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Neurobehavioral effects and brain GFAP modulation by *Mimosa pudica* extract in zebrafish exposed to zinc oxide nanoparticles

Sourav Pal^{1*} , Brisanka Tarafder², Debashish Dey² and Sayed Mohammed Firdous³

Abstract

Background Alzheimer's disease (AD), marked by amyloid β peptide buildup leading to neurodegeneration and cognitive decline, is a growing health concern. This study explores the neuroprotective potential of *Mimosa pudica*, a plant with traditional medicinal uses, focusing on its antioxidant properties and effects in a zinc-induced zebrafish model of AD.

Methods AD-like symptoms were induced in zebrafish using zinc oxide nanoparticles (ZnO NPs). The hydromethanolic extract of *M. pudica* (HMEMP) was analyzed for antioxidant activity through DPPH assays, functional group identification via FTIR, and immunohistochemical staining to observe brain astroglial cells. Behavioral tests were conducted to assess cognitive and social impairments, including open field, mirror biting, light–dark preference, social preference, object familiarity, and novel object recognition tests.

Results HMEMP showed strong, dose-dependent antioxidant activity and contained functional groups linked to neuroprotection. In zebrafish exposed to ZnO NPs, HMEMP significantly improved cognitive, social, and exploratory behaviors. Histological analysis revealed reduced neuroinflammation and tissue degeneration, supporting the extract's neuroprotective effects against ZnO NP-induced AD-like symptoms.

Conclusion HMEMP exhibits strong antioxidant and neuroprotective properties, suggesting its potential to treat AD and other neurodegenerative diseases. Further research is needed to elucidate the specific bioactive compounds and mechanisms involved in its neuroprotective effects.

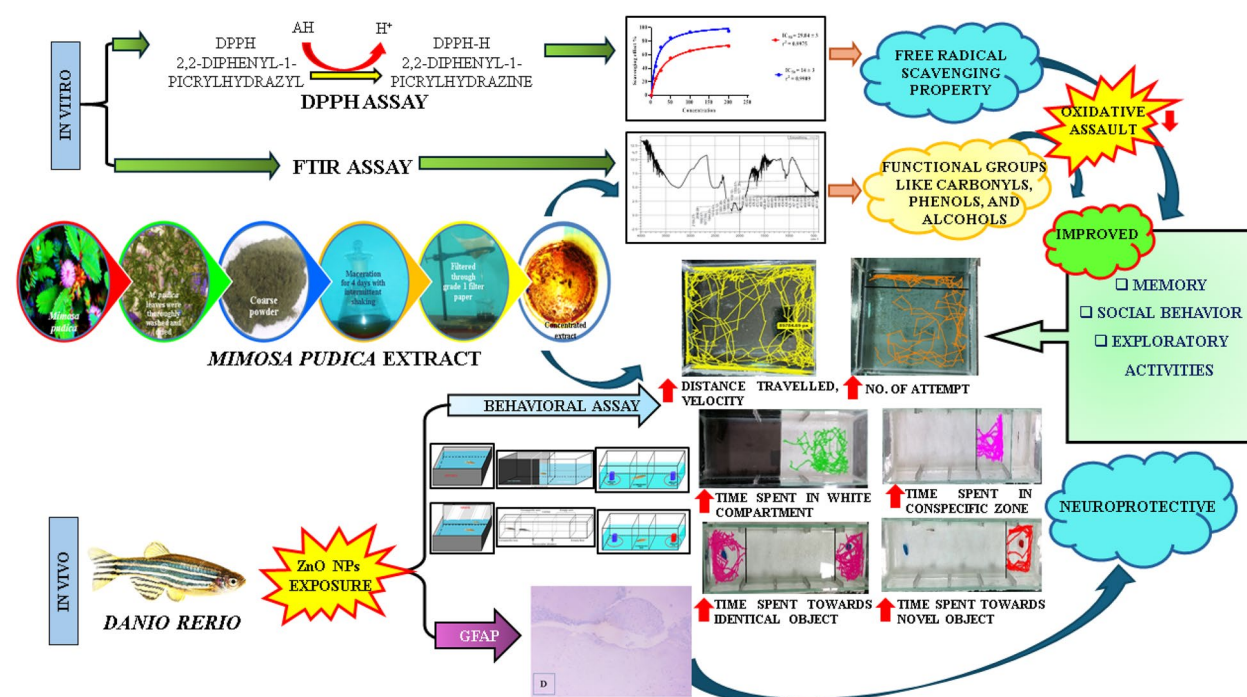
Keywords Neurodegeneration, *Mimosa Pudica*, Oxidative stress, Behavioral assessment, Glial fibrillary acidic protein

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Graphical abstract



Background

Alzheimer's disease (AD) stands as a significant global health challenge, and its impact is continually expanding. It is marked by the accumulation of fibrillar amyloid β (A β) peptides in the brain, triggering a cascade of molecular disturbances (Firdous et al., 2024a; b). The resultant A β plaques and tau tangles disrupt normal neuronal function, heralding neurodegeneration and cognitive decline, the very essence of AD symptoms. The staggering prevalence of dementia, primarily driven by AD, underscores its ominous trajectory (Mukadam et al., 2024). Current estimates suggest a populace afflicted by dementia numbering as high as 55 million globally, with over 60% residing in low- and middle-income countries. Projections indicate this number will nearly double every two decades, reaching 78 million by 2030 and 139 million by 2050 (Jain & Hogervorst, 2023). Age emerges as a potent determinant, with AD's prevalence escalating exponentially. The leap from age 60 to 85 witnesses a nearly 15-fold increase, painting a grim picture of its relentless advance. Regions like North America and Western Europe bear the brunt, boasting at age 60, trailed by Latin America and China/developing Western Pacific areas. Various studies depict fluctuating dementia prevalence rates (Cao et al., 2020). Addressing modifiable

risk factors, even in midlife, emerges as a promising strategy to stave off or mitigate AD's onset. By marshaling resources toward preemptive measures, there lies a potential avenue to reshape the trajectory of this insidious disease.

Medicinal plants represent a compelling avenue in AD treatment, presenting a natural alternative with potential therapeutic benefits (John et al., 2022). Their efficacy in improving cognitive function, reducing neurotoxicity, and enhancing memory in AD patients has garnered attention. Recent research has spotlighted several promising candidates, including *Saururus chinensis* (Oh et al., 2024), *Syagrus romanzoffiana* (El-Hawary et al., 2021), *Lavandula angustifolia* (Arasteh et al., 2023), *Melissa officinalis* (Noguchi-Shinohara et al., 2023), *Crocus sativus* (Yang et al., 2023), and *Magnolia officinalis* (Li et al., 2024). These plants harbor bioactive compounds with diverse pharmacological actions, such as antioxidants, anti-inflammatory agents, anti-cholinesterase effects, and anti-amyloidogenic properties. These mechanisms target the underlying pathophysiology of AD, offering potential avenues for disease modification.

Conventional pharmaceuticals frequently induce adverse effects such as gastrointestinal discomfort, sleep disturbances, and muscle pains, coupled with potential

drug interactions (Doroszkievicz & Mroczko, 2022). Conversely, herbal medicines, sourced from plants, are often perceived as safer due to their natural composition, with a reduced likelihood of adverse effects. Herbal remedies operate holistically, bolstering the body's innate healing mechanisms without the typical side effects of synthetic drugs. This variance in side effect profiles underscores the burgeoning interest in natural and plant-based remedies as potentially safer alternatives for conditions like AD. Their perceived safety and efficacy propel research and interest in integrating them into mainstream healthcare, offering a promising avenue for the holistic management of various health ailments while minimizing the risks associated with conventional pharmaceuticals.

Mimosa pudica, commonly known as the "sensitive plant" or "sleepy plant," is a versatile creeping herb belonging to the Mimosaceae family (Majeed et al., 2021). This plant serves as a rich biological source of medicinal compounds and has a long history of traditional use across various cultures for treating a wide array of ailments (Rizwan et al., 2022). The root is traditionally employed to alleviate bilious fevers, piles, jaundice, leprosy, dysentery, asthma, leucoderma, and blood disorders (Majeed et al., 2021). The leaves and stems are utilized for their properties in relieving itchiness, skin wounds, and diarrhea (Ramakrishna & Lake, 2020). Moreover, *M. pudica* is believed to possess mood-enhancing, blood circulation-promoting, and hair growth-stimulating attributes (Meesakul et al., 2023). Chemically, *M. pudica* is known to contain a variety of bioactive compounds, including alkaloids, flavonoid C-glycosides, sterols, terpenoids, tannins, saponins, fatty acids, and other constituents, like phthalic acid dioctyl ester, α -linolenic acid, cinnamaldehyde, and adrenaline-like substances (Khan et al., 2021). These chemical constituents contribute to the plant's medicinal properties and therapeutic potential.

Zinc oxide nanoparticles (ZnO NPs) exhibit dual functionality, acting both as oxidative stress inducers and therapeutic agents through antimicrobial and antioxidant mechanisms. Their capacity to disrupt biofilms (e.g., *P. aeruginosa*) and mitigate pathogen-induced oxidative damage, demonstrated via camphene conjugation, highlights their role in combating antibiotic resistance and ROS-mediated cytotoxicity (Issac et al., 2024). Concurrently, rutin trihydrate conjugation stabilizes gut microbiota and reduces neurodegeneration by scavenging free radicals and enhancing antioxidant gene expression (SOD, CAT) (Issac & Velumani, 2024). ZnO NPs' redox duality enables pathogen inhibition and cytoprotection, modeling gut-brain dysregulation and Alzheimer's pathways via oxidative stress-induced tau

hyperphosphorylation (GSK3 β /ERK activation), offering a tunable platform for combinatorial therapies targeting microbial imbalance and neurotoxicity (Tian et al., 2015). ZnO NPs disrupt mitochondrial function, triggering caspase-mediated neuronal apoptosis, mimicking AD-associated neurodegeneration (Mousavi et al., 2021). They bind tau proteins, altering their structure into β -sheet-rich aggregates resembling neurofibrillary tangles. Behavioral outcomes include reduced locomotor activity, velocity, and time spent in light compartments, linked to oxidative stress, apoptosis, and neurodevelopmental toxicity (Chen et al., 2014). These effects highlight the utility of zebrafish for studying AD-related mechanisms and screening neuroprotective interventions. Our investigation integrates the established role of ZnO NPs in the progression of AD using a ZnO NPs-induced AD model. The effectiveness of antioxidants was assessed via DPPH assays. Furthermore, Fourier-transform infrared (FTIR) spectroscopy was performed on the hydromethanolic extract of *Mimosa pudica* (HMEMP) to identify functional groups pertinent to AD diagnosis. Subsequently, brain tissue was isolated and subjected to glial fibrillary acidic protein (GFAP) immunohistochemical staining to examine and visualize astroglial cells within the zebrafish brain.

Methods

Collection and identification of plant materials

The *M. pudica* leaves were collected from Rashkundu, Paschim Medinipur district of West Bengal. The taxonomic identification and authentication of the plant material were conducted at the Central National Herbarium, Botanical Survey of India, B. Garden, Howrah. For future reference, the voucher specimen has been preserved under the reference number (CNH/Tech. II/2024/73). Preceding the drying phase, the seeds underwent a meticulous cleansing with running tap water, ensuring the removal of any extraneous debris. Before drying, the seeds were rinsed with running tap water to remove debris. The leaves were then cut into small pieces and dried in the shade at 40 °C for one week.

Extract preparation

The described extraction method employed the maceration technique, utilizing hydroalcohol as the solvent. Freshly collected *M. pudica* leaves underwent drying, pulverization, and subsequent dual maceration in hydroalcohol, prepared by dissolving 20 g of powdered material in a 1:2 mixture of methanol and distilled water (Koodkaew et al., 2018). Maceration lasted 3–4 days, with intermittent shaking to ensure thorough extraction of plant constituents. The extract obtained from successive macerations underwent meticulous filtration

and concentration using a Rota-evaporator apparatus. Subsequently, the concentrated HMEMP was placed in vacuum desiccators to eliminate residual water content, improving stability and extending shelf life. Finally, the concentrated extract was transferred to labeled bottles and stored under refrigeration to preserve potency and integrity for subsequent utilization.

