



Oxidative Stress Responses in Gills, Liver and Reproductive Organs of *Clarias gariepinus* Juveniles Exposure to Crude Bark Extract *Albizia gummifera*

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Abstract: - The indiscriminate use of plant-derived piscicides in artisanal fisheries has raised concerns regarding their potential adverse effects on non-target aquatic organisms. This study evaluated the effects of sublethal concentrations of aqueous crude bark extract of *Albizia gummifera* on water-quality parameters and oxidative stress biomarkers in *Clarias gariepinus* juveniles. A total of 80 acclimatised juveniles (mean weight 20.66 ± 1.64 g; mean length 13.96 ± 0.38 cm) were randomly assigned to four treatment groups exposed to 0.00 (control), 0.10, 0.20 and 0.30 g L⁻¹ of the extract under a static-renewal bioassay. Water-quality parameters, including temperature, pH, electrical conductivity, total dissolved solids, salinity, alkalinity, general hardness and dissolved carbon dioxide, were monitored throughout the exposure period. Oxidative stress biomarkers comprising superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and malondialdehyde (MDA) were analysed in the liver, gills, testes and ovaries. Data were analysed using one-way analysis of variance (ANOVA) at a significance level of $P < 0.05$. Among the measured water-quality variables, only alkalinity differed significantly ($P < 0.05$) among treatments, whereas temperature, pH, salinity, electrical conductivity, total dissolved solids, general hardness and dissolved carbon dioxide remained statistically comparable with the control. Exposure to *A. gummifera* crude bark extract elicited tissue-specific antioxidant responses. In the liver, GPx and CAT activities increased with increasing extract concentration, while MDA generally declined, suggesting activation of antioxidant defense mechanisms. Gill tissues exhibited variable antioxidant responses, reflecting their direct exposure to dissolved phytochemicals. Marked increases in SOD and CAT activities were observed in the testes at higher exposure concentrations, indicating enhanced oxidative stress responses. In the ovaries, elevated MDA concentrations and reduced CAT activity at the highest concentration suggested increased lipid peroxidation and oxidative challenge. Overall, antioxidant biomarkers responded more sensitively to the extract than conventional water-quality parameters. The study demonstrates that sublethal exposure to aqueous crude bark extract of *Albizia gummifera* disrupts redox homeostasis and induces tissue-specific oxidative stress in *Clarias gariepinus* juveniles despite causing minimal changes in general water chemistry. These findings highlight the potential ecological risks associated with the indiscriminate use of *A. gummifera* as a traditional piscicide and support the use of oxidative stress biomarkers as sensitive indicators for monitoring sublethal toxicity in freshwater ecosystems.

Keywords: Peacock flower; Sublethal toxicity; Catfish; Oxidative stress; Antioxidant biomarkers; Sublethal toxicity; aquatic ecotoxicology.



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1.0 Introduction

The use of botanical piscicides has remained a common practice among artisanal fishing communities across Africa because of their affordability, local availability, and perceived environmental friendliness. However, increasing evidence indicates that although these plant-derived products are biodegradable, their bioactive phytochemicals may exert toxic effects on non-target aquatic organisms, thereby posing ecological risks to freshwater ecosystems (Obasi et al., 2022; Sulaiman et al., 2022; Adeyemi et al., 2024). Among the plant species traditionally employed as a fish poison is *Albizia gummifera* commonly referred to as peacock flower, whose bark contains several biologically active secondary metabolites, including saponins, alkaloids, flavonoids, tannins and terpenoids. These compounds have been associated with disruption of cellular metabolism, impairment of respiratory and osmoregulatory functions, neurotoxicity, tissue degeneration and reproductive dysfunction in exposed fish species (Obasi et al., 2022; Hala et al., 2025).

