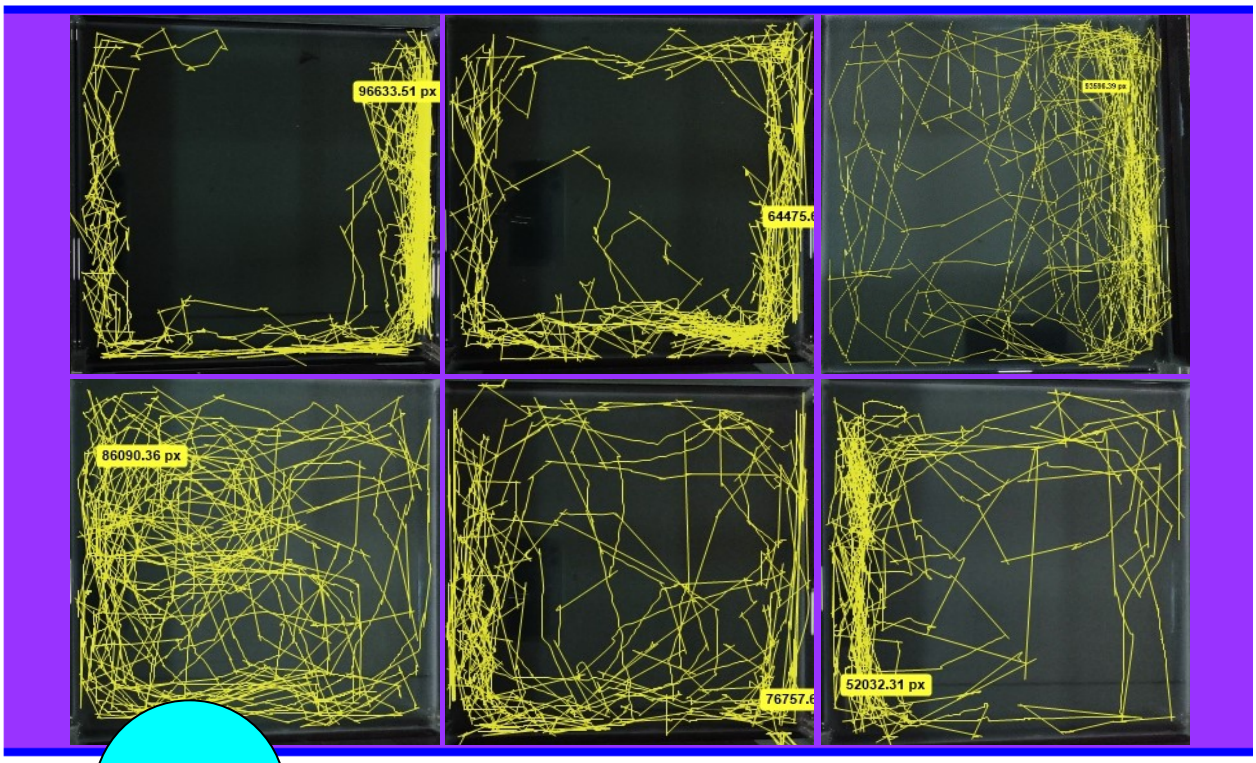

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Neurobehavioral assessment of seed extract of *Swietenia mahagoni* on zebrafish model

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Abstract

Traditional medicine in tropical areas often includes the use of *Swietenia mahagoni* to treat a wide range of conditions. However, preclinical testing is crucial to ensure their safety profile and address any remaining concerns about their efficacy. The zebrafish model of open field test shows that decreased dopamine and cholinergic transmission causes impaired locomotion and diminished exploratory behavior. In addition to the elevation of 5-HT and GABA reported in the mirror bite test findings, the extract may have suppressed dopamine expression. The mirror-bite test and the light-dark test both showed elevated levels of GABA expression. Findings from the predator avoidance test are very suggestive of a neurotransmitter-regulating action of the extract.

