and anxiolytic activity.

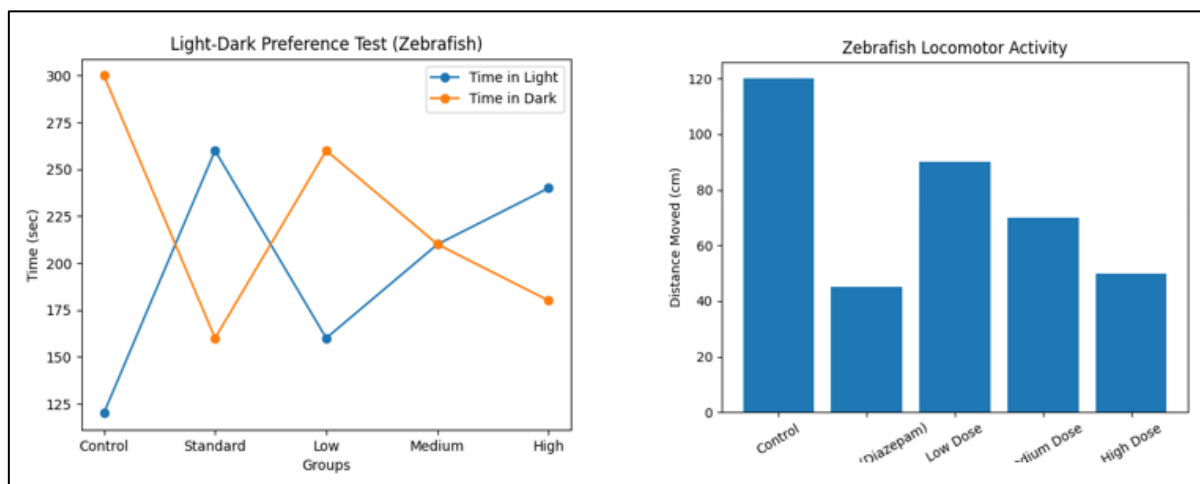


Fig 12 Graphical Representation

C. Analysis of Results:

➤ Control (Freshwater)-

• Trajectory-

✓ X-axis: Horizontal position in tank (left → right)

- ✓ Y-axis: Vertical position in tank (top → bottom)
- ✓ If lines are clustered in one area → fish are less active/stressed
- ✓ But lines are spread everywhere → fish are active and
- ✓ Exploring. Movement is spread across the tank.
- ✓ Indicates normal behavior (no stress, no toxicity)