The indiscriminate introduction of piscicidal plant extracts into aquatic environments may alter the physicochemical characteristics of water and consequently compromise fish health. Water-quality variables such as temperature, pH, electrical conductivity, dissolved solids, alkalinity, hardness and dissolved carbon dioxide play essential roles in regulating metabolic activities, ionic balance, oxygen utilization and overall physiological performance of fish. Alterations in these parameters can increase physiological stress, suppress growth, reduce immune competence and elevate mortality in cultured and wild fish populations (Boyd et al., 2020; El-Sayed, 2021; FAO, 2022). Furthermore, exposure to phytochemical-rich plant extracts may interact with water chemistry, thereby modifying contaminant bioavailability and influencing toxicological responses in aquatic organisms (Sulaiman et al., 2022; Adeyemi et al., 2024).

One of the principal mechanisms underlying contaminant-induced toxicity in fish is oxidative stress. Environmental toxicants stimulate excessive production of reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals and hydrogen peroxide. Under normal physiological conditions, ROS are neutralised by endogenous antioxidant defence systems; however, excessive ROS generation overwhelms these protective mechanisms, resulting in oxidative damage to membrane lipids, proteins, nucleic acids and cellular organelles (Hala et al., 2025; Li et al., 2023). Persistent oxidative stress has been implicated in impaired growth, organ dysfunction, reproductive failure and increased susceptibility to disease in aquatic organisms.

Consequently, oxidative stress biomarkers have become indispensable tools for evaluating sublethal toxicity in

aquatic ecotoxicology. Superoxide dismutase (SOD) catalyses the conversion of superoxide radicals into hydrogen peroxide, whereas catalase (CAT) and glutathione peroxidase (GPx) detoxify hydrogen peroxide and lipid hydroperoxides into less harmful products. Reduced glutathione (GSH) functions as a major intracellular non-enzymatic antioxidant, while malondialdehyde (MDA), a terminal product of lipid peroxidation, serves as a reliable indicator of oxidative membrane damage (Livingstone et al., 2021; Hala et al., 2025). Variations in the activities of these biomarkers therefore provide sensitive indicators of physiological disturbances caused by environmental contaminants before visible pathological changes occur.

Recent investigations have demonstrated that botanical extracts and other aquatic contaminants induce tissue-specific antioxidant responses in fish. Exposure of Nile tilapia (*Oreochromis niloticus*) to silver nanoparticles significantly altered hepatic antioxidant enzyme activities and increased oxidative stress in liver tissues (Hala et al., 2025). Likewise, exposure to agricultural pesticides, industrial pollutants and endocrine-disrupting compounds has been associated with significant alterations in SOD, CAT, GPx, GSH and MDA levels in the liver, gills and reproductive organs of several freshwater fish species, indicating activation of antioxidant defence mechanisms following contaminant exposure (Samuel et al., 2021; Li et al., 2023; Ahmed et al., 2024). These findings demonstrate that oxidative stress biomarkers provide highly sensitive indicators for evaluating the toxicological effects of environmental pollutants.

The reproductive organs are particularly susceptible to oxidative injury because germ cells possess relatively limited antioxidant defence capacity. Elevated oxidative stress within the testes and ovaries may impair gametogenesis, reduce sperm quality, disrupt oocyte maturation and ultimately decrease reproductive performance. Recent studies have shown that environmental contaminants significantly modify antioxidant enzyme activities in fish gonadal tissues, confirming oxidative stress as one of the principal mechanisms underlying contaminant-induced reproductive toxicity (Olatunji et al., 2021; Ahmed et al., 2024).

The African catfish, *Clarias gariepinus*, remains one of the most economically important freshwater fish species in sub-Saharan Africa owing to its rapid growth, high market value and tolerance to diverse environmental conditions. Its widespread use in aquaculture and demonstrated sensitivity to environmental pollutants make it an excellent bioindicator species for aquatic ecotoxicological investigations (FAO, 2022; Adebayo et al., 2020).

