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Evaluation of Toxic Impact of Mercuric Chloride on Some Behavioural and Neurological Indices of *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

Mercury chloride ($HgCl_2$) is a harmful pollutant that accumulates in fish tissues, and this study examines its effects on the behaviour and neurological health of *Clarias gariepinus*. Juvenile fishes (120) procured from the same brood were used in this study. The fish were acclimatised for 14 days before exposure to $HgCl_2$ concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/L in a 96-hour acute toxicity test. Mortality and behavioural responses were recorded, and the LC_{50} value from Probit regression informed the selection of sublethal concentrations of 0.067, 0.130, 0.200, and 0.267 mg/L for a 28-day sublethal test. After exposure, fish were anaesthetised and dissected, with brain samples analysed for biochemical markers such as acetylcholinesterase, glutamate dehydrogenase, malondialdehyde, glutathione, succinate dehydrogenase, superoxide dismutase, and nitric oxide. Data were analysed using one-way ANOVA and LSD post-hoc tests ($p<0.05$). Histological analysis assessed neurodegeneration. Behavioural symptoms included erratic swimming, balance loss, and increased mucus production, worsening with higher mercury concentrations and prolonged exposure. Mortality rates rose with mercury levels, reaching 100% at 1.2 mg/L within 48 hours. The LC_{50} for $HgCl_2$ was 0.267 mg/L. Biochemical results showed increased activities of acetylcholinesterase, glutamate dehydrogenase, malondialdehyde, and nitric oxide and decreased levels of glutathione, succinate dehydrogenase, and superoxide dismutase in exposed fish. Histological examination revealed no brain changes in controls but significant neurodegeneration in mercury-exposed fish, especially at 0.200 and 0.267 mg/L. These findings highlight $HgCl_2$'s severe neurotoxicity to *Clarias gariepinus*, affecting brain function and inducing oxidative stress.

Keywords: Mercury, *Clarias gariepinus*, Brain, Acute, Sublethal, Toxicity.

Introduction

Despite extensive evidence of its detrimental effects, pollution continues to increase globally due to human activities[1], with Nigeria facing significant water quality issues from industrial effluents, agricultural pesticides, petroleum activities, and other contaminants.[2] The rise in industrial and agricultural activities leads to the release of various pollutants, particularly metals, into aquatic ecosystems.[3] In aquatic environments, mercury can bioaccumulate in fish and other aquatic organisms, leading to potential toxicity and adverse health effects.[4] Mercury chloride, a highly toxic and water-soluble compound, poses significant threats to aquatic life due to its ready availability for uptake by organisms like fish.[5] Mercury chloride readily penetrates the blood-brain barrier, accumulating in brain tissues, where it triggers oxidative stress and disrupts neurotransmitter functions, thereby targeting the central nervous system.[6]

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Mercury compounds produce reactive oxygen species, impair antioxidant defences, and result in oxidative damage to neuronal cells, which in turn leads to behavioural and neurological alterations in aquatic organisms.[7] Heavy metals can induce neurotoxic effects in fish species, resulting in histopathological changes that affect both the structure and function of their nervous system.[8] The African sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), is a widely researched freshwater fish known for its broad geographic range, economic significance, and ecological versatility.[9] Fish toxicity testing is an efficient method for assessing the impact of environmental pollutants on aquatic ecosystems, with *Clarias gariepinus* being a suitable test organism due to its well-studied biology and resilient nature.[10]

Although the toxic effects of mercury compounds on aquatic organisms are well documented, there is limited understanding of the specific neurotoxicity of mercury chloride in *Clarias gariepinus*. Exploring these effects will enhance knowledge of mercury toxicity mechanisms and help develop strategies to mitigate its environmental impact. This study aimed to investigate the neurotoxic effects of mercury chloride on *Clarias gariepinus* by evaluating changes in neurological enzyme levels, oxidative stress biomarkers, and behavioural responses. It examined both acute and sub-lethal toxicities through static and renewal bioassays, as well as neurohistopathological changes such as neuronal degeneration, gliosis, and inflammation.

Materials and Methods

Collection of the Experimental Animals

A total of 120 juvenile *Clarias gariepinus* were procured from the same brood and utilised in this study. The fish were obtained from Super Fish Farm located in Ogbomoso, Nigeria (Latitude: 8.1383° N, Longitude: 4.2400° E) in March 2023 and transported to the fisheries laboratory at the Department of Pure and Applied Biology, Ladoke Akintola

University of Technology, Ogbomoso. The average total length and body weight of the fish were 28.2 ± 0.72 cm and 350.27 ± 2.01 g, respectively. Before the collection, the laboratory was prepared, and all necessary materials were organised for the study.

Ethical Approval
All experimental procedures and materials were approved by the Faculty of Basic Medical Sciences' Ethical Research Committee of Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The ERC Approval Number is ERCFBMSLAUTECH:065/09/2024.

Reagents
Reagents used include anhydrous mercury chloride and Mercuric chloride powder (98% purity) (Sigma-Aldrich, Sigma Chemicals Co., St. Louis, MO, USA), Glutamate Dehydrogenase (GDH) Activity Assay Kit (KA3787) (Abnova, CA, USA), Acetylcholinesterase Activity Assay Kit (Sigma Aldrich, St. Louis, MO, USA)

Experimental Designs
The fish were divided into two main categories: the first category consisted of six treatment groups and a control group for the acute toxicity test. The second category included four treatment groups and a control group for the sublethal toxicity test, with each group encompassing both treatment and control groups, contained ten fish.

Acclimatisation
The fish were housed in plastic containers measuring 28 x 24 x 42.5 cm, filled halfway with 30 litres of water. They were acclimated to the laboratory environment for 14 days, during which the water was changed daily to avoid the buildup of waste metabolites and food debris. The fish were fed conventional fish-feed pellets twice a day.

Acute Toxicity Testing
The acute toxicity test followed the methods recommended by the American Society for Testing of Materials using a static bioassay technique where the test media were renewed every 24 hours at the same concentration.[11] After the acclimatisation period, a range-finding test was conducted using concentrations of mercury chloride between 0.2 mg/L and 1.5 mg/L, following the methodology of Guedenon *et al*.[12] This resulted in the establishment of a concentration gradient of 0.2 mg/L, 0.4 mg/L, 0.6 mg/L, 0.8 mg/L, 1.0 mg/L, and 1.2 mg/L with the control group for the acute toxicity test. Ten juvenile catfish were selected for each of the seven groups. The groups were placed in separate plastic containers and exposed to varied concentrations for 96 hours. The plastic containers were covered with mosquito mesh nets to prevent the fish from jumping out or moving between bowls. The fish were fasted for 24 hours before and during the 96-hour exposure period. The setup was monitored hourly to observe changes in fish behaviour, and dead fish were removed. A fish was considered dead when immobile with no opercular movement when probed with a glass rod. The 96-h LC_{50} of $HgCl_2$ on *Clarias gariepinus* was calculated using Finney Probit regression analysis of the mortality results.