In vitro study

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The free radical scavenging activity was evaluated using the DPPH assay. To do this, 5 ml of a 0.04% DPPH radical solution were combined with HMEMP leaf extract solutions at concentrations ranging from 12 to 200 µg/ml. For comparative purposes, ascorbic acid was also tested at the same concentrations. Extract concentrations were chosen based on preliminary screening and literature, ensuring detectable antioxidant activity and dose–response, while avoiding solubility issues or matrix-related interferences (Stasiłowicz-Krzemień et al., 2023). These mixtures were vortexed and then kept in a dark environment for 30 min. All DPPH assay measurements were performed in triplicate (n=3 technical replicates) for each concentration. The optical density (OD) was recorded at 517 nm following standard procedures, and the scavenging activity was subsequently calculated (Pal et al.,).

Fourier transform infrared spectroscopy (FTIR) assay of HMEMP

FTIR spectroscopy was performed to identify the functional groups present in the HMEMP extract. The previously prepared dried extract was finely powdered using a mortar and pestle. Approximately 2 mg of the powdered sample was placed on the FTIR sample holder for analysis. The spectra were recorded using an FTIR over a defined wavelength range. The resulting absorption peaks were analyzed and compared with standard reference spectra to identify the functional groups in the extract (Patle et al., 2020). These peaks were meticulously compared with reference spectra to ascertain the presence of specific chemical bonds and functional groups within the extract.

In vivo study

Experimental animals

Adult fish were procured from a local distributor in Kolkata, West Bengal, India. The animals are kept in groups of 20–25 in 40-L tanks with filtered water at a temperature of 25–27 °C. The tanks were illuminated using fluorescent lights on a 14-h light: 10-h dark cycle, which closely mimics natural photoperiods and supports normal circadian rhythms in zebrafish. Fluorescent lighting was chosen for its ability to provide uniform, stable illumination

with minimal heat output, thereby minimizing temperature fluctuations and stress for the animals. The animals are around 3–4 months old. Two times a day, fish that have never been in an experiment are fed. All the experiments were carried out as per the guidelines of zebrafish care and husbandry (Ref. No. PGIMS/2024/07-76).

Oral acute toxicity study

Fish were exposed to the HMEMP leaf extract for 96 h under static conditions following OECD 203 guidelines, with daily monitoring of mortality. For the 14-day behavioral assessment of ZnO NP-induced AD-like symptoms, HMEMP extracts were administered under semi-static conditions with solution renewal every 48 h (Pulido-Reyes et al., 2024). LC₅₀ values were calculated only when mortality data allowed statistical estimation. To assess behavioral alterations in zebrafish exposed to ZnO NPs with AD-like symptoms, HMEMP exposure was prolonged to 14 days.

Synthesis and utilization of ZnO NPs for inducing AD-like symptoms in the zebrafish model

ZnO NPs were synthesized by preparing separate solutions of zinc acetate and 1 mol NaOH in 2-propanol, which were then combined to yield ZnO NPs (Ahmed et al., 2020). NaOH was added to the zinc acetate solution and stirred at 700–1200 rpm until a milky mixture formed. To obtain ZnO NPs, the mixture was filtered and then heated at 600 °C for 2 h (Ahmed et al., 2020). Symptoms similar to Alzheimer's disease were induced in adult zebrafish when exposed to 0.69 mg/L of ZnO NPs, which is one-fifth of the LC₅₀ value (3.48 mg/L). The induction procedure involved acclimatizing adult zebrafish to laboratory conditions for 1 week, followed by a 2-week exposure to 0.69 mg/L of ZnO NPs (Farag et al., 2023). Behavioral tests and histological staining were conducted to assess the development of AD-like pathology, including cognitive impairment and altered expression of disease-related proteins.

Experimental design

After acclimatization with the experimental condition for 1 week, zebrafish of either sex were randomly divided into four groups to determine whether the HMEMP extract has neuroprotective and cognitive-enhancing properties in an AD model produced by ZnO NPs. The objective of extending the treatment duration was to take advantage of the extract's possible long-term effects in improving the zebrafish model's behavioral abnormalities linked to AD. After 48 h intervals, water should be changed, and animals were exposed to the extract of each experimental group for 48 h.

Grouping of animals

Animals were divided into four groups ($n=5$). Animals in Group I were provided with a standard feed and housed in freshwater. Animals in Group II were subjected to a concentration of 0.69 mg/L of ZnO NPs. Animals in Group III were exposed to ZnO NPs and then administered HMEMP at a 20 mg/L concentration. Animals belonging to Group IV were exposed to ZnO NPs and subsequently administered HMEMP at a concentration of 40 mg/L.

Behavioral analysis

Zebrafish were tested for behavioral changes in response to ZnO NPs exposure and subsequent treatment with HMEMP at concentrations of 20 and 40 mg/L using a series of tests, including the open field test (OFT), mirror biting test (MBT), light–dark preference test (LDPT), social preference test (SPT), object familiarity test (OBFT), and novel object recognition test (NORT) evaluations. The video recordings of zebrafish movements were obtained using a high-resolution camera positioned directly above each experimental setup. This setup allowed for the capture of individual fish movements within a controlled environment. To minimize external interference, the manual recordings were conducted in a noise-free zone, ensuring that any observed behavioral changes were not influenced by external stimuli. Due to the fast throughput and vast amount of data created, manual observations may not be possible. To automatically assess and record multiple endpoints on days 0, 7, and 14, video-tracking software (kinovea version 0.9.5) is utilized (Pal et al., 2023a; b).

OFT

Anxiety and exploratory behavior in zebrafish were assessed using the OFT. After acclimatization, each zebrafish was individually placed in a tank containing 6 cm of water. Due to the complexity of manual observation, video tracking was employed to quantify endpoints such as exploration and thigmotaxis (wall-hugging). The arena was divided into predefined zones to facilitate behavioral analysis. Over 5 min, variables including time spent in each zone, distance traveled, and velocity were recorded and analyzed (Firdous et al., 2024a; b).

MBT

A mirror was attached to the side wall of the tank to observe aggressive behavior in zebrafish. Two zones were delineated by drawing lines: one at 0.5 cm from the mirror to define the "contacting the mirror" zone, and another at 2.5 cm to define the "approach to the mirror" zone. Behavioral activity was recorded for 5 min. Video

tracking software was used to create virtual boundaries, and manual scoring was performed to quantify the number of mirror contacts and approaches, as well as latency to first contact and approach (Firdous et al., 2024a; b).

LDPT

The LDPT was conducted using a tank divided into distinct light and dark compartments. Zebrafish were transferred to the experimental tank at least 60 min before testing for acclimatization. Fish were gently netted from the maintenance tank and placed into the main compartment, with sliding doors closed for 3–5 min to facilitate acclimation. The doors were then opened to allow free exploration. Behavioral parameters such as time spent in the light zone, distance traveled, swimming speed, and frequency and duration of freezing episodes were recorded during a 5-min test session using video analysis (Firdous et al., 2024a; b).

SPT

The SPT apparatus consisted of five 10×10 cm compartments separated by a 50-cm Plexiglas corridor. A conspecific stimulus fish was placed in a compartment separated by a transparent partition. Test zebrafish were introduced individually into the central zone, separated from the stimulus compartments by transparent sliding doors. After a 30-s acclimatization period, the sliding doors were raised, allowing free movement for 5 min. Behavioral parameters, including the number of entries and the duration spent in the central zone, conspecific arm, and empty arm, were recorded manually. Additionally, the corresponding video recordings were analyzed using Kinovea software (kinovea version 0.9.5) (Firdous et al., 2024a; b).

OBFT

Two identical objects, each secured with intact glass slides, were placed in the segmented enclosure (Fig. 1). A zebrafish was introduced into the middle segment and allowed to acclimate for 2–3 min. Following acclimatization, the glass slides were removed, permitting free exploration for 5 min. The duration of time spent exploring

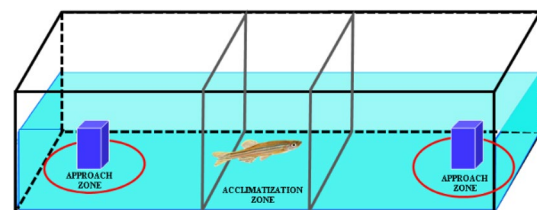


Fig. 1 OBFT apparatus

the two identical objects was recorded as an indicator of behavioral response (Wallace & Hofmann, 2021).

NORT

The NORT utilized the same tank as the OBFT but replaced one of the familiar objects with a novel one (Fig. 2). Zebrafish were acclimated in the middle segment for 2–3 min. After the removal of the glass slides, fish were allowed to explore freely for 5 min. The behavioral response was quantified by measuring the time spent investigating the novel object relative to the familiar one (Gaspary et al., 2018).

Histopathology

The telencephalon and optic tectum were prioritized due to their conserved roles in neurodegenerative pathology and astroglial reactivity in zebrafish models (Dietel et al., 2020). Brains were dissected intact in ice-cold PBS (pH 7.4, 4 °C) within 2 min post-euthanasia to preserve tissue integrity. Dissected tissues were immediately fixed in 4% paraformaldehyde (PFA) at 4 °C for 24 h (Schwarzer et al., 2020). Following fixation, tissues were transferred to 30% sucrose-PBS (w/v) at 4 °C for 24–48 h until gravitational sinking occurred, ensuring complete cryoprotection. Tissues were mounted dorsally in 2% low-melting agarose (prepared in PBS, 37 °C) to standardize horizontal alignment of the optic tectum and telencephalon. Agarose-embedded blocks were equilibrated at −20 °C, and 20 µm horizontal sections were cut using a Zeiss Microm cryostat (HM650 blade, −20 °C chamber). Sections were collected on gelatin-coated coverslips (0.1% gelatin, 0.01% chromium potassium sulfate) to prevent detachment during subsequent staining. For paraffin-embedded tissues, sections were dewaxed in xylene, rehydrated through graded alcohols, and stained with Mayer's hematoxylin. Differentiation was performed in 0.5% acid alcohol, followed by counterstaining with eosin Y and mounting with DPX medium. Cryosections designated for GFAP immunohistochemistry underwent antigen retrieval in citrate buffer (pH 6.0, 95 °C, 20 min). GFAP was visualized using a commercial anti-GFAP kit (Abcam ab7260) following the manufacturer's protocol (Koke et al., 2010). Analysis via microscopy techniques,

such as confocal microscopy, enables assessment of GFAP expression and distribution, offering insights into astroglial cell populations within the zebrafish CNS (Zabegalov et al., 2021). Image analysis was performed using ImageJ (version 1.53).

Statistical analysis

The behavioral results in this study are expressed as mean ± S.E.M. However, histological data in Table 8 are represented as mean ± standard deviation (SD) to better capture within-group variability due to the morphometric nature and smaller sample size. All data were analyzed using GraphPad Prism version 6. Statistical comparisons between groups were performed using Two-way ANOVA followed by Dunnett's post hoc test. Differences were considered statistically significant at $p \leq 0.05$.

Results

DPPH assay

One common way to test a sample's capacity to scavenge free radicals is to see how well it reacts with the stable DPPH radical. This includes plant extracts. At concentrations of 12, 25, 50, 100, and 200 µg/ml, the scavenging antioxidant activity of the HMEMP leaf extract was 25.27, 36.86, 55.32, 65.97, and 73.33%, correspondingly. The scavenging effect was determined to be 73.33% when comparing the extract at the same dose with ascorbic acid (200 µg/ml) (Fig. 3).

FTIR assay of HMEMP

The infrared spectrum exhibits peak at specific wavenumbers (cm^{-1}), corresponding to distinct functional groups. Peaks across various wavenumbers, such as C–H stretching (around 3000–2800 cm^{-1}), C=O stretching (1654 cm^{-1}), N–H bending (1559–1540 cm^{-1}), and C–Cl stretching (668–650 cm^{-1}), further indicate the presence of alkane, carbonyl, amine, and alkyl chloride groups (Fig. 4).