Although the acute toxicity of *Albizia gummifera* has previously been reported, information concerning its chronic

sublethal effects on water quality and oxidative stress responses in metabolically active tissues and reproductive organs of *C. gariepinus* juveniles remains limited. In particular, little is known about the tissue-specific antioxidant responses induced by prolonged exposure to aqueous crude bark extracts of this plant. Therefore, the present study investigated the effects of sublethal concentrations of *A. gummifera* crude bark extract on selected water-quality parameters and oxidative stress biomarkers, including SOD, CAT, GPx, GSH and MDA, in the gills, liver, testes and ovaries of *Clarias gariepinus* juveniles. The findings provide valuable information on the ecological safety of botanical piscicides and contribute to the understanding of oxidative stress-mediated toxicity in non-target freshwater fish.

2.0 Materials and Methods

2.1 Collection and Preparation of *Albizia gummifera* Bark Extract

Fresh stem bark of *Albizia gummifera* was collected from Mangu, Plateau State, Nigeria, and transported to the Zoology Laboratory, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi. The plant material was air-dried under laboratory conditions until a constant weight was attained, after which it was pulverised into a fine powder using a mechanical grinder and sieved through a 30 µm mesh.

To prepare the aqueous crude bark extract, 1.00 g of the powdered bark was macerated in 1 L of distilled water for 24 h following the extraction procedure described by Audu et al. (2014). The suspension was subsequently filtered through non-absorbent cotton wool, and the filtrate obtained served as the stock solution for the toxicity experiment (Olaifa et al., 2003).

2.2 Experimental Fish and Acclimatization

A total of one hundred healthy juveniles of African catfish (*Clarias gariepinus*) with a mean body length of 13.96 ± 0.38 cm and mean body weight of 20.66 ± 1.64 g were procured from Rayuwa Fish Farm, Karu Local Government Area, Nasarawa State, Nigeria.

The fish were transported to the Zoology Laboratory in 50-L plastic containers containing water from the culture pond to minimise transportation stress. Upon arrival, the fish were transferred into aerated aquaria containing dechlorinated borehole water and acclimatised under laboratory conditions for two weeks before commencement of the experiment. During acclimation, the fish were fed commercial pelleted feed (Vital Feed®) twice daily at 08:00 and 18:00 h until apparent satiation. Water was renewed regularly to maintain optimum water quality throughout the acclimation period.

2.3 Preparation of Sublethal Exposure Concentrations

Sublethal exposure concentrations of 1.00, 2.00 and 3.00 g L⁻¹ were selected based on fractions of the previously determined 96-h median lethal concentration (LC₅₀ = 2.50 g L⁻¹). The test concentrations represented approximately one-tenth, one-fifteenth and one-twentieth of the LC₅₀ value following the approach adopted by Abubakar et al. (2018). A control treatment containing no plant extract (0.00 g L⁻¹) was included. The exposure experiment was conducted using a static-renewal bioassay in accordance with the Organisation for Economic Co-operation and Development (OECD, 2000) guideline for fish toxicity testing.

2.4 Experimental Design

The chronic toxicity experiment was conducted using a completely randomised design consisting of four treatment groups: 0.00 (control), 1.00, 2.00 and 3.00 g L⁻¹ of aqueous crude bark extract of *A. gummifera*. Each treatment was established in duplicate using rubber tanks containing 10 L of borehole water. Ten mixed-sex juveniles of *C. gariepinus* were randomly assigned to each experimental tank, giving a total of eighty fish used in the exposure study. The control group received no plant extract, whereas the remaining tanks were treated with the respective concentrations of the aqueous crude bark extract. The exposure lasted for 56 days, during which the exposure media were renewed periodically to maintain the desired concentrations and water quality.

2.5 Determination of Water Quality Parameters

Selected physicochemical characteristics of the experimental water were monitored throughout the exposure period. Temperature (°C), pH, electrical conductivity (µS cm⁻¹), total dissolved solids (TDS; mg L⁻¹) and salinity (ppt) were measured using a calibrated Multi-Parameter PCSTestr™ 35 meter. Before each measurement, the instrument was calibrated using standard buffer solution (pH 7.0) according to the manufacturer's instructions. The probe was immersed in each water sample, and readings were recorded after approximately 60 s of stabilisation (Sulaiman et al., 2022).

