

Evaluation of Central Nervous System Depressant and Sedative Activities of Cold, Hot and Ethanolic Extracts of Parijata Flowers Using Zebrafish Model

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Abstract: Parijata, which is also called night jasmine, usually blooms at nighttime. It is a medicinal tree or shrub that has been extensively used in the traditional medicinal systems, like the folk system of medicine and the Ayurvedic system. The shrub was used to treat neurological diseases due to the presence of bioactive phytoconstituents that possess therapeutic properties. The study evaluates the central nervous system depression and sedative activity of different extracts of parijata flowers in the zebra fish model. The zebrafish is susceptible to medications that act centrally, and they are suitable for easy assessment of locomotor activity in the current study. The fish were exposed to different extracts of parijata flowers, which were pretreated with caffeine to induce hyperactivity, which is a sign of CNS stimulation. The study findings showed a significant positive result. There is a noticeable reduction of caffeine-infused hyperactivity, suggesting depression and a sedative effect on the central nervous system. The behavioral responses of zebrafishes were observed by monitoring swimming speed, freezing behavior, and locomotor activity. This effect was extract-dependent, and bioactive compounds like flavonoids and iridoid glycosides are responsible for these activities, which act through modulation of the neurotransmitter system involved in locomotor activity. The study implies scientific support for the traditional medicinal use of parijata.

Keywords: *Parijata; CNS Depressant Activity; Sedative Activity; Zebrafish; Locomotor Behavior; Ethanolic Extract; Behavioral Study; Plant Extract; Neuroactive Compounds.*

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I. INTRODUCTION

Parijata is a small medicinal tree or shrub that belongs to the family Oleaceae and genus Nyctanthes. The plant is native to South Asia, and it is widely distributed across India and other Southeast Asian countries. It is commonly known as night-blooming jasmine, coral jasmine, and tree of sorrow. It grows in dry, deciduous forests and hillsides. The shrub grows up to 10m tall; the flowers are delicate, small, soft, and

star-shaped with petals arranged around an orange tube. The plant has gained attention due to its unique morphological characteristics and pleasant aroma. The orange dye from the corolla, nyctanthin, is used to dye silk. Its flowers, leaves, seeds, and bark have been investigated for various pharmacological and therapeutic properties [4,5,13,14]. Among its reported activities, the plant's central nervous system effects are particularly interesting, suggesting possible sedative and calming actions.[6]

➤ *Macroscopic Characters:*

Table 1 Macroscopic Characters of Parijata

PARAMETER	CHARACTERISTICS
Color	White petals with orange tubular center
Odor	Pleasant and aromatic
Taste	Slightly bitter
Shape	Star-shaped flowers with fused tubular base
Texture	Soft and delicate
Size	Small flowers measuring about 1.5–2 cm in diameter

The central nervous system regulates various activities like movement, alertness, sleep, and emotional behavior. These activities are depressed by plant extracts or synthetic drugs that usually produce a sedative effect by reducing locomotion and excitability. As a result, natural products are screened and tested for CNS depressant activity, which has become an important area of pharmacological research [3,10]. Medicinal plants are more valued since they offer safe and affordable alternatives to synthetic sedatives [5,12,13]. Zebra fish have become a popular model for neurobehavioral research as they are small, cost-effective, and easy to maintain. They are highly responsive to pharmacological agents. Its behavioral responses can be measured easily, so it's suitable for conducting depressant and sedative studies. The study uses caffeine-induced hyperactivity as a screening method to assess the activity of the plant extracts to counter CNS stimulation and produce sedation and depression [1,2,9,11].

Earlier studies have suggested that parijata possesses CNS depressant and tranquilizing properties [5,6,12,13]. Flowers of the parijata are considered to contain rich sources of bioactive phytochemicals like flavonoids, iridoids, and other secondary metabolites [4,5,12,13]. These compounds contribute to CNS depression by enhancing the GABA A receptor activity and modulating the neurotransmitter pathways. The present study aimed to observe whether the different extracts could reduce hyperactivity produced by the treatment of caffeine. However, more experimental evidence is needed to support the traditional claims and to understand the sedative potential of the flower extract in an appropriate animal model. The findings of this work may help in establishing the pharmacological basis of Parijata flowers and may contribute to the development of natural CNS-active agents [10].