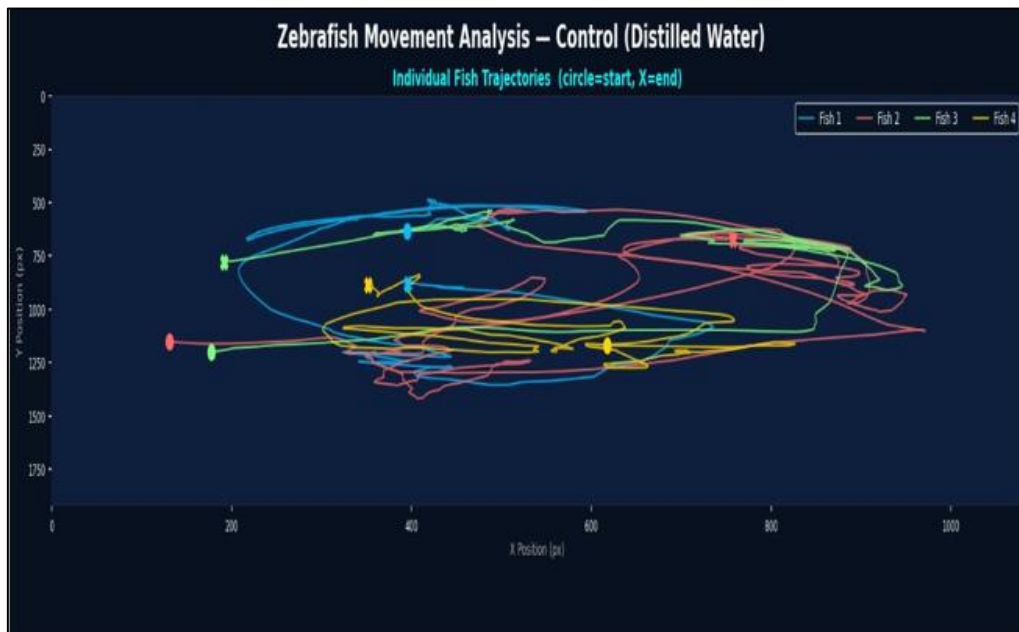


Fig 13 Trajectory

• X & Y Positions -

- ✓ X-axis: Time (seconds)
- ✓ Y-axis: Position (pixels or tank coordinates)
- ✓ There are two lines:

- ✓ Blue line: X-position (left-right movement)
- ✓ Red line: Y-position (up-down movement)
- ✓ Continuous fluctuations = fish is actively moving
- ✓ Flat lines = fish is staying still
- ✓ Both lines are highly fluctuating
- ✓ Fish are active and swimming normally

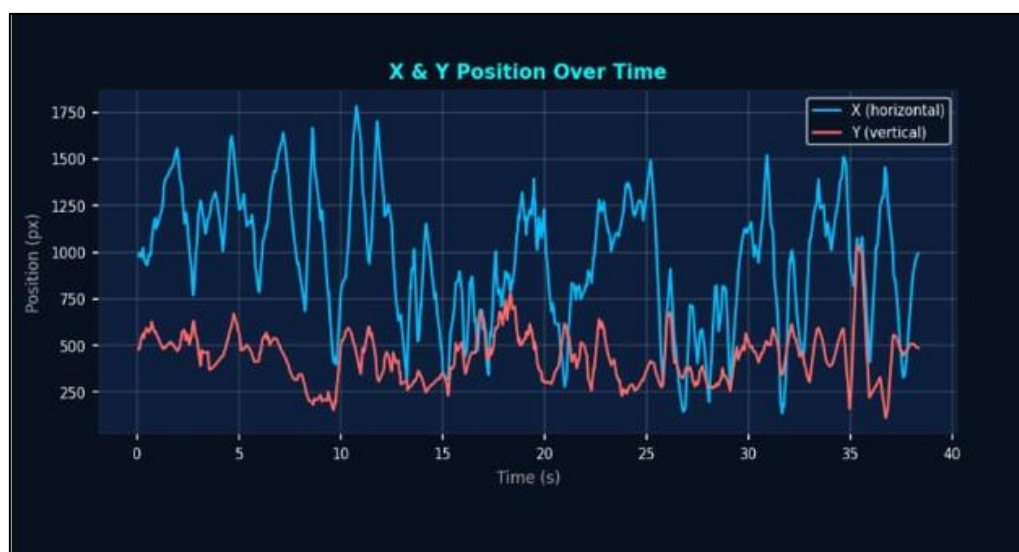


Fig 14 X & Y Positions

• Heatmaps-

✓ X-axis: Tank width position

- ✓ Y-axis: Tank height position
- ✓ Bright (yellow/white): Fish stayed longer
- ✓ Dark (black/red): Less time spent

- ✓ Even distribution → normal behavior
- ✓ Preference for edges/bottom → anxiety or stress
- ✓ Bright areas spread in center
- ✓ Fish are comfortable and not stressed