In vivo study

Oral acute toxicity study

Acute toxicity of the HMEMP was tested in the zebrafish model as per the OECD guidelines 203. The study conducted on acute oral toxicity revealed that the leaf extracts of HMEMP were non-toxic to *Danio rerio*. LC_{50} is considered the most accepted basis for determining the acute toxicity test. In the toxicity study, no mortality was observed, which indicates that the extract was non-toxic. At a dose of 200 and 400 mg/L, no mortality was observed in zebrafish. The lethal dose to kill 50% of experimental fish is more than 400 mg/L at a 96-h exposure period.

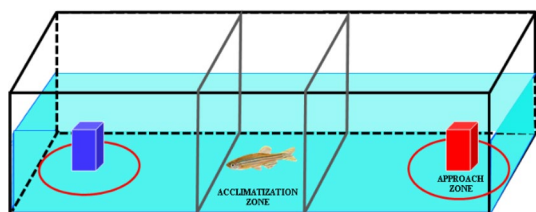


Fig. 2 NORT apparatus

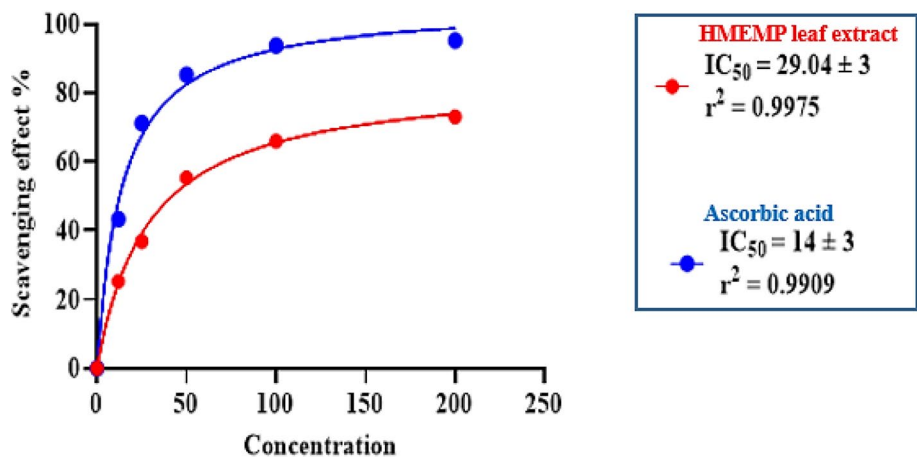


Fig. 3 DPPH radical scavenging activity of HMEMP leaf extract and ascorbic acid at concentrations of 12–200 μg/mL

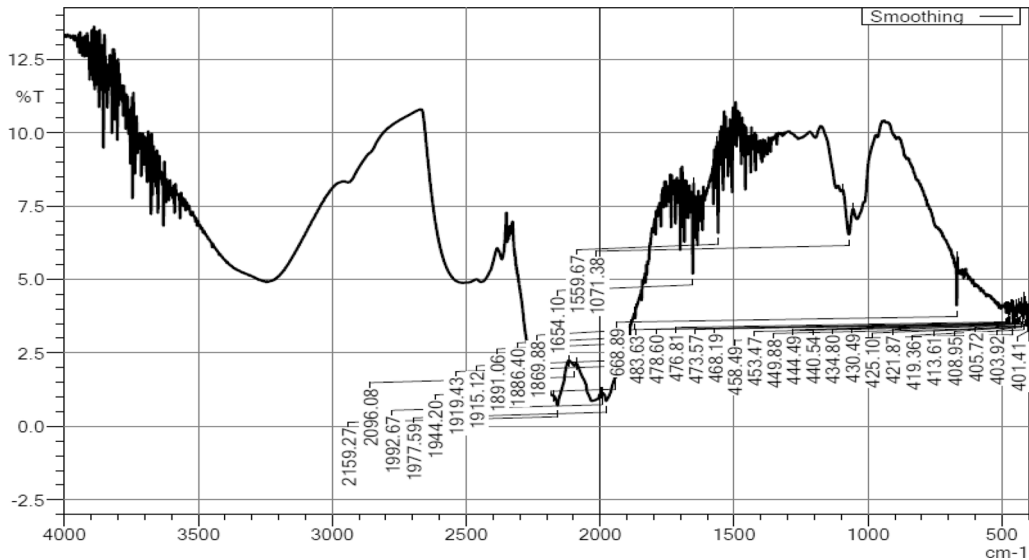


Fig. 4 FTIR analysis of the leaf extract of *M. pudica*

The exposure period for the HMEMP was extended to 14 days to evaluate its effects on the ZnO NPs-induced zebrafish model. Two doses of HMEMP were selected for the study: 20 mg/L (1/20th of 400 mg/L) and 40 mg/L (1/10th of 400 mg/L). Throughout the study period, no mortality was observed among the zebrafish, indicating that these doses were safe for the duration of the experiment.

Behavioral analysis
OFT

Exposure to ZnO NPs significantly impaired zebrafish locomotion, as evidenced by reductions in TDT and velocity on Days 7 and 14 compared to the normal

Table 1 Effect of HMEMP on TDT (in cm) of ZNO NPs-induced AD model in Zebra fish in OFT

GROUP	DAY 0	DAY 7	DAY 14
I	1903.51 ± 19.03	2180.54 ± 111.40	2060.74 ± 74.14
II	2089.11 ± 16.36 [#]	1155.39 ± 122.48 ^{##}	435.54 ± 55.69 ^{##}
III	1974.56 ± 16.96	1935.57 ± 34.06 ^{**}	2866.13 ± 114.05 ^{**}
IV	2088.44 ± 59.75	2452.90 ± 39.46 ^{**}	2635.87 ± 82.42 ^{**}

Values are expressed as Mean ± S'EM, n' = 5; [#]p' < 0.05 and ^{##}p < 0.01 considered statistically significant when compared to the control group; ^{*}p < '0.05 and ^{**}p < '0.01 when compared to the positive control group

Table 2 Effect of HMEMP on the velocity (in m/sec) of ZnO NPs-induced AD model in zebrafish in OFT

GROUP	DAY 0	DAY 7	DAY 14
I	9.15 ± 0.59	7.70 ± 0.34	9.49 ± 0.46
II	7.91 ± 0.35	3.43 ± 0.06 ^{##}	2.60 ± 0.17 ^{##}
III	9.67 ± 0.29*	8.21 ± 0.55**	9.16 ± 0.37**
IV	10.79 ± 0.19**	8.96 ± 0.26**	11.45 ± 0.63**

Values are expressed as Mean ± S'EM, $n=5$; * $p < 0.05$ and ^{##} $p < 0.01$ considered statistically significant when compared to the control group; * $p < 0.05$ and ^{**} $p < 0.01$ when compared to the positive control group

control (Tables 1 and 2). Two-way ANOVA revealed a significant interaction between treatment and time for both TDT ($F(6, 48)=42.76$, $p < 0.0001$) and velocity ($F(6, 48)=31.52$, $p < 0.0001$), confirming time-dependent neurotoxicity (Fig. 5). Co-treatment with HMEMP extract (20 and 40 mg/L) significantly restored locomotor activity, particularly at 40 mg/L, which showed marked improvement in both parameters ($p < 0.01$ vs. ZnO NP group).

MBT

Zebrafish exposed to ZnO NPs exhibited a significant reduction in the time spent in the approach zone on Days 7 and 14 compared to the normal control group (Group I), indicating diminished exploratory and aggressive behavior associated with neurobehavioral impairment (Table 3 and Fig. 6). Statistical analysis revealed a significant interaction between treatment and time ($F(6, 48)=19.84$, $p < 0.0001$), confirming the time-dependent behavioral toxicity of ZnO NPs. Co-treatment with HMEMP extract led to a dose-dependent improvement in behavioral responses. Both the 20 mg/L (Group III) and 40 mg/L (Group IV) groups showed a significant increase in the time spent in the approach zone compared to the ZnO NP-exposed group ($p < 0.05$).

LDPT

According to the findings, zebrafish exposed to ZnO NPs exhibited a significant, time-dependent reduction in the time spent in the light compartment on Days 7 and 14 compared to the normal control group, reflecting increased anxiety-like behavior (Table 4). Statistical analysis confirmed a significant interaction between treatment and time ($F(6, 48)=27.63$, $p < 0.0001$), highlighting the progressive anxiogenic effect of ZnO NPs. In contrast, treatment with HMEMP at both 20 and 40 mg/L significantly enhanced exploratory behavior in ZnO NP-challenged animals. Notably, both doses increased the time spent in the light compartment on

Day 14 ($p < 0.01$ vs. Group II), with the higher dose showing a consistent and more pronounced effect across time points (Fig. 7).

SPT

Zebrafish exposed to ZnO NPs exhibited a notable reduction in the time spent toward the conspecific arm of the social preference apparatus on Days 7 and 14 compared to the normal control group, indicating impaired social behavior (Table 5). Findings revealed a significant interaction between treatment and time ($F(6, 48)=16.42$, $p < 0.0001$), confirming the time-dependent social deficits induced by ZnO NP exposure. However, co-treatment with HMEMP at 20 and 40 mg/L significantly improved social preference behavior throughout the study period. The 40 mg/L group showed a more pronounced effect, with a significant increase in time spent toward the conspecific arm on Day 14 ($p < 0.01$ vs. Group II), approaching normal control levels, as depicted in Fig. 8.

OBFT

The object familiarity test evaluates zebrafish behavior by measuring the time spent exploring identical objects. In this study, exposure to ZnO NPs resulted in a significant, time-dependent reduction in the time spent near the identical object on Days 7 and 14 compared to the normal control group (Group I), indicating impaired object recognition and memory performance (Table 6). Findings revealed a significant interaction between treatment and time ($F(6, 48)=14.97$, $p < 0.0001$), confirming the progressive cognitive and behavioral decline induced by ZnO NP exposure. Conversely, zebrafish treated with HMEMP following ZnO NP challenge demonstrated a dose-dependent behavioral improvement. Notably, the 40 mg/L group showed a significant increase in exploration time toward identical objects on both Days 7 and 14 compared to the positive control group ($p < 0.01$), as depicted in Fig. 9, indicating potential memory-preserving and neuroprotective effects of HMEMP.