Sublethal Procedure
The median lethal concentration (LC_{50}) obtained from probit analysis of the acute toxicity test was used to design the sublethal study. The probit analysis showed that the 96-hour LC_{50} of mercury chloride for *C. gariepinus* was 0.267 mg/L, which was set as the highest dose for the sublethal test. Fifty fish were randomly divided into five equal groups of ten fish each. Group 1 served as the control, while Groups 2 to 5 were exposed to sublethal concentrations of 0.067 mg/L, 0.130 mg/L, 0.200 mg/L, and 0.267 mg/L of mercuric chloride, respectively, for 28 days. Behavioural responses and mortalities were recorded throughout the exposure period.

Sample Collection
After the 28-day chronic test, the fish were given a 24-hour resting period before sample collection began. They were anaesthetised with ketamine and then dissected to remove their brains from the cranial cavities. Each brain was quickly excised upon sacrifice, weighed, and stored in sterile plain bottles. The brain tissues designated for histological analysis were preserved in formalin within sterile bottles.

Tissue Homogenisation
Five whole brains from each sublethal concentration were placed in separate plain bottles for homogenisation. The brain samples were homogenised using a mortar and pestle on an ice bath to maintain enzyme activity during the process. A 0.1M phosphate buffer at pH 7.4 was prepared for homogenisation. The resulting homogenised brain samples were stored in clean sample bottles and placed in a freezer to prevent autolysis and microbial contamination.

Tissue Centrifugation
After homogenisation, the brain samples were centrifuged at 10,000 x g for 15 minutes.

Biochemical Analysis
Determination of Acetylcholinesterase (AChE)
Acetylcholinesterase (AChE) activity was determined using the Ellman method,[13] which involves the reaction of thiocholine produced by AChE with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) to form a yellow colour proportional to the AChE activity in the sample. The Acetylcholinesterase Activity Assay Kit, based on this method, was used. The test samples were diluted in an assay buffer, and a series of dilutions was prepared. Each dilution (75 µL) was pipetted into a 96-well plate, followed by the addition of substrate solution (75 µL) to each well. After incubation for 10 minutes at room temperature, 150 µL of working reagent was added to each well and mixed thoroughly. The plate was incubated for 15 minutes at room temperature and protected from light. Stop solution (75 µL) was then added to each well, mixed gently, and the absorbance was measured at 412 nm using a microplate reader.

Determination of Glutamate Dehydrogenase (GDH)
Glutamate dehydrogenase (GDH) activity was measured using the Glutamate Dehydrogenase (GDH) Activity Assay Kit (KA3787) from Abnova, United States. This colourimetric assay quantifies GDH activity by measuring the conversion of NADH to NAD+, resulting in a decrease in absorbance at 450 nm; the rate of this decrease is directly proportional to the GDH activity in the sample. Test samples were added to a 96-well plate along with assay buffer to achieve a total volume of 50 µL per well. Then, 50 µl of reaction mix was added to each well and mixed gently before incubating at room temperature for 30 minutes. The absorbance was measured at 450 nm using a microplate reader, and GDH activity was calculated using the formula provided in the kit manual.
The GDH activity was calculated using the following formula presented in Equation 1:

$$\text{GDH Activity } (\mu U/mg) = \left(\frac{\Delta A450}{\text{Reaction Time(min)} \times \text{Vsample(ml)}}\right) \times \left(\frac{\text{Vtotal}}{\textbf{Sample Concentration } (\mu g/ml)}\right) \quad (1)$$

Where: ΔA450 represents the change in absorbance at 450 nm, Vsample denotes the volume of the sample used, Vtotal indicates the total reaction volume, and Sample Concentration refers to the concentration of the sample in the well.

Determination of the Nitric Oxide Concentration
Nitric oxide (NO) concentration was determined using the Griess method, which measures nitrite production as an indicator of gas production through a diazotisation reaction.[14] The Griess Reagent System employs a chemical reaction involving sulfanilamide and N-1-naphthylethylenediamine dihydrochloride (NED) in acidic conditions (phosphoric acid) to detect nitrite (NO_2^-) in various biological and experimental liquid samples, including plasma, serum, urine, and tissue culture medium. The sensitivity to nitrite varies depending on the matrix, with a detection limit of 2.5 µM (125 pmol) in ultrapure, deionised distilled water.

For the assay, 50 mL of the test sample was placed in a 96-well microplate in quadruplicate, and Griess solution (1% sulfanilamide in 5% phosphoric acid and 0.1% NED in water) was added at room temperature. After 10 minutes, the absorbance was measured at 550 nm using an enzyme-linked immunosorbent assay (ELISA) plate reader. Nitric oxide concentrations were calculated from a standard curve of sodium nitrite and reported in millimolars (mM).

Determination of Malondialdehyde (MDA) Activity
The estimation of lipid peroxidation was conducted according to the method established by Varshney and Kale.[15] This process relies on the reaction between malondialdehyde (MDA) and thiobarbituric acid (TBA), which forms an MDA-TBA adduct that exhibits strong absorbance at 532 nm. For the assay, 0.4 mL of the samples was mixed with 1.6 mL of Tris-KCl buffer, followed by the addition of 0.5 mL of 30% trichloroacetic acid (TCA). Next, 0.5 mL of 0.75% TBA was incorporated, and the mixture was incubated for 45 minutes at 80°C, resulting in a pink-coloured reaction mixture. After incubation, the mixture was centrifuged at 3000 rpm for 15 minutes, and the absorbance of the clear pink supernatant was measured at 532 nm using a spectrophotometer.

