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RESEARCH ARTICLE

Toxicological evaluation of *Calotropis gigantea* (L.) W. T. Aiton (Apocynaceae) stem extract in zebrafish: A chronic exposure study

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Abstract

Calotropis gigantea is widely used in traditional medicine across rural and tribal regions for treating various ailments. The safety profile of this plant especially in concerning long term or high dose exposure, remains inadequately studied. This study aims to scientifically assess the toxicity of *C. gigantea* stem extract using zebrafish (*Danio rerio*) as a model organism. Chronic exposure over 10, 20 and 30 days revealed significant oxidative stress, mitochondrial impairment and histopathological alterations in vital organs. Key antioxidant enzymes glutathione reductase (GR), glutathione S-transferase (GST), succinate dehydrogenase (SDH), catalase (CAT) and superoxide dismutase (SOD) were analysed in the liver, gills, brain and muscles. Enzyme activity has initially increased but declined by the 30th day, indicating progressive oxidative damage. The liver and gills exhibited the most substantial biochemical and structural changes. The histological analysis confirmed cellular degeneration, inflammation and necrosis. These findings highlight the potential risks associated with unregulated therapeutic use of *C. gigantea* and emphasize the need for scientific validation and public awareness to ensure safe application.

Keywords: histopathological alterations; oxidative stress; stress induced damage; zebrafish toxicity

Introduction

Since prehistoric times, plants and their derivatives have been used for forensic purposes (1). *Calotropis gigantea* (L.) W.T. Aiton belongs to the family Apocynaceae, also known as "crown flower" or "giant milkweed," is a widely distributed non cultivable weed in Asia and Africa. *Calotropis gigantea* has been traditionally used in the treatment of bronchitis, asthma and gastrointestinal disorders due to its purgative properties. The latex is employed as a purgative, while root bark and its inspissated juice treat leprosy, secondary syphilis (2). The milky white latex secreted in this plant is rich in bioactive compounds such as glycosides, alkaloids, flavonoids, tannins and cardenolides, Despite the medicinal significance, several of its phytochemicals have been linked to toxic effects (3).The numerous environmental factors have an impact on the growth, development and productivity of plants. These stressors frequently cause osmotic stress, which increases the buildup of reactive oxygen species (ROS) and alter the homeostasis and ion distribution in plant cells (2). Plants have evolved a variety of defence mechanisms, including enzymatic techniques to scavenge free ROS within plant cell (4). These enzymes not only protect different cell components from damage, but also regulate cellular and subcellular processes such as mitosis, tissue degradation, cell destruction, which are crucial for plant growth and development (5). Despite its traditional use in various indigenous medicinal practices, the market viability of *Calotropis gigantea* based formulations remains constrained due to the absence of rigorous toxicity profiling. Future research should emphasize sub-acute and chronic toxicity models, along with detailed organ specific toxicity assessments that correspond to the therapeutic targets of the bioactive constituents. *Calotropis gigantea* includes poisonous cardenolides that have been related to cardiotoxic effects, including calotropin, calactin, calotoxin and uscharin (6). The secretory substances cause serious cardiac failure by blocking the Na^+/K^+-ATPase pump, which interferes with ion transport across cell membranes. The research using animal models has shown that consuming *Calotropis gigantea* extracts can cause tissue damage, arrhythmias and cardiotoxicity (7). Furthermore, eye and cutaneous toxicity have been linked to latex exposure (8). Alkaloids such as gigantin, calotoxin and calotropin are the latex can cause severe kerato-conjunctivitis, characterized by corneal edema, Descemet's folds and sudden dimness of vision (9). As the corneal epithelium seems to be quite resilient, the endothelium cells are extremely vulnerable to harm, which causes the number of endothelial cells to gradually decrease over time. The *Calotropis gigantea* poisoning usually does not result in uveitis or subsequent glaucoma, unlike other chemical burns. but chronic exposure can result in permanent endothelial damage (10).

Danio rerio are often used as model organisms in toxicity studies, due to its genetic resemblance to humans, rapid development and transparent embryos, enabling real-time assessment of toxic effects. Ideal for environmental monitoring, it is extremely sensitive to contaminants such as pesticides, heavy metals and hormone-altering substances (11). Compared to traditional rodent models, zebrafish are cost effective, ethically preferable and well suited for long term toxicity screening. Despite the known acute toxicity of *Calotropis gigantea* in rodents and *in vitro* systems, there is a lack of chronic toxicity data using aquatic vertebrates like zebrafish, which are ecologically more relevant for plant-based compound exposure. Plants produce and accumulate ROS, which severely destroys cell organelles and causes membrane peroxidation, which damages the cell membrane, breaks down biological macromolecules and eventually kills the cell. Plant tolerance to various stressors appears to be mostly determined by their ability to scavenge the harmful effects of ROS. Antioxidants are essential for plant cells to function at their best and are the first line of defence against any damage that free radicals may inflict (12). Measurable oxidative stress responses in zebrafish include DNA damage and variations in the activity of antioxidant enzymes. Based on the available literature, this is one of the few studies to investigate the chronic toxicity of *Calotropis gigantea* stem extract in zebrafish over an extended exposure period (10, 20 and 30 days), by assessing biochemical markers of oxidative stress and histological changes in key organs.

Material and Methods

Preparation of stem extract

Calotropis gigantea was collected from Tumkur district, Karnataka, India. The voucher specimen (accession no. FRLHT-6806) was submitted to Foundation for Revitalisation of Local Health Tradition (FRLHT), Bengaluru. The stems of the plant were shade dried and grind into a fine powder. The extracts were prepared using the Soxhlet method, with 5 g of the dried powdered extracted in 50 mL of methanol. The extracts were then filtered using a rotary evaporator and various concentrations of the test solutions were stored at 4 °C until further use (13).

Acute toxicity of methanolic stem extract of *Calotropis gigantea*

Acute toxicity of methanolic stem extract of *Calotropis gigantea* was tested for acute toxicity in the zebrafish model as per the OECD guidelines 203 (14). Five chemical free glass tubs, each containing 4 L of dechlorinated tap water, were used for exposure. The water was aerated 24 hr prior to the experiment to stabilize dissolved oxygen levels. Adult *Danio rerio* were acclimatized for 7 days before the experiment under laboratory conditions (26 ± 1 °C; 14:10 hr light-dark cycle). At 24, 48, 72 and 96 hr after exposure, fish were examined. Concentrations of 0.5, 0.25, 0.125, 0.075 and 0.050 g/L were selected as effective concentrations for conducting the main toxicity tests of the plant extracts. Feeding was halted 24 hr before exposure to avoid faecal contamination. A control group was maintained under identical conditions without extract exposure. The fish were monitored for mortality at 12 hr intervals over a 96 hr period. Mortalities were recorded at 24, 48 and 96 hr and the concentrations required to cause 50 % lethality in the fish were documented (15). Fish were examined and considered dead if there was no visible movement and no reaction upon touching the caudal peduncle. Probit analysis was used to calculate the LC_{50} value. where mortality data were statistically analysed to estimate the dose required to cause 50 % mortality in the exposed population. The median lethal concentration (LC_{50}) of the acute toxicity experiment was calculated using the probit analysis and was analysed by IBM SPSS Statistics 21.0 software with 95 % confidence limits.

All procedures involving zebrafish were approved by the Institutional Animal Ethics Committee of CHRIST (Deemed to be University), Bengaluru, in accordance with CPCSEA guidelines (IAEC approval no. CU-RAC - 2370082).

Chronic toxicity of methanolic stem extract of *Calotropis gigantea*

Zebrafish were subjected to chronic exposure to *Calotropis gigantea* stem extract for 30 days to assess its detrimental effect (16). Based on the LC_{50} value, a sublethal concentration equivalent to $1/10^{th}$ of the LC_{50} was selected for the study. For the chronic toxicity study, zebrafish were exposed to a sublethal concentration ($1/10^{th}$ of the determined LC_{50} value) of *Calotropis gigantea* stem extract over a 30-day period to assess long term physiological effects. Pre-acclimatized fish were divided into three groups (n = 20 per group) and housed in 8 L tanks containing dechlorinated tap water under static renewal conditions. The exposure tanks received fresh extract daily to ensure consistent dosing, while water quality parameters were maintained through frequent renewal and aeration. A control group was maintained under identical conditions without the extract. Fish were fed twice daily with commercial feed and fecal matter and debris were promptly removed to avoid contamination. Throughout the experiment, mortality was recorded daily and any dead individuals were immediately removed.

Measurement of TPC and TFC

The TPC of the stem extract were calculated (17). 2 mL of a 20 % (w/v) sodium carbonate solution were combined with 0.5 mL of each extract and the mixture was vortexed. After 6 min, 1 mL of 50 % Folin-Ciocalteu's phenol reagent was added and the mixture was left to incubate for half an hour at room temperature. The absorbance was then measured at 760 nm. Total phenolic contents of extracts were expressed as milligram gallic acid equivalent (GAE)/g dry weight. Gallic acid was used as standard and calibration curve was plotted ($y = 0.0092x + 0.0207$, $R^2 = 0.9972$). Every sample underwent triple analysis. For TFC measurement 1 mL of the crude extract was combined with 1 mL of 10 % methanolic aluminium chloride, allowed to rest for 40 min at room temperature in darkness and the absorbance of the samples was recorded at 430 nm. The standard curve was created using quercetin ($y = 0.0084x + 0.0285$, $R^2 = 0.9928$) and the TFC value was expressed as µg quercetin (QE) equivalence per gram of dry extract weight (18).

