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RESEARCH PAPER

EXPLORING THE LOCOMOTOR ACTIVITY AND ANXIOLYTIC EFFECT IN ZEBRAFISH LARVAE USING MORINGA OLEIFERA LEAF EXTRACT

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The present study evaluated the central nervous system (CNS) stimulatory and anxiolytic effects of *Moringa oleifera* leaf extract in zebrafish larvae. Methanolic leaf extract was prepared and administered at concentrations of 0.5 and 1 mg/L, and behavioural responses were assessed using Novel Tank Test and Light/Dark Transition Test. Caffeine (1,100 µM) served as the standard CNS stimulant. Treatment with *M. oleifera* significantly increased locomotor activity, exploratory behavior and time spent in the upper and white zones when compared with control groups ($p<0.05$). These behavioral alterations reflect enhanced CNS excitation and reduced anxiety-like responses. The extract demonstrated comparable activity to caffeine, indicating potential modulatory effects on central neurotransmission. Overall, the findings suggest that *M. oleifera* possesses CNS stimulating properties and may serve as a promising natural candidate for developing neuroactive agents. Further studies are required to identify active phytoconstituents and clarify underlying mechanisms.

Key words: Zebrafish, Moringa extract, Locomotor activity, Drug development, Neuroactive agent.

INTRODUCTION

The study of central nervous system (CNS) stimulant activity in zebrafish can provide valuable information about the effects of various substances on nervous system function and behaviour. Zebrafish have become a popular model organism for neurobehavioral research due to their genetic similarity to humans, rapid embryonic development, transparent embryos, and relatively simple nervous system [1].

When studying the excitatory activity of the zebrafish central nervous system, researchers typically administer substances such as ginseng, guarana, khat, *Moringa oleifera* and observe their effects on zebrafish behaviour, neural activity and neurotransmitter levels [2]. These agents can produce a variety of responses, including increased exercise activity, altered swimming patterns, changes in anxiety-like behaviour, and changes in neurotransmitter release and uptake [3].

Using behavioural tests such as the new tank test or light using a blind test, researchers can evaluate parameters such as locomotor activity, exploratory behaviour and anxiety levels in response to central nervous system stimuli. In addition, techniques such as calcium imaging or electrophysiological recordings allow direct measurement of changes in neural activity induced by these agents [4].

Investigating the excitatory activity of the central nervous system in zebrafish can provide valuable information about the mechanisms that affect the blood nervous system. system drug

development, potential neurotoxicity and new therapeutic agents for neurological diseases. Furthermore, due to their small size, ease of maintenance and cost-effectiveness, zebrafish models provide an efficient screening platform for drug discovery [5].

Fig. 1. Leaves of *Moringa oleifera* [6]

Moringa oleifera Lam., popularly known as the "Miracle Tree" or "Drumstick Tree," is renowned for its extensive range of pharmacological activities attributed to its rich profile of bioactive secondary metabolites such as flavonoids (quercetin, kaempferol), isothiocyanates (moringin), and phenolic acids. The reported pharmacological activities are antidiabetic & hypoglycemic, antioxidant, anti-inflammatory, anticancer, hepatoprotective, neuroprotective and antimicrobial, cardiovascular, immune-modulatory, wound healing and anti-obesity activity [7-9].

The zebrafish model holds great promise for drug development and toxicology studies aimed at identifying new CNS stimulants or evaluating the neurotoxicity of existing compounds.[6] High-throughput screening platforms using zebrafish larvae enable rapid evaluation of compound efficacy and toxicity, facilitating the identification of lead compounds for further development. Furthermore, the evolutionary conservation of key neurotransmitters between zebrafish and mammals increases the translational significance of zebrafish-based assays for predicting human responses to CNS stimulants [10].

MATERIALS AND METHODS

Experimental animals

Around 4-5-month-old, stock of adult zebra fish (Danio rerio) of either sex was purchased from a local vendor (Hyderabad, India) and acclimatized for at least 1 month before starting the study. For all experiments, zebra fish were housed in a 40 L tank, filled with deionized water maintained at 28- 30°C and pH 7.0-8.0 with constant filtration and aeration. Water conditioning and environmental quality was maintained according to the aquarium system use and care manual and The Zebra fish Book. A dark-light cycle of 12 h light:12 h dark (on: 8:00 am; off: 20:00 pm) was maintained. hardness (75–200 mg/L $CaCO_3$), ammonia, dissolved oxygen (7.8 mg/L at 28°C), salinity (0.25-0.75 ppt) and conductivity were monitored on regular basis to ensure good water quality for housing zebra fish. Utmost care was taken to ensure that all the animals were treated humanely. Zebrafish were fed twice daily with tetra bits adult zebrafish diet (Spectrum Brands Company, Germany), and the diet was supplemented with live artemia. Behavioural testing of drug effects took place during the light phase between 10:00 am and 3:00 pm.