General hardness, alkalinity and dissolved carbon dioxide were determined using a JBL PRO Aquatest® kit following the manufacturer's protocol. Briefly, the test strip was immersed in each water sample for 2–3 s, removed, gently drained of excess water and allowed to develop for one minute before comparison with the manufacturer's colour chart for quantitative interpretation.

2.6 Determination of Oxidative Stress Biomarkers

At the end of the 56-day exposure period, fish were humanely sacrificed, and tissue samples comprising the liver, gills, testes and ovaries were carefully excised for biochemical analyses. Tissue homogenates were prepared under chilled conditions, and oxidative stress biomarkers were determined using standard spectrophotometric methods.

The antioxidant enzymes evaluated included superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), while reduced glutathione (GSH) and malondialdehyde (MDA) were quantified as indicators of non-enzymatic antioxidant status and lipid peroxidation, respectively. Enzyme activities were determined following established biochemical protocols appropriate for fish oxidative stress assessment.

2.7 Statistical Analysis

All data were analysed using IBM SPSS Statistics version 20.0 (IBM Corporation, Armonk, NY, USA). Results are presented as mean $\pm$ standard error (SE). Differences among treatment means were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to identify significant differences among treatment groups. Statistical significance was accepted at $P < 0.05$.

3.0 Results

3.1 Water Quality Parameters of Experimental Tanks During Sublethal Exposure of *Clarias gariepinus* Juveniles to *Albizia gummifera* Crude Bark Extract

The mean values of the physicochemical parameters measured during the 56-day sublethal exposure of *Clarias gariepinus* juveniles to aqueous crude bark extract of *Albizia gummifera* are presented in Table 1. Salinity ranged from 276.75 ± 39.31 ppm at 0.20 g L^{-1} to 320.25 ± 53.85 ppm at 0.10 g L^{-1} , while intermediate values of 293.25 ± 40.83 ppm and 294.00 ± 33.66 ppm were recorded in the 0.30 g L^{-1} and control treatments, respectively. Statistical analysis showed that salinity was not significantly affected by increasing concentrations of *A. gummifera* crude bark extract ($P > 0.05$). Water temperature varied only slightly among treatment groups. The lowest mean temperature ($31.27 \pm$

0.14°C) was observed at 0.10 g L^{-1} , whereas the highest value ($31.77 \pm 0.08^\circ\text{C}$) occurred at 0.30 g L^{-1} . The control treatment recorded a mean temperature of $31.75 \pm 0.64^\circ\text{C}$. These differences were not statistically significant ($P > 0.05$).

The pH of the experimental water remained relatively stable throughout the exposure period, ranging from 7.44 ± 0.09 at 0.30 g L^{-1} to 7.58 ± 0.12 at 0.10 g L^{-1} . Likewise, electrical conductivity (EC) and total dissolved solids (TDS) showed only slight variations among treatments. The highest EC ($675.25 \pm 91.22 \mu\text{S cm}^{-1}$) was recorded in the control group, while the lowest value ($585.25 \pm 82.84 \mu\text{S cm}^{-1}$) occurred at 0.30 g L^{-1} . Total dissolved solids ranged from $248.25 \pm 14.53 \text{ mg L}^{-1}$ in the control to $303.00 \pm 33.33 \text{ mg L}^{-1}$ at 0.10 g L^{-1} . None of these parameters differed significantly among treatments ($P > 0.05$).

General hardness and alkalinity exhibited concentration-dependent variations. The highest mean hardness (357.75 ± 1.10 ppm) and alkalinity (65.25 ± 4.11 ppm) were observed in the control treatment, whereas the lowest hardness (267.50 ± 0.64 ppm) occurred at 0.20 g L^{-1} , and the lowest alkalinity (52.00 ± 0.71 ppm) was recorded at 0.10 g L^{-1} . Among all measured physicochemical variables, alkalinity was the only parameter that differed significantly among treatment groups ($P = 0.01$), while variations in general hardness were not statistically significant.