In vitro antioxidant assay

The antioxidant properties of *Calotropis gigantea* stem extracts were assessed through the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method (19). A stock solution of DPPH (24 mg in 100 mL methanol) was diluted to

create a working solution with an absorbance of 0.99 ± 0.02 at 515 nm. Various extract concentrations (0.2-1 mg/mL) were combined with 3 mL of DPPH solution, stirred and left to incubate in the dark at room temperature for 30 min. Absorbance was measured at 515 nm, including a control (without extract) and standards (BHA, ascorbic acid). The DPPH scavenging effect was evaluated using the formula:

Scavenging Activity = [(Control OD -Sample OD)/control OD] ×100 Eqn.1

The FRAP assay was conducted following a standard protocol (20). Different concentrations (20 %, 40 %, 60 %, 80 % and 100 %) of the plant extract were prepared. To each sample, 2.5 mL of 0.2 mol phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide were added. The mixture was incubated at 50 °C for 20 min. After incubation, 2.5 mL of 10 % trichloroacetic acid was added and the solution was centrifuged at 3000 rpm for 10 min. The supernatant was collected and 2.5 mL of distilled water was mixed with 2.5 mL of the collected solution. Subsequently, 0.5 mL of 0.1 % ferric chloride ($FeCl_3$) solution was added. The absorbance was recorded at 700 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) with ascorbic acid serving as the standard.

Gas Chromatography- Mass Spectrometry (GC-MS) analysis

GC-MS analysis of the extract was conducted using a Shimadzu Nexis GCMS-TQ8040NX gas chromatograph coupled with a TurboMass quadrupole mass spectrometer. The system was equipped with an Rtx-5 fused silica capillary column (30 m × 0.25 mm, film thickness 1 µm). The oven temperature was programmed to increase from 100 °C to 320 °C at a rate of 100 °C/ min, with a final hold time of 10 min. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was set at 250 °C, with an injection volume of 1 µL in neat form and a split ratio of 1:10 was set. The interface and ion source temperatures were maintained at 320 °C and 200 °C, respectively. Mass spectra were acquired at an ionization energy of 70 eV over a scan range of 40-700 amu. Data acquisition and analysis were carried out using GCMS solution software.

Biochemical assay for oxidative stress

Antioxidant and detoxification enzymes were analysed in the muscle, brain, liver and gills tissues of zebrafish using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Tissues were dissected, weighed, homogenized in a cold buffer and centrifuged at 10000 × g for 45 min at 4 °C. The supernatant was used immediately or stored at -20 °C.

GR activity

GR activity was measured using NADPH oxidation by GSSG at 340 nm (30 °C) (21). Tissue homogenates (20 %) from muscle, brain, liver and gills were prepared in 50 mM phosphate buffer (pH 7.6) with 3 mM EDTA. The reaction mixture (1.7 mL) contained 1 mL phosphate buffer, 0.2 mL EDTA, 0.2 mL NADPH, 0.2 mL GSSG and 0.1 mL enzyme extract. Absorbance was recorded for 5 min and the activity was calculated using the extinction coefficient ($\varepsilon = 6.22\ mM^{-1}\ cm^{-1}$). One unit of GR activity was defined as the enzyme required to oxidize 1 µM of NADPH/min/g tissue at 30 °C, expressed as µM/min/mg protein.

CAT activity

CAT activity was measured by the decomposition of H_2O_2, recorded as a decrease in absorbance at 240 nm (25 °C). Tissue homogenates (5 %) were prepared in 0.05 mol potassium phosphate buffer (pH 7). The reaction was initiated by adding 0.2 mL enzyme extract to 2.8 mL of 0.05 % H_2O_2 and the absorbance was recorded for 5 min. Activity was calculated using $\varepsilon = 6.93 \times 10^{-3}\ mM^{-1}\ cm^{-1}$ and expressed as mM/min/mg protein (22).

SOD activity

SOD activity was measured by its inhibition of NBT reduction at 540 nm. Tissue homogenates (25 %) were prepared in 50 mM sodium carbonate buffer (pH 10). The reaction mixture included NBT, Triton X-100 and hydroxylamine hydrochloride, with enzyme extract added. Activity was expressed as U/min/ mg protein based on 50 % NBT reduction inhibition (23).

SDH activity

SDH activity was measured by monitoring the reduction of potassium ferricyanide at 420 nm. A 15 % tissue homogenate was prepared in 0.2 mol potassium phosphate buffer (pH 7.8) with 0.1 % BSA. The reaction mixture contained assay buffer, succinic acid (0.6 mol, pH 7.8), BSA (1 % w/v) and enzyme solution. Absorbance was recorded at 420 nm for 5 min at 30 ° C. Enzyme activity (U/g tissue) was determined using a standard curve with 200-1000 µM of ferricyanide and the values were expressed as U/mg protein (24).

GST activity

GST activity was measured following a standard protocol with modifications. A 10 % tissue homogenate was prepared in 0.1 mol sodium phosphate buffer (pH 7.6) containing 1 mM PTU. The reaction mixture (1 mL) consisted of 0.775 mL phosphate buffer, 0.1 mL ethanolic CDNB, 0.1 mL GSH (50 mM) and 0.025 mL enzyme sample. Absorbance was recorded at 340 nm for 5 min at 25 ± 1 °C. Enzyme activity (U/g tissue) was calculated using an extinction coefficient of 9.6 $mM^{-1}cm^{-1}$ and expressed as µM/min/mg protein (25).

Histopathological analysis

Brain, liver, gills and muscle tissues were analysed for histological changes. Tissues were fixed in 10 % formalin, dehydrated in graded ethanol, embedded in paraffin and sectioned at 5 µm thickness. Sections were stained with haematoxylin and eosin (H&E) and examined under a light microscope for histopathological alterations.

Results and Discussion

Quantitative phytochemical analysis

The results of TPC and TFC of stem extract are provided in Table 1. In stem maximum total phenolic content (59.56 ± 0.26 mg GAE/g dry weight) was observed in the methanolic extract of the stem and minimum in chloroform(45.63 ± 0.18 mg GAE/g dry weight) and total flavonoid content (54.65 ± 0.24 mg QE/g dry weight) was observed in the methanolic extract of the stem and minimum in chloroform (39.56 ± 0.16 mg QE/g dry weight). This result is consistent with previous studies indicates that methanol, as a polar solvent, is very efficient in extracting phenolic and flavonoid compounds, which are typically water soluble and ability to breakdown cell wall (18).

Table 1. Quantitative phytochemical concentration of *Calotropis gigantea* stem extracts

Solvent	Total phenols (mg GAE/g Dry weight)	Total flavonoid (mg QE/g Dry weight)
Methanol	59.56 ± 0.26	54.65 ± 0.24
Chloroform	45.63 ± 0.18	41.19 ± 0.18
Water	50.43 ± 0.23	39.56 ± 0.16

GC-MS analysis

The results revealed the presence of various bioactive compounds and their relative concentrations in *C. gigantea* (Fig 1). olean-12-en-3-ol, acetate (3β-), urs-12-en-23-oic acid, 3-(acetyloxy)- (4β)- and epilupeol were the three most abundant compounds detected, with peak area percentages of 10.01 %, 7.76 % and 6.86 %, respectively. Meanwhile, the least three detected compounds were n-hexadecanoic acid, n-nonadecanol-1 and squalene, with peak area percentages of 1.15 %, 0.39 % and 0.73 %, respectively (Table 2). In general, the major and minor chemical compounds found in *C. gigantea* extract have minimal toxicity profiles, especially at the amounts usually seen in natural extracts (26). However, under certain physiological situations or at larger concentrations, some chemicals may have negative consequences. Therefore, comprehensive *in vivo* studies are necessary to confirm their safety for any pharmacological or therapeutic uses and to fully evaluate their toxicological consequences.

Antioxidant activity assay

The antioxidant potential of the methanolic stem extract of *Calotropis gigantea* was evaluated through the DPPH scavenging assay, assessment of its reducing power and determination of its total antioxidant capacity. The extract exhibited a strong dose-dependent inhibition of DPPH activity, achieving 58.96 % inhibition at a concentration of 100 µg/mL, comparable to the standard antioxidant, ascorbic acid. The reducing power of methanolic stem extract of *Calotropis gigantea* increased with increasing amount of sample. Reducing power of methanolic stem extract were found 57.8 mg Ascorbic acid equivalent per gram of extract. These findings indicate that the stem extract has strong electron-donating substances that can scavenge free radicals (27). The presence of phytoconstituents such flavonoids, tannins and cardiac glycosides that have been previously identified in *Calotropis gigantea* may be the cause of the antioxidant action (28). The significant antioxidant capacity supports the traditional use of this plant, while also raising the need for toxicity profiling due to its bioactivity.