NORT

In the behavioral landscape shaped by ZnO NP exposure, zebrafish exhibited a pronounced decline in time spent exploring novel objects on Days 7 and 14 compared to the normal control group (Table 7), reflecting cognitive disruption. Findings confirmed a significant relation between treatment and time interaction ($F(6, 48)=18.64$, $p < 0.0001$), underscoring time-dependent neurocognitive impairment. Remarkably, HMEMP-treated groups at 20 and 40 mg/L defied this trajectory, showing a dose-dependent resurgence in novel object exploration. The 20 mg/L group demonstrated significant improvement ($p < 0.01$ and $p < 0.05$ vs. Group II), while the 40 mg/L

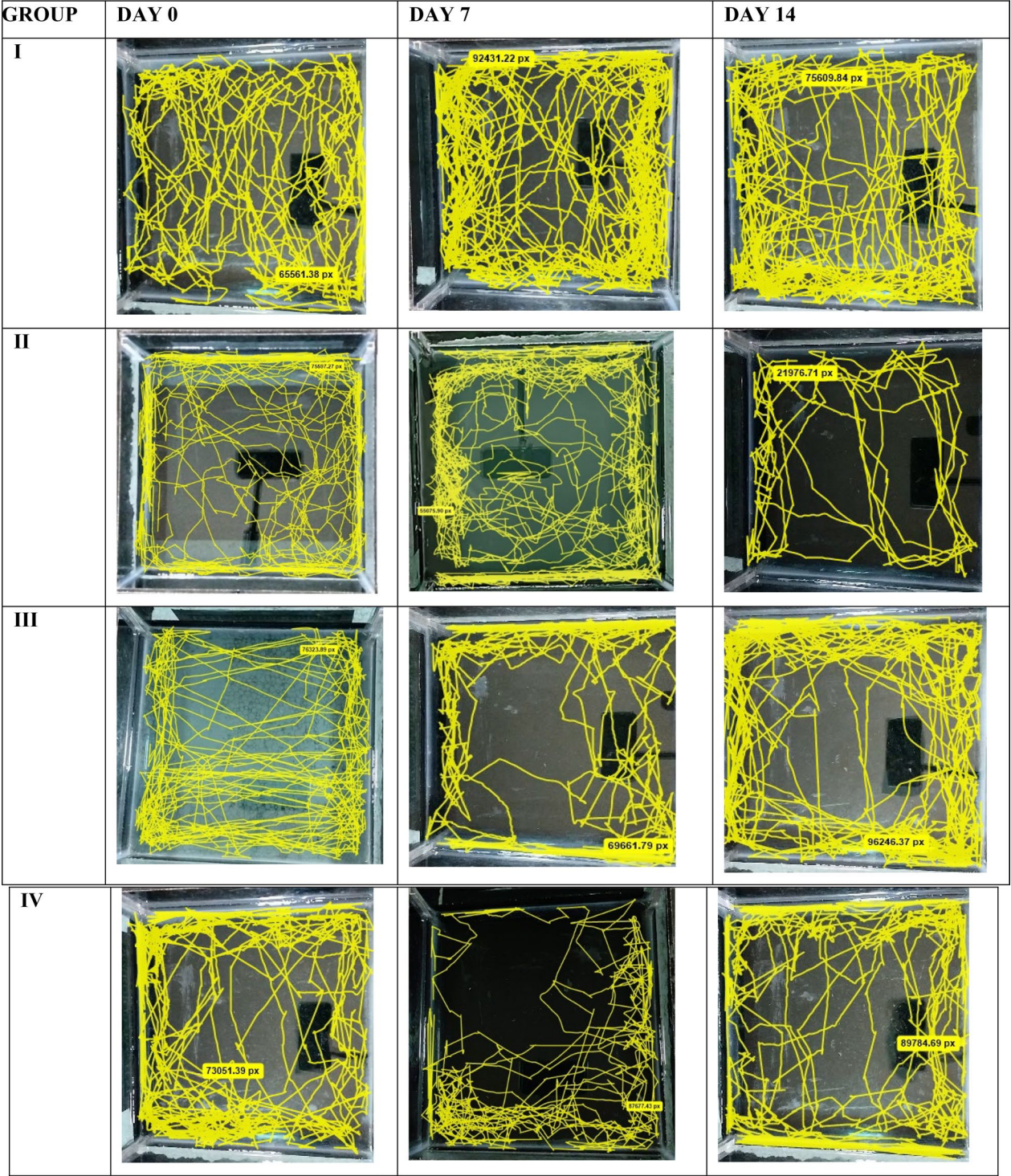


Fig. 5 Effect of HMEMP on total distance traveled (in pixels) by ZnO NPs-induced zebrafish in the OFT based on video-graphic analysis using Kinovea software on Days 0, 7, and 14

group displayed a robust and sustained enhancement ($p<0.01$), with Day 14 values nearing baseline levels (Fig. 10), suggesting strong memory-restorative potential.

Behavioral correlations in the context of extract exposure

The Pearson correlation heatmap illustrates the associations among key behavioral measures in *M. pudica* across

Table 3 Effect of HMEMP on time spent (min) in the approach zone of the ZnO NPs-induced AD model in zebrafish in MBT

GROUP	DAY 0	DAY 7	DAY 14
I	4.55 ± 0.10	4.35 ± 0.22	4.53 ± 0.11
II	4.48 ± 0.02	2.83 ± 0.20 [#]	2.50 ± 0.32 [#]
III	4.27 ± 0.01	3.36 ± 0.03	3.22 ± 0.06*
IV	4.48 ± 0.13	3.49 ± 0.02*	3.32 ± 0.16*

Values are expressed as Mean ± S'EM, $n=5$; [#] $p < 0.05$ and ^{##} $p < 0.01$ considered statistically significant when compared to control group; * $p < 0.05$ and ** $p < 0.01$ when compared to the positive control group

diverse experimental circumstances (Fig. 11). Strong positive correlations were observed, particularly in the OFT, where both TDT and Velocity showed high consistency across different concentrations. For example, a perfect correlation ($R^2=1.00$) was found between OFT measures at concentrations III and IV, indicating a stable pattern of exploratory behavior. Similarly, the OBFT demonstrated high positive correlations, with OBFT (Time Spent) showing a perfect correlation between concentrations III and IV, suggesting consistent activity levels across these conditions. In the LDPT, significant positive correlations were also observed, particularly between measures at lower concentrations, reflecting consistent anxiety-related behaviors. On the other hand, strong negative correlations were identified between certain tests. For instance, a strong inverse relationship was found between OFT and the MBT, with a correlation of $R^2=-0.99$, indicating that higher exploratory activity is associated with reduced aggression. Similarly, negative correlations between OBFT and the SPT suggest that increased exploration corresponds with decreased social interaction, highlighting the potential anxiolytic effects of *M. pudica*. Overall, the correlation data underscore the interconnectedness of exploratory, anxiety-related, and social behaviors in zebrafish. The extract of *M. pudica* shows significant neuroprotective and anxiolytic potential, modulating these behaviors in a consistent and concentration-dependent manner.

Histopathological analysis

GFAP assay

The figure illustrates the results of the GFAP assay, offering insights into the effects of ZnO NPs on zebrafish brain tissue across the different treatment groups (Fig. 12). In the positive control group exposed to ZnO NPs, GFAP immunostaining revealed a marked increase in reactive astrocytes characterized by intensified GFAP expression, loosely organized cells, and regions with low cellular density compared to the normal control. The astrocytes exhibited hypertrophic morphology, with

enlarged cell bodies and thickened processes, indicative of astrogliosis. This reactive gliosis suggests a neuroinflammatory response to ZnO NP-induced oxidative stress and neuronal injury. The distribution of reactive astrocytes was focal, primarily localized in regions associated with neurotoxicity.

GFAP quantification showed significant astrogliosis in ZnO NP-exposed zebrafish, with elevated GFAP-positive area ($26.7 \pm 2.9\%$) and cell count ($87.2 \pm 4.5/\text{mm}^2$) versus the control group (Table 8). HMEMP co-treatment reduced these markers dose-dependently. Group III (20 mg/L) showed partial improvement, while Group IV (40 mg/L) approached control levels ($10.6 \pm 1.6\%$, $49.8 \pm 2.7/\text{mm}^2$). Statistical analysis confirmed significant group differences in GFAP-positive area with F value 41.28 and p value less than 0.0001, and in GFAP⁺ cell count with F value 52.64 and p value less than 0.0001.

H&E staining

The figure presents the investigation of ZnO NPs effects on zebrafish brain tissue architecture and the therapeutic potential of *M. pudica* extract, as assessed through H&E staining across the different treatment groups (Fig. 13). H&E staining of the Positive control group demonstrated subtle but distinct histopathological alterations, including mild neuronal degeneration, vacuolization, and occasional cellular disorganization within the brain parenchyma. These changes corroborate the GFAP findings, reflecting tissue damage and reactive gliosis. In contrast, treatment groups receiving ZnO NPs combined with low and high doses of HMEMP showed a dose-dependent attenuation of these histopathological changes. GFAP staining intensity was reduced, with fewer hypertrophic astrocytes and a more preserved tissue architecture, suggesting the neuroprotective effect of HMEMP against ZnO NP-induced damage.

Discussion

AD is linked to the accumulation of β -amyloid plaques and oxidative stress in the brain. Oxidative stress is a key factor in AD and other neurodegenerative disorders (Firdous et al., 2024a; b). The bioactive compounds in *M. pudica* extract, known for their antibacterial activity (Gopinath et al., 2020) may also possess antioxidant properties. By reducing oxidative stress through its antioxidant effects, *M. pudica* extract could offer neuroprotective benefits, aiding in the management of AD and other conditions associated with oxidative damage in the brain.

The DPPH radical scavenging assay is a widely utilized method to evaluate the free radical scavenging ability of various samples, including plant extracts (Baliyan et al., 2022). The HMEMP leaf extract has demonstrated

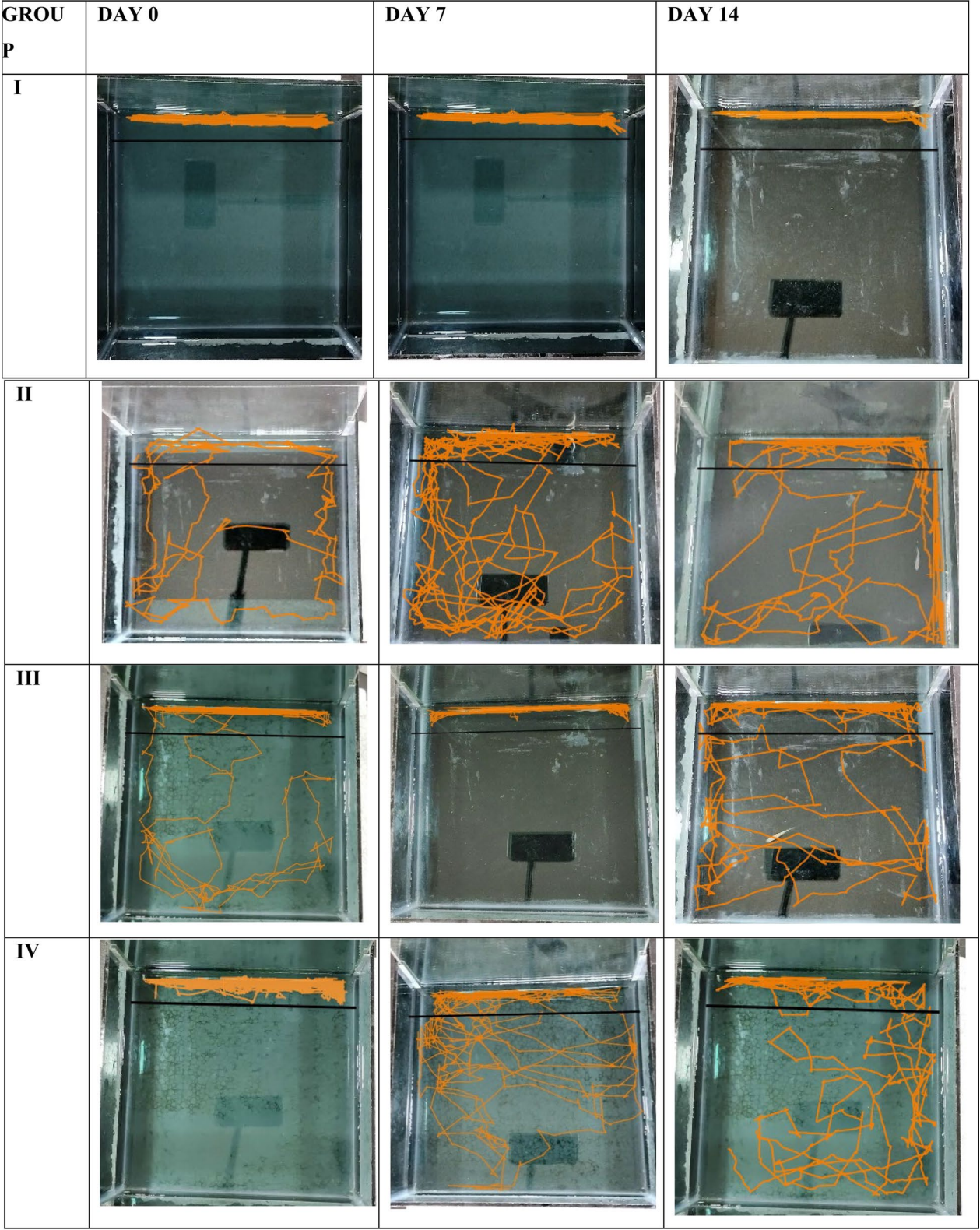


Fig. 6 Effect of HMEMP on time spent (min) in the approach zone (in pixels) by ZnO NPs-induced zebrafish in the MBT based on video-graphic analysis using Kinovea software on Days 0, 7, and 14