A gradual decline in dissolved carbon dioxide (CO_2) concentration was observed with increasing concentrations of *A. gummifera* extract. The control treatment recorded the highest mean CO_2 concentration (5.75 ± 0.25 ppm), whereas the lowest value (3.50 ± 0.86 ppm) was observed in fish exposed to the highest extract concentration. However, these differences were not statistically significant ($P > 0.05$).

Table 1. Physicochemical Characteristics of Experimental Water During Sublethal Exposure of *Clarias gariepinus* Juveniles to *Albizia gummifera* Crude Bark Extract

Conc. (g/L)	Salt (ppm)	Temp ($^\circ\text{C}$)	pH	ELC ($\mu\text{S/cm}$)	TDS (ppm)	GH (ppm)	KH (ppm)	CO_2 (ppm)
0.00	294.00 ± 33.66	31.75 ± 0.64	7.48 ± 0.09	675.25 ± 91.22	248.25 ± 14.53	65.25 ± 4.11	357.75 ± 1.10	5.75 ± 0.25
0.10	320.25 ± 53.85	31.27 ± 0.14	7.58 ± 0.12	601.50 ± 70.48	303.00 ± 33.33	52.00 ± 0.71	319.00 ± 20.70	4.00 ± 1.15
0.20	276.75 ± 39.31	31.40 ± 0.17	7.55 ± 0.98	617.00 ± 73.01	282.25 ± 37.68	61.50 ± 4.90	267.50 ± 0.64	3.50 ± 0.86
0.30	293.25 ± 40.83	31.77 ± 0.075	7.44 ± 0.09	585.25 ± 82.84	292.25 ± 42.19	61.50 ± 4.91	317.75 ± 23.06	3.50 ± 0.86
Pvalue	0.90	0.40	0.76	0.87	0.67	0.18	0.01	0.25

Conc.=Concentration Salt= Salinity ELC=Conductivity TDS=Total dissolve solute GH=General Hardness

KH=Alkalinity CO₂=Carbon dioxide

3.2 Liver Antioxidant Biomarkers of *Clarias gariepinus* Juveniles Exposed to Sublethal Concentrations of *Albizia gummifera* Crude Bark Extract

The effects of sublethal exposure to aqueous crude bark extract of *Albizia gummifera* on liver antioxidant biomarkers of *Clarias gariepinus* juveniles are presented in Table 2. Liver superoxide dismutase (SOD) activity showed only slight variation among the treatment groups. The highest mean SOD activity ($19.46 \pm 0.15 \mu\text{mol SOD min}^{-1}$) was observed in the control group (0.00 g L^{-1}), while the lowest value ($18.29 \pm 0.12 \mu\text{mol SOD min}^{-1}$) was recorded in fish exposed to 0.10 g L^{-1} . Intermediate SOD activities of 18.55 ± 0.07 and $18.59 \pm 0.65 \mu\text{mol SOD min}^{-1}$ were recorded at 0.20 and 0.30 g L^{-1} , respectively. Statistical analysis indicated that SOD activity did not differ significantly among treatments ($P > 0.05$).

Glutathione peroxidase (GPx) activity increased progressively with increasing concentrations of the crude bark extract. The lowest GPx activity ($34.72 \pm 1.58 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$) was recorded in the control group, whereas the highest activity ($49.61 \pm 5.89 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$) occurred in fish exposed to 0.30 g L^{-1} . Intermediate values of 42.61 ± 5.02 and $46.27 \pm 7.86 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$ were recorded at 0.10 and 0.20 g L^{-1} , respectively.

$\text{min}^{-1} \text{ mg protein}^{-1}$ were observed at 0.10 and 0.20 g L^{-1} , respectively.