Biomarker assay

There is a significant lack of research examining the histological and enzymatic alterations caused by plant toxins especially regarding *Calotropis gigantea*. Although numerous studies have examined its phytochemical composition and overall toxicity. Studies focusing directly on its effects on oxidative stress markers (SOD, CAT, GST, etc.) and histopathological changes in various organs remains limited. This gap in the literature underscores the necessity for additional research to thoroughly comprehend the toxicological impacts of *Calotropis gigantea,* particularly in models such as zebrafish. Assessing the impact of pollutants on organisms through alterations in essential enzymes during particular reactions is a commonly employed technique to examine oxidative stress levels (29). The SOD can initiate the mutation of O_2 and transform it into H_2O and H_2O_2, making it the first line of defence against oxidative stress. The interaction of xenobiotics' electrophilic groups with GSH's sulfydryl groups can be catalysed by GST, a phase II detoxification metabolic enzyme, increasing GSH's hydrophobicity (30). Enzymes linked to glutathione, such as GST, GPx and GSH, are important players in coordinating the body's defence against oxidative stress. These enzymes are particularly important when the cell is experiencing moderate oxidative stress (31). The SOD and CAT are regarded as the first line defences against oxidative stress among the antioxidant enzymes. They are essential for the transformation of ROS into nontoxic metabolites and serve related purposes (32). The impact of sub chronic exposure to *Calotropis gigantea* on specific oxidative stress markers and the activity of the detoxifying enzyme presented display the stress markers along with the activity of the detoxifying enzyme (Fig. 2-5). According to the present study, fish exposed to *Calotropis gigantea* showed significant changes in the activity of CAT, SOD, SDH and glutathione enzyme. SOD and CAT are regarded as the first line defences against oxidative stress among the antioxidant enzymes. They are essential for the transformation of ROS into nontoxic metabolites and serve related purpose.

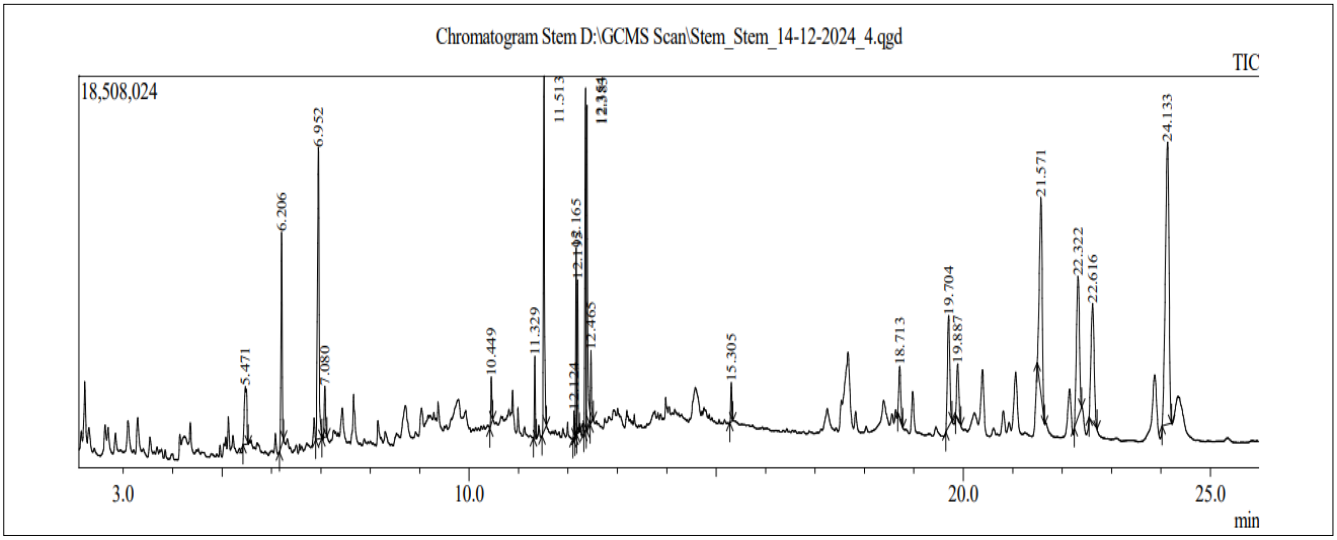

Fig. 1. GC-MS chromatogram of *Calotropis gigantea* stem extract.

Table 2. GC-MS data- compounds of *Calotropis gigantea* methanolic stem extract

SL No	Compound Name	Retention Time	Peak Area %	Molecular Formula
1	maltol	5.471	2.68	$C_6H_6O_3$
2	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl.	6.206	4.65	$C_6H_8O_4$
3	5-hydroxymethylfurfural	6.952	8.75	$C_6H_6O_3$
4	1,2,3-propanetriol, 1-acetate	7.080	1.18	$C_5H_{10}O_4$
5	(E)-4-(3-hydroxyprop-1-en-1-yl)-2-methoxyphenol	10.449	0.87	$C_{10}H_{12}O_3$
6	hexadecanoic acid, methyl ester	11.329	1.15	$C_{17}H_{34}O_2$
7	n-hexadecanoic acid	11.513	7.10	$C_{16}H_{32}O_2$
8	n-nonadecanol-1	12.124	0.39	$C_{19}H_{40}O$
9	9,12-octadecadienoic acid (Z,Z)-, methyl ester	12.165	2.78	$C_{19}H_{34}O_2$
10	11,14,17-eicosatrienoic acid, methyl ester	12.195	2.30	$C_{18}H_{32}O_2$
11	9,12-octadecadienoic acid (Z,Z)-	12.354	7.26	$C_{18}H_{32}O_2$
12	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	12.383	6.69	$C_{18}H_{30}O_2$
13	octadecanoic acid	12.465	1.33	$C_{18}H_{36}O_2$
14	squalene	15.305	0.73	$C_{30}H_{50}$
15	campesterol	18.713	1.84	$C_{28}H_{48}O$
16	γ-sitosterol	19.704	4.80	$C_{29}H_{50}O$
17	stigmasta-5,24(28)-dien-3-ol, (3β,24Z)-	19.887	2.15	$C_{29}H_{48}O$
18	olean-12-en-3-ol, acetate, (3β)-	21.571	10.01	$C_{32}H_{52}O_3$
19	urs-12-en-23-oic acid, 3-(acetyloxy)-, (4β)-	22.322	7.76	$C_{32}H_{50}O_4$
20	epilupeol	22.616	6.86	$C_{30}H_{50}O$

Enzyme activity

The peroxisomes contain the enzyme CAT, which helps to remove hydrogen peroxide, which is then converted to oxygen and water (33). CAT activity increased significantly in the liver, gills and muscle after the first 10-20 days of exposure (Fig. 2). According to findings, groups on the twentieth day had greater levels of CAT activity, the liver responded the most strongly, rising from 127.59 ± 6.38 U/mg at 10 days to 233.247 ± 9.42 U/mg at 20 days. At first, CAT activity rose in the liver and muscle, reaching a maximum at 20 days (liver: 233.247 ± 9.48 U/mg), suggesting an enhancement of antioxidant defences to combat oxidative stress. In the current study, CAT activity in gills was observed to be reduced across chronic exposures of both 20th and 30th day of the toxicant. This might be attributed to the surge of superoxide radicals that impede CAT activity and the failure to establish sufficient compensation when exposed to the toxic substance (34, 35). Other species, including *Cyprinus carpio* exposed to diazinon (36), *Oncorhynchus mykiss* exposed to propiconazole (37) and *Cyprinus carpio* treated to prometryne (38), also showed a decrease in CAT activity in their gills. As the main organs for detoxification, the liver and gills had the strongest reactions, although the brain's susceptibility to oxidative stress was highlighted by a delayed but notable rise at 20 days (124.69 ± 6.12U/mg). These results highlight the need for more research into the ecotoxicological consequences of *Calotropis gigantea* extract by showing that it causes oxidative stress in zebrafish, with early adaptive responses followed by potential damage. The SOD activity in zebrafish tissues was significantly altered by prolonged exposure to *Calotropis gigantea* stem extract, suggesting an active response to oxidative stress (Fig. 3). A robust antioxidant defense was suggested by the liver's consistent SOD activity, which ranged from 66.218 ± 8.22 at 10 days to 59.627 ± 4.859 at 30 days. SOD activity decreased significantly in all the organ after the exposure of stem extract (30). Comparable findings were reported that the enzymes' inhibitive reaction may have indicated that the antioxidant system was unable to maintain the balance of antioxidant defence, which may have been brought on by the exposure to PFDDs, which increased ROS generation and caused oxidative chemicals to build up in the cells (32). In contrast, the gills showed a significant decrease at 30 days (32.03 ± 9.09) after initially increasing (63.978 ± 1.584 at 10 days to 68.027 ± 3.345 at 20 days), signifying potential cellular damage or enzyme inhibition from prolonged exposure. The results were caused by excessive ROS formation in fish following exposure, which was more than SOD's capacity

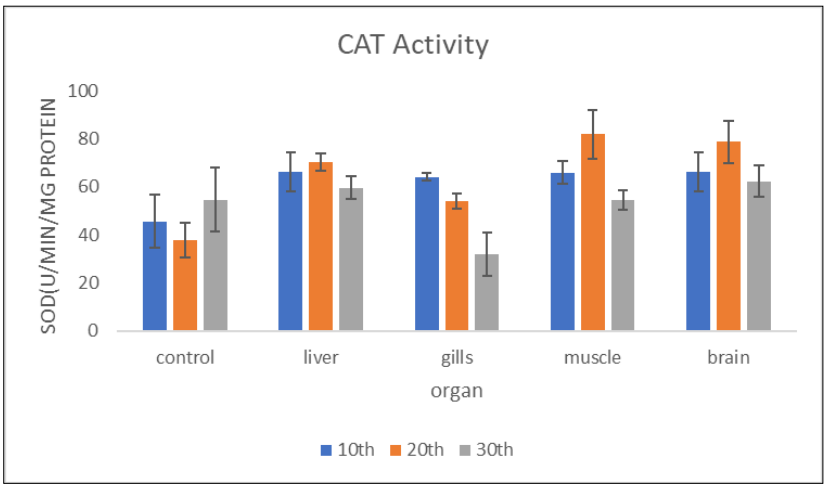

Fig. 2. Assessment of CAT response in zebrafish over 10, 20 and 30 days exposure to *Calotropis gigantea.*