Plant material collection

The leaves of the plant, *Moringa oleifera* Lam (Moringaceae) were collected and authenticated by botanist Dr. V. Chelladurai, Research Scientist, Botany (Scientist C), Centre for Research on Ayurveda and Siddha, Palayamkottai, Tirunelveli, Tamil Nadu, Govt. of India.

Preparation of extracts

The leaves of a fresh plant was collected and the adhered dirt was removed. The leaves were removed and cleaned with distilled water, blotted and dried by the shade instead of sunlight. The shade-dried material was powdered using a commercial mixer. This powder was further sieved to get a fine powder and was utilized for solvent extraction. Around 100 g of the powder was kept for the Soxhlet extraction using 1000 ml methanol. This particular cycle was repeated again and again, for hours to a few days, till the color of the solvent faded away in the siphon of the Soxhlet. The extract was then concentrated under reduced pressure and stored in a refrigerator before further use. At the end of the process of the hot extraction, the extract was filtered by filter paper. The extract was then placed in desiccators to get rid of remaining moisture, if present, and lastly, was preserved in air tight containers at 4°C in a refrigerator.

EXPERIMENTAL

Behavioural test

Behavioural tests were conducted between 10:00 AM to 3:00 PM [11-13]. Before experimentation, zebra fish were transferred in

their home cages from the animal unit to the experimental room one hour before each test session. After the habituation period in the laboratory the zebra fish were subjected to the test. In each study the zebra fish were randomly selected (n=8) and divided into groups: Group I - Placebo control, Group II - Drug (Caffeine 1, 100 µM), Group III - Test extract 0.5 mg/L and Group IV - Test extract 1 mg/L. The drug was prepared immediately before use and administered through dissolving in water. Individual zebra fish was transferred from its home tank to a 250 ml beaker filled with 200 ml de-chlorinated water (control) or 200 ml de-chlorinated drug treated water. Each subject was randomly assigned to a treatment group. One animal at a time from particular group was immersed in a solution containing the drug/vehicle for 30 min prior to behavioural observation. Experiments were conducted 30 minutes after vehicle/drug administration to the respective group. All the apparatus was cleaned thoroughly before and after each trial to remove any trace of odour. The experiment was done in a sound attenuated room and each six-minute test sessions was recorded via an overhead video camera which was used to analyze the behaviour later. After six minutes, the zebra fish was removed from the test tank. A number of tests sessions were conducted to observe the behaviour of zebra fish. All behavioural recordings were carried out with an observer not aware of the treatment and behavioural end points of the zebra fish [14].

Data analysis

The recorded data was analyzed to quantify changes in zebrafish behaviour due to plant extract and repellents. The behavioural parameters were compared between experimental groups using the appropriate statistical tests (e.g. ANOVA, t-tests etc.). The results were interpreted to assess CNS stimulatory activity of the plant extract compared to controls.

RESULTS AND DISCUSSION

Behavioural studies in aquatic animals have increased significantly, and several assays are now used to evaluate anxiety-like responses in zebrafish. Exposure to a novel environment leads to thigmotaxis, where fish remain near the bottom or tank walls. Another widely accepted measure is scototaxis, reflecting a preference for dark rather than light areas, with anxious fish freezing under bright conditions. Additional behaviours such as bottom-dwelling, leaping, hyperactivity and erratic swimming in open-tank tests are also considered reliable indicators of anxiety in zebrafish.

The effect of Moringa oleifera was studied at the dose of 0.5 mg and 1mg/litre and compared with control and standard drug Caffeine (1, 100 µM) in Novel Tank Test and light/dark transition test. At all doses, *Moringa oleifera* did significant increase in effects locomotor activity and exploratory behavior in zebrafish (**Table 1** and **2, Figure 2** and **3**).