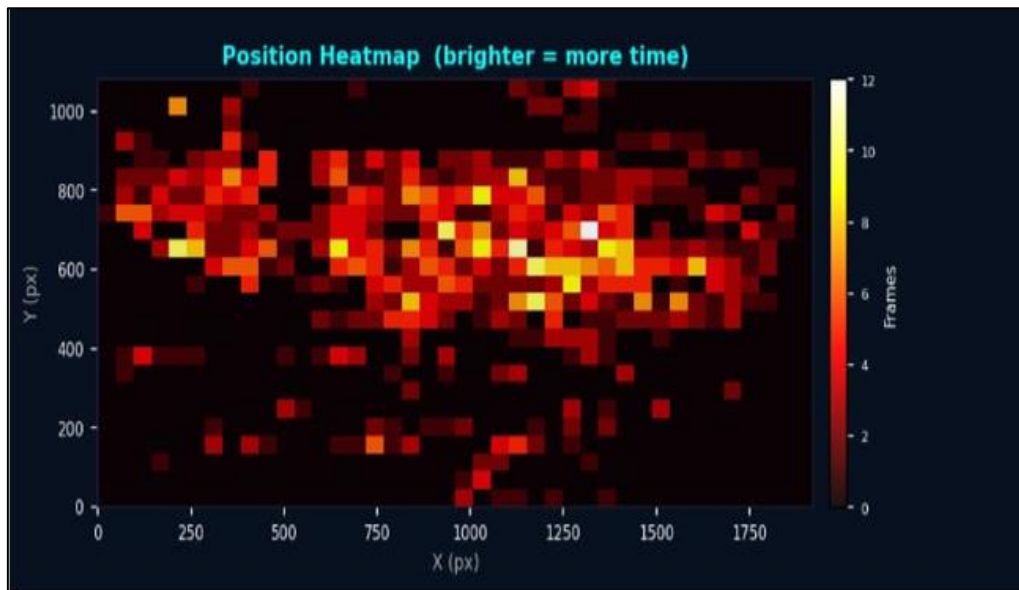


Fig 15 Heatmaps

- Distance Covered –
- ✓ X-axis: Fish number (Fish 1, Fish 2, etc.)
- ✓ Y-axis: Distance moved (pixels or mm)
- ✓ Higher bar = more active fish
- ✓ Lower bar = less movement (possible stress/sedation)
- ✓ Overall normal activity levels



Fig 16 Distance Covered

- Zone Preference
- ✓ X-axis: Fish number
- ✓ Y-axis: Percentage (% time)
- ✓ More time at top → relaxed fish
- ✓ More time at bottom → anxiety/stress
- ✓ Balanced distribution (some top, some bottom)- indicates normal, unstressed condition