Data are presented as mean ± standard error (SE), with **n = 3** per group. Statistical analysis was performed using one way ANOVA to assess differences in enzyme activity across time points. A significance level of $p < 0.05$ was considered statistically significant

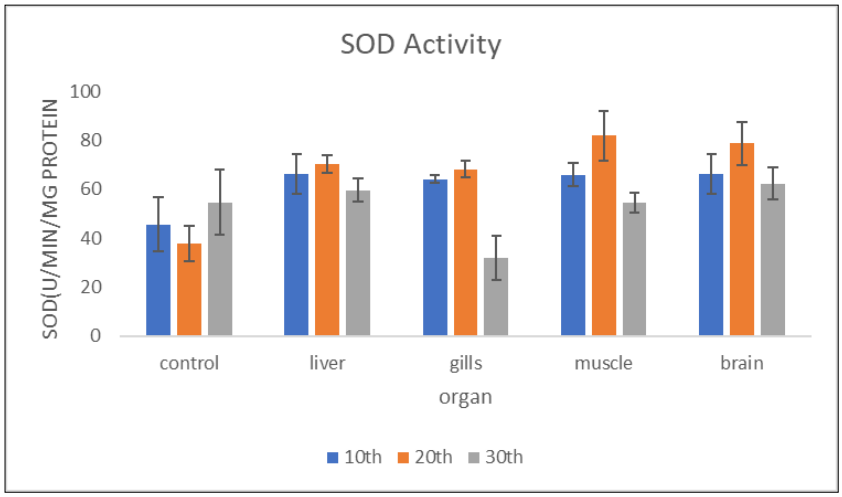

Fig. 3. Assessment of SOD response in zebrafish over 10, 20 and 30 days exposure to *Calotropis gigantea.*

Data are presented as mean ± standard error (SE), with n = 3 per group. Statistical analysis was performed using one way ANOVA to assess differences in enzyme activity across time points. A significance level of $p < 0.05$ was considered statistically significant

to eliminate ROS. Consistent SOD activity in the control group confirmed the extract's effects. These findings highlight the particular organ and time-related oxidative stress that *Calotropis gigantea* causes, highlighting its potential negative impacts on aquatic life. GR activity in zebrafish tissues was significantly impacted by prolonged exposure to *Calotropis gigantea* stem extract, suggesting an organ and time dependent response to oxidative stress. At all-time points the liver showed the highest GR activity (0.057 ± 0.004 at 10 days to 0.054 ± 0.003 at 30 days $p<0.05$), indicating its potent antioxidant capacity to maintain glutathione homeostasis (Fig. 4). GR activity in the gills increased gradually over time (from 0.021±0.001 at 10 days to 0.045±0.003 at 30 days), indicating an adaptive response to oxidative stress. On the other hand, GR activity decreased in the brain and muscle by 30 days (brain: 0.0054 ± 0.0002, muscle: 0.037 ± 0.0001), which may be a sign of cellular damage or a reduction in antioxidant stores. The control group's consistent GR activity demonstrated that the extract was responsible for the observed alterations. GSH depletion produced by oxidative stress from xenobiotics can lower the body's capacity to scavenge free radicals, increasing the cells overall oxidative potential. Fish GSH level variation has been used as an indicator of their level of xenobiotic exposure (39). It is non-enzymatic, low molecular weight antioxidant whose sulfhydryl group helps remove oxyradicals from cells (40). The alteration of GST activity in zebrafish tissues after prolonged exposure to *Calotropis gigantea* stem extract shows unique organ specific and time related reactions to oxidative stress (Fig. 5) (41). The liver showed a significant rise in GST activity over time (0.046 ± 0.008 at 10 days rising to 0.108 ± 0.0011 at 30 days), emphasizing its vital function in detoxifying and conjugating reactive metabolites. Similarly, the gills demonstrated an incremental increase in GST activity (0.021 ± 0.007 at 10 days to 0.060 ± 0.007 at 30 days), suggesting a responsive adaptation to oxidative stress. According to the research, fish exposed to stem extract showed increased GST activity in their gills with longer exposure times and greater amounts of the toxicant. On the other hand, the muscle showed very constant GST activity (0.0262 ± 0.005 at 30 days compared to 0.0278 ± 0.004 at 10 days), indicating a limited role in detoxifying processes. Additionally, the brain's susceptibility to oxidative stress was highlighted by a rise in GST activity (from 0.0334 ± 0.0010 at 10 days to 0.0551 ± 0.008 at 30 days). The control group's consistent GST activity demonstrated that the extract was responsible for the observed alterations. The liver and gills functions in detoxifying and the brain's vulnerability to extended exposure are highlighted by these findings, which show the organ specific antioxidant responses

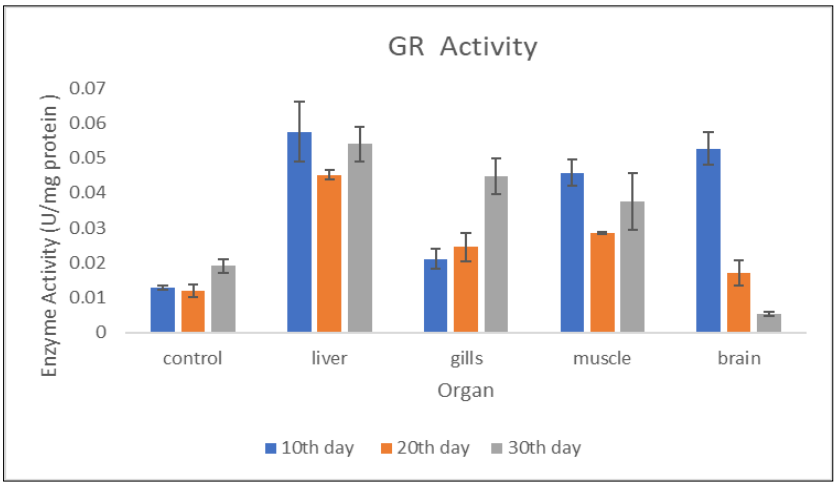

Fig. 4. Assessment of GR response in zebrafish over 10, 20 and 30 days exposure to *Calotropis gigantea*.

Data are presented as mean ± standard error (SE), with **n = 3** per group. Statistical analysis was performed using one way ANOVA to assess differences in enzyme activity across time points. A significance level of $p < 0.05$ was considered statistically significant

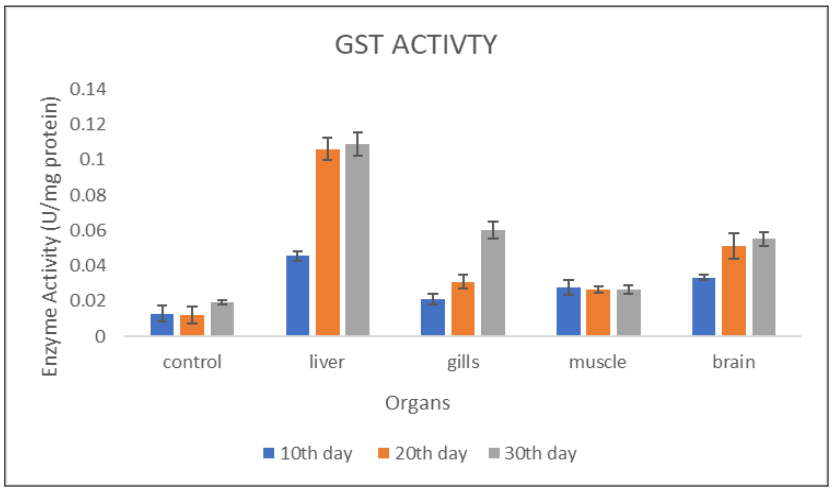

Fig. 5. Assessment of GST response in zebrafish over 10, 20 and 30 day exposure to *Calotropis gigantea*.

Data are presented as mean ± standard error (SE), with **n = 3** per group. Statistical analysis was performed using one way ANOVA to assess differences in enzyme activity across time points. A significance level of $p < 0.05$ was considered statistically significant

to oxidative stress caused by *Calotropis gigantea.* The exposure of *Oreochromis niloticus* and *Cyprinus carpio* to 2,4-D and azinophosmethyl, *Brycon cephalus* to methyl parathion and *Danio rerio* to atrazine have all been shown to have similar effects (42, 43). SDH is a part of SDH (complex II) in the electron transport chain and is found in the inner mitochondrial membrane (44). When the enzyme SDH is inactive, succinate can establish up in the mitochondria and leak into the cytosol, as a result, certain prolyl hydroxylase enzymes (PHDs) may be inhibited (45). In this study, the effect of *Calotropis gigantea* extract on SDH activity in zebrafish tissues shows unique patterns of mitochondrial reaction as time progresses (46). SDH activity in liver and gills, reached its maximum at 10 days (liver: 0.472 ± 0.024; gills: 0.541 ± 0.036), reflecting an early increase in mitochondrial function to manage oxidative stress (Fig. 6). Nevertheless, at 30 days, activity noticeably decreased (liver: 0.362 ± 0.0018; gills: 0.376 ± 0.028), indicating potential mitochondrial dysfunction or cellular injury from extended exposure (47). The control group exhibited consistent SDH activity, corroborating the effects of the extract (48). These findings emphasize that liver and gills as the main sites of mitochondrial dysfunction caused by *Calotropis gigantea*, illustrating their essential functions in detoxification and stress response (49). These findings underline the ecological risk of *Calotropis gigantea* stem extract in aquatic environments. Prolonged exposure disrupts redox homeostasis, impairs mitochondrial function and induces organ specific oxidative damage, raising concerns about its environmental persistence and toxicity.