Introduction

Zebrafish (*Danio rerio*) has become a prominent model organism in the fields of developmental biology and neurodegenerative disease modeling due to its numerous advantages. One of the key reasons for its popularity is the high degree of genetic and functional similarity between zebrafish and humans (Ablain and Zon, 2013). These disorders can arise from various conditions such as traumatic brain injury, stroke, multiple sclerosis, dementia, neuro-oncological conditions, and metabolic or encephalopathic impairments. Neurobehavioral disorders encompass a range of behavioral disabilities that result from these brain diseases (Firdous et al., 2012). Traumatic brain injury is often associated with significant behavioral problems. These can include changes in mood, cognition, personality, and social behavior (McAllister, 2008).

Swietenia mahagoni (commonly known as mahogany) is indeed a large, deciduous timber tree belonging to the Meliaceae family. It is primarily grown in tropical areas like India, Malaysia, and Southern China. It is indigenous to the West Indies. *S. mahagoni* is highly valued for its timber, which is used in various industries, including furniture, cabinetry, and boat building (Sahgal et al., 2009). Additionally, the tree has a long history of traditional medicinal use in Africa. They have been claimed to have therapeutic efficacy for the therapy of intestinal parasitism, amoebiasis, cancer, malaria, diabetes, hypertension, and other conditions (Eid et al., 2013). However, it is important to note that the scientific evidence supporting the safety and efficacy of these traditional uses is lacking. Determining the toxicity of *S. mahagoni* crude methanolic seed extract (SMME) in brine shrimp and zebrafish models is the purpose of the current investigation. Utilizing experimental paradigms of anxiety and depression, SMME was examined to assess the anxiolytic and antidepressant efficacy at two distinct dosages in Swiss Albino male mice (Sahgal et al., 2010). According to reports, *S. mahagoni* is used for a variety of traditional medical purposes. The current study was done to exa-

mine this plant's *in vitro* antioxidant and antibacterial properties.

The literature review reveals that the *S. mahagoni* extract was investigated to evaluate its antianxiety and antidepressant activity, and antidiabetic activity (Panda et al., 2010). However, there is no report of the effect of this plant on neurobehavioral aspects in aquatic models. Hence, the objective of this current investigation aimed to assess the impact of the methanolic extract derived from *S. mahagoni* seeds on lethality and neurobehavioral features in brine shrimp and zebrafish models, respectively.

Materials and Methods

Plant materials

S. mahagoni seeds were obtained from Paschim Medinipur, West Bengal. Before drying, the seeds have been rinsed with tap water to eliminate dirt. Cut into little pieces, the seeds were dried at 40ºC for a week. A blender was used to turn the seeds into powder.

Extract preparation

Maceration with methanol was used to extract the powdered seeds for four days. After filtering the extract with Whatman filter sheets, the filtrate was collected and concentrated in a rotary evaporator at 40ºC. Three days were spent drying the concentrated extract in a 40º C oven before it was placed in the fridge for later usage (Abubakar et al., 2020).

Experimental animal

Wild-type zebrafish were used for the experiment and they were maintained under controlled conditions of temperature at 26-28.5°C, with a 14:10 hour (light: dark). They were placed in a circulatory system to continuously filter and ventilate the water in the system to maintain the quality of water required for a healthy aquatic environment. Water changes and extracts were given every 24 hours (Maximino et al., 2012).

Oral acute toxicity study

According to the OECD 203 guidelines, the fish are exposed to the test chemical for 96 hours while in static, semi-static, or flow-through settings, and mortalities are then recorded. The concentrations needed to kill 50% of the fish (LC_{50}) are identified whenever feasible (Magyary, 2018).

Grouping of animals

Fish of Group I received a normal diet and were kept in fresh water. Fish of Group II were exposed to the extract (10 mg/L). Fish of Group III were exposed to the extract (20 mg/L).