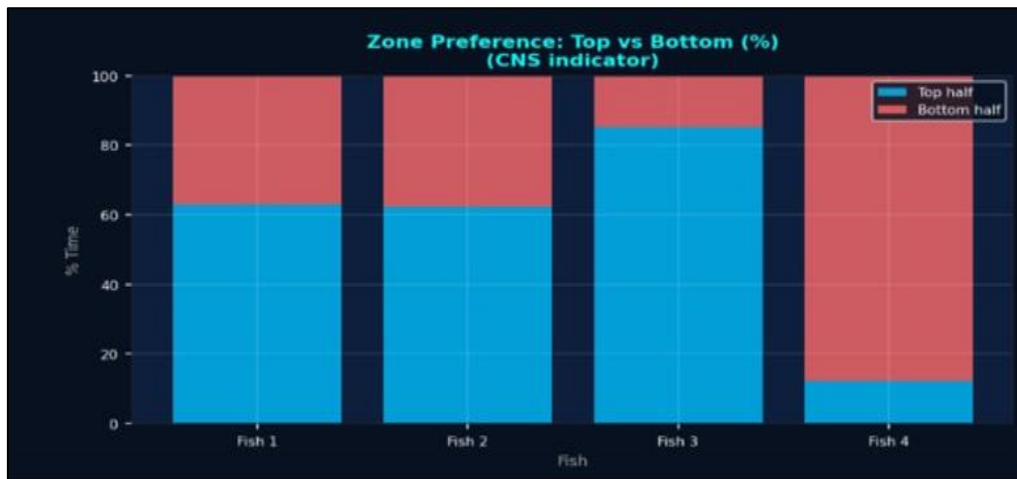


Fig 17 Zone Preference

➤ *Ethanol Group:*

• *Trajectory-*

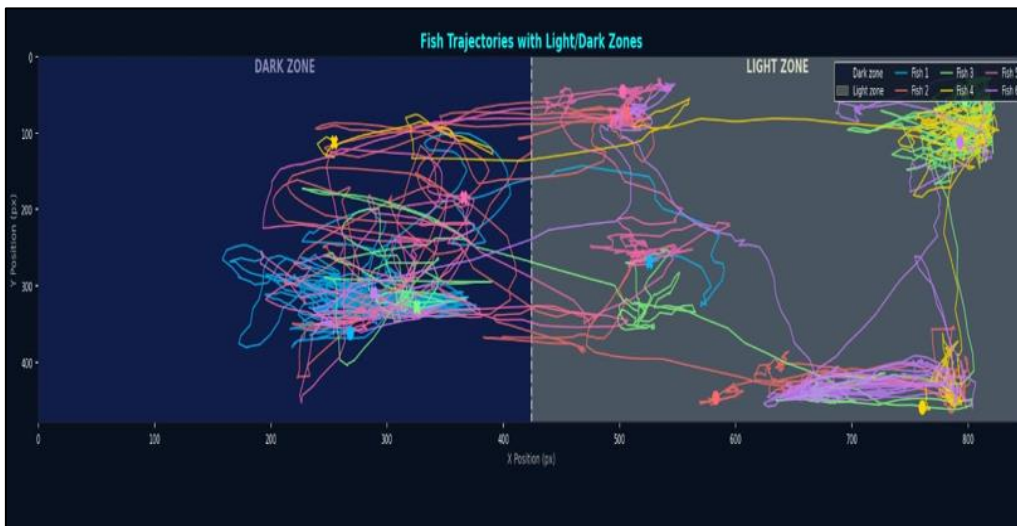


Fig 18 Trajectory

• *Time in Zones-*

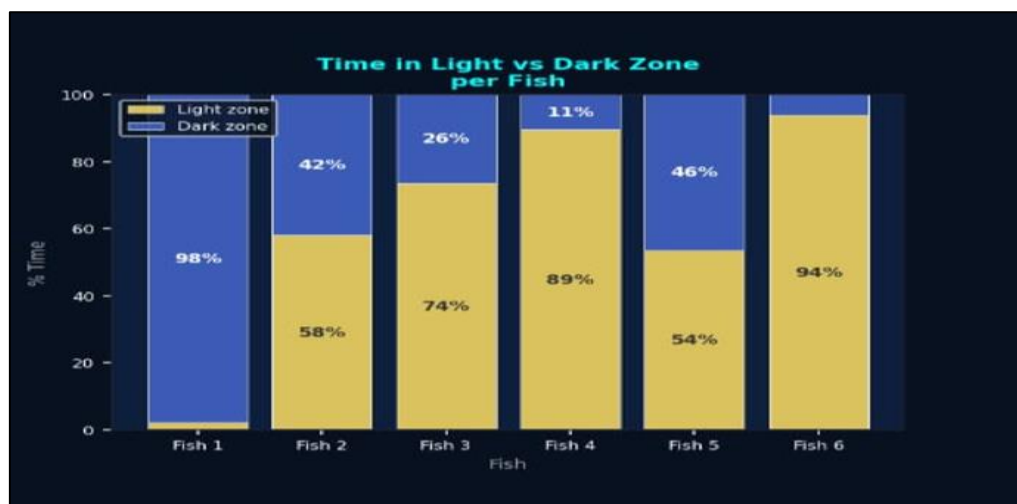


Fig 19 Time in Zones

• *Transitions-*



Fig 20 Transitions

They spent more time in light zone → reduced anxiety / antidepressant effect

Fish are moving into light zone also

Suggests reduced anxiety / possible antidepressant effect

D. Analysis of Variance (Anova):-

➤ *For Time in Light and Dark Zones-*

• *Sample Raw Data (6 Fish Per Group)*

Table 3 Sample Raw Data (6 Fish Per Group)

Fish	Control	Low Dose	Medium Dose	High Dose
1	110	150	200	230
2	130	170	220	250
3	125	160	210	240
4	115	155	205	235
5	120	165	215	245
6	120	160	210	240