Table 4 Effect of HMEMP on time spent (min) in light compartment in ZnO NP-induced AD model in zebrafish in LDPT

GROUP	DAY 0	DAY 7	DAY 14
I	3.42 ± 0.04	3.42 ± 0.03	3.07 ± 0.14
II	3.14 ± 0.04	1.49 ± 0.14 ^{##}	0.51 ± 0.13 ^{##}
III	3.53 ± 0.13	1.45 ± 0.14	2.52 ± 0.39 ^{**}
IV	3.87 ± 0.15 [*]	4.15 ± 0.25 ^{**}	4.34 ± 0.08 ^{**}

Values are expressed as Mean ± S'EM, n'=5; [#]p'<0.05 and ^{##}p<0.01 considered statistically significant when compared to control group; ^{*}p<'0.05 and ^{**}p<'0.01 when compared to the positive control group

potential as a natural antioxidant through this assay. Results indicate that HMEMP leaf extract exhibits concentration-dependent DPPH radical scavenging activity. The antioxidant properties of *M. pudica* leaf extract, as demonstrated by the DPPH radical scavenging assay, have significant implications for the treatment of AD and oxidative stress-related conditions. The bioactive compounds present in *M. pudica* extract, which exhibit antioxidant activity, may offer neuroprotective benefits by reducing oxidative stress in the brain. By neutralizing free

radicals and inhibiting oxidative damage, the extract may help maintain cellular homeostasis and promote overall health. Previous studies also demonstrate the antioxidant properties of *M. pudica* extract. In one study, the DPPH radical scavenging activity of *M. pudica* extract and *L-Mimosine*, a major bioactive compound in the plant, was compared (Fernandes et al., 2023). Both the extract and *L-Mimosine* exhibited concentration-dependent free radical scavenging activity. However, *M. pudica* is known to contain a complex profile of phytochemicals including,

Table 5 Effect of HMEMP on time spent (min) toward the conspecific arm of the SPT

GROUP	DAY 0	DAY 7	DAY 14
I	4.46 ± 0.02	4.43 ± 0.05	4.34 ± 0.06
II	4.15 ± 0.14	3.52 ± 0.40 [#]	3.00 ± 0.20 ^{##}
III	3.56 ± 0.15 [*]	3.37 ± 0.03	3.19 ± 0.04
IV	3.37 ± 0.04 ^{**}	4.02 ± 0.24	4.06 ± 0.18 ^{**}

Values are expressed as Mean ± S'EM, n'=5; [#]p'<0.05 and ^{##}p<0.01 considered statistically significant when compared to control group; ^{*}p<'0.05 and ^{**}p<'0.01 when compared to the positive control group

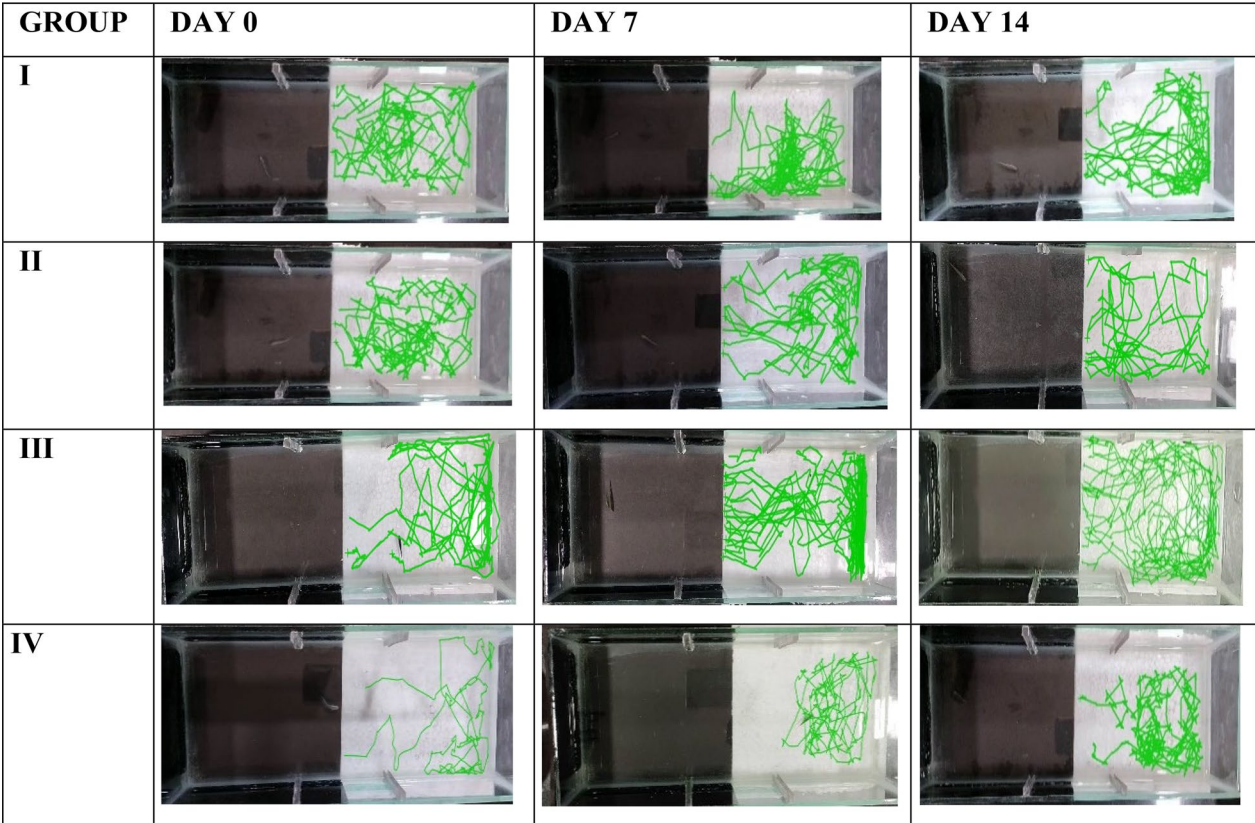


Fig. 7 Effect of HMEMP on time spent (min) in the light compartment (in pixels) by ZnO NPs-induced zebrafish in the LDPT based on video-graphic analysis using Kinovea software on Days 0, 7, and 14

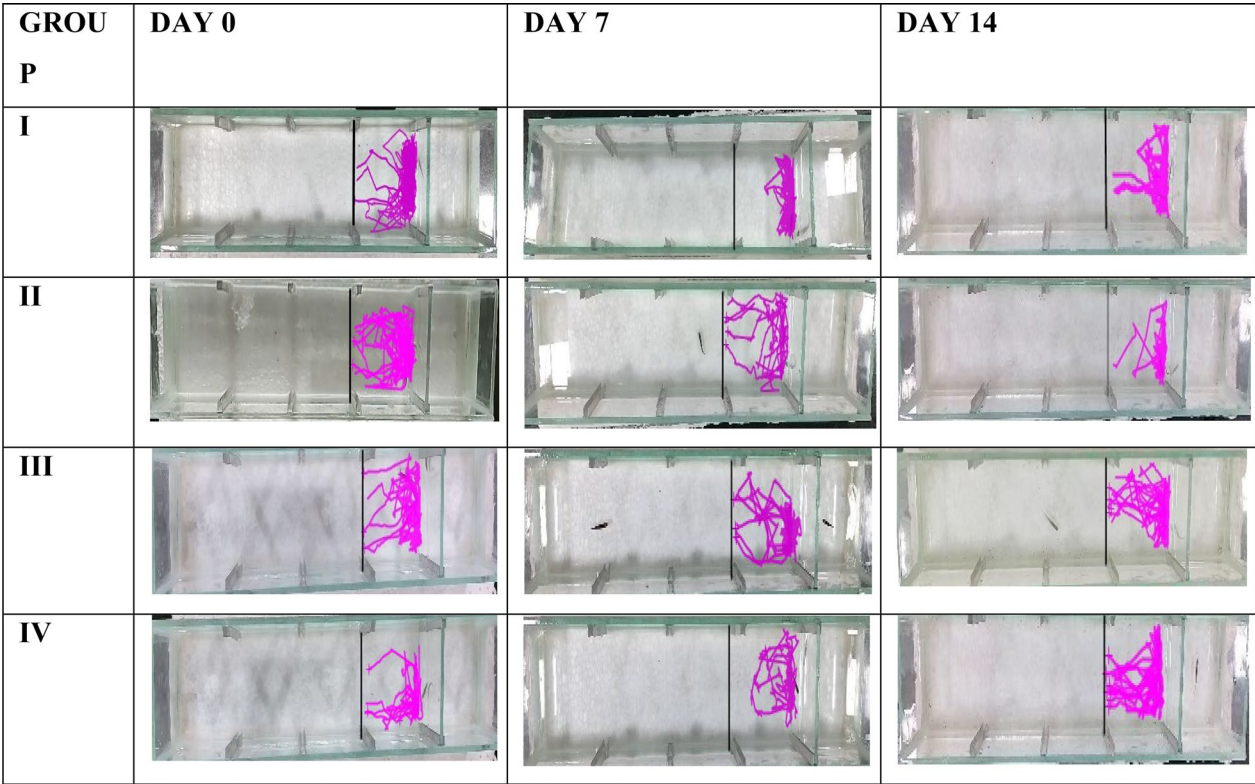


Fig. 8 Effect of HMEMP on time spent (min) toward the conspecific arm (in pixels) by ZnO NPs-induced zebrafish in the SPT based on video-graphic analysis using Kinovea software on Days 0, 7, and 14

phenolics, flavonoids, alkaloids, and others, with established antioxidant and neuroprotective effects. While L-mimosine is one possible contributor, the overall antioxidant activity observed in our HMEMP extract likely reflects the combined or synergistic effects of multiple constituents. Accordingly, further research, such as bioactivity-guided fractionation and characterization, is needed to identify the specific bioactive compounds responsible for the antioxidant and neuroprotective activities in our extract.

Table 6 Effect of HMEMP on time spent (min) toward the identical object of ZnO NPs-induced AD model in zebrafish in OBFT

GROUP	DAY 0	DAY 7	DAY 14
I	1.39 ± 0.05	1.09 ± 0.13	1.23 ± 0.05
II	1.22 ± 0.03 [#]	0.59 ± 0.14	0.40 ± 0.05 ^{##}
III	1.17 ± 0.03	0.56 ± 0.15	1.36 ± 0.06 ^{**}
IV	1.33 ± 0.03	1.19 ± 0.05 [*]	1.42 ± 0.21 ^{**}

Values are expressed as Mean ± S'EM, n' = 5; [#]p' < 0.05 and ^{##}p < 0.01 considered statistically significant when compared to control group; ^{*}p < '0.05 and ^{**}p < '0.01 when compared to the positive control group

Potential treatment strategies for disorders related to oxidative stress and AD can be better understood with the use of IR spectroscopy. Carbonyl groups, as seen by peaks at 1713 cm⁻¹ in our analysis, probably come from chemicals like ketones or aldehydes. It is well-documented that these substances amplify oxidative stress by encouraging the production of ROS, which in turn harms cells (Oyerinde et al., 2023). Reducing oxidative stress and protecting neurons from its harmful effects in AD patients may be possible by targeting these carbonyl molecules (Singh et al., 2019). Possible antioxidant characteristics are also indicated by peaks in the 3200–3600 cm⁻¹ and 1090 cm⁻¹ areas, which indicate the existence of alcohol or phenol functional groups. Compounds containing alcohol and phenol have antioxidant properties, meaning they can neutralize free radicals and lessen the effects of oxidative stress (Santos-Sánchez et al., 2019). Neurodegenerative diseases are characterized by oxidative damage; using these chemicals in treatment plans may mitigate this effect. Peaks at 3016 cm⁻¹ and 2978 cm⁻¹, which show the presence of alkane functional groups, further suggest the presence of hydrophobic substances. It is within the realm of possibility that these substances can pass the blood–brain barrier and