II. METHADODOLOGY

➤ *Procurement of Plant Material:*

Fresh flowers of Parijata were collected during the flowering season from nearby cultivated plants. The plant material was carefully examined for purity and cleaned to eliminate unwanted dirt particles and contaminants.

➤ *Processing of Plant Material:*

The collected flowers were evenly spread on a sheet and shade-dried under ambient conditions to preserve their active phytoconstituents. Once fully dried, the flowers were powdered with an electric blender and sieved to achieve a

uniform coarse powder. The resultant powder was then stored in sealed containers for subsequent extraction procedures.



Fig 1 Collection of Flowers from Cultivated Plants



Fig 2 Day 1 of Drying



Fig 3 Day 15 of Drying



Fig 4 Dried Flower Ground into Powder



Fig 5 Powdered Drug

III. PREPARATION OF FLOWER EXTRACTS

➤ Cold Aqueous Maceration Extract

The powdered flower sample of required quantity was immersed in distilled water and kept undisturbed for 48 hours at room temperature with intermittent shaking. The mixture was filtered and the obtained filtrate was concentrated to produce the crude cold extract [4].



Fig 6 Cold Aqueous Extract

➤ Hot Aqueous Extract

About a few grams of flower powder were immersed in distilled water and heated at controlled temperature for a fixed duration of time. After cooling, the extract was filtered and concentrated for experimental applications [4].



Fig 7 Boiling of Extract



Fig 8 Hot Aqueous Extract

➤ Ethanolic Extract

The powdered material was placed in the Soxhlet apparatus with ethanol and water as solvents in 70:30 ratio. This solvent mixture is used for extraction purposes because it can extract a wide range of both polar and non-polar compounds. Continuous extraction was carried out until the solvent became colorless. The obtained extract was concentrated by evaporation and stored in an airtight container for further use [4,14].



Fig 9 Starting of Cycle



Fig 10 End of Cycle



Fig 11 Ethanolic Extract

IV. PRELIMINARY PHYTOCHEMICAL INVESTIGATION

➤ Test for Saponins:

- Procedure: 1ml of extract and 5ml of Distilled Water was shaken well for 10-15 sec
- Observation: Stable froth presence for 1min indicated presence of Saponins.

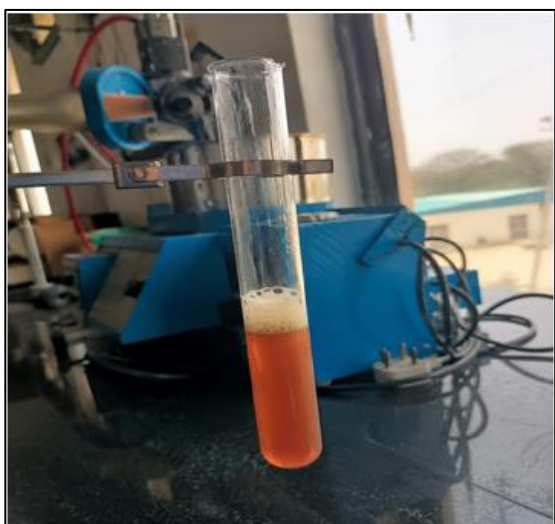


Fig 12 Saponins✓

➤ Tannin:

- Procedure: To 1ml of plant extract 5% 1ml ferric chloride was added and mixed.
- Observation: Appearance of Dark black color - presence of tannins

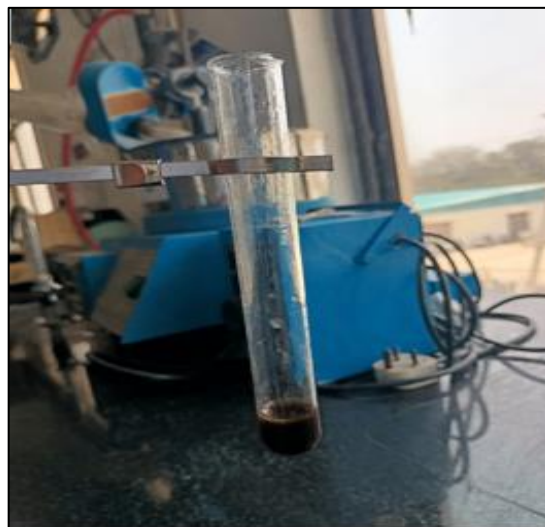


Fig 13 Tannins ✓

➤ Test for Flavonoid:

- Procedure: To 2ml of dilute NaOH, 50µl of sample was added.
- Observation: Yellowish/greenish colour indicates flavonoids present



Fig 14 Flavonoids ✓

➤ Test for Steroid & Terpenoid:

- Procedure: To 1 ml of extract, 2ml chloroform and 1ml of conc. HCL was added by sides of the test tube.
- Observation: Upper layer – reddish color- terpenoids present Lower layer - green fluorescence – steroids present



Fig 15 Steroid & Terpenoids ✓

➤ *Test for Phenol:*

- Procedure: 500µl plant sample, 2ml of NaOH solution
- Observation: Formation red color -presence of phenols

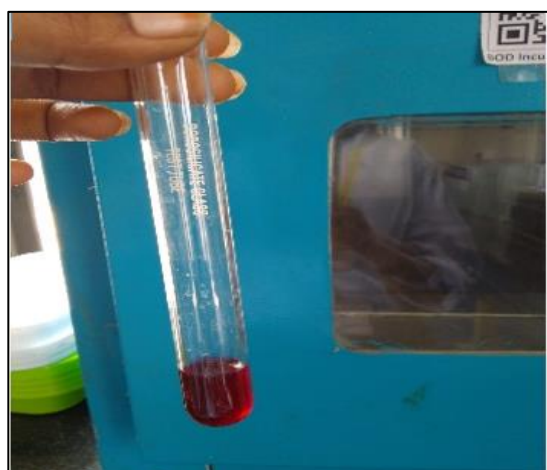


Fig 16 Phenol ✓

➤ *Test for Glycosides*

- Procedure- 1 ml extract, 1 ml glacial acetic acid, 1–2 drops of 1% FeCl_3 , few drops of conc. H_2SO_4 along the side of test tube.
- Observation: Brown ring at junction - glycosides present [4,5,13]



Fig 17 Glycosides ✓

➤ *Maintenance of Experimental Animals*

Adult zebrafish were selected for neurobehavioral evaluation due to their sensitivity towards CNS-active compounds. The fishes were housed in aerated tanks containing dechlorinated water under standardized environmental conditions [2,8,11].

• *Laboratory Conditions:*

- ✓ Water temperature maintained at 26–28°C
- ✓ Continuous aeration provided
- ✓ Controlled photoperiod maintained
- ✓ Fish fed twice daily with commercial feed
- ✓ Prior to experimentation, zebrafish were acclimatized to the laboratory environment for one week.

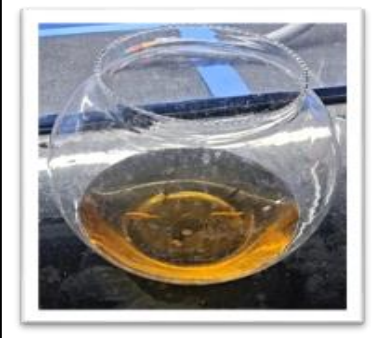
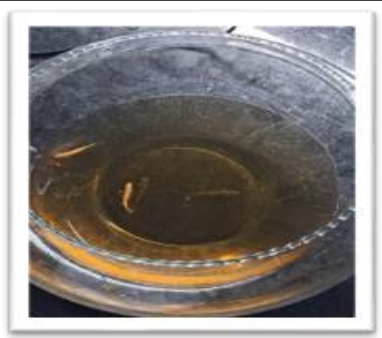
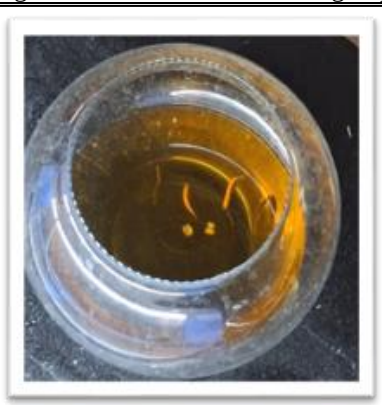
• *Experimental Protocol:*

Zebrafish were divided into the following groups:

Table 1 Grouping of Animals

Group	Treatment	
Group I	Normal control	

Fig 18 Normal control group

Group II	Caffeine-treated control	 Fig 19 Caffeine-treated control group
Group III	Caffeine + Cold extract	 Fig 20 Caffeine & Cold extract group
Group IV	Caffeine + Hot extract	 Fig 21 Caffeine & Hot extract group
Group V	Caffeine + Ethanolic extract	 Fig 22 Caffeine & Ethanolic extract group

➤ **Procedure:**

After grouping the animals, caffeine solution was prepared freshly in aquarium water. Aquarium water was selected because the fish are adapted to it. Zebrafish were exposed to caffeine solutions for a fixed duration of time to induce hyperactive behavior. After caffeine administration,

the fish exhibited increased swimming activity, rapid locomotor movements, enhanced exploration, and increased responsiveness. This is a sign of hyperactivity in fish resembling CNS stimulation. Following induction of hyperactivity, zebrafish from the caffeine treatment groups were transferred into separate tanks containing different

Parijata flower extracts, such as hot, cold, and ethanolic. The fish were observed for a fixed period, and their behavioral responses were carefully recorded.

Parameters such as swimming speed, locomotor activity, distance travelled, exploratory behavior, resting

duration, freezing response, and reaction to external stimuli were monitored throughout the experiment. Reduction in caffeine-induced hyperactivity and locomotor activity, along with prolonged resting behavior and reduced responsiveness after treatment with the extracts, were considered indicators of CNS depressant and sedative activity.

➤ *Observations:*

Table 2 Parameters Observed

PARAMETER OBSERVED	NORMAL GROUP	CAFFEIN TREATED	COLD EXTRACT	HOT EXTRACT	ETHANOLIC EXTRACT	STANDARD
Swimming speed	Normal	Very high	Slightly reduced	Moderately reduced	Markedly reduced	Highly reduced
Locomotor activity	Normal	Increased	Mild decrease	Moderate decrease	Significant decrease	High decreased
Exploratory behavior	Normal	Hyperactive	Reduced slightly	Reduced	Strongly reduced	Significant reduction
Resting duration	Normal	Very less	Mild increase	Moderate increase	Maximum increase	Maximum increase
Sedative behavior	Absent	Absent	Mild	Moderate	Strong	Very strong
CNS depressant effect	Absent	Absent	Mild	Moderate	Significant	Highly Significant

V. RESULTS

➤ *Swimming Speed [Distance Travelled] [Cm/Min]:*

Table 3 Swimming Speed of Fishes

GROUP	FISH 1	FISH 2	FISH 3	FISH 4	FISH 5	FISH 6	Mean $\pm$ SD (s/min)
Control	188	192	195	190	194	193	192.0 $\pm$ 2.6
Caffeine	342	350	348	355	346	349	348.3 $\pm$ 4.4
Diazepam	100	106	112	109	114	107	108.0 $\pm$ 5.0
HAE	158	162	166	164	161	163	162.3 $\pm$ 2.
CAE	145	149	151	154	148	153	150.0 $\pm$ 3.4
Ethanolic Extract	120	124	128	131	126	127	126.0 $\pm$ 3.7

➤ *ANOVA of Swimming Speed (cm/min)*

Table 4 ANOVA of Swimming Speed

Source of Variation	Sum of Squares (SS)	df	Mean Square (MS)	F-value	P-value
Between Groups	226,709.90	5	45,341.98	480.3	<0.0001
Within Groups	2,831.66	30	94.39	-	-
Total	229,541.56	35	-	-	-

The effect of parijata flower extracts on the swimming speed of zebrafish is presented in Table 8.1. The control group exhibited a mean swimming speed of 192.0 $\pm$ 2.6 cm/min. The caffeine-treated group showed a significant increase in swimming speed (348.3 $\pm$ 4.4 cm/min) compared to the control, indicating enhanced locomotor activity. In contrast, the diazepam-treated group exhibited the lowest swimming speed (108.0 $\pm$ 5.0 cm/min), confirming its CNS depressant effect. Among the extract-treated groups, the ethanolic extract produced the greatest reduction in

swimming speed (126.0 $\pm$ 3.7 cm/min), followed by the hot aqueous extract (150.0 $\pm$ 3.4 cm/min) and the cold aqueous extract (162.3 $\pm$ 2.9 cm/min).

One-way analysis of variance (ANOVA) revealed a highly significant difference among the six experimental groups ($F(5,30) = 480.37$, $P < 0.0001$), indicating that the treatments significantly affected locomotor activity in zebrafish.