Behavioral analysis

Open field test

The open field test is a common experimental method for studying animals' curiosity and emotions, especially in rats and fish. Locomotion, exploration, anxious-like behavior, and reactions to unexpected environments can all be better understood by analyzing several factors. Thigmotaxis, in which subjects gravitate towards the arena's walls or boundaries, has been observed in both adult zebrafish and rodents. There's speculation that this reflects an innate need for safety and protection when confronted by a novel setting. It has been linked to anxious feelings and the desire to keep out of danger as much as possible. Using the open field test in zebrafish research has been a boon to our understanding of the neurobiology behind behaviors as diverse as curiosity and fear in a genetically tractable organism (Souza et al., 2021).

Here's a step-by-step procedure for conducting the open field test in zebrafish:

A minimum of 5 min and up to 10 min should be allotted before testing begins so that the zebrafish can become acclimated to the test environment. This eases tension and makes it easier for them to adapt to their new surroundings. To conduct your experiment, each zebrafish should be moved from its holding tank to the center of the testing tank in its container or net. The zebrafish should be recorded while being observed for a set amount of time, usually 5 min. Automatic video-tracking devices can collect data about the fish. Endpoints include time spent in the periphery or center of the tank (s), distance travelled in each zone, and velocity.

Video-tracking software (Kinovea version 0.9.5) (Alzweiri et al., 2022) is used to automatically analyze and record these endpoints on days 0, 7, and 14 as manual observations may be impractical due to the high throughput and large amount of data generated (Souza et al., 2021).

Mirror biting test

The mirror bite test, also known as the mirror image stimulation test, is a typical behavioral assay used to evaluate aggressive or territorial zebrafish behavior in scientific studies. Zebrafish are made hostile when they are shown their reflection in a mirror (Ogi et al., 2021).

Generally, this is how you should go about performing a mirror bite test: Prepare a tank or aquarium with clear walls and fill it with water to an appropriate level for zebrafish. Attach a mirror to one side of the tank, facing inward. Ensure that the mirror is securely fixed and positioned at an angle that allows the zebrafish to see their reflection. Allow the zebrafish to acclimate to the tank and the presence of the mirror for a while, typically at least 5 min to 10 min, to reduce stress and habituate them to the environment.

Transfer one zebrafish at a time into the tank, and observe the zebrafish's behavior for 5 min when they encounter their reflection in the mirror. Use video recording to document their responses on days 1, 7, and 14. Responses include mirror biting, rapid movements, etc (Ogi et al., 2021).

Light-dark preference test

The light-dark test is a behavioral assay commonly used in zebrafish research to assess anxiety-like behaviors and light/dark preference. It involves exposing zebrafish to a tank divided into two distinct zones - one brightly lit and the other dark to observe their behavior in response to different light conditions (Maximino et al., 2012).

Here is a general procedure for conducting the light-dark test: Prepare a tank or aquarium divided into two compartments - one brightly lit (light zone) and the other dark (dark zone). Allow the zebrafish to acclimate to the experimental room and holding tanks for some time (e.g., 5 min to 10 min) to reduce stress and allow them to adjust to the environment. Transfer one zebrafish at a time into the test tank, and observe the zebrafish's behavior when placed in the light-dark tank.

Use video recording to document their responses on days 0, 7, and 14. Record the time the zebrafish spends in each zone—the light zone and the dark zone—during a specific observation period, typically 5 min. This indicates their preference for light or dark environments. Responses include transitions between zones, freezing behavior, and time spent in the light zone (Maximino et al., 2012).

Predator avoidance test

The zebrafish predator avoidance test is a commonly used experimental paradigm in behavioral neuroscience to study the escape responses and predator avoidance behaviors of zebrafish. Zebrafish are small freshwater fish native to South Asia and are widely used as a model organism in scientific research. The predator avoidance test typically involves exposing zebrafish to a simulated predatory threat or a real predator in a controlled laboratory setting. The purpose of the test is to observe and analyze the fish's behavioral responses and assess their ability to detect and evade potential predators (Gerlai, 2010).