Determination of Glutathione (GSH) Concentration
Glutathione (GSH) concentration was estimated using the method described by Kuo *et al*.[16] GSH reduces hydrogen peroxide (H_2O_2) to water (H_2O). 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), a water-soluble aromatic disulfide, reacts with thiol (SH) compounds at pH 8.0 to produce one mole of coloured complex per mole of thiol. The colour intensity of this complex anion is measured at 412 nm to determine the thiol (SH) concentration. GSH levels were expressed as μmol/mL of tissue.
To measure GSH, 0.4 mL of the whole sample was mixed with 1.6 mL of phosphate buffer and 2.0 mL of trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 15 minutes. Then, 1.0 mL of the homogenate was added to 1.5 mL of Na_2HPO_4 solution and 0.25 mL of DTNB. The colour intensity was measured at 412 nm against a reagent blank.

Determination of Succinate Dehydrogenase (SDH) Activity
Succinate dehydrogenase (SDH) levels in brain tissues were quantitatively measured using the method described by Beatty *et al*.[17] The assay mixture consisted of 1 mL of 0.1 M sodium succinate, 1 mL of 0.2 M phosphate buffer at pH 7.5, 1 mL of 0.1% tetrazolium salt (INT) solution, and 0.1 mL of brain homogenate. After thorough mixing, the tubes were incubated at 37°C for 1 hour, and the reaction was stopped by adding 0.1 mL of 30% trichloroacetic acid (TCA) to the assay mixture. Then, 7 mL of ethyl acetate was added to each tube, and they were centrifuged for 4 minutes after mixing to extract the colour. The optical density was measured at 420 nm using a Shimadzu UV/visible spectrophotometer. The results were calculated using the molar extinction coefficient of the chromophore ($1.36\times104\ M^{-1}$) and expressed as a percentage of the control.

The formula for calculating SDH activity in this assay is expressed in Equation 2:

$$\text{SDH activity (\%)} = \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100 \qquad (2)$$

Determination of Superoxide Dismutase (SOD) Activity
Superoxide dismutase (SOD) activity was determined using the method of Misra and Fridovich,[18] which relies on the ability of SOD to inhibit the auto-oxidation of epinephrine at pH 10.2 as the basis for a simple assay. In this assay, 1 mL of the sample was diluted in 9 mL of distilled water to obtain a 1 in 10 dilution. An aliquot of 0.2 mL of the diluted sample was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) and equilibrated in the spectrophotometer. The reaction was initiated by adding 0.3 mL of 0.3 M adrenaline to the mixture. The absorbance of the sample was measured at 480 nm against the blank and monitored from 0 seconds to 3 minutes. The formula for determining SOD activity is presented in Equation 3.

$$\text{SOD activity (U/mg protein)} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control} \times 50}\right) \times \text{Dilution factor} \qquad (3)$$

Tissue Processing for Histological Studies
Grossing
The tissues were examined and cut into small pieces no thicker than 4 mm, then placed in pre-labelled cassettes. They were immersed in 10% formal saline for 24 hours for fixation.

Tissue Processing
Tissue processing was performed automatically using a Leica TP 1020 tissue processor. The tissues passed through various reagents, starting with stations 1 and 2 containing 10% formal saline, followed by stations 3 to 7 with increasing concentrations of alcohol (70%, 80%, 90%, 95%, absolute 1, and absolute 2) for dehydration. The tissues then went through two changes of xylene at stations 8 and 9 for clearing before being transferred to three wax baths for infiltration. The entire process was programmed to run for 12 hours, with each station lasting 1 hour.

Embedding
Each processed tissue was embedded in paraffin wax using a semi-automatic tissue embedding centre. The molten wax was poured into a metal mould, where the tissue was orientated and buried. A pre-labelled cassette was placed on top, and the assembly was transferred to a cold plate to solidify. The resulting tissue block was then removed from the mould.

Microtomy
The blocks were trimmed to expose the tissue surface using a rotary microtome set to 6 micrometres. The surfaces were cooled on ice before sectioning, which was done at 4 micrometres to create ribbon sections.

Floating
The sections were floated on a water bath (Raymond Lamb) set at 45°C and then picked up using clean slides, which were labelled accordingly.

Drying
The slides were dried on a hot plate (Raymond Lamb) set at 60°C for 1 hour.

Staining
The staining technique employed was the Haematoxylin and Eosin method, following the procedure outlined by Avwioro.[19]

Procedure for Haematoxylin and Eosin (H and E) Staining
The tissue sections were dewaxed in xylene for 15 minutes, followed by passage through a series of alcohol solutions (absolute, 95%, and 70%) and rinsed with water. They were then stained with Harris haematoxylin for 5 minutes, briefly differentiated in 1% acid alcohol, blued under running tap water for 10 minutes, and counterstained with 1% aqueous eosin for 2 minutes. Finally, the sections were dehydrated in ascending grades of alcohol, cleared in xylene, and mounted using DPX.

Statistical Analysis
All mortality results were analysed in relation to the varying concentrations of mercury chloride. Finney's Probit regression analysis was employed to calculate the median lethal concentration (LC_{50}). The brain biochemical parameters for each fish in the control and experimental groups were measured, analysed, and reported as the mean ± SEM. One-way analysis of variance (ANOVA) was performed on the data to determine significance at $p < 0.05$. A Fisher's Least Significant Difference (LSD) post-hoc test was used to make pairwise comparisons between the control and each treatment group for each biochemical parameter. The analyses were conducted using SPSS version 25 (2017), while charts were plotted using GraphPad Prism 5 (2010).

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Results and Discussion

During acute toxicity tests on juvenile *Clarias gariepinus*, erratic swimming, sluggishness, and excessive mucus production were observed, particularly at mercury chloride concentrations of 0.8 mg/L, 1.0 mg/L, and 1.2 mg/L. Symptoms such as hyperactivity, respiratory distress, and loss of balance were noted, consistent with findings from Guedenon *et al*.[11] Longer exposure times were linked to higher mortality rates, indicating significant neurotoxic effects from mercury concentration. Increased mucus secretion may result from toxicity, while elevated opercula movement suggests respiratory distress due to gill impairment.[20] Skin peeling indicates epithelial damage, and vomiting reflects impaired gut motility. Overall, mercury exposure causes severe behavioural changes and increased mortality in aquatic organisms.