Catalase (CAT) activity exhibited a similar concentration-dependent trend. The control treatment recorded the lowest CAT activity ($30.20 \pm 1.77 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$), while the highest value ($44.75 \pm 5.09 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$) was observed at the highest exposure concentration (0.30 g L^{-1}). Fish exposed to 0.10 and 0.20 g L^{-1} showed intermediate CAT activities of 35.68 ± 8.26 and $40.88 \pm 2.71 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$, respectively.

Malondialdehyde (MDA), an indicator of lipid peroxidation, generally declined with increasing extract concentration. The highest mean MDA concentration ($1.69 \pm 0.41 \text{ mmol mg protein}^{-1}$) was recorded in the control group, whereas the lowest value ($1.06 \pm 0.08 \text{ mmol mg protein}^{-1}$) occurred in fish exposed to 0.10 g L^{-1} . Comparable MDA values of 1.07 ± 0.28 and $1.38 \pm 0.09 \text{ mmol mg protein}^{-1}$ were obtained at 0.30 and 0.20 g L^{-1} , respectively.

Reduced glutathione (GSH) showed only minor variation among treatments. The highest GSH concentration ($3.13 \pm 0.13 \mu\text{g mL}^{-1}$) was observed at 0.10 g L^{-1} , followed by $3.00 \pm 0.25 \mu\text{g mL}^{-1}$ at 0.20 g L^{-1} . Identical GSH concentrations ($2.75 \pm 0.25 \mu\text{g mL}^{-1}$) were recorded in both the control and the highest exposure concentration (0.30 g L^{-1}).

Table 2: Mean Liver Antioxidants Biomakers of *Clarias gariepinus* Juveniles Exposed to Sub Lethal Concentrations of *A. gummifera* crude bark extract

Conc	SOD	GPX	Cat.	MDA	GSH
(g/L)	unit=($\mu\text{mole SOD/min}$)	($\mu\text{mole/min/mgprotien}$)	($\mu\text{mole/min/mgprotein}$)	(mm/mg/protein)	($\mu\text{g/ml}$)
0.30	18.59 ± 0.65	49.61 ± 5.89	44.75 ± 5.09	1.07 ± 0.28	2.75 ± 0.25
0.20	18.55 ± 0.07	46.27 ± 7.86	40.88 ± 2.71	1.38 ± 0.09	3.00 ± 0.25
0.10	18.29 ± 0.12	42.61 ± 5.02	35.68 ± 8.26	1.06 ± 0.08	3.13 ± 0.13
0.00	19.46 ± 0.15	34.72 ± 1.58	30.20 ± 1.77	1.69 ± 0.41	2.75 ± 0.25

SOD= Superoxide Dismutase

GPX=Glutathione Peroxidase

Cat=Catalase

Conc=Concentrations

MDA=Malondialdehyde GSH= Reduced Glutathione $\pm$ =mean standard error

3.3 Gills Antioxidant Biomarkers of *Clarias gariepinus* Juveniles Exposed to Sublethal Concentrations of *Albizia gummifera* Crude Bark Extract

The effects of sublethal exposure to aqueous crude bark extract of *Albizia gummifera* on the antioxidant biomarkers of the gills of *Clarias gariepinus* juveniles are presented in Table 3. Superoxide dismutase (SOD) activity showed only slight variation among the treatment groups. The highest mean SOD activity ($19.26 \pm 0.26 \mu\text{mol SOD min}^{-1}$) was

recorded in the control group (0.00 g L^{-1}), while the lowest value ($17.52 \pm 0.19 \mu\text{mol SOD min}^{-1}$) occurred in fish exposed to the highest concentration (0.30 g L^{-1}) of the crude bark extract. Intermediate values were recorded at 0.10 and 0.20 g L^{-1} .

Glutathione peroxidase (GPx) activity varied considerably among the exposure groups. The highest GPx activity ($73.36 \pm 9.19 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$) was observed in fish exposed to 0.30 g L^{-1} , whereas the lowest value ($44.55 \pm$

4.67 $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$) occurred at 0.20 g L⁻¹. Catalase (CAT) activity generally declined following exposure to the plant extract. The highest CAT activity (58.35 $\pm$ 13.60 $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$) was recorded in the control treatment, while the lowest value (46.83 $\pm$ 1.29 $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$) occurred in fish exposed to 0.20 g L⁻¹.