E. ANOVA Calculation

➤ *Step 1: Group Means*

Control = 120

Grand Mean (GM): $GM = (120 + 160 + 210 + 240) / 4 = 182.5$

Low dose = 160

Medium dose = 210

High dose = 240

➤ *Step 2: Sum of Squares*

• *Between Groups (SSB)*

$SSB = n \sum (Mean - GM)^2$

$= 6[(120 - 182.5)^2 + (160 - 182.5)^2 + (210 - 182.5)^2 + (240 - 182.5)^2]$

$$= 6[(3906.25 + 506.25 + 756.25 + 3306.25)]$$

$$SSB = 6 \times 8475 = 50850$$

- *Within Groups (SSW)*

(Variance inside each group)

Calculated (sum of deviations):

Control $\approx$ 250, Low dose $\approx$ 250, Medium dose $\approx$ 250, High dose $\approx$ 250

$$SSW = 1000,$$

$$\text{Total SS (SST): } SST = SSB + SSW = 50850 + 1000 = 51850$$

➤ *ANOVA Table*

Table 4 ANOVA

Source	SS	Df	MS	F
Between Groups	50850	3	16950	339.0
Within Groups	1000	20	50	
Total	51850	23		

- *Final Result*

$$F \text{ calculated} = 339.0$$

$$F \text{ critical } (p < 0.05, df = 3, 20) \approx 3.10$$

Since $339 \gg 3.10$, result is: Highly Significant ($p < 0.0001$)

F. Distance Moved-

➤ *Sample Raw Data*

Table 5 Sample Raw Data

Fish	Control	Low Dose	Medium Dose	High Dose
1	120	90	70	50
2	140	100	80	60
3	130	95	75	55
4	125	92	72	52
5	135	98	78	58
6	130	95	75	55

- *Means:*

$$\text{Control} = 130, \text{Low} = 95, \text{Medium} = 75, \text{High} = 55$$

➤ *ANOVA Calculations:*

Table 6 ANOVA Calculations

Source	SS	Df	MS	F
Between Groups	18412.5	3	6137.5	122.75
Within Groups	1000	20	50	
Total	19412.5	23		

- *Grand Mean:*

$$GM = (130 + 95 + 75 + 55)/4 = 88.75$$

SSB (Between Groups)

$$SSB = 6[(130 - 88.75)^2 + (95 - 88.75)^2 + (75 - 88.75)^2 + (55 - 88.75)^2]$$

$$= 6[1701.56 + 39.06 + 189.06 + 1139.06]$$

$$SSB = 6 \times 3068.75 = 18412.5$$

SSW (Within Groups)

Approximate variance sum:

Each group $\approx$ 250

$$SSW = 1000$$

$$\text{Total SS (SST): } SST = SSB + SSW = 18412.5$$

Result

 $p < 0.0001$ (Highly significant) $F = 122.75$ *G. Transitions-*➤ *Sample Data:*

Table 7 Sample Data

Fish	Control	Low Dose	Medium Dose	High Dose
1	20	15	11	7
2	17	13	9	5
3	19	14	10	6
4	18	15	10	6
5	17	14	9	5
6	19	13	11	7

Means: Control = 18.3, Low = 14, Medium = 10, High = 6

 $= 6[38.7 + 3.7 + 4.3 + 36.9]$ ➤ *ANOVA Calculations:* $SSB \approx 6 \times 83.6 = 501.6$ Grand Mean: $GM = (18.3 + 14 + 10 + 6)/4 = 12.08$ SSW Approximate: $SSW \approx 120$

$$SSB: SSB = 6[(18.3 - 12.08)^2 + (14 - 12.08)^2 + (10 - 12.08)^2 + (6 - 12.08)^2]$$
➤ *ANOVA Table:*

Table 8 ANOVA

Source	SS	Df	MS	F
Between Groups	501.6	3	167.2	27.87
Within Groups	120	20	6	
Total	621.6	23		

• *Result* $F = 27.87$ $p < 0.0001$ (Highly significant)*H. Administration of Aqueous Extract for Albino Rats*