The study assessed the mortality rates of *Clarias gariepinus* (n=10) exposed to varying concentrations of mercury chloride ($HgCl_2$) over 96 hours (Table 1). The control group had 0% mortality, while higher concentrations and longer exposure times increased mortality, reaching 100% at 1.2 mg/L within 48 hours. The first death was observed 45 minutes after exposure at the highest concentration, and no deaths occurred in the control group, confirming that fatalities were due to mercury toxicity. Mortality rates were 50% at 0.2 mg/L, 60% at 0.4 mg/L, 70% at 0.6 mg/L, 80% at 0.8 mg/L, and 100% at 1.0 mg/L, demonstrating a dose-dependent response. This suggests that as the concentration increases, the likelihood of mortality also rises, which aligns with findings from previous studies on heavy metal toxicity in aquatic organisms.[21, 22] Although *C. gariepinus* generally shows resistance to various toxicants,[11, 23] exhibited limited resistance to mercury. The estimated median lethal concentration (LC_{50}) for mercury chloride was found to be 0.267 mg/L, with a 95% confidence interval ranging from 0.067 mg/L to 0.401 mg/L (Table 2). The relatively low LC_{50} value may be attributed to the juvenile stage of the organisms, aligning with findings from Ishikawa *et al*.[24] and Shyong and Chen[25] in other fish species.

Table 1: Mortality of *Clarias gariepinus* (n=10) exposed to acute mercury chloride ($HgCl_2$)

$HgCl_2$ (mg/L)	Fish (n=10)	No of Deaths at 24, 48, 72 and 96 hours				Mortality
		24h	48h	72h	96h	
Control	10	0	0	0	0	0
0.20	10	1	2	1	1	5
0.40	10	2	2	1	1	6
0.60	10	2	1	2	1	6
0.80	10	3	3	1	1	8
1.00	10	3	4	3	-	10
1.20	10	4	6	-	-	10

Table 2: Probability-Concentration Estimates and Confidence Intervals for LC_{50} of Mercury Chloride in *Clarias gariepinus* at 96 Hours

Probability	Concentration		
	Estimate	Lower Bound	Upper Bound
.010	0.025	0.000	0.083
.050	0.050	0.001	0.129
.100	0.073	0.003	0.163
.200	0.114	0.008	0.218
.500	0.267*	0.067	0.401
.600	0.347	0.146	0.506
.900	0.985	0.651	3.676
.990	2.847	1.361	60.157

* is the median lethal concentration (LC_{50}) estimate at 96h
Lower and Upper bounds indicate 95% Confidence Limits for Concentration.

The mean and standard error values for each biochemical parameter across four exposed groups and a control group are presented in Table 3. The study revealed a dose-response relationship between mercury chloride concentration and acetylcholinesterase (ACHE) activity in the brain of *Clarias gariepinus* (Figure 1), with higher mercury levels increasing ACHE activity compared to the control (0.06±0.01 µmol/g tissue), indicating neurotoxic effects on cholinergic neurotransmission and potential erratic swimming, consistent with findings in other fish [26] Glutamate dehydrogenase (GDH) activity increased with mercury concentration (Figure 2).

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Table 3: Dose-Dependent Effects of Mercury Chloride on Biochemical Parameters in the Brain Tissue of *Clarias gariepinus*

Biochemical Parameters	Control	0.067	0.130	0.200	0.267	P-value
ACHE µmol/gtiss	0.06±0.01	0.09±0.01	0.08±0.03	0.12±0.01	0.15±0.02	0.016*
GDH µmol/gtiss	2.26±0.65	2.58±0.40	3.23±0.51	3.55±0.60	4.19±0.40	0.107
MDA nmol/gtis	19.05±1.74	26.15±1.20	29.81±1.07	33.33±0.46	37.85±1.45	0.000*
GSH µmol/gtiss	1.93±0.21	1.52±0.23	1.07±0.08	0.98±0.06	0.88±0.15	0.001*
SOD µmol/gtiss	53.23±5.68	49.00±6.23	46.27±6.71	38.81±7.68	24.88±2.70	0.028*
SDH µmol/gtiss	14.40±0.39	13.99±0.88	12.89±1.09	9.37±1.76	10.05±1.24	0.016*
NO µmol/gtiss	13.70±1.27	17.14±1.53	20.01±1.36	25.12±2.01	25.29±0.70	0.000*

*indicate significance at p-value < 0.05

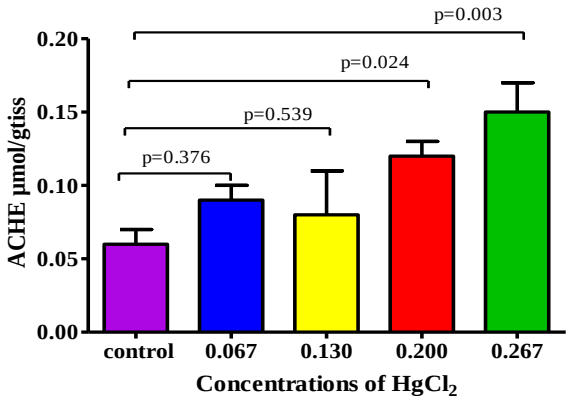

Figure 1: Acetylcholinesterase activities in the brain of *Clarias gariepinus* exposed to varying concentrations of Mercury Chloride.
The bar and error bar represent mean ± SEM p-values were determined by One-way ANOVA and LSD Post Hoc Test for multiple comparisons, and $p<0.05$ were considered significantly different.

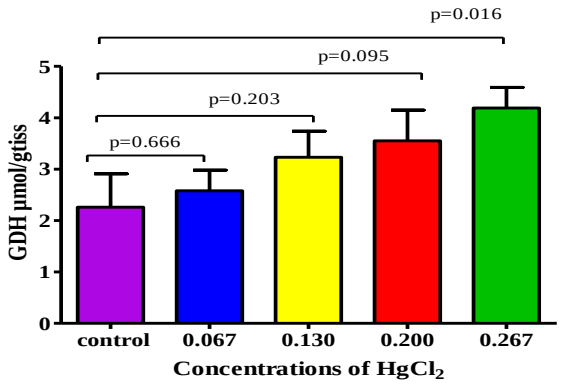

Figure 2: Glutamate Dehydrogenase activities in the brain of *Clarias gariepinus* exposed to varying concentrations of Mercury Chloride.
The bar and error bar represent mean ± SEM, p-values were determined by One-way ANOVA and LSD Post Hoc Test for multiple comparisons, $p<0.05$ were considered significantly different.

Still, differences were not statistically significant ($p > 0.05$), except between the control group (2.26±0.65 µmol/g tissue) and the highest sublethal dosage of 0.267 mg/L (4.19±0.40 µmol/g tissue). Mercury exposure may elevate extracellular glutamate levels,[27] disrupting neuronal function and causing oxidative stress in fish.[28]Malondialdehyde (MDA) levels significantly increased with mercury exposure (Figure 3), peaking at 0.267 mg/L (37.85±1.45 nmol/g tissue), indicating oxidative damage and lipid peroxidation.