Here is a general procedure for conducting the light-dark test: Ensure that the zebrafish are acclimated to their environment for a sufficient period (5-10 min) before the experiment. Prepare a testing tank or a divided section within the main tank to create a choice or avoidance scenario. Decide on the type of predator stimulus you will use such as cichlids (e.g., *Nile tilapia*), pufferfish, or catfish. Introduce the predator stimulus into the tank or the divided section. Ensure that the predator stimulus is visible to the zebrafish but cannot physically harm them.

Transfer one zebrafish at a time into the test tank, and observe the time spent in the approach zone for 5 min. Use video recording equipment or automated tracking systems to monitor their movements on days 0, 7, and 14 (Bass and Gerlai, 2008).

Open field test, mirror biting test, light-dark preference test, predator avoidance test

Statistical analysis

The findings are presented as a mean ± S.E.M. GraphPad Prism (v3.1) (Lama et al., 2014), which was used to examine the data. One-way analysis of variance (ANOVA) was used for comparison across all groups, with Dunnett's post hoc test serving as a secondary analysis. Statistical significance was assumed at $p \leq 0.05$.

Results

Zebrafish model

The zebrafish were exposed to the extract for 14 days to assess the behavioral changes in the animals if any. In the study, no mortality was observed at a dose of 100 and 200 mg/L, indicating that the extract was non-toxic. The lethal dose to kill 50% of experimental fishes is more than 100 and 200 mg/L at 96 hours. exposure period. The extract was exposed for 14 days at the dose of 10 mg/L ($1/20^{th}$ of 200 mg/L; PGLE) and 20 mg/L ($1/10^{th}$ of 200 mg/L; PGLE), the conclusions gained from the lethality assay performed on brine shrimp are supported by these findings. No mortality was observed during the study period.

Open field test

After comparing the data of extract-exposed group with the normal control group, it was found that exposure to the extract at both experimental doses increased the total distance traveled as well as exploratory activity prominently on days 7 and 14. The extract (10, 20 mg/L) had shown a significant decrease in the total distance traveled on days 7 and 14 (Table I; Figure 1).

Mirror biting test

Zebrafish exposed to the extract at both experiment doses (10 mg/L and 20 mg/L) showed a significant decrease in number of attempts for mirror biting on day 14 compared to the control group (Table I).

Light-dark model

As per the literature in the light-dark model, fish find comfort in the dark compartment when fish is anxious, in pain, or fear, and time spent or exploration in the light compartment is an indication of boldness or else reduction of pain and anxiety.

On day 7, the finding discovered that zebrafish given a

larger dose of the extract (20 mg/L) spent significantly more time in the light. On day 14, extract exposure boosted explorer behavior. On days 7 and 14, the extract (10 and 20 mg/L) significantly increased the amount of time exposed to light. Table I and Figure 2 both show an uptick in adventuring. Similar results have been seen with the extract at doses of 10 and 20 mg/L, with much less time in the dark on days 7 and 14.

Predator avoidance test

Zebrafishes treated with the SMME at the dose of 10 and 20 mg/L showed a significant increase in time spent in the approach zone when compared to the control group throughout the study period (Table I; Figure 3), indicating a decrease in anxiety and an increase in the boldness.

Discussion

To evaluate the efficacy of any medicinal plant, the first step is to ascertain the nature and significance of any adverse effects, as well as the neurobehavioral level at which these effects first become apparent (Sofowora et al., 2013). Upon conducting a comparative analysis between the obtained results and those derived from the control group, it was observed that the exposure to the extract at the experimental doses led to a significant reduction in both the total distance travelled and the level of exploratory activity. According to Maqbool et al. (2019), an increase in dopaminergic activity has been observed to enhance exploration and locomotor activity in the open field test. The exploration behavior of zebrafish is observed to decline when there is a blockade of dopaminergic activity or receptors. According to Prut and Belzung (2003), there is evidence