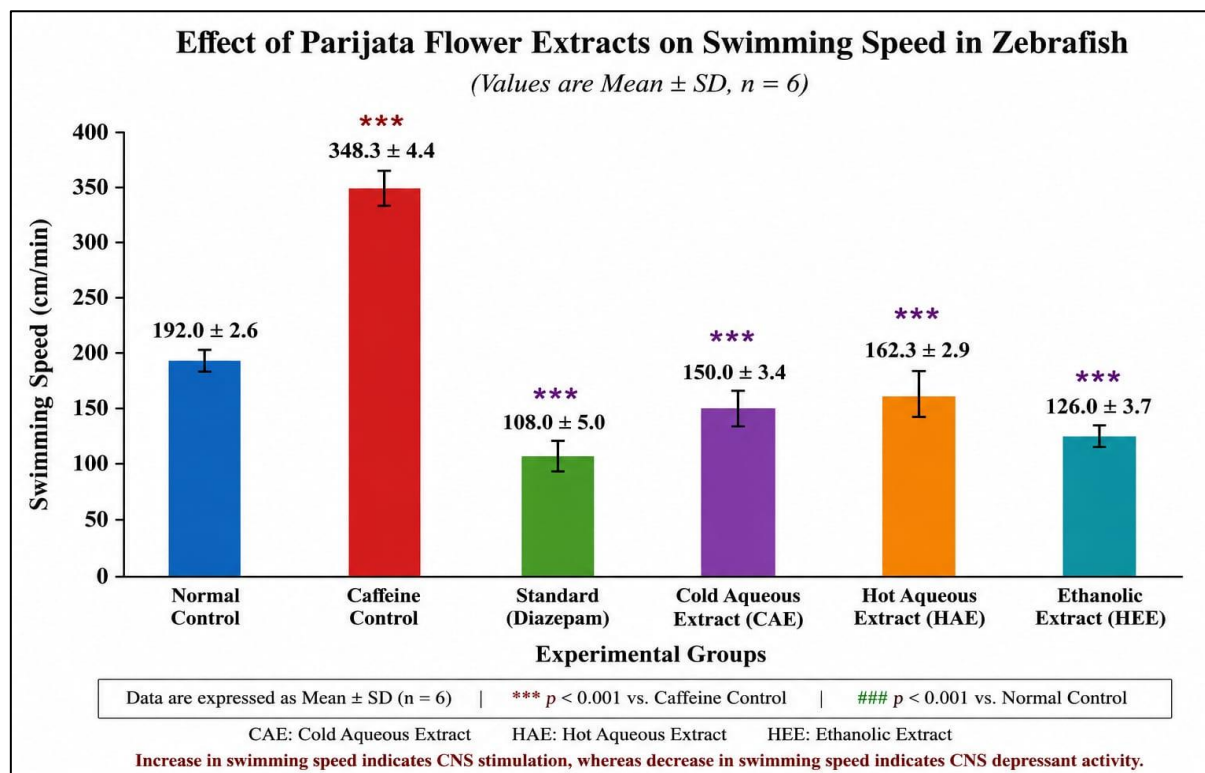


Fig 23 Effect of Parijata Flower Extract on Swimming Speed in Zebrafishes.

➤ Freezing Time [Sec/Min]:

Table 5 Freezing Time

GROUP	FISH 1	FISH 2	FISH 3	FISH 4	FISH 5	FISH 6	Mean $\pm$ SD (s/min)
Control	4	5	4	3	5	4	4.2 $\pm$ 0.8
Caffeine	1	2	1	2	1	2	1.5 $\pm$ 0.5
Diazepam	9	13	10	12	14	9	11.2 $\pm$ 2.1
HAE	7	8	6	9	8	9	7.8 $\pm$ 1.2
CAE	5	6	5	7	6	6	5.8 $\pm$ 0.7
HEE	8	10	12	9	11	9	9.8 $\pm$ 1.6

➤ ANOVA of Freezing Time {sec/min}

Table 6 ANOVA of Freezing Time

Source of Variation	Sum of Squares (SS)	df	Mean Square (MS)	F-value	P-value
Between Groups	391.557	5	78.311	49.28	< 0.0001
Within Groups	47.665	30	1.589	-	-
Total	439.222	35	-	-	-