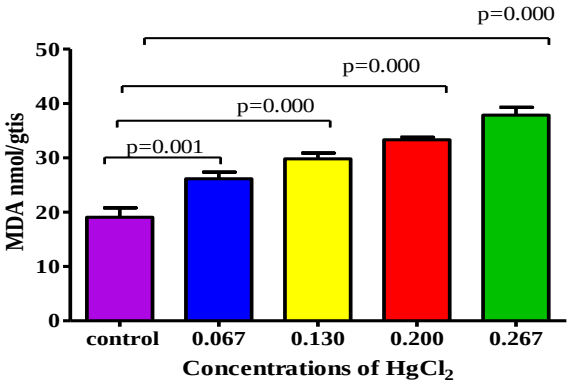

Figure 3: Malondialdehyde concentrations in the brain of *Clarias gariepinus* exposed to varying concentrations of Mercury Chloride.
The bar and error bar represent mean ± SEM p-values were determined by One-way ANOVA and LSD Post Hoc Test for multiple comparisons, and $p<0.05$ were considered significantly different.

These findings align with other studies and may explain physiological symptoms like skin peeling and excess mucus production.[29] Superoxide dismutase (SOD) activity declined at higher mercury concentrations, with the control group exhibiting the highest activity (53.23±5.68 µmol/g tissue) and the lowest at 0.267 mg/L (24.88±2.70 µmol/g tissue), as shown in Figure 4. This decrease suggests a reduced capacity to neutralise superoxide radicals and increased cellular damage.[30] Glutathione (GSH) levels also decreased with rising mercury concentration, from 1.93±0.21 µmol/g tissue in the control group to 0.88±0.15 µmol/g tissue at 0.267 mg/L, as shown in Figure 5, indicating compromised antioxidant defences and greater vulnerability to oxidative stress.[31]

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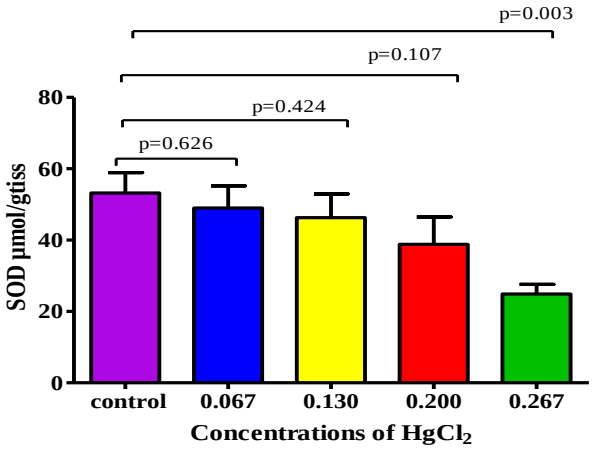

Figure 4: Superoxide dismutase concentrations in the brain of *Clarias gariepinus* exposed to varying concentrations of Mercury Chloride.
The bar and error bar represent mean ± SEM p-values were determined by One-way ANOVA and LSD Post Hoc Test for multiple comparisons, and $p<0.05$ were considered significantly different.

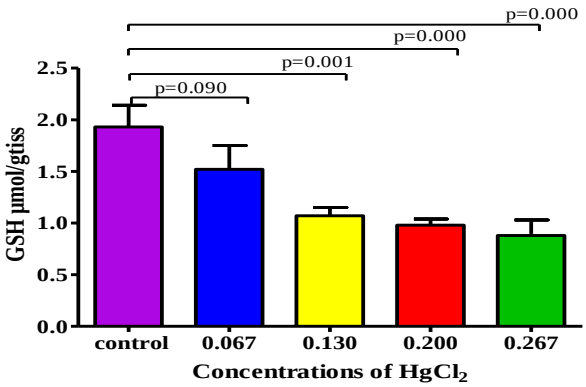

Figure 5: Glutathione concentration in the brain of *Clarias gariepinus* exposed to varying concentrations of Mercury Chloride. The bar and error bar represent mean ± SEM p-values were determined by One-way ANOVA and LSD Post Hoc Test for multiple comparisons, and $p<0.05$ were considered significantly different.

Nitric oxide (NO) levels significantly increased with mercury exposure, reaching 25.29±0.70 µmol/g tissue at 0.267 mg/L compared to 13.70±1.27 µmol/g tissue in the control (Figure 6). This increase in nitric oxide level indicates inflammation and cellular distress, which supports findings that elevated NO is a typical response to heavy metal stress.[32] Succinate dehydrogenase (SDH) activity significantly decreased with mercury exposure (Figure 7), indicating impaired mitochondrial function and reduced energy supply for cells.[33]
Neurohistology effectively assesses brain integrity and early neurotoxicity in aquatic organisms.[34] This study found that the control group of juvenile *Clarias gariepinus* exhibited normal histological features (Figure 8), including an intact diencephalon and granular layer in the cerebellum, indicating preserved neuronal structures essential for sensory processing and motor control,[35] with no significant lesions present. This aligns with previous studies on healthy fish.[36] In contrast, exposure to 0.067 mg/L $HgCl_2$ revealed significant neurotoxic effects, with damaged neuronal cells in the cortex indicating mercury exposure (Figure 9). Atrophic glial cells and mild gliosis suggested a reactive response, although excessive gliosis may worsen neuroinflammation.[37]

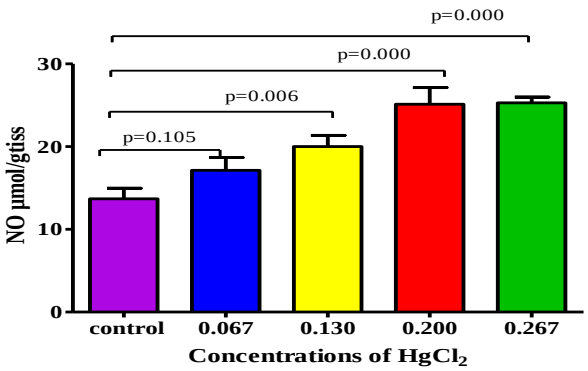

Figure 6: Nitric Oxide concentrations in the brain of *Clarias gariepinus* exposed to varying concentrations of Mercury Chloride.
The bar and error bar represent mean ± SEM p-values were determined by One-way ANOVA and LSD Post Hoc Test for multiple comparisons, and $p<0.05$ were considered significantly different.