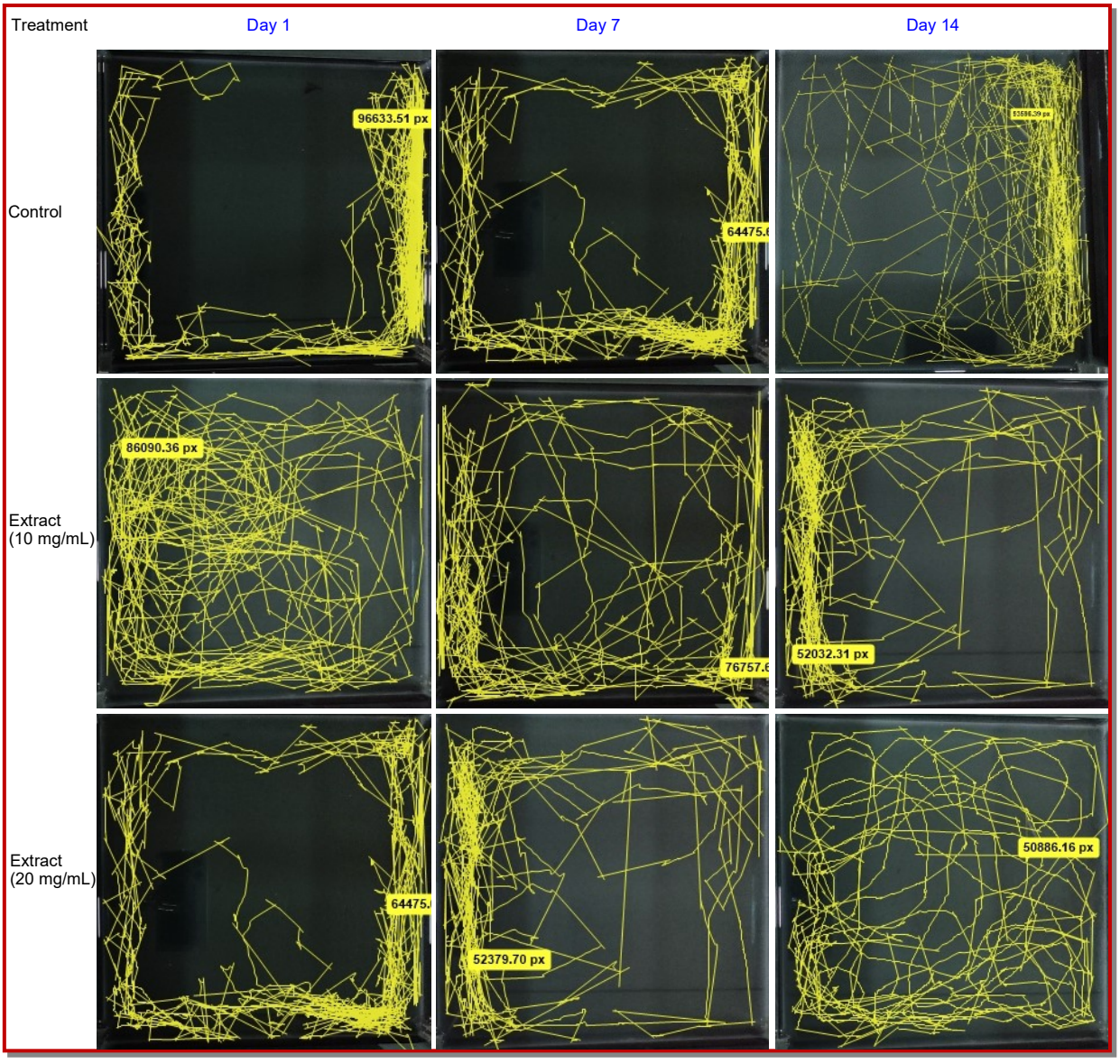

Figure 1: Video-graphic analysis of days 1, 7, and 14 showing total distance travelled in open field arena in pixels (px) using ki-novea software