The effect of parijata flower extracts on freezing time is presented in Table 8.3. The control group exhibited a freezing time of 4.2 $\pm$ 0.8 s/min, whereas the caffeine-treated group showed the lowest freezing time (1.5 $\pm$ 0.5 s/min), indicating increased locomotor activity. The diazepam-treated group demonstrated the highest freezing time (11.2 $\pm$ 2.1 s/min), confirming its sedative effect. Among the extract-treated groups, the ethanolic extract produced the highest freezing time (9.8 $\pm$ 1.6 s/min), followed by the hot aqueous extract

(7.8 $\pm$ 1.2 s/min) and the cold aqueous extract (5.8 $\pm$ 0.7 s/min)

One-way analysis of variance (ANOVA) demonstrated a highly significant difference among the six experimental groups (F(5,30) = 49.28, P < 0.0001), indicating that the treatments significantly influenced freezing behavior in zebrafish.

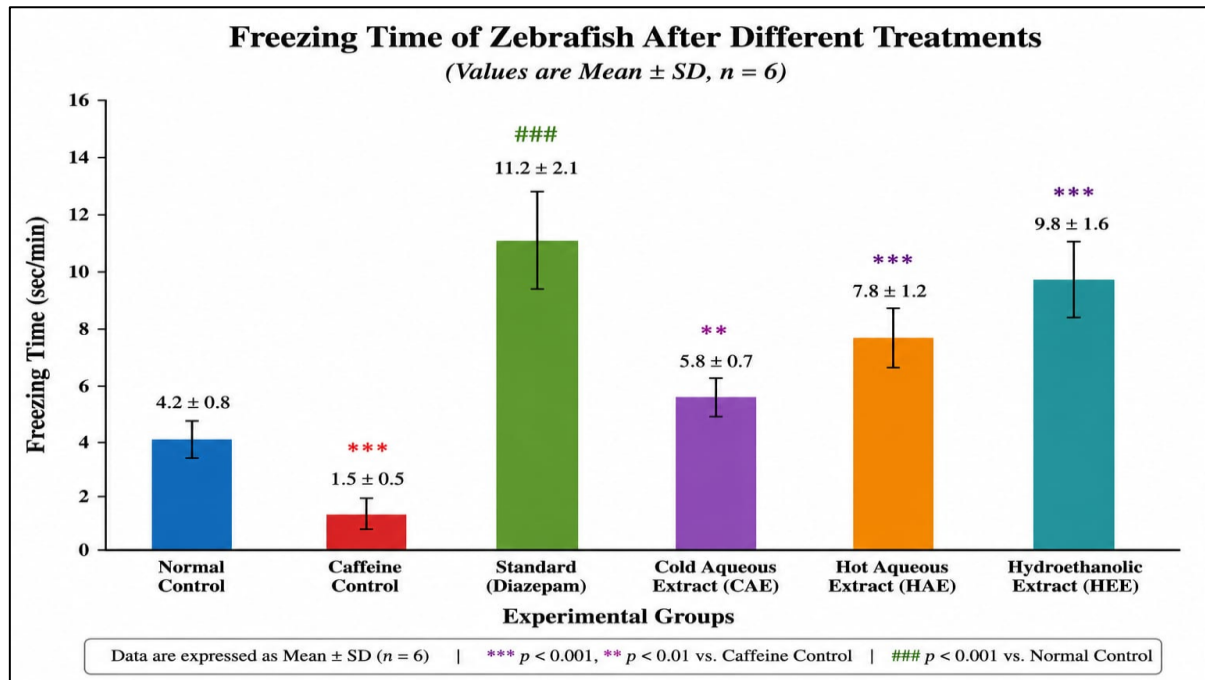


Fig 24 Freezing Time of Zebrafish After Different Extract Treatments

➤ Tail and Head Movement:

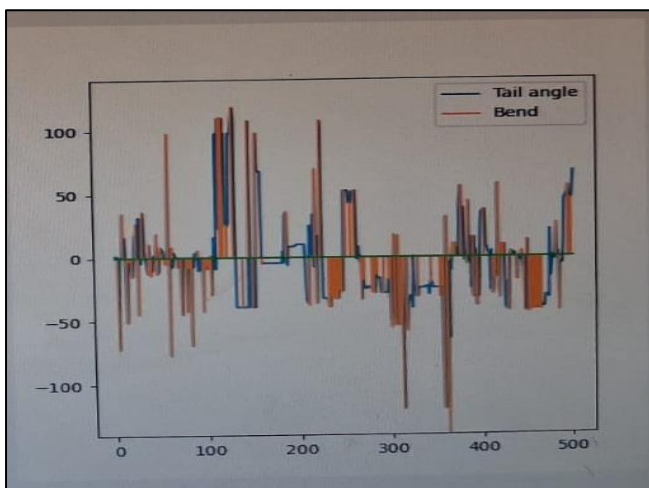


Fig 25 Control Group

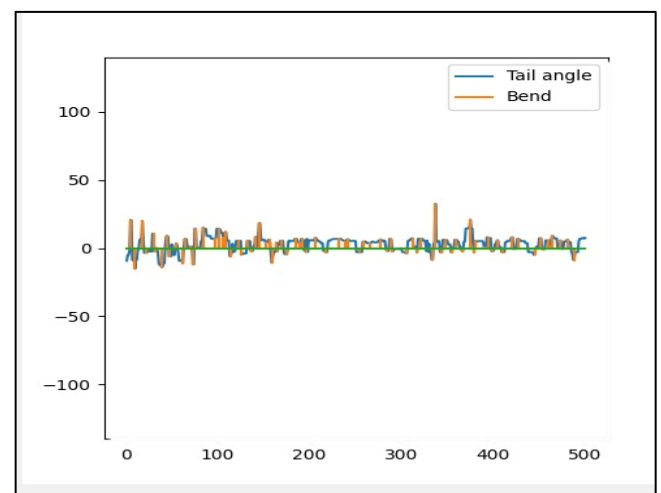


Fig 27 Diazepam (Standard)