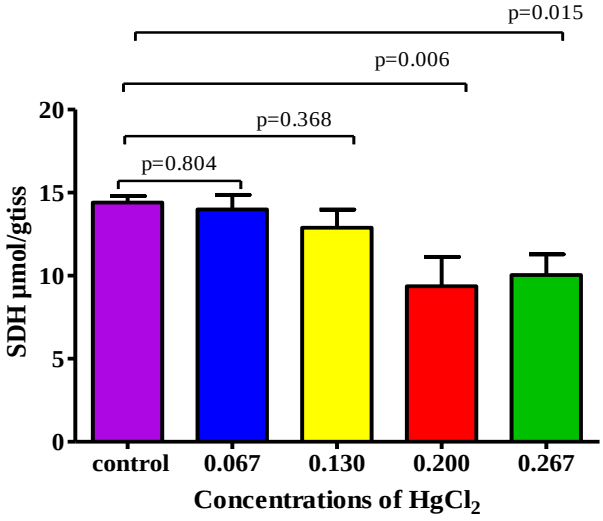

Figure 7: Succinate dehydrogenase activities in the brain of *Clarias gariepinus* exposed to varying concentrations of Mercury Chloride. The bar and error bar represent mean ± SEM p-values were determined by One-way ANOVA and LSD Post Hoc Test for multiple comparisons, and $p<0.05$ were considered significantly different.

Photomicrographs of fish exposed to 0.130 mg/L $HgCl_2$ showed significant alterations in the diencephalon, particularly in the cortex, indicating structural damage and neuronal cell degeneration (Figure 10). Higher concentrations (0.200 mg/L and 0.267 mg/L) resulted in further structural changes, including disorganisation and damage to neuronal layers (Figures 11 and 12), consistent with neurotoxic effects from mercury exposure.[38] At 0.267 mg/L, extensive gliosis and severe neuronal degeneration were observed, highlighting significant neurotoxic effects (Figure 12). This extensive gliosis indicates a pronounced inflammatory response, potentially disrupting neuronal circuits.[39] Severe degenerative changes in neuronal morphology due to mercury exposure, including cellular shrinkage and vacuolation, along with disrupted cerebellar architecture, may lead to behavioural abnormalities in affected fish.[40]

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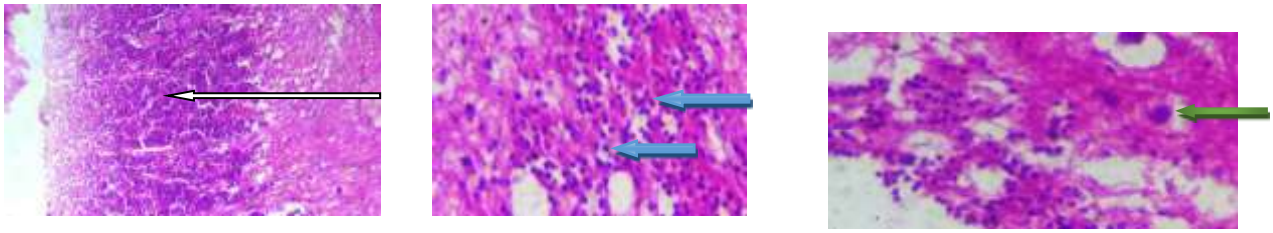

Figure 8: Photomicrographs of the brain tissue sections of non-treated *Clarias gariepinus* showing a normal diencephalon with a normal granular layer and monocellular areas (white arrow), cortex with normal neuronal cells (blue arrow), and typical glial cells (green arrow).

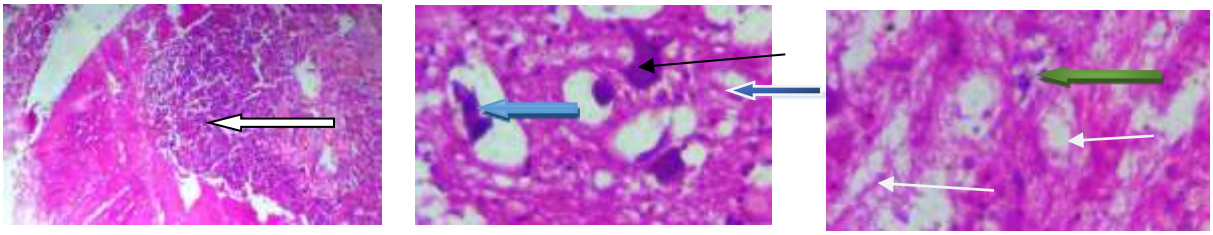

Figure 9: Photomicrographs of the brain tissue sections of *Clarias gariepinus* exposed to 0.067 mg/L $HgCl_2$ showing a diencephalon area with normal cerebellum (white arrow), moderately deteriorated and depleted neuronal cells (green arrow), an area of mild gliosis (slender white arrow), and a few atrophic glial cells (blue arrows)

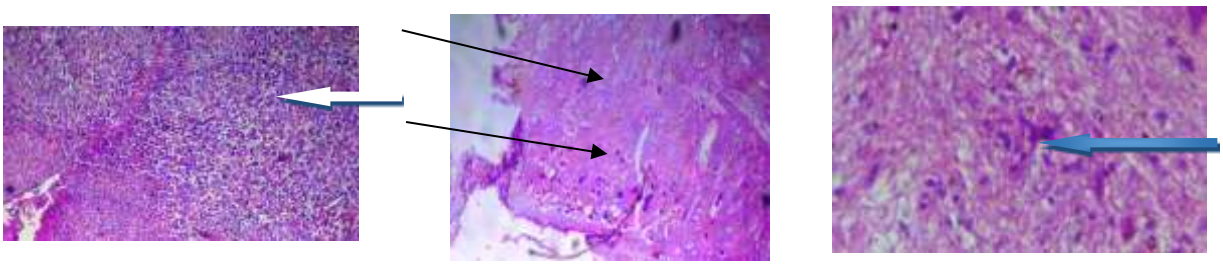

Figure 10: Photomicrographs of the brain tissue sections of *Clarias gariepinus* exposed to 0.130 $HgCl_2$ revealing the diencephalon with poor cortex structure (white arrow), neuronal cell degeneration and the population is very low (black arrows); and a few atrophic glial cells (blue arrow).