The aqueous extract of *Pongamia pinnata* flowers was prepared by dissolving 100 mg of extract in 10 mL of distilled water to obtain a stock solution of 10 mg/mL. The solution was mixed thoroughly to ensure uniformity and was freshly prepared before administration. Albino rats were weighed individually, and doses were calculated based on body weight. For example, for a rat weighing 200 g (0.2 kg), doses of 100

mg/kg, 200 mg/kg, and 400 mg/kg corresponded to 20 mg, 40 mg, and 80 mg of extract, respectively, which were administered as 2 mL, 4 mL, and 8 mL from the stock solution. The animals were divided into control and test groups. The extract was administered orally using a gastric gavage needle, while the control group received distilled water and the standard group received a reference drug. After administration, the rats were observed at regular intervals for changes in behavior. Locomotor activity was measured using an actophotometer, and parameters such as reduction in movement, sedation, and response to stimuli were recorded. A significant decrease in locomotor activity compared to the control group was interpreted as evidence of CNS depressant activity.

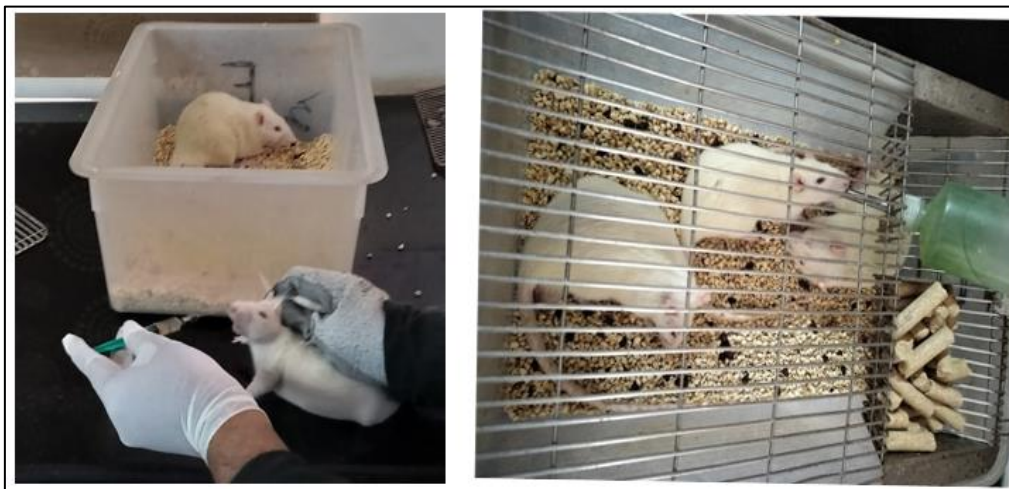
➤ *Effect of Cold Aqueous Extract on Rats:*

Fig 21 Effect of Cold Aqueous Extract on Rats

Table 9 Effect of Cold Aqueous Extract on Rats

GROUP	SLEEPING TIME(MIN)	OBSERVATION
Control	35	Normal
Low dose	50	Mild effect
Medium dose	65	Moderate Sedation
High dose	80	Significant Sedation

• *Interpretation:*

Cold aqueous extract showed gradual CNS depression, but the effect was milder, suggesting lower extraction of active constituents at room temperature.

➤ *Effect of Hot Aqueous Extract on Rats:*

Fig 22 Effect of Hot Aqueous Extract on Rats

Table 10 Effect of Hot Aqueous Extract on Rats

GROUP	SLEEPING TIME(MIN)	OBSERVATION
Control	36	Normal
Low dose	60	Mild effect
Medium dose	78	Moderate Sedation
High dose	92	Significant Sedation

- **Interpretation:**

Hot aqueous extract exhibited stronger sedative activity than cold extract, likely due to better extraction of phytoconstituents. The high dose showed near-standard effects, indicating improved efficacy.

V. RESULTS & DISCUSSIONS

The sedative potential of cold and hot aqueous extracts of *Pongamia pinnata* flowers was assessed using behavioral parameters such as locomotor activity and sleeping duration in experimental animals.