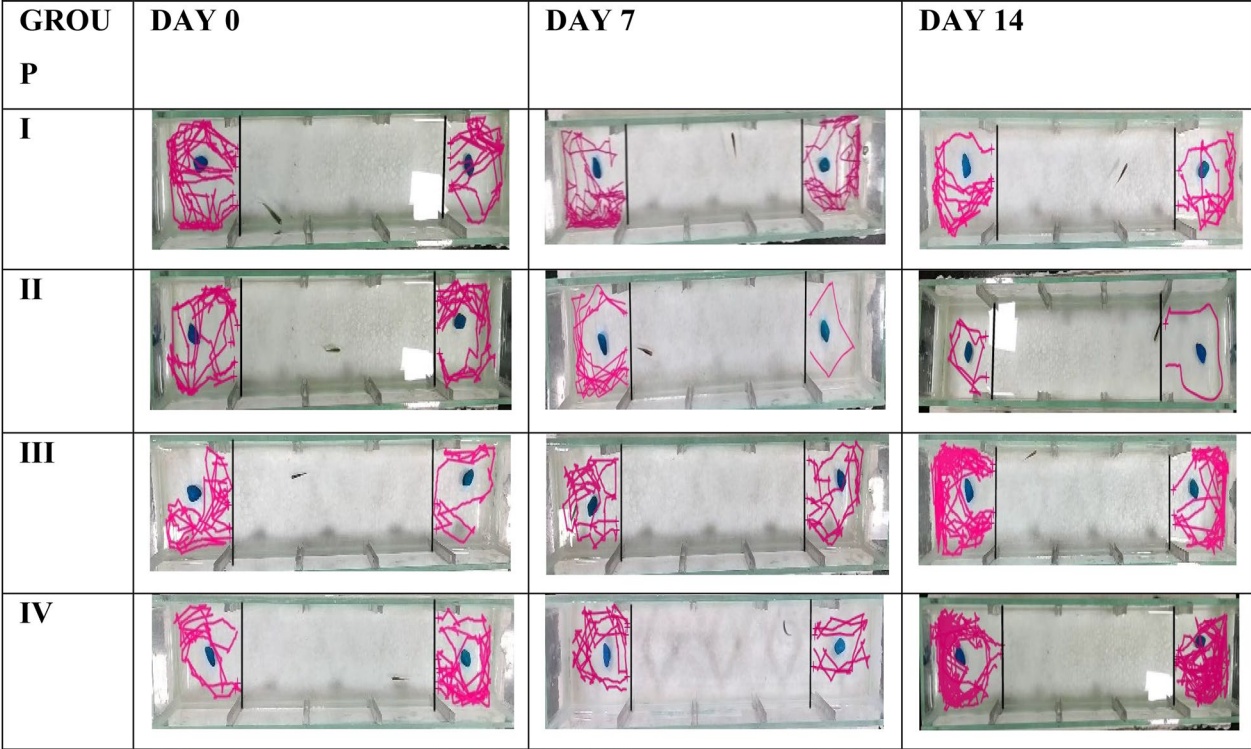


Fig. 9 Effect of HMEMP on time spent (min) toward the identical objects (in pixels) by ZnO NPs-induced zebrafish in the OBFT based on video-graphic analysis using Kinovea software on Days 0, 7, and 14

safeguard neurons. Hydrophobic chemicals have the potential to slow down the evolution of AD by reducing neuronal damage and dysfunction caused by oxidative stress (Perluigiet al., 2024).

Exposure to ZnO NPs caused a significant reduction in both the overall distance traveled and velocity in adult zebrafish. These findings indicate that ZnO NPs could adversely affect the movement and regulation of neurotransmitters in mature zebrafish. Nanoparticles such as ZnO NPs can hinder the activity of acetylcholinesterase (AChE), which is responsible for breaking down the neurotransmitter acetylcholine (Essa et al., 2019). This

interference can affect cognitive function and motor activity. Moreover, studies have demonstrated that exposure to zinc chloride hampers the levels of brain acetylcholine, has neurotoxic effects, and impairs memory and motor functions in adult zebrafish (Toni et al., 2023). The 48-h exposure period for ZnO NPs is critical for assessing acute toxicity, aligning with OECD guidelines and modeling real-world pollution spikes. Recent studies confirm that 48 h captures immediate hazards like ROS generation (Annangi et al., 2016), while longer exposures risk confounding factors like NPs aggregation, higher mortality (Kaya et al., 2017). In our investigation, we observed that the exposure of ZnO NPs to the animals resulted in an elevation of oxidative stress and a reduction in antioxidant defenses. This led to neuroinflammatory effects and DNA damage in the brain, which are believed to be the underlying mechanisms of Zinc poisoning. On the same days, the animals showed a substantial increase in both the TDT and velocity when treated with the extract at both doses. This implies that the extract could potentially regulate the neurotransmitter activity in response to exposure to ZnO NPs, hence mitigating the adverse effects found in the positive control groups.

Zebrafish exhibit mirror-biting behavior by biting their reflection and tracing the mirror image by quickly

Table 7 Effect of HMEMP on time spent (min) toward the novel objects in Zno NPs induced AD model in zebrafish in NORT

GROUP	DAY 0	DAY 7	DAY 14
I	1.52 ± 0.13	1.21 ± 0.03	1.14 ± 0.15
II	1.02 ± 0.12 [#]	0.29 ± 0.05 ^{##}	0.56 ± 0.14 [#]
III	1.25 ± 0.07	1.11 ± 0.16 ^{**}	1.13 ± 0.01 [*]
IV	1.12 ± 0.02	1.04 ± 0.14 ^{**}	1.31 ± 0.08 ^{**}

Values are expressed as Mean ± S'EM, n'=5; [#]p'<0.05 and ^{##}p<0.01 considered statistically significant when compared to control group; ^{*}p<'0.05 and ^{**}p<'0.01 when compared to the positive control group

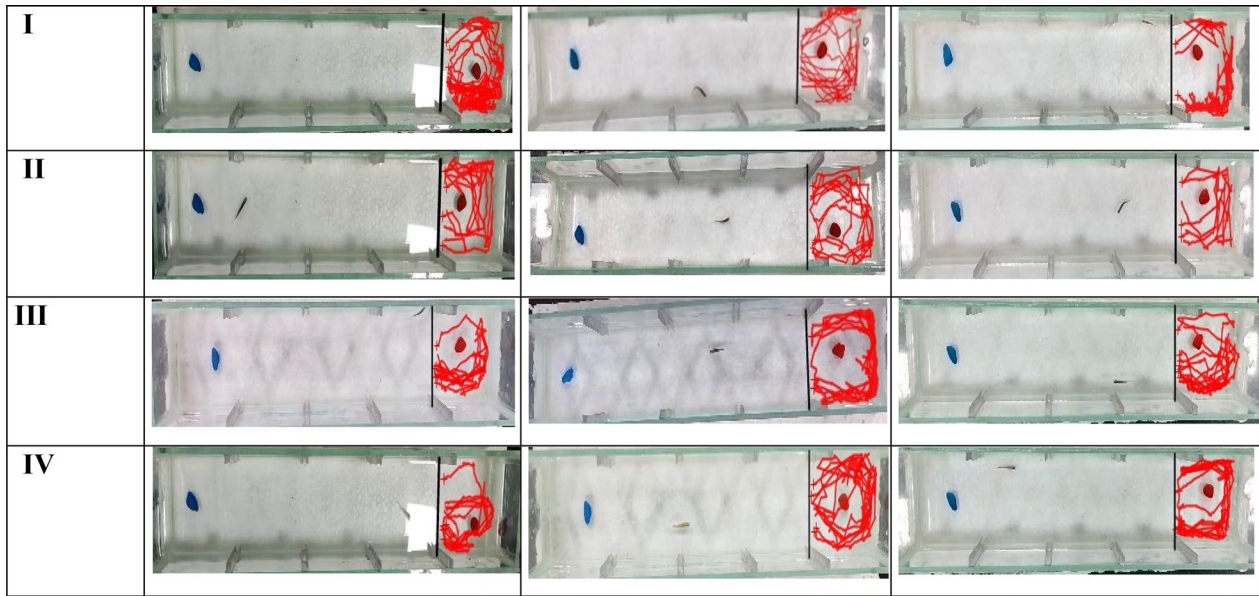


Fig. 10 Effect of HMEMP on time spent (min) toward the novel objects (in pixels) by ZnO NPs-induced zebrafish in the NORT based on video-graphic analysis using Kinovea software on Days 0, 7, and 14