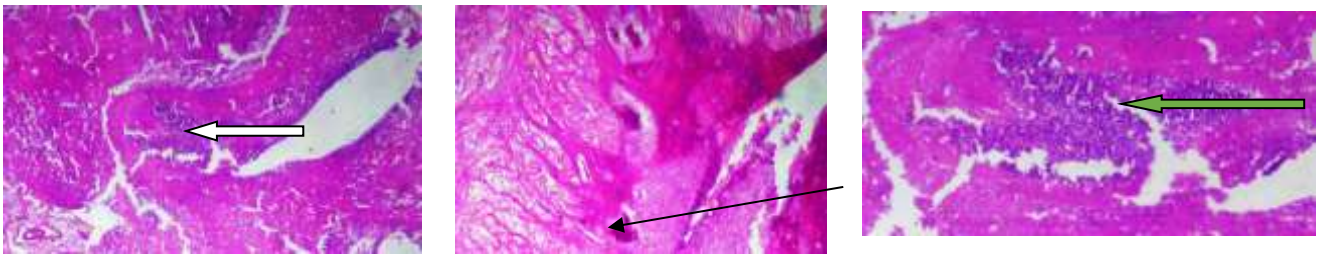

Figure 11: Photomicrographs of the brain tissue sections of *Clarias gariepinus* exposed to 0.200 $HgCl_2$ revealing a diencephalon with poor cortex structure (white arrow) and a normal cerebellar granular layer (green arrow), and a few shrunken neurons (slender arrow).

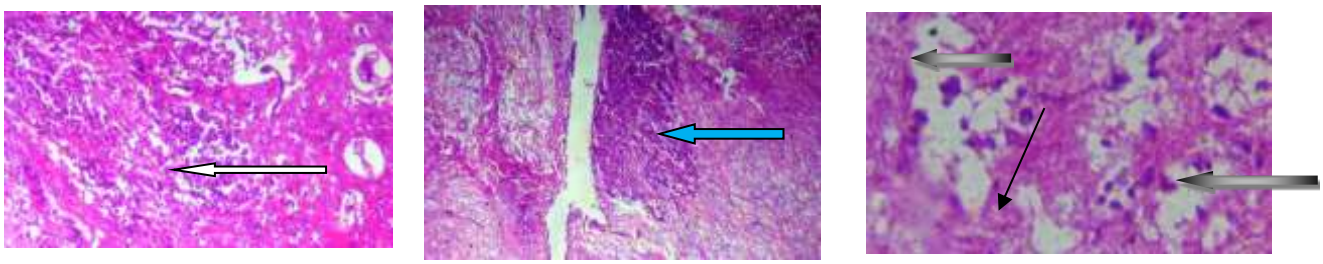

Figure 12: Photomicrographs of the brain tissue sections of *Clarias gariepinus* exposed to 0.267 $HgCl_2$ show a diencephalon with poor cortex organization (white arrow), a restricted granular layer of the cerebellum with significant chromatolysis (blue arrow), extensive gliosis (slender arrow), and severe degenerative alterations in neuronal cells (black arrows).