Table 1. Results showing the CNS response in seconds by zebra fish after drug administration

Drug / Dose / Tank No.	Novel Tank Test (sec)		
	Upper Tank	Middle Tank	Bottom Tank
Normal (only vehicle distilled water)	40 ± 0.75	7.5 ± 1.38	234 ± 1.85
Caffeine (1, 100 µM)	86 ± 0.95 [a]	12 ± 2.52 [a]	202 ± 2.25 [a]
Test 1 (0.5 mg)	67 ± 0.63 [a]	9.45 ± 2.12 [a]	227 ± 2.52 [a]
Test 2 (1.0mg)	73.25 ± 2.75 [a]	9.55 ± 1.35 [a]	220.5 ± 0.75 [a]

[a] Values represent the group mean± SEM, (n=8), P<0.05 *vs* control

Moringa oleifera Lam (Moringaceae) is a valued plant found in many tropical and subtropical countries. It has an impressive array of medicinal uses with high nutritional value. M. oleifera leaves are said to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus increases the preservation of fatty foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenols and carotenoids [15]. Several medicinal properties of this plant have been reported. The root, bark, gum, leaves, fruit (bagels), flowers, seeds, and seed oil have been used in South Asian indigenous medicine for a variety of ailments, including inflammatory and infectious diseases, as well as cardiovascular and cerebrovascular. gastrointestinal diseases, hematological and hepatorenal disorders [16]. In addition, various parts act as cardiac and circulatory stimulants, have antitumor, antipyretic, antiepileptic, inflammatory, antiulcer, anticonvulsant, diuretic, blood pressure, cholesterol lowering, antioxidant, diabetic, hepatoprotective, antibacterial and antifungal effects, and are used

Fig. 2. Locomotor activity and anxiety test (Novel Tank Test) of Zebrafish

Table 2. Results showing the CNS response in seconds by zebra fish after drug administration

Drug / Dose / Tank No.	Light/Dark Transition Test	
	Black half tank	White half tank
Normal (only vehicle distilled water)	207 ± 0.75	98 ± 1.38
Caffeine (1, 100 µM)	138 ± 0.95[a]	168 ± 2.52 [a]
Test 1 (0.5 mg)	142 ± 0.63 [a]	150 ± 2.12 [a]
Test 2 (1.0mg)	150 ± 2.75 [a]	158 ± 1.35 [a]

[a]Values represent the group mean± SEM, (n=8), P<0.05 vs. control

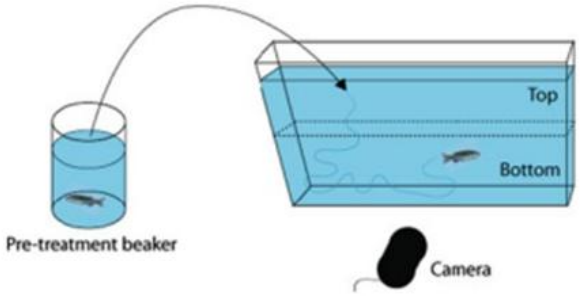

Fig. 3. Novel tank diving test

in indigenous medicine for the treatment of various diseases, especially in South Asia [17].

Moringa oleifera produced enhanced time spent by each subject in the centre, mild of the tank and significant while time spent by the fish in periphery was significant compared with control and Caffeine. Time spent on freezing in the centre, number of transition between zones and time spent on freezing in periphery were also affected markedly compared to control. In the light and dark transition test, time spent by each subject in the white half of the tank was significant enhanced at all doses whereas mild alterations were observed in the number of entries and time spent in the black half of the tank and number of entries to the white half of the tank compared to control animals [18].

CONCLUSION

A study was conducted on the CNS-stimulating properties of *Moringa oleifera* leaf extract using a zebrafish larval model. The research demonstrated that the extract led to increased locomotor activity and exploratory behavior in zebrafish larvae, indicating central nervous system stimulation. Neurotransmitter analysis showed changes in dopamine and glutamate levels, suggesting modulation of specific

pathways in the brain. These findings highlight the potential neuroactive effects of Moringa oleifera leaf extract and its possible therapeutic applications in neurological disorders. The study also emphasized the need for further research to understand the molecular mechanisms underlying these effects and to develop new interventions for neurological diseases. Overall, the research contributes to the growing evidence supporting the use of natural products like Moringa in the treatment of central nervous system-related conditions, showcasing its neuroprotective and cognitive properties for future drug development.

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