Histology

In this study, the histopathology of zebrafish treated with plant extracts and a control group was examined. The histological examinations performed on the gills, intestines, liver and brain of zebrafish gills subjected to *Calotropis gigantea* extracts revealed considerable structural harm in comparison to the control group. During the 30-day exposure to stem extract, the gill structure showed disorganized lamellae, elevated epithelium and slight fusion of secondary lamellae, suggesting initial signs of tissue strain (Fig. 7). Fish can adjust to alterations in their surroundings by enhancing the diffusion range to their haemoglobin. They achieve this by creating a barrier through the multiplication of epithelial cells and the merging of primary and secondary lamellar cells (50). The existence of enlarged epithelial cells indicates a protective reaction to toxic exposure, possibly decreasing oxygen diffusion efficiency. Many studies suggested that chemical exposure will harms the fish's gills and cause physiologically significant ions to "leak" out of the fish into the surrounding water (51). More evident effects are observed in the gills treated with stem extract, where

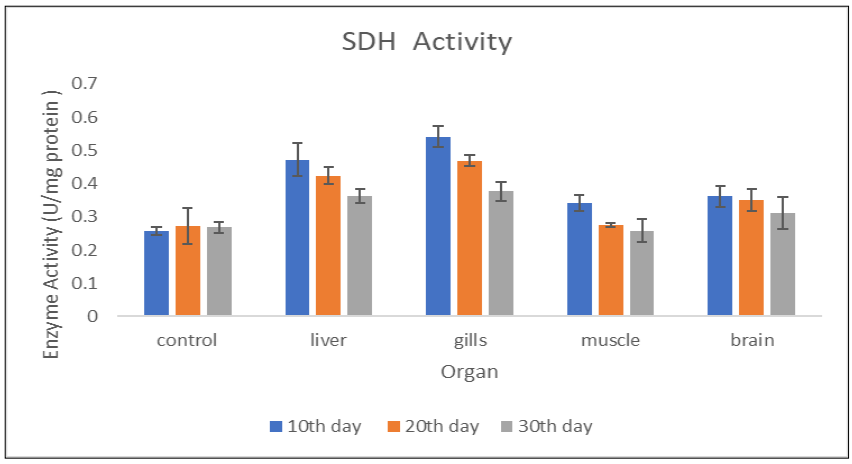

Fig. 6. Assessment of SDH response in zebrafish over 10, 20 and 30 day exposure to *Calotropis gigantea*.

Data are presented as mean ± standard error (SE), with **n = 3** per group. Statistical analysis was performed using one way ANOVA to assess differences in enzyme activity across time points. A significance level of $p < 0.05$ was considered statistically significant

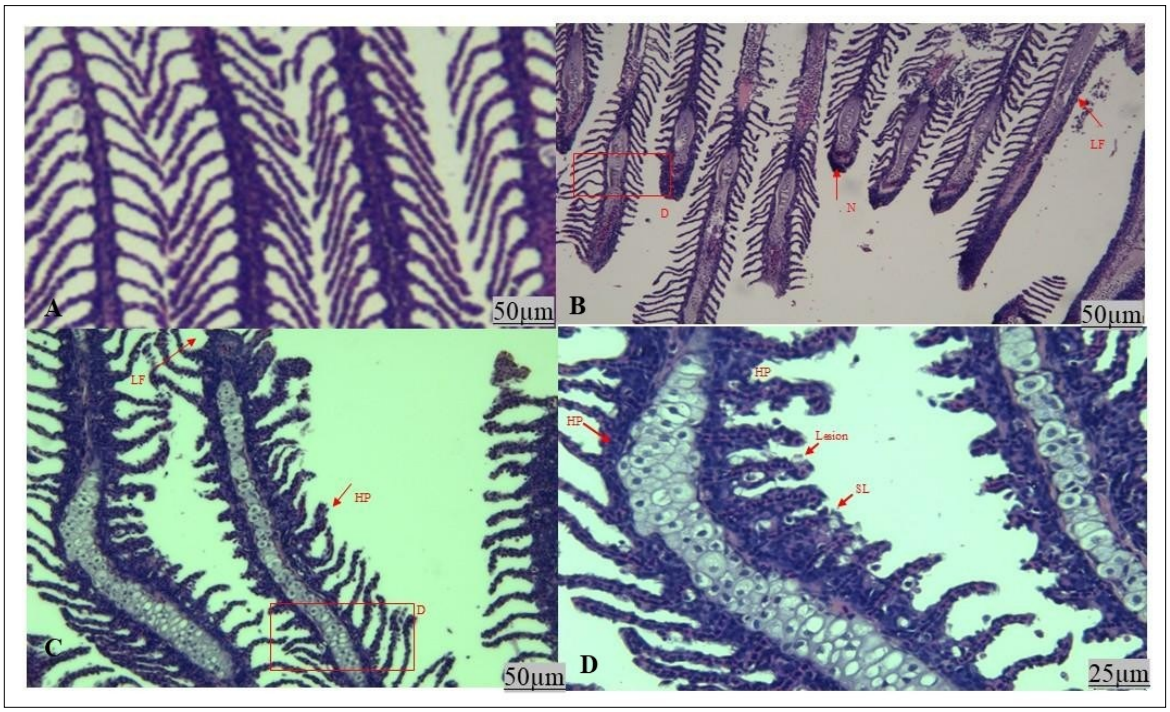

Fig. 7. Histopathological changes in the gills of *Danio rerio* to stem extract.

A- control; B- 10 days of the exposure mild HP beginning D; C - 20 days of exposure, N, moderate SL fusion (LF), HP; D - 30 days of exposure, severe LF, N, tissue lesion.

SL-secondary lamellae, N- necrosis, HP- hyperplasia of epithelial cells in the secondary lamellae, D- disorganization of the secondary lamellae, Lesion - an area of abnormal or damaged tissue, LF - lamilar fusion

hypertrophy, epithelial detachment and lamellar clubbing are apparent, indicating significant toxicity. When the gills of catfish (*Clarias gariepinus*) were exposed to extracts of *Parkia biglobosa* fruits, it displayed the appearance of lesions (52).

The control liver sample has intact cellular morphology and compact hepatocytes, demonstrating normal hepatic architecture. However, noticeable pathological alterations appear as exposure time rises. The control liver sample retained cell morphology and dense hepatocytes, indicating normal liver structure. But significant pathological changes are seen as exposure duration increases (Fig. 8) (53). The occurrence of vacuolization and hyperplasia in certain areas indicates a compensatory reaction to extended chemical stress, potentially affecting respiratory efficiency. After 10 days of exposure, however, the overall structure remained the same, with mild hepatocellular alterations such as mild vacuolization and cellular enlargement. After 20 days, the hepatocellular dysfunction, aberrant nuclear structure and enhanced vacuolization become increasingly apparent. The toxic plant exposure can induce cytoplasmic vacuolization and nuclear pyknosis (54). In addition, sinusoidal congestion and mild inflammatory infiltration point to the first signs of poisoning. Severe hepatotoxic effects, including necrotic patches, hepatocellular degeneration and widespread vacuolation, become apparent after 30 days of exposure. These study findings may be connected to previous studies on *Danio rerio* subjected to sublethal pesticide dose (55). With increasing sinusoidal congestion and inflammatory cell infiltration, the hepatic cord structure becomes disturbed, indicating advanced liver injury. These observations are seen in the chronic exposure with certain medications, like phenytoin and diclofena. Chronic toxicity is further indicated by the appearance of fibrosis-like alterations (56, 57). These histopathological findings suggest that prolonged exposure to *Calotropis gigantea* stem extract leads to progressive liver degeneration, correlating with oxidative stress-induced hepatotoxicity. The results of degeneration of liver tissue study could be linked to previous studies (58).