Conclusion

This study highlights the significant toxic effects of mercury chloride on the behaviour and neurological health of *Clarias gariepinus*, particularly in juvenile fish. The observed behavioural changes and neurotoxic biomarkers indicate the urgent need for effective management strategies to mitigate mercury pollution and protect aquatic ecosystems. Future research should focus on the long-term ecological impacts of mercury exposure, explore the mechanisms underlying neurotoxicity, and assess the effectiveness of remediation strategies. In addition, studies should investigate the effects of combined pollutants to understand better their cumulative impact on aquatic biodiversity and ecosystem health.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Ali H, Khan E, Ilahi I. Heavy Metals in Water, Soil and Plants: A Review. J Chem. 2019; 1-14.
2. Aladesanmi T, Oladipo O, Ali G. Aquatic Environmental Contamination: The fate of Asejire Lake in South-Western Nigeria. J Environ Sci Technol. 2013; 7:482-489. doi:10.5897/AJEST11.221.
3. Fatiha B, Omar K, El Azhari M. Mercury-Induced Acute Nephrotoxicity in Rats: Treatment with Aqueous Extract of *Pistacia atlantica* (Desf). Trop J Nat Prod Res. 2021; 5(12):2063–2067. doi.org/10.26538/tjnpr/v5i12.3
4. Clarkson TW, Magos, L. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol. 2006; 36(8):609–662. https://doi.org/10.1080/10408440600845619
5. Mahmoud, UM, Mekkawy, IA, Ibrahim, AA. Biochemical response of the African catfish *Clarias gariepinus* (Burchell, 1822) to sublethal concentrations of mercury chloride with supplementation of selenium and vitamin E. Toxicol Environ Health Sci. 2012; 4(4):218–234. https://doi.org/10.1007/s13530-012-0136-3
6. Branco V, Aschner M, Carvalho C. Neurotoxicity of mercury: An old issue with contemporary significance. Adv Neurotoxicol. 2021; 5:239–262. https://doi.org/10.1016/bs.ant.2021.01.001
7. Farina M, Aschner M, Rocha JBT. Oxidative stress in MeHg-induced neurotoxicity. Toxicol Appl Pharmacol. 2011; 256(3):405–417. https://doi.org/10.1016/j.taap.2011.05.001
8. Gobe G, Crane D. Mitochondria, reactive oxygen species and cadmium toxicity in the kidney. Toxicol Lett. 2010; 198(1):49–55. https://doi.org/10.1016/j.toxlet.2010.04.013
9. Turan F, Turan F. A general view of African catfish Clarias gariepinus (Burchell, 1822) from the Asi River. J Anim Vet Adv. 2016; 15(11):2222-2227.
10. Ayandiran AT, Ogundiran MA, Olayinka AS, Olaniyi OA, Olanipekun AS, Oluwaseun OT. Histological Response of *Clarias gariepinus* to Varying Concentrations of Pharmaceutical Effluents. Ann Res Rev Biol. 2020; 68-75.
11. ASTM (American Society for Testing of Materials). Method 729-90, Guide for Conducting Acute Toxicity Test with Fishes, Macroinvertebrates and Amphibians. ASTM, Philadelphia, 1991; 403-422.
12. Guedenon P, Edorh AP, Hounkpatin ASY, Alimba CG, Ogunkanmi AB, Nwokejiegbe EG, Boko M. Acute Toxicity of Mercury ($HgCl_2$) to African Catfish, *Clarias gariepinus*. Res J Chem Sci. 2012; 41-45.
13. Ellman GL, Courtney DK, Andreas V, Featherstone RM. A new and rapid colourimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7:88–95.
14. Griess P. Remarks on the paper by Weselsky and Benedikt. "On some azo compounds." Chem Ber. 1879; 12:426-428. https://doi.org/10.1002/cber.187901201117
15. Varshney R, Kale RK. Effects of Calmodulin Antagonists on Radiation-Induced Lipid Peroxidation in Microsomes. Int J Radiat Biol. 1990; 58:733-743. http://dx.doi.org/10.1080/09553009014552121
16. Kuo C, Keizo M, Stuart DS, Jerry BH. Lipid peroxidation: A possible mechanism of cephaloridine-induced nephrotoxicity. Toxicol Appl Pharmacol. 1983; 67:78-88. https://doi.org/10.1016/0041-008X(83)90246-6.
17. Beatty CH, Basinger GM, Dully CC, Bocek RM. Comparison of red and white voluntary skeletal muscles of several species of primates. J Histochem Cytochem. 1966; 14:590.
18. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247:3170–3175.
19. Avwioro OG. Histochemistry and Tissue Pathology, Principles and Techniques. Claverianum Press, Nigeria, 2010.
20. Hassaninezhad L, Safahieh A, Salamat N, Savari A, Majd NE. Assessment of gill pathological responses in the tropical fish yellowfin seabream of Persian Gulf under mercury exposure. Toxicol Rep. 2014; 1:621–628. https://doi.org/10.1016/j.toxrep.2014.07.016
21. Stanković AR, Dragićević S, Pavić D, Gačić Z. Heavy metals in water, sediment, and fish in the Danube River in Serbia. Environ Monit Assess. 2018; 190(11):665.
22. Kwok KWH, Leung KMY, Ng CKY. Effects of mercury contamination on fish behaviour: A critical review. Environ Sci Pollut Res. 2020; 27(15):17389-17403.
23. Okomoda J, Ayuba VO, Omeji S. Haematological Changes of *Clarias gariepinus* (Burchell, 1822) Fingerlings Exposed to Acute Toxicity of Formalin. PAT. 2010; 6(1):92-100.
24. Ishikawa NM, Ranzani-Paiva MJT, Lombardi JV. Acute toxicity of silver (HgCl2) to Tilapia fish, *Oreochromis leucostictus*. B Inst Pesca Sao Paulo. 2007; 33(1):99-104.
25. Shyong WJ, Chen HC. Acute toxicity of selenium, cadmium, and silver to the freshwater fish *Varicorhinus barbatus* and *Zacco barbata*. Acta Zool Taiwanica. 2000; 11(1):33-45.
26. Yulianto B, Soegianto A, Affandi M, Payus CM. The impact of various periods of mercury exposure on the osmoregulatory and blood gas parameters of tilapia (*Oreochromis niloticus*). Emerg Contam. 2023; 9(3):100244. https://doi.org/10.1016/j.emcon.2023.100244
27. Porciúncula LO, Rocha JB, Tavares RG, Ghisleni G, Reis M, Souza DO. Methylmercury inhibits glutamate uptake by synaptic vesicles from rat brains. Neuroreport. 2003; 14(4):577–580. https://doi.org/10.1097/00001756-200303240-00010
28. Ur Rehman MZ, Ullah I, Abdullah S. Toxic effects of a mixture of heavy metal pollutants on freshwater fish species *Cirrhina mrigala L*. Orig Res. 2016; 1:63-68.
29. Zhu X, Cai L, Meng S, Chen S, Zhou Y. Protective effects of *Lycium barbarum* polysaccharide on mercury-induced hippocampal oxidative damage in rats. Int J Environ Res Public Health. 2018; 15(11):2421.
30. Li, ZH, Li, P, Randak, T. Evaluating the toxicity of environmental concentrations of waterborne chromium (VI) to a model teleost, *Oncorhynchus mykiss*: a comparative study of in vivo and in vitro. Comp Biochem Physiol C Toxicol Pharmacol. 2016; 167:51-59.

31. Farina M, Avila DS, da Rocha JB, Aschner M. Metals, oxidative stress and neurodegeneration: A focus on iron, manganese and mercury. Neurochem Int. 2018; 113:490-498.
32. Zhang Z, Tang J, Chen X, Wang X, Hu J. Chronic mercury exposure induces neuroinflammation via NOX2/ROS/NF-κB pathway in Dabry's sturgeon (*Acipenser dabryanus*). Fish Shellfish Immunol. 2021; 113:178-187.
33. Lin Q, Yang X, Sun L, Guo L, Huang J. Effects of acute copper exposure on mitochondrial function, gene expression and enzyme activity of antioxidant systems in zebrafish. Aquat Toxicol. 2019; 207:93-102.
34. Pérez-Cadahía B, Laffon B, Pásaro E. Review on the effects of exposure to spilled oils on human health. J Appl Toxicol. 2019; 39(4):701-718.
35. Rodríguez F, López JC, Vargas JP, Gómez Y, Broglio C, Salas C. Conservation of spatial memory function in the pallial forebrain of reptiles and ray-finned fishes. J Neurosci. 2017; 37(32):7712-7725.
36. Quagio-Grassiotto I, Pelegrini DR, Fanta E. Structural and ultrastructural study of the brain of the Characiformes fish, *Astyanax altiparanae*: Histological evidence for telencephalic pallium in fish. Micron. 2015; 73:1-10.
37. Nascimento JM, Martins NR, Gonçalves CL, Lopes LG, Leite S, Rodrigues JL, Da Cunha MA. Neuroprotective effect of fructose-1, 6-bisphosphate against brain ischemic damage in rats. Mol Neurobiol. 2020; 57(6):2671-2682.
38. Olsson A, Söderström S, Gauthier D. Methylmercury effects on brain and muscle histology of zebrafish (*Danio rerio*) embryos. Aquat Toxicol. 2020; 229:105654.
39. Sahin SA, Doğan Z, Şimşek A. Mercury-induced neurotoxic effects on human and animal cells: a review. Environ Sci Pollut Res. 2020; 27(25):31557-31570.
40. García-Hernández M, Bernal-Hernández YY, Rodríguez-Ruiz HA, Domínguez-López ML. Mercury and arsenic alter the behaviour of juvenile goldfish (*Carassius auratus*): a comparative study. Chemosphere. 2019; 235:714-722.