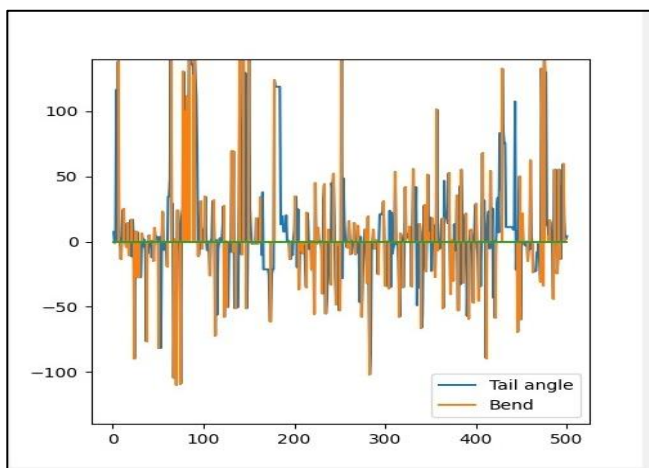


Fig 26 Caffeine Treated Group

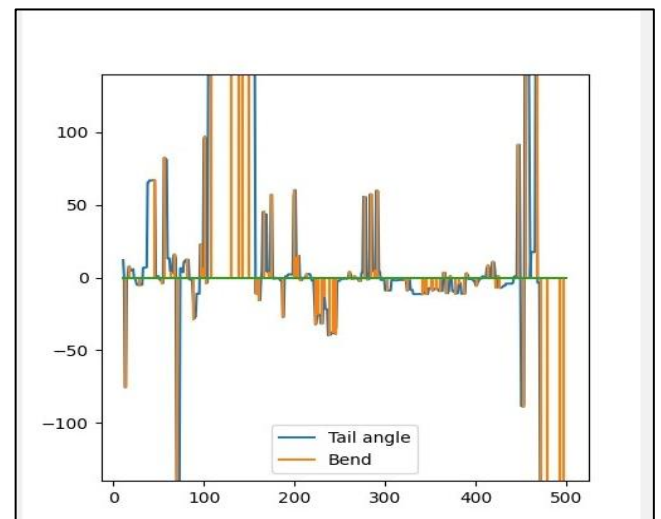


Fig 28 CAE

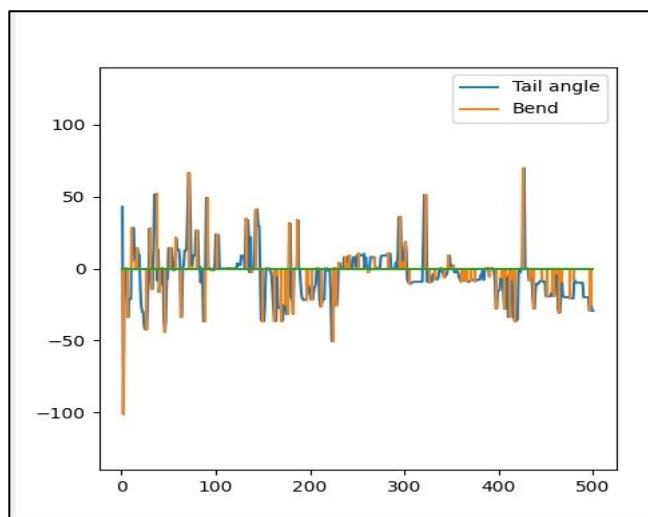


Fig 29 HAE

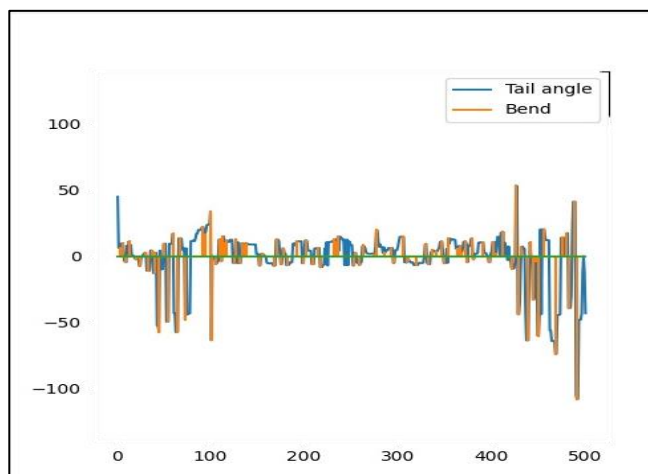


Fig 30 Ethanolic Extract

VI. DISCUSSIONS

Caffeine infused hyperactivity methods is the most suitable method to perform CNS depressant and sedative activity [1,2,7,8]. The present study was conducted to evaluate the depressant and sedative activities of hot, cold, ethanolic extracts of Parijata flower using caffeine infused hyperactivity.

Caffeine infusion produced enhanced locomotor activity, increased swimming speed and rapid exploratory behavior conforming the stimulation of CNS. [1,7,8] Treatment with extracts exhibited reduction in the swimming speed, reduced locomotor activity indicating depression. Among the treated groups ethanolic showed significant effect followed by hot and cold extracts. The extracts have also showed sedative effect in the fishes. The treated groups showed prolonged resting time, calming behavior, delayed responses to external stimuli. The ethanolic extract produced a more efficient sedative effect which may be a attribute to effective extraction of bio constituents.

The reduction in caffeine-induced hyperactivity suggests possible modulation of central nervous system pathways responsible for stimulation and alertness [3,10]. The hot extract demonstrated moderate activity whereas the cold extract showed comparatively milder effects, indicating that the extraction method influences recovery of active constituents.

The superior activity of ethanolic extract may be due to its ability to extract both polar and moderately non-polar phytoconstituents more effectively [4,14]. The findings of the present study support the traditional medicinal use of Parijata flowers in calming and sleep-related disorders [5,6,12,13]. The study also confirms the usefulness of zebrafish as an effective experimental model for neurobehavioral evaluation of herbal extracts [2,3,11].

VII. CONCLUSION

The present investigation demonstrated that the flower extracts of Parijata possess significant CNS depressant and sedative activities in zebrafish models. Administration of the extracts effectively reduced caffeine-induced hyperactivity and produced calming behavioral responses in treated fishes. Among the different extracts evaluated, the ethanolic extract showed the highest CNS depressant and sedative activity followed by hot extract and cold extract.

The observed effects may be attributed to the presence of neuroactive phytoconstituents such as flavonoids, alkaloids, phenolic compounds, and terpenoids. The study suggests that Parijata flowers may serve as a potential natural source for the development of herbal CNS depressant and sedative agents with possible therapeutic application in stress, insomnia, and neurological disorders [4,13,15].

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