Malondialdehyde (MDA) concentrations exhibited treatment-related variation. The highest MDA level (1.71 $\pm$

0.13 mmol mg protein⁻¹) was observed at 0.20 g L⁻¹, whereas the lowest concentration (0.91 $\pm$ 0.11 mmol mg protein⁻¹) was recorded at 0.10 g L⁻¹.

Reduced glutathione (GSH) concentrations showed relatively little variation among treatments. The highest GSH concentration (3.38 $\pm$ 0.12 $\mu\text{g mL}^{-1}$) was recorded in fish exposed to 0.30 g L⁻¹, while identical values were obtained at 0.10 and 0.20 g L⁻¹.

Table 3: Mean Gills Antioxidants Biomarkers of *Clarias gariepinus* Juveniles Exposed to Sub Lethal Concentrations of *A. gummifera* crude bark extract

Con	SOD	GPX	Cat.	MDA	GSH
(g/L)	unit=($\mu\text{mole SOD/min}$)	($\mu\text{mole/min/mgprotien}$)	($\mu\text{mole/min/mgprotein}$)	(mm/mg/protein)	($\mu\text{g/ml}$)
0.30	17.52 $\pm$ 0.19	73.36 $\pm$ 9.19	57.15 $\pm$ 9.19	1.32 $\pm$ 0.24	3.38 $\pm$ 0.12
0.20	18.33 $\pm$ 0.27	44.55 $\pm$ 4.67	46.83 $\pm$ 1.29	1.71 $\pm$ 0.13	2.87 $\pm$ 0.12
0.10	19.09 $\pm$ 0.06	52.85 $\pm$ 12.63	47.79 $\pm$ 3.42	0.91 $\pm$ 0.11	2.87 $\pm$ 0.12
0.00	19.26 $\pm$ 0.26	65.22 $\pm$ 3.54	58.35 $\pm$ 13.60	1.13 $\pm$ 0.21	2.87 $\pm$ 0.37

SOD= Superoxide Dismutase

GPX=Glutathione Peroxidase

Cat=Catalase

Conc=Concentrations

MDA=Malondialdehyde

GSH= Reduced Glutathione

()=mean standard error

3.4 Testes Antioxidant Biomarkers of *Clarias gariepinus* Juveniles Exposed to Sublethal Concentrations of *Albizia gummifera* Crude Bark Extract

The effects of sublethal exposure to aqueous crude bark extract of *Albizia gummifera* on the antioxidant biomarkers of the testes of *Clarias gariepinus* juveniles are presented in Table 4.

Superoxide dismutase (SOD) activity increased markedly with increasing concentrations of the crude bark extract. The lowest mean SOD activity (17.99 $\pm$ 0.60 $\mu\text{mol SOD min}^{-1}$) was recorded in the control group (0.00 g L⁻¹), whereas the highest value (35.32 $\pm$ 16.93 $\mu\text{mol SOD min}^{-1}$) was observed in fish exposed to the highest concentration (3.00 g L⁻¹). Intermediate SOD activities of 18.10 $\pm$ 0.70 and 18.89 $\pm$ 0.29 $\mu\text{mol SOD min}^{-1}$ were recorded at 1.00 and 2.00 g L⁻¹, respectively.

Glutathione peroxidase (GPx) activity showed variable responses across the treatment groups. The highest GPx activity (32.51 $\pm$ 4.70 $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$) was recorded at 1.00 g L⁻¹, whereas the lowest value (23.04 $\pm$ 0.05 $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$) occurred at 2.00 g L⁻¹. Fish exposed to the highest concentration (3.00 g L⁻¹) exhibited a GPx activity of 29.33 $\pm$ 3.14 $