Fish muscles are often polluted with dangerous toxins and pesticides (59). The histological examination of zebrafish muscle tissues subjected to *Calotropis gigantea* stem extract for varying durations showed advancing toxic effects (Fig. 9). In the control group, the muscle fibers exhibited a well-structured arrangement with little inter-fiber gaps and no evidence of inflammation or necrosis. However, with increasing exposure duration, noticeable degenerative changes were observed, these similar results were observed (60). On the 20th day of exposure, muscle fibers exhibited disorganization, fragmentation and edema, signalling initial indications of oxidative stress and structural decline. Histological and ultrastructural studies on adult zebrafish demonstrated that bioaccumulation of toxins leads to skeletal muscle damage (61). The effect on the muscular regeneration process was evaluated at two levels: first, on the proliferative capacity of myoblasts and second, on myogenic differentiation. The sarcolemma seemed damaged, indicating possible cytotoxic effects. Muscle deterioration was evident by day 30, with extensive vacuolization, necrotic areas and infiltration of inflammatory cells. The fibers' structural integrity was severely damaged and they were atrophied, which could indicate persistent poisoning. Mitochondrial abnormalities such as changes in their shape are seen in histology (62). These histological alterations indicate that *Calotropis gigantea* stem extract induces muscle toxicity in a time-dependent manner, potentially through oxidative stress, protein denaturation and inflammatory responses. The findings highlight the need for further molecular studies to assess oxidative stress biomarkers and apoptotic pathways involved in muscle toxicity, providing deeper insights into the mechanisms of toxicity associated with

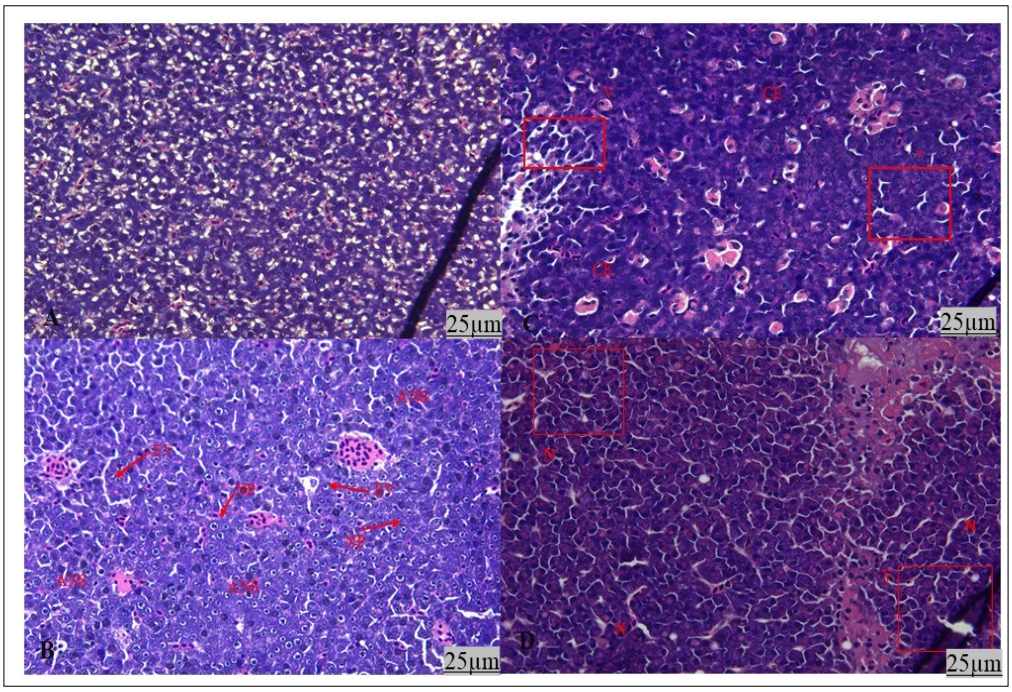

Fig. 8. Histopathological changes in the liver of *Danio rerio* to stem extract.

A- control; B- 10 days of the exposure, v- mild vacuolation, CE - cellular enlargement; C - 20 days of exposure, EV - enlarged vacuolization, ABS- aberrant nuclear structure, NP -nuclear pyknosis; D - 30 days of exposure, N- necrotic patches, hepatocellular degeneration and increased vacuolation are present

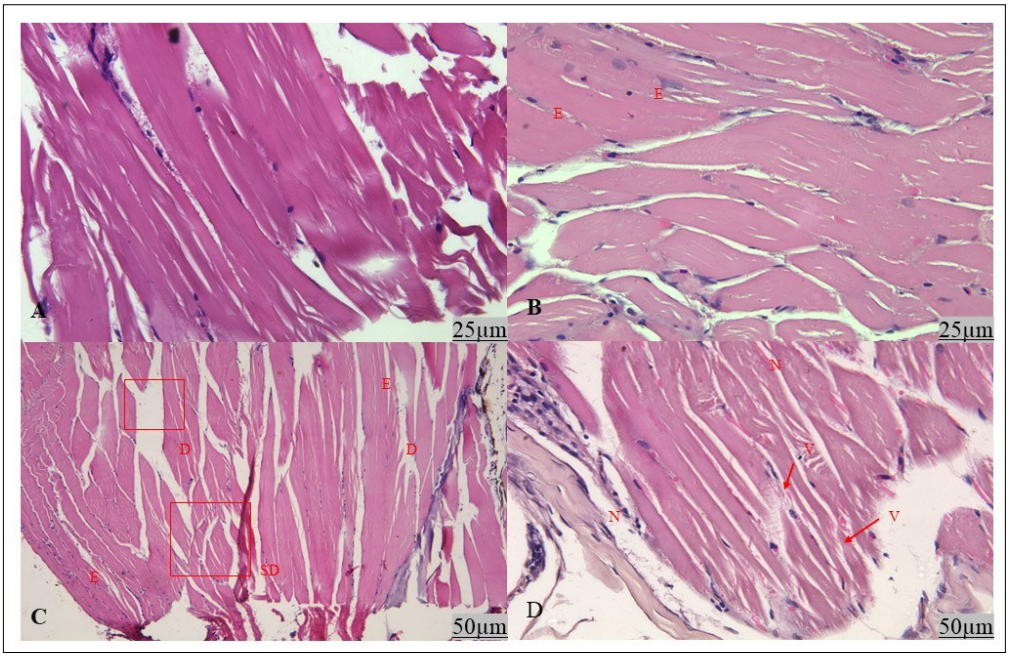

Fig. 9. Histopathological changes in the muscles of *Danio rerio* to stem extract.

A- control; B- 10 days of the exposure, E- edema; C- 20 days of exposure, E, disorganisation, fragmentation, SD- structural decline; D- 30 days of exposure, N- necrotic areas and infiltration of inflammatory cells are present

Calotropis gigantea. Histopathological examination of zebrafish brain tissue exposed to extract from *Calotropis gigantea* across several time periods shows gradual neurotoxic effects (Fig. 10). The brain tissue in the control group (0 days, leaf extract) shows no evidence of inflammation, necrosis or degeneration and a well-organized neural architecture with distinct neuronal cell bodies. Early indications of neurotoxicity, such as minor neuronal swelling, vacuolization and a small disarray in the brain tissues, possibly brought on by oxidative stress, appear after ten days of exposure to the stem extract. Dose-dependent histopathological changes in the brain were also evident (58).

The brain tissue had significant vacuolization, neuronal loss, gliosis and indications of neuroinflammation, including microglial activation, after 20 days of treatment. After 30 days, significant neurodegeneration was noted, marked by widespread neuronal loss, areas of necrosis and altered brain structure. The occurrence of bleeding and enlarged perivascular spaces indicates vascular injury. Exposure of *Epinephelus coioides* to toxins derived from *Gambusia affinis* resulted in notable neuropathological alterations, including haemorrhagic lesions, necrotic regions, nuclear fragmentation (nuclear dust), hyperchromatic nuclei, cellular vacuolation, endothelial hypertrophy, cloudy swelling, hydropic degeneration and

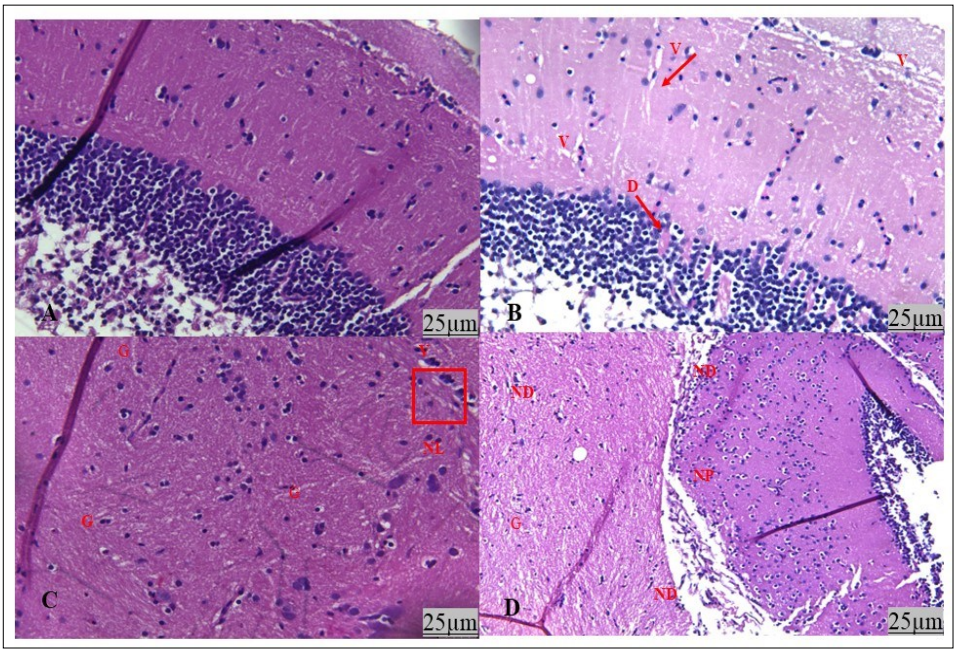

Fig. 10. Histopathological changes in the Brain of *Danio rerio* to stem extract.

A- control; B- 10 days of the exposure, V- vacuolization D- disarray of brain; C- 20 days of exposure, V, NL- neuronal loss, G- gliosis; D- 30 days of exposure, N- neurodegeneration, altered brain structure

ectopic accumulation of granular substances in brain tissues (63). These histopathological changes are likely correlated with oxidative stress enzyme activity (SOD, CAT, GST) and confirming the neurotoxic effects of *Calotropis gigantea* extracts (64). The oxidative stress enzyme activities (SOD, CAT, GST) initially increased during early exposure (10-20 days), indicating a defensive response. This correlates with mild tissue changes such as vacuolization in the liver, disorganization of muscle fibers and early signs of neurotoxicity. By 30 days, enzyme levels declined, coinciding with severe histopathological damage hepatic necrosis, muscle degeneration and neuroinflammation confirming the time-dependent, oxidative stress-mediated toxicity of *Calotropis gigantea* stem extract.