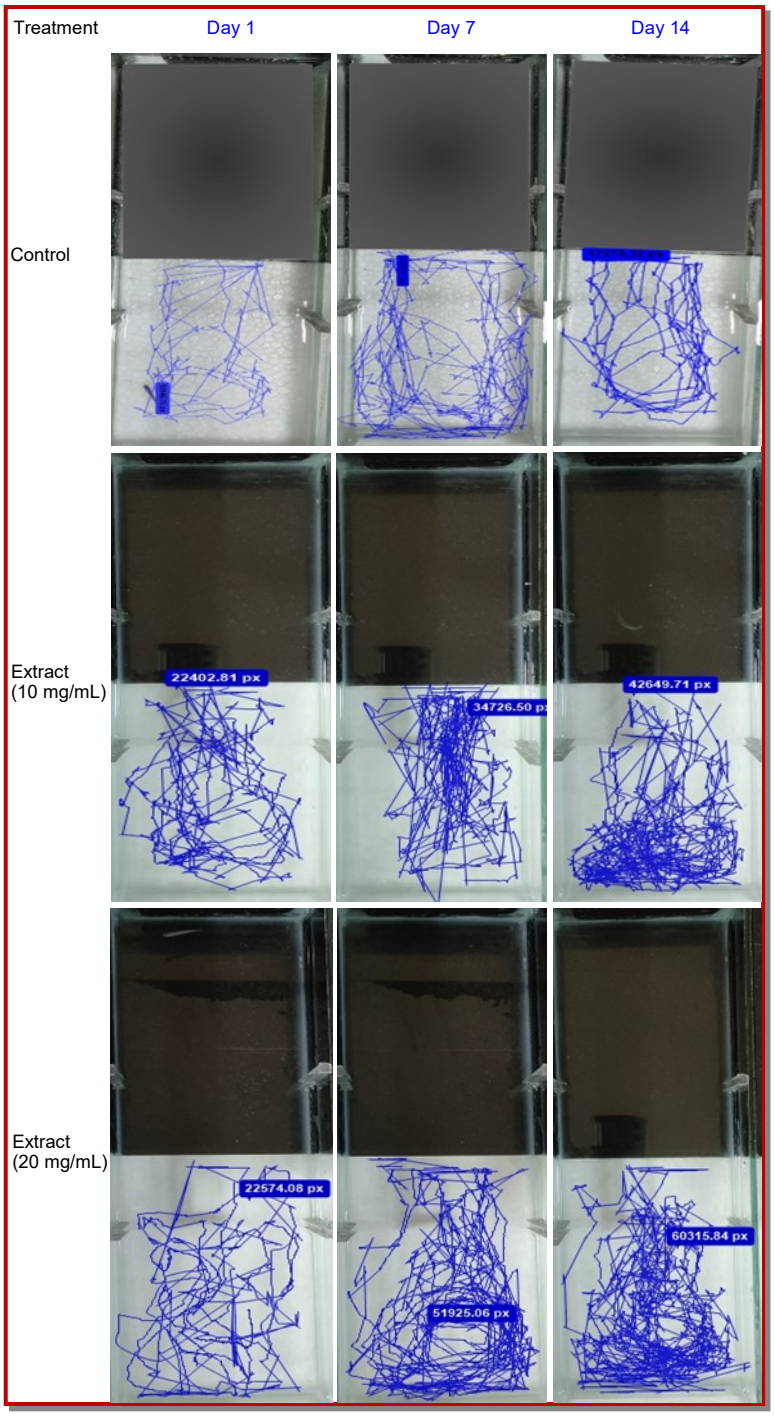

Figure 2: Video-graphic analysis of days 1, 7, and 14 showing total distance traveled in the light-dark model in pixels (px) using Kinovea software

to suggest that glutamatergic activity can both enhance and inhibit exploration. The extract may exert regulatory effects on neurotransmitter systems, neuronal signaling pathways, and gene expression, as evidenced by the observed associations between reduced locomotor activity and exploratory behavior, decreased dopamine and acetylcholine signaling, and alterations in the expression of genes involved in neuronal plasticity.