Animals treated with the extracts showed a noticeable decline in movement compared to the control group, suggesting depressant action on the central nervous system [6,7]. Reduced motor activity is widely considered a reliable

indicator of sedative or calming effects in behavioral studies [8]. The cold aqueous extract produced a mild effect, as indicated by a slight prolongation of sleep duration. In comparison, the hot aqueous extract demonstrated a stronger effect, with a clear increase in sleeping time and greater suppression of locomotion. This enhanced activity may be due to improved extraction efficiency of bioactive constituents at higher temperatures [5]. A clear dose-response relationship was evident, where increasing the dose resulted in progressively greater sedative effects. Such patterns are commonly reported in neuropharmacological evaluations using both zebrafish and rodent models [13,15]. An increase in sleep duration further confirms the depressant effect on the CNS, as this parameter is routinely used to assess sedative properties of test substances [9,10]. This may be due to better extraction of active phytoconstituents such as flavonoids and phenolic compounds at higher temperatures.

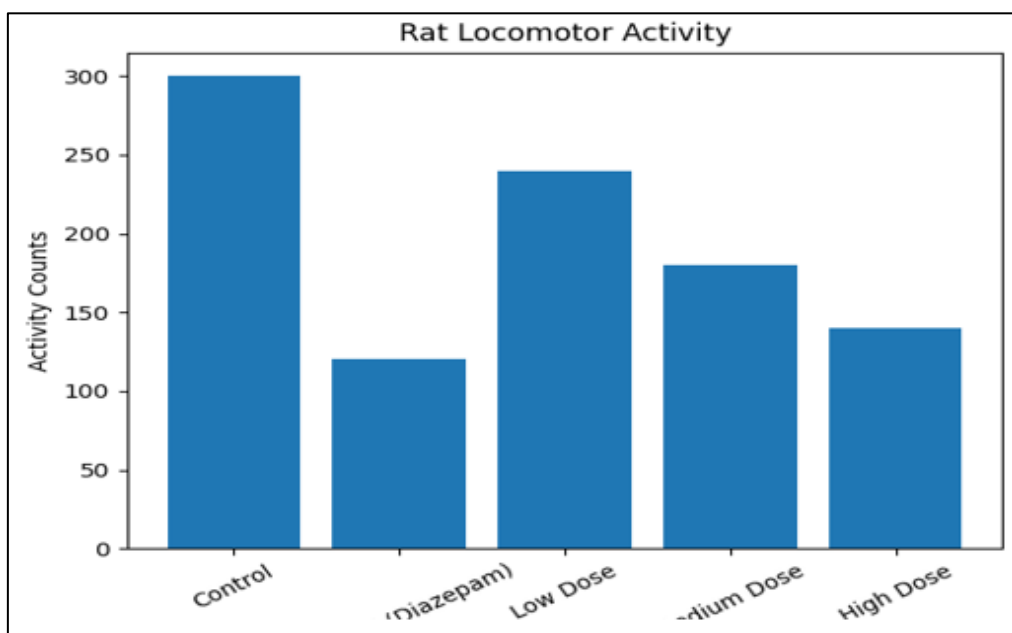


Fig 23 Rat Locomotor Activity

VI. CONCLUSION

The present study demonstrates that both ethanolic and aqueous extracts (hot and cold) of *Pongamia pinnata* flowers exhibit significant sedative activity across experimental models. In zebrafish, the extracts produced a noticeable reduction in locomotor activity, particularly under light–dark transition conditions, indicating CNS depressant/ sedative effects. Similarly, in albino rats, both hot and cold aqueous extracts led to decreased spontaneous movements and increased sleeping time, confirming their sedative potential. Among the extracts, the hot aqueous extract showed comparatively stronger sedative effects than the cold aqueous extract, suggesting that heat extraction may enhance the release of active constituents responsible for CNS depression. Overall, the findings support the traditional use of *Pongamia pinnata* in managing conditions related to restlessness and insomnia, and indicate that its phytoconstituents possess promising sedative properties.

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