Conclusion

In conclusion, chronic exposure to *Calotropis gigantea* stem extract results in significant biochemical and histological alterations in zebrafish, especially impacting the liver, gills, brain and muscles. The observed decline in antioxidant enzyme activity and mitochondrial function over time suggests progressive oxidative stress and tissue damage. Histological analyses corroborated the biochemical findings, revealing tissue level anomalies such as hepatocyte degeneration, gill lamellar disorganization, neuronal damage in the brain and muscular atrophy. Collectively, these changes suggest that prolonged or high dose exposure to *C. gigantea* stem extract poses substantial health risks, potentially compromising organ integrity and systemic health. These findings highlight the potential health risks associated with prolonged or high dose exposure to this plant extract. Further research is needed to identify the specific bioactive compounds responsible and to understand their mechanisms of toxicity in biological systems.

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Authors' contributions

APD was responsible for sample collection, sample preparation, conducting the enzyme-based assays, data acquisition, analysis and interpretation, performed the statistical analysis and drafted the manuscript. Both authors read and approved the final manuscript.

KAP conceived and designed the study, provided administrative, technical and material support, supervised the work and critically revised the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Borgia V, Carlin MG, Crezzini J. Poison, plants and palaeolithic hunters. An analytical method to investigate the presence of plant poison on archaeological artefacts. Quat Int. 2017;427:94–103. https://doi.org/10.1016/j.quaint.2015.12.025
2. Rajput VD, Harish, Singh RK, Verma KK, Sharma L, Quiroz-Figueroa FR, et al. Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. Biology (Basel). 2021;10(4):267. https://doi.org/10.3390/biology10040267

3. Rani R, Sharma D, Chaturvedi M, Yadav JP. Phytochemical analysis, antibacterial and antioxidant activity of *Calotropis procera* and *Calotropis gigantea*. Nat Prod J. 2019;9(1):47–60. https://doi.org/10.2174/2210315508666180608081407
4. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage and antioxidative defense mechanism in plants under stressful conditions. J Bot. 2012;2012:1–26. https://doi.org/10.1155/2012/217037
5. Liu N, Lin Z, Guan L, Gaughan G, Lin G. Antioxidant enzymes regulate reactive oxygen species during pod elongation in *Pisum sativum* and *Brassica chinensis*. PLoS One. 2014;9(2):e87588. https://doi.org/10.1371/journal.pone.0087588
6. You H, Lei M, Song W, Chen H, Meng Y, Guo D, et al. Cytotoxic cardenolides from the root bark of *Calotropis gigantea*. Steroids. 2013;78(10):1029–34. https://doi.org/10.1016/j.steroids.2013.06.002
7. Ahmed OM, Fahim HI, Boules MW, Ahmed HY. Cardiac and testicular toxicity effects of the latex and ethanolic leaf extract of *Calotropis procera* on male albino rats in comparison to abamectin. Springerplus. 2016;5:1644. https://doi.org/10.1186/s40064-016-3326-7
8. Basak SK, Bhaumik A, Mohanta A, Singhal P. Ocular toxicity by latex of *Calotropis procera* (Sodom apple). Indian J Ophthalmol. 2009;57(3):232–4. https://doi.org/10.4103/0301-4738.49402
9. Manjunatha SA, Venkataramana PA, Rajshekar, Siddegowda S. Ocular toxicity due to accidental exposure to plant latex by *Calotropis procera* and *Calotropis gigantea*. J Clin Diagn Res; 2021. https://doi.org/10.7860/jcdr/2021/49510.15334
10. Goyal A, Kumar D. Ocular toxicity by latex of *Calotropis procera* in Rajasthan. J Evol Med Dent Sci. 2014;3(56):12808–12. https://doi.org/10.14260/jemds/2014/3699
11. Segner H. *Danio rerio* as a model organism for investigating endocrine disruption. Comp Biochem Physiol C Toxicol Pharmacol. 2009;149(2):187–95. https://doi.org/10.1016/j.cbpc.2008.10.099
12. Rajput VD, Minkina T, Yaning C, Sushkova S, Chapligin VA, Mandzhieva S. A review on salinity adaptation mechanism and characteristics of *Populus euphratica*, a boon for arid ecosystems. Sheng Tai Xue Bao. 2016;36(6):497–503. https://doi.org/10.1016/j.chnaes.2016.08.001
13. Sabry MM, El-Halawany AM, Fahmy WG, Eltanany BM, Pont L, Benavente F, et al. Evidence on the inhibitory effect of *Brassica* plants against *Acinetobacter baumannii* lipases: phytochemical analysis, *in vitro* and molecular docking studies. BMC Complement Med Ther. 2024;24:164. https://doi.org/10.1186/s12906-024-04460-y
14. OECD. OECD guidelines for the testing of chemicals, section 2 test no 203: fish, acute toxicity test. Paris: OECD Publishing; 2019.
15. Islam MA, Amin SMN, Brown CL, Juraimi AS, Uddin MK, Arshad A. Determination of median lethal concentration for endosulfan, heptachlor and dieldrin pesticides to African catfish and their impact on its behavioral patterns and histopathological responses. Toxics. 2021;9(12):34. https://doi.org/10.3390/toxics9120340
16. Saleh Alanazi SH, Farooq Khan M, Alazami AM, Baabbad A, Ahmed Wadaan M. *Calotropis procera*: a double edged sword against glioblastoma, inhibiting glioblastoma cell line growth by targeting histone deacetylases and angiogenesis. Heliyon. 2024;10(2):e24406. https://doi.org/10.1016/j.heliyon.2024.e24406
17. Rajha HN, Louka N, Darra NE, Hobaika Z, Boussetta N, Vorobiev E, et al. Multiple response optimization of high-temperature, low-time aqueous extraction process of phenolic compounds from grape byproducts. Food Nutr Sci. 2014;5(4):351–60. https://doi.org/10.4236/fns.2014.54042
18. Ahmad Nejhad A, Alizadeh Behbahani B, Hojjati M, Vasiee A, Mehrnia MA. Identification of phytochemical, antioxidant, anticancer and antimicrobial potential of *Calotropis procera* leaf aqueous extract. Sci Rep. 2023;13:14716. https://doi.org/10.1038/s41598-023-42086-1
19. Clarke G, Ting KN, Wiart C, Fry J. High correlation of DPPH radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian Rainforest. Antioxidants (Basel). 2013;2(1):1–10. https://doi.org/10.3390/antiox2010001
20. Griffin SP, Bhagooli R. Measuring antioxidant potential in corals using the FRAP assay. J Exp Mar Bio Ecol. 2004;302(2):201–11. https://doi.org/10.1016/j.jembe.2003.10.008
21. Hamelet J, Seltzer V, Petit E, Noll C, Andreau K, Delabar JM, et al. Cystathionine beta synthase deficiency induces catalase-mediated hydrogen peroxide detoxification in mice liver. Biochim Biophys Acta. 2008;1782(7-8):482–8. https://doi.org/10.1016/j.bbadis.2008.05.003
22. Alici E, Arabaci G. Determination of SOD, POD, PPO and CAT enzyme activities in *Rumex obtusifolius* L. Annu Res Rev Biol. 2016;11(3):1–7. https://doi.org/10.9734/arrb/2016/29809
23. Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc. 2010;5(1):51–66. https://doi.org/10.1038/nprot.2009.197
24. Jones AJY, Hirst J. A spectrophotometric coupled enzyme assay to measure the activity of succinate dehydrogenase. Anal Biochem. 2013;442(1):19–23. https://doi.org/10.1016/j.ab.2013.07.018
25. Vontas JG, Enayati AA, Small GJ, Hemingway J. A simple biochemical assay for glutathione S-transferase activity and its possible field application for screening glutathione S-transferase-based insecticide resistance. Pestic Biochem Physiol. 2000;68(3):184–92. https://doi.org/10.1006/pest.2000.2512
26. Devanesan S, AlSalhi MS. Identification, evaluation of the source of natural bioactive compounds from *Calotropis gigantea* L. flowers and their anticancer potential. J King Saud Univ Sci. 2024;36(2):103038. https://doi.org/10.1016/j.jksus.2023.103038
27. Lalhminghlui K, Jagetia GC. Evaluation of the free-radical scavenging and antioxidant activities of *Schima wallichii* Korth *in vitro*. Future Sci OA. 2018;4(2):FSO272. https://doi.org/10.4155/fsoa-2017-0086
28. Mbinda W, Musangi C. Antioxidant activity, total phenolic and total flavonoid contents of stem bark and root methanolic extracts of *Calotropis procera*. J Phytopharm. 2019;8(4):161–6. https://doi.org/10.31254/phyto.2019.8403
29. Zucchi S, Blüthgen N, Ieronimo A, Fent K. The UV-absorber benzophenone-4 alters transcripts of genes involved in hormonal pathways in zebrafish (*Danio rerio*) eleuthero-embryos and adult males. Toxicol Appl Pharmacol. 2011;250(2):137–46. https://doi.org/10.1016/j.taap.2010.10.001
30. Huang X, Li Y, Wang T, Liu H, Shi J, Zhang X. Evaluation of the oxidative stress status in zebrafish liver induced by three typical organic UV filters (BP-4, PABA and PBSA). Int J Environ Res Public Health. 2020;17(2):651. https://doi.org/10.3390/ijerph17020651
31. Bayır M, Bayır A, Uzun BN, Turhan S. Investigating the genomic and biochemical effects of dalapon on antioxidant systems in zebrafish. Toxicol Mech Methods. 2025:1–16. https://doi.org/10.1080/15376516.2025.2473525
32. Li C, Qin L, Qu R, Sun P, Wang Z. Responses of antioxidant defense system to polyfluorinated dibenzo-p-dioxins (PFDDs) exposure in liver of freshwater fish *Carassius auratus*. Ecotoxicol Environ Saf. 2016;126:170–6. https://doi.org/10.1016/j.ecoenv.2015.12.036
33. van der Oost R, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol. 2003;13(2):57–149. https://doi.org/10.1016/s1382-6689(02)00126-6
34. Sayeed I, Parvez S, Pandey S, Bin-Hafeez B, Haque R, Raisuddin S. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish *Channa punctatus* Bloch. Ecotoxicol Environ Saf. 2003;56(2):295–301. https://doi.org/10.1016/s0147-6513(03)00009-5
35. Li ZH, Velisek J, Zlabek V, Grabic R, Machova J, Kolarova J, et al. Hepatic antioxidant status and hematological parameters in