After 14 days, it was noted that zebrafish exposed to the extract at both experimental doses showed a significant reduction in their tendency to engage in biting behavior toward the mirror. Nevertheless, following this time frame, the frequency of attempts to bite the mirror increased to a degree that was similar to that observed in the control group. The provided data indicate that the extract under investigation can reduce dopamine expression while concurrently augmenting the expre-

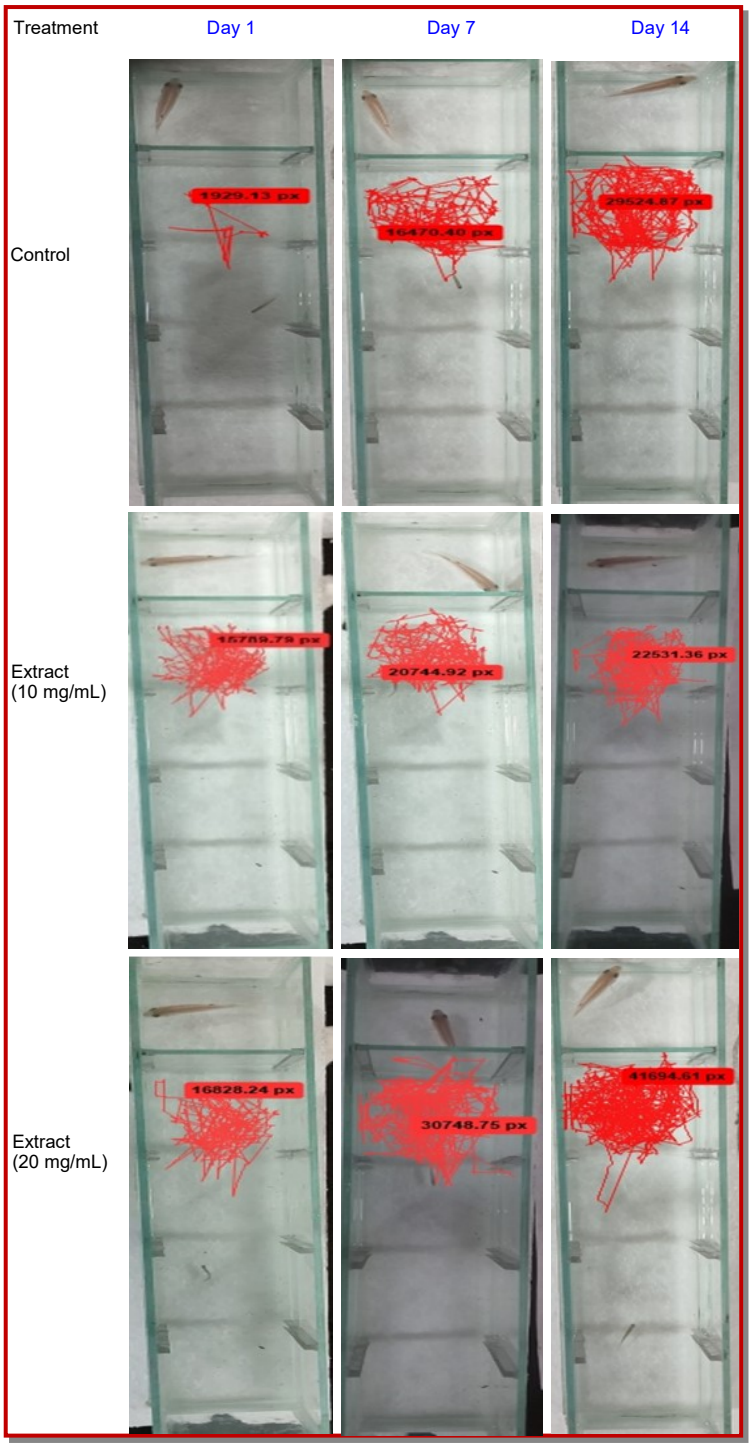

Figure 3: Video-graphic analysis of days 1, 7, and 14 showing total distance traveled in predator avoidance model in pixels (px) using Kinovea software

ssion of 5-HT and GABA. The modulation of aggressive behavior in zebrafish has been attributed to the dopamine, 5-HT, and GABA systems (Jones and Norton, 2015). Specifically, heightened dopamine signaling in the brain has been linked to escalated aggressive behavior, whereas augmented expression of GABA and 5-HT is associated with reduced aggression (Liu et al., 2021). Further investigation is necessary to understand the underlying processes of this activity.

The results of the light-dark paradigm demonstrate a significant rise in the duration of light exposure for the animals subjected to the extract at both experimental dosages. The association between heightened dopaminergic activity and a reduced scotaxis preference, as well as an enhanced preference for the light side in the light-dark test, has been established. The study conducted by Mhalhel et al. (2023) has shown that an inclination towards the light side in the light-dark test,

Table I

Effect of *S. mahagoni* extract on different parameter of zebrafish

Names	Day 1	Day 7	Day 14
Total distance travel open field (cm)			
Control	1926.8 ± 4.6	1935.9 ± 5.2	1941 ± 6.4
Extract (10 mg/L)	1932.2 ± 4.4	1862.4 ± 5.3[b]	1746.9 ± 8.3[b]
Extract (20 mg/L)	1915.9 ± 9.0	1837.5 ± 8.6[b]	1776.1 ± 9.4[b]
Time spent in approach zone (sec)			
Control	236.7 ± 2.9	222.3 ± 3.0	218.4 ± 2.8
Extract (10 mg/L)	232.6 ± 2.4	167.6 ± 6.9	142.9 ± 6.7[b]
Extract (20 mg/L)	228.8 ± 2.4	154.6 ± 1.7	131.6 ± 1.2[b]
Time spent in light (sec)			
Control	76.8 ± 6.8	95.7 ± 5.0	133.5 ± 6.45
Extract (10 mg/L)	84.6 ± 2.3	103.9 ± 8.3	167.0 ± 7.26[a]
Extract (20 mg/L)	87.4 ± 5.9	122.6 ± 3.59[a]	165.9 ± 9.9[a]
Time spent in dark (/sec)			
Control	218.2 ± 6.9	205.65 ± 2.36	166.8 ± 4.2
Extract (10 mg/L)	210.5 ± 4.6	196.03 ± 8.42	132.2 ± 8.5[a]
Extract (20 mg/L)	208.3 ± 7.6	177.3 ± 3.7[a]	135.6 ± 2.4[a]
Time spent in approach zone (sec)			
Control	70.4 ± 5.8	73.5 ± 6.7	84.9 ± 7.4
Extract (10 mg/L)	75.3 ± 5.4	98.4 ± 3.7	108.5 ± 9.6
Extract (20 mg/L)	81.1 ± 7.1	108.7 ± 8.4[a]	132.1 ± 9.4[a]

which is associated with a decrease in scotaxis behavior, is observed to rise after augmentation of GABAergic activity or the administration of GABA agonists. Furthermore, these findings provide additional support for the conclusions derived from the mirror bite test, which identified an augmentation in GABA expression.

According to Stewart et al. (2012), the memory of rodents is negatively affected by protracted restraint and exposure to a predator or its odors. Predator exposure, as an ecologically significant stressor, has the potential to elicit an intrinsic anxiety response in zebrafish. Spinello et al. (2019) observed heightened motility and erratic behavior in zebrafish, resembling the stress response seen by rodents in the presence of predators, following exposure to the Indian leaf fish for durations of 24 and 72 hours. According to the results, zebrafish exposed to the extract exhibit an increased duration of proximity to the food source. This phenomenon may be attributed to the evolutionary development of the animals' fearlessness and audacity.

Conclusion

Methanolic extract of *S. mahagoni* extract may have reduced dopamine expression alongside the up-regulation of 5-HT and GABA seen in these neurobehavioral findings.

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Ethical Issue

All experiments have been completed with the CPCSEA, and experimental ethics (Ref. No. PGIMS/2023/06-51). Every attempt has been made to decrease animal struggle and to lessen the range of animals utilized in all experiments

Conflict of Interest

Authors declare no conflict of interest

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