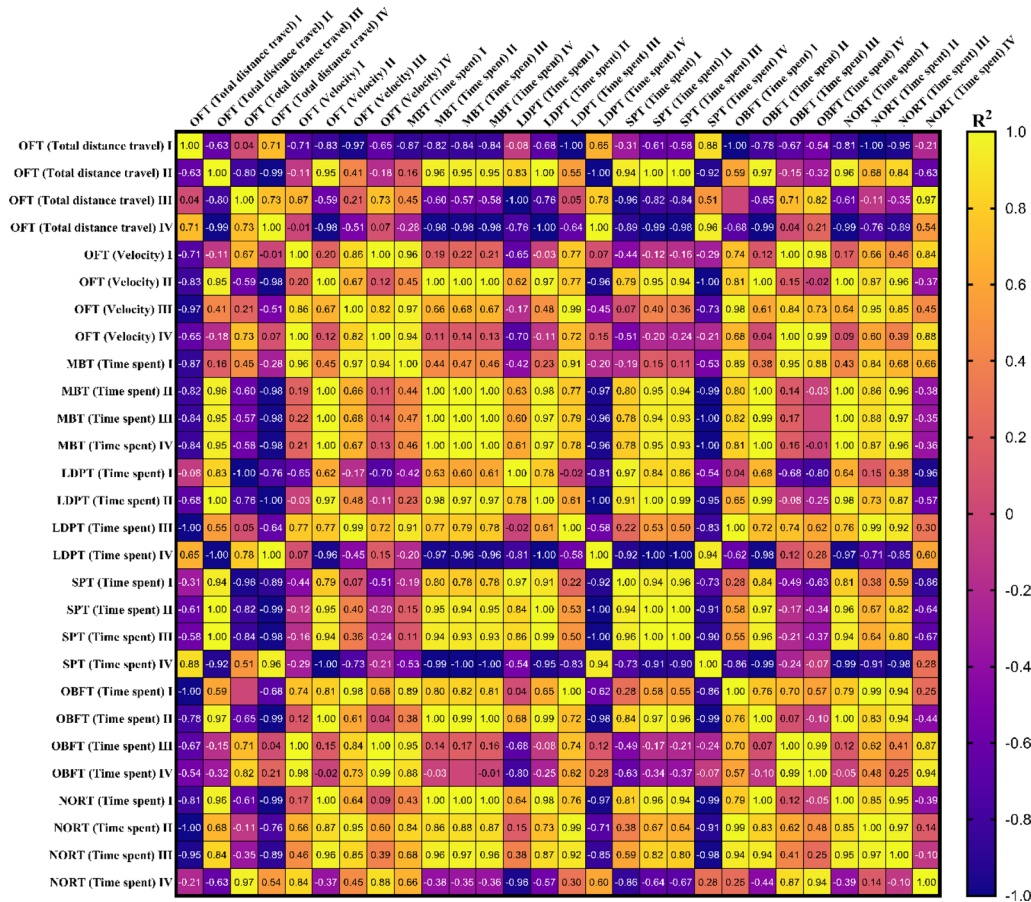


Fig. 11 Pearson correlation heatmap among all studied parameters

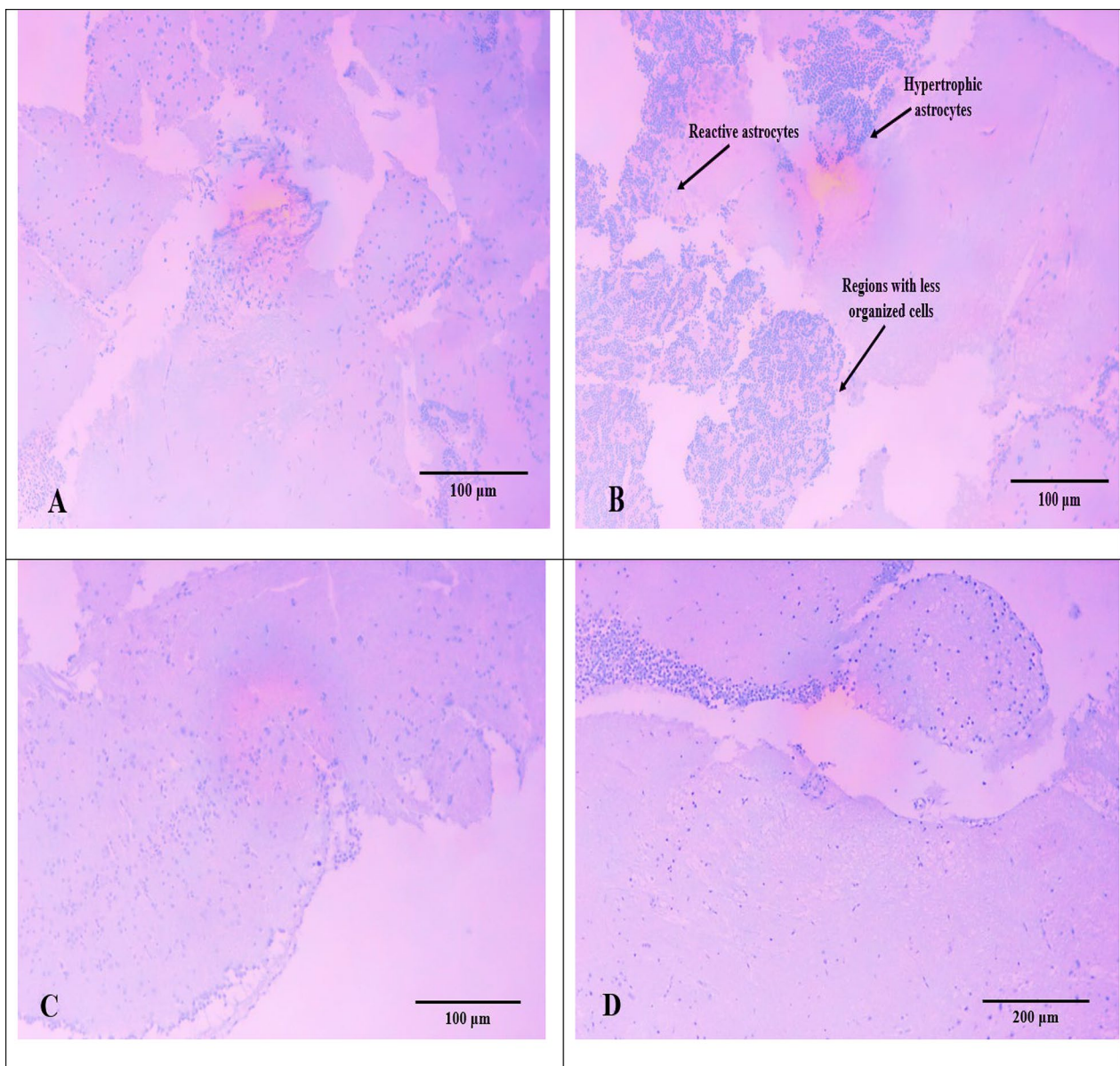


Fig. 12 Effect of HMEMP on GFAP in zebrafish brain. **a** Normal Control (Left); **b** Positive Control (Zebrafish treated with ZnO NPs) (Right); **c** Zebrafish exposed to ZnO NPs and treated with lower dose (20 mg/L) of HMEMP (Left); **d** Zebrafish exposed to ZnO NPs and treated with higher dose (40 mg/L) of HMEMP (Right);

swimming back and forth. The amount of time spent close to the mirror and the number of mirror bites are used to quantify aggression and social interaction (Midtun et al., 2020). The MBT has been employed to study the effects of various neuropsychiatric and neuropsychological diseases, such as schizophrenia, personality disorders, and autism, which show some form of social deficiency. It has also been utilized in studies investigating the effects of neurotoxic compounds like zinc and lead on zebrafish behavior. In a neurotoxicity model

using $PbCl_2$, exposure reduced the fish's locomotor activity and aggressive behaviors in a 3D LAT and mirror-biting test, respectively (Bui et al., 2020). This indicates that neurotoxic compounds can lead to a decrease in aggressive behaviors, such as mirror biting, in zebrafish models. The Zn-induced AD model in zebrafish also displayed an elevation of ROS levels, lipid peroxidation markers and a reduction of melatonin and antioxidant enzyme activities in the brain (Surai et al., 2024). These neurochemical changes can contribute to the observed behavioral

Table 8 Histological quantification of GFAP expression across treatment groups

GROUP	GFAP-Positive Area (%)±SD	GFAP + Cell Count (/mm ²)±SD
I	8.2±1.3	42.5±3.1
II	26.7±2.9 ^{##}	87.2±4.5 ^{##}
III	17.4±2.1 [*]	61.3±3.9 ^{**}
IV	10.6±1.6 ^{**}	49.8±2.7 [*]

Values are expressed as Mean ± S'D, n'=5; ^{*}p'<0.05 and ^{##}p<0.01 considered statistically significant when compared to normal control group

alterations, including decreased aggression. Regarding the regulation of neurotransmitters, the Zn-induced AD model in zebrafish showed increased AChE activity, dopamine, glutamate, and GABA levels while decreasing glycine and histidine levels (Benchoula et al., 2019). These changes in neurotransmitter systems, particularly the cholinergic system, are associated with the cognitive and behavioral deficits observed in AD (Bekdash, 2021). Therefore, it is reasonable to predict that the mirror-biting behavior in the Zn-induced AD model of adult zebrafish would gradually decrease from Day 0 to Day 14, as the cognitive impairment and neurochemical changes associated with disease progression manifest in this

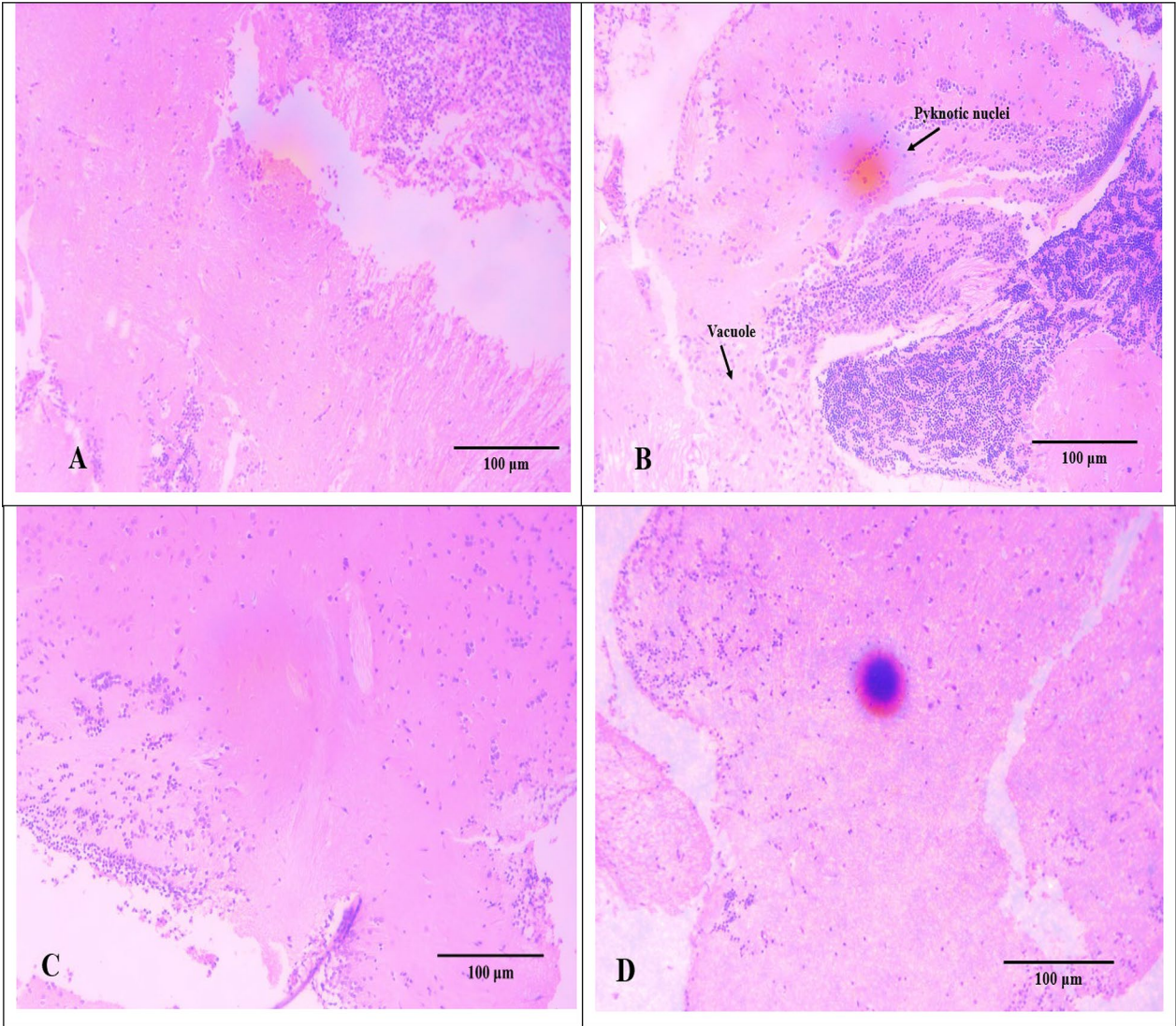


Fig. 13 Effect of HMEMP on zebrafish brain (H&E staining; magnification 100x). **a** Normal Control (Left); **b** Positive Control (Zebrafish treated with ZnO NPs) (Right); **c** Zebrafish Exposed to ZnO NPs and treated with lower dose (20 mg/L) of HMEMP (Left); **d** Zebrafish Exposed to ZnO NPs and treated with higher dose (40 mg/L) of HMEMP (Right)

model. Zebrafish exposed to ZnO NPs exhibited a notable reduction in the number of attempts at mirror biting compared to the normal control group. Conversely, the extract-treated groups demonstrated a significant improvement or increase in the number of attempts compared to the ZnO NPs-induced group.

Typically, the LDPT is employed to evaluate anxiety-like behavior in zebrafish, with an extended duration in the dark area indicating heightened anxiety (Johnson et al., 2023). AD is commonly linked to changes in neurotransmitter systems, namely a reduction in acetylcholine levels, which may affect anxiety-related behaviors. Our study found that the introduction of zinc in zebrafish created a model of AD. The results indicate that the zebrafish exposed to ZnO NPs showed a decreased ability to remember information and spent a longer duration in the dark area, which is associated with higher levels of anxiety-like behavior. Previous research has demonstrated that in other models of neurotoxicity, such as those caused by lead, zebrafish have also exhibited a decrease in the time it takes for them to enter the dark compartment in the IAT, which suggests a decline in memory function (Liaquat et al., 2019; Wang et al., 2022). This provides more evidence that being exposed to toxins that cause AD results in a reduction in the amount of time spent in the light compartment during behavioral tests. The results of our study indicate that the Zn-induced AD model shows comparable trends, with a gradual decrease in exploration of the light compartment over time. This supports the idea that the model accurately represents certain features of AD-related anxiety and cognitive loss. These findings indicate that exposure to ZnO NPs may adversely affect exploratory behavior and the modulation of neurotransmitters in the animal model. Nevertheless, administration of the HMEMP extract at both doses in animals exposed to zinc resulted in a notable enhancement in exploratory behavior. Significantly, HMEMP exhibited a notable increase in the duration of time spent in the light compartment on day 14 at both experimental doses, indicating an elevated level of exploratory behavior.