rainbow trout, *Oncorhynchus mykiss*, after chronic exposure to carbamazepine. Chem Biol Interact. 2010;183(1):98–104. https://doi.org/10.1016/j.cbi.2009.09.009

36. Costantini D. Oxidative stress and hormesis in evolutionary ecology and physiology: a marriage between mechanistic and evolutionary approaches. Springer. 2014:359
37. Schlenk D, Benson WH. Target organ toxicity in marine and freshwater teleosts: organs. CRC Press. 2003:426
38. Stara A, Kristan J, Zuskova E, Velisek J. Effect of chronic exposure to prometryne on oxidative stress and antioxidant response in common carp (*Cyprinus carpio* L.). Pestic Biochem Physiol. 2013;105(1):18–23. https://doi.org/10.1016/j.pestbp.2012.11.002
39. Feng M, Qu R, Wang C, Wang L, Wang Z. Comparative antioxidant status in freshwater fish *Carassius auratus* exposed to six current-use brominated flame retardants: a combined experimental and theoretical study. Aquat Toxicol. 2013;140-41:314–23. https://doi.org/10.1016/j.aquatox.2013.07.001
40. Lana JV, Rios A, Takeyama R, Santos N, Pires L, Santos GS, et al. Nebulized glutathione as a key antioxidant for the treatment of oxidative stress in neurodegenerative conditions. Nutrients. 2024;16(15):2476. https://doi.org/10.3390/nu16152476
41. Tierbach A, Groh KJ, Schönenberger R, Schirmer K, Suter MJF. Glutathione S-transferase protein expression in different life stages of zebrafish (*Danio rerio*). Toxicol Sci. 2018;162(2):702–12. https://doi.org/10.1093/toxsci/kfx293
42. Oruc EO, Sevgiler Y, Uner N. Tissue-specific oxidative stress responses in fish exposed to 2,4-D and azinphosmethyl. Comp Biochem Physiol C Toxicol Pharmacol. 2004;137(1):43–51. https://doi.org/10.1016/j.cca.2003.11.006
43. Monteiro DA, de Almeida JA, Rantin FT, Kalinin AL. Oxidative stress biomarkers in the freshwater characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). Comp Biochem Physiol C Toxicol Pharmacol. 2006;143(2):141–9. https://doi.org/10.1016/j.cbpc.2006.01.004
44. Yang Y, Liu W, Mu X, Qi S, Fu B, Wang C. Biological response of zebrafish embryos after short-term exposure to thifluzamide. Sci Rep. 2016;6:38485. https://doi.org/10.1038/srep38485
45. King A, Selak MA, Gottlieb E. Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. Oncogene. 2006;25(34):4675–82. https://doi.org/10.1038/sj.onc.1209594
46. Van Vranken JG, Bricker DK, Dephoure N, Gygi SP, Cox JE, Thummel CS, et al. SDHAF4 promotes mitochondrial succinate dehydrogenase activity and prevents neurodegeneration. Cell Metab. 2014;20(2):241–52. https://doi.org/10.1016/j.cmet.2014.05.012
47. Shi S, Wang L, van der Laan LJW, Pan Q, Verstegen MMA. Mitochondrial dysfunction and oxidative stress in liver transplantation and underlying diseases: new insights and therapeutics. Transplantation. 2021;105(11):2362–73. https://doi.org/10.1097/TP.0000000000003691
48. Campbell RJ, Jasmin BJ, Michel RN. Succinate dehydrogenase activity within synaptic and extrasynaptic compartments of functionally-overloaded rat skeletal muscle fibers. Pflugers Arch. 1996;431(5):797–9. https://doi.org/10.1007/BF02253847
49. Sawong S, Pekthong D, Suknoppakit P, Winitchaikul T, Kaewkong W, Somran J, et al. *Calotropis gigantea* stem bark extracts inhibit liver cancer induced by diethylnitrosamine. Sci Rep. 2022;12(1):12151. https://doi.org/10.1038/s41598-022-16321-0
50. Flores-Lopes F, Thomaz AT. Histopathologic alterations observed in fish gills as a tool in environmental monitoring. Braz J Biol. 2011;71(1):179–88. https://doi.org/10.1590/s1519-69842011000100026
51. van den Heuvel MR, Power M, Richards J, MacKinnon M, Dixon DG. Disease and gill lesions in yellow perch (*Perca flavescens*) exposed to oil sands mining-associated waters. Ecotoxicol Environ Saf. 2000;46(3):334–41. https://doi.org/10.1006/eesa.1999.1912
52. Stancova V, Plhalova L, Tichy F, Doubkova V, Marsalek P, Hostovsky M, et al. Oxidative stress indices and histopathological effects of the nonsteroidal antiinflammatory drug naproxen in adult zebrafish (*Danio rerio*). Neuro Endocrinol Lett. 2015;36(Suppl 1):73–8.
53. Wilson JM, Laurent P. Fish gill morphology: inside out. J Exp Zool. 2002;293(3):192–211. https://doi.org/10.1002/jez.10124
54. Xavier J, Jose J, Reddy J, Ka P. Histopathological and toxicological studies on zebrafish using white-fruited and green-fruited varieties of. Scientifica (Cairo). 2024;2024:4689625. https://doi.org/10.1155/2024/4689625
55. Zulfahmi I, Batubara AS, Perdana AW, Rahmah A, Nafis B, Ali R, et al. Chronic exposure to palm oil mill effluent induces oxidative stress and histopathological changes in zebrafish (*Danio rerio*). J Hazard Mater. 2025;490:137844. https://doi.org/10.1016/j.jhazmat.2025.137844
56. Mullick FG, Ishak KG. Hepatic injury associated with diphenylhydantoin therapy. A clinicopathologic study of 20 cases. Am J Clin Pathol. 1980;74(4):442–52. https://doi.org/10.1093/ajcp/74.4.442
57. Scully LJ, Clarke D, Barr RJ. Diclofenac induced hepatitis. 3 cases with features of autoimmune chronic active hepatitis. Dig Dis Sci. 1993;38(4):744–51. https://doi.org/10.1007/BF01316809
58. Sun S, Wang Y, Du Y, Sun Q, He L, Zhu E, et al. Oxidative stress-mediated hepatotoxicity in rats induced by ethanol extracts of different parts of *S.* Pharm Biol. 2020;58(1):1277–89. https://doi.org/10.1080/13880209.2020.1859552
59. Joystu D. Bioaccumulation of toxic heavy metals in the edible fishes of eastern Kolkata wetlands (EKW), the designated Ramsar site of West Bengal, India. Int J Aquac Fish Sci. 2017:18–21. https://doi.org/10.17352/2455-8400.000023
60. Roemhildt ML, Beynnon BD, Gauthier AE, Gardner-Morse M, Ertem F, Badger GJ. Chronic *in vivo* load alteration induces degenerative changes in the rat tibiofemoral joint. Osteoarthritis Cartilage. 2013;21(2):346–57. https://doi.org/10.1016/j.joca.2012.10.014
61. de Oliveira Ribeiro CA, Nathalie MD, Gonzalez P, Yannick D, Jean-Paul B, Boudou A, et al. Effects of dietary methylmercury on zebrafish skeletal muscle fibres. Environ Toxicol Pharmacol. 2008;25(3):304–9. https://doi.org/10.1016/j.etap.2007.10.033
62. Dubińska-Magiera M, Daczewska M, Lewicka A, Migocka-Patrzałek M, Niedbalska-Tarnowska J, Jagla K. Zebrafish: a model for the study of toxicants affecting muscle development and function. Int J Mol Sci. 2016;17(11):1941. https://doi.org/10.3390/ijms17111941
63. Alkshab AA, Taha AM. Histological effect of lead chloride on the brain of *Gambusia affinis*. J Phys Conf Ser. 2021;1818(1):012024. https://doi.org/10.1088/1742-6596/1818/1/012024
64. Gupta RC, Lall R, Srivastava A. Nutraceuticals: efficacy, safety and toxicity. Academic Press. 2021:1397.

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