ZnO NPs have been reported to upregulate neuronal excitability and induce glutamate release, potentially affecting the balance of neurotransmitters in the CNS (Dai et al., 2021). This imbalance in neurotransmitters such as dopamine, serotonin, and glutamate can lead to disruptions in social behavior, as these neurotransmitters play crucial roles in regulating social interactions and cognition (Fernández et al., 2018). Specifically, exposure to ZnO NPs has been associated with impaired learning and memory abilities, altered spatial cognition, and enhanced long-term potentiation in animal models. Additionally, ZnO NPs have been shown to affect

dopamine, 5-HT, and their metabolites' levels, potentially impacting behavior and cognitive function. Furthermore, studies have demonstrated that ZnO NPs can induce changes in brain monoamines such as dopamine, norepinephrine, and serotonin, as well as ions like Ca^{2+} , Na^{+} , K^{+} , and Zn^{2+} (Alimohammadi et al., 2019). These alterations in neurotransmitter levels and signaling can lead to cognitive impairment, memory deficits, and behavioral changes in response to ZnO NP exposure. In our study, zebrafish exposed to ZnO NPs exhibited a notable reduction in the time spent toward the conspecific arm of the SPT compared to the normal control group. The reduction in time spent toward the conspecific arm in the social preference test indicates that ZnO NPs exposure impairs the ability of zebrafish to recognize and interact with their peers. This social recognition memory deficit is likely due to the alterations in neurotransmitter levels and signaling caused by ZnO NPs in the brain regions responsible for social behavior, such as the telencephalon and hypothalamus (Teleanu et al., 2022).

When zebrafish were treated with HMEMP, they showed a notable increase in the amount of time spent in the conspecific arm compared to the positive control group. This suggests that HMEMP positively influenced social behavior, which had been disrupted by ZnO NPs. Possible processes responsible for the protective effects of HMEMP include the presence of chemicals that can restore the equilibrium of important neurotransmitters like dopamine, serotonin, and glutamate. These neurotransmitters play a critical role in social behavior and cognition. This modulation is anticipated to alleviate the social behavior deficits caused by ZnO NPs. The extract's ability to counteract oxidative stress and neuroinflammation generated by ZnO NPs, as demonstrated by DPPH and FTIR assays, indicates its potential to safeguard brain circuits responsible for social behavior. HMEMP has the potential to improve the ability of synapses to change and strengthen, as well as promote the functioning of neurons in brain areas involved in social recognition, such as the telencephalon and hypothalamus. This can help counterbalance the harmful effects caused by ZnO NPs.

Assessing the amount of time that each fish spends exploring two distinct items is crucial to the neurobehavioral and neurotransmitter components of the novel object recognition test. This assessment measures the fish's spatial awareness and memorization abilities (Wallace & Hofmann, 2021). OBFT allows investigators to compare the amount of time fish spend exploring known objects with that of unfamiliar ones. This comparison provides valuable insights regarding the fish's cognitive ability and recognition memory. Furthermore, by comparing the two, we can learn more about the neurobehavioral and neurotransmitter systems that

underlie zebrafish recognition memory, which in turn helps us comprehend the fish's responses to novel stimuli and retention of previously encountered objects. The reduced exploration time toward identical objects in ZnO NP-exposed zebrafish reflects these neurobiological disruptions (as summarized above). Treatment with HMEMP significantly improved exploration time, likely by mitigating oxidative stress and restoring neurotransmitter balance. Exposure to ZnO NPs in zebrafish induces oxidative stress and neuroinflammation, which cause disruptions in multiple neurotransmitter systems, including glutamate, GABA, dopamine, and serotonin. These neurochemical alterations impair synaptic plasticity, neurogenesis, and the expression of neurotransmitter transporters and receptors, collectively leading to deficits in exploratory behavior, recognition memory, and cognitive function (Alimohammadi et al., 2019; Bernhardt et al., 2018; Dai et al., 2021). Conversely, fish challenged with ZnO NPs and treated with HMEMP at higher concentrations displayed a significant increase in exploration time toward identical objects. This increase suggests a potential modulation of neurotransmitter regulation by HMEMP. The antioxidant properties of HMEMP counteract oxidative stress induced by Zn NPs, while its ability to balance glutamate and GABA levels enhances synaptic plasticity and reduces anxiety-like behaviors. HMEMP also promotes dopaminergic signaling, regulates serotonin levels, and reduces stress, collectively contributing to increased exploration behavior. The alignment of these results with those of the light–dark preference test and social preference test underscores the comprehensive impact of ZnO NPs exposure on neurotransmitter regulation and behavior.

The NORT assesses exploratory behavior by measuring the time spent investigating a novel object versus a familiar one (Wallace & Hofmann, 2021). In zebrafish exposed to ZnO NPs, the decline in exploratory behavior suggests disruptions in neurotransmitter regulation. Similarly, the decline in novel object exploration observed in ZnO NP-exposed zebrafish is attributable to the aforementioned mechanisms of oxidative stress-induced neurotransmitter dysregulation and impaired synaptic plasticity. HMEMP treatment counteracted these effects, enhancing exploratory behaviour (Zhang et al., 2022). Impaired dopaminergic signaling affects motivation and reward-based behaviors, and altered serotonergic systems may induce anxiety or depression-like states (Callaghan et al., 2018). Conversely, treatment with HMEMP enhances exploratory behavior, surpassing even the positive control group exposed to ZnO NPs. This suggests that HMEMP counteracts the neurotoxic effects of ZnO NPs through various mechanisms, including antioxidant properties, modulation of neurotransmitter systems, reduction of

neuroinflammation, promotion of neurogenesis and synaptic plasticity, and stress reduction. HMEMP's multifaceted effects highlight its potential as a therapeutic agent in mitigating neurotoxicity and supporting cognitive and behavioral outcomes in zebrafish.

The GFAP assay provides insight into the impact of ZnO NPs on zebrafish brain tissue architecture, with implications for neurodegeneration and potential therapeutic interventions (Jin et al., 2021). In the normal control group (A), astrocytes exhibit typical morphology with evenly distributed GFAP activity, indicating intact tissue architecture. In contrast, the positive control group (B), representing zebrafish treated with ZnO NPs for AD development, displays significant tissue alterations characterized by increased GFAP activity, hypertrophic astrocytes, and neurodegenerative changes. Treatment with a lower dose of *M. pudica* extract (C) results in partial improvement, although less pronounced compared to the higher dose. Despite some remaining irregularities, reduced GFAP activity and improved tissue architecture indicate a degree of neuroprotective effects. Similarly, treatment with a higher dose of *M. pudica* extract (D) shows moderate improvement, with reduced GFAP activity and less pronounced astrocytic activation. The tissue structure demonstrates signs of recovery, suggesting potential therapeutic efficacy. These findings highlight the potential of *M. pudica* extract as a therapeutic agent for mitigating ZnO NPs-induced neurodegeneration, offering promise for future neuroprotective interventions.

The effects of ZnO NPs on zebrafish brain tissue architecture and the therapeutic potential of *M. pudica* extract were investigated using H&E staining. In the normal control group (A), zebrafish displayed normal tissue architecture with well-preserved neuronal structures, intact blood vessels, and no signs of inflammation or degeneration. Conversely, zebrafish treated with ZnO NPs (B) exhibited significant tissue disruption, marked by neuronal loss, vacuolation, pyknotic nuclei, and irregular cell boundaries, alongside signs of inflammation and gliosis. Blood vessels in this group showed evidence of leakage or hemorrhage. Treatment with a higher dose of *M. pudica* extract (C) resulted in noticeable improvement, with more intact neuronal structures, reduced vacuolation, and regular cell boundaries. There was also a decrease in inflammation and gliosis, and blood vessels appeared more intact. Zebrafish treated with a lower dose of *M. pudica* extract (D) showed partial improvement, with some recovery in neuronal structures and moderate reductions in vacuolation and inflammation. However, cell boundaries were not as well-defined as in the higher dose group, and minor blood vessel damage persisted.

From the findings, ZnO NP exposure significantly increased GFAP-positive area and cell count, indicating astrogliosis. HMEMP co-treatment dose-dependently reduced these markers, with Group IV showing near-control levels, suggesting HMEMP mitigates ZnO NP-induced neuroinflammation and astrogliosis. These findings suggest that *M. pudica* extract, particularly at higher doses, has neuroprotective effects against ZnO NP-induced neurodegeneration in zebrafish. The analysis provides a comparative overview of the histological changes observed in the GFAP assay and H&E staining across different treatment groups in adult zebrafish, highlighting the effects of ZnO NPs and the potential therapeutic benefits of *M. pudica* extract. HMEMP exerts rapid neuroprotective effects primarily through its potent antioxidant activity. The bioactive compounds in the extract, including phenols and alcohol groups, scavenge free radicals and reduce oxidative stress induced by ZnO NPs. This antioxidant action mitigates ROS-mediated neuronal damage and neuroinflammation, preserving neurotransmitter balance and enhancing synaptic plasticity. Consequently, HMEMP restores cognitive and behavioral functions by protecting brain regions involved in memory, social interaction, and anxiety regulation.

Research constraint

The principal limitation of this study lies in the lack of a detailed mechanistic exploration of the neuroprotective actions of HMEMP. While the current work demonstrates significant behavioral improvement and histological recovery in the ZnO NP-induced zebrafish model of AD, it does not identify the specific bioactive compounds responsible for these effects. Furthermore, the precise molecular pathways, such as those involved in oxidative stress regulation, neurotransmitter modulation, neuroinflammation, or synaptic plasticity, were not investigated. The use of whole-plant extract, though practical for preliminary screening, limits the ability to attribute effects to defined chemical constituents. Additionally, the study is limited by the absence of molecular biomarkers that could validate behavioral and histopathological findings. This restricts deeper insight into the extract's mechanism of action. Future studies should incorporate targeted biochemical and molecular assays to elucidate the signaling cascades involved, as well as isolate and characterize active phytoconstituents. Moreover, the results are model-specific, and translation to mammalian systems or clinical contexts will require further validation.

Conclusion

This study demonstrated that HMEMP significantly mitigated neurobehavioral impairments in a zebrafish model of AD induced by ZnO NPs. Behavioral assessments revealed improvements in locomotor activity, social interaction, anxiety-like responses, and recognition memory across all paradigms following HMEMP treatment, particularly at the higher dose. These outcomes suggest that HMEMP may exert neuroprotective effects by reducing oxidative stress and modulating neurotransmitter-related behavior. Histological analysis further supported its role in attenuating astroglial activation and preserving brain tissue integrity. Overall, HMEMP shows promising therapeutic potential for neurodegenerative conditions like AD. Further research is warranted to identify the active compounds and clarify the underlying mechanisms.

Abbreviations

AD	Alzheimer's disease
ZnO NPs	Zinc oxide nanoparticles
DPPH	2,2-Diphenyl-1-picrylhydrazyl
HMEMP	Hydromethanolic extract of <i>Mimosa pudica</i>
FTIR	Fourier-transform infrared
GFAP	Gial fibrillary acidic protein
OD	Optical density
LC ₅₀	Lethal concentration 50
OFT	Open field test
MBT	Mirror-biting test
LDPT	Light-dark preference test
SPT	Social preference test
OBFT	Object familiarity test
NORT	Novel object recognition test
ANOVA	Analysis of variance
OECD	The organization for economic co-operation and development
TDT	Total distance traveled
AChE	Acetylcholinesterase
GABA	Gamma-aminobutyric acid
IAT	Implicit association test
CNS	Central nervous system
5-HT	5-Hydroxy tryptamine

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None.

Author contributions

The study plan was developed by SPM writing was carried out by SMF and SP. BT and DD contributed to data collection, as well as the construction of figures and tables. Data interpretation was performed by SMF and SP. The manuscript was revised to its final form by SMF.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethical approval and consent

All the experiments were carried out per the guidelines of zebrafish care and husbandry (Ref. No. PGIMS/2024/07-76).

Consent for publication

Not applicable.

Competing interest

The authors declare no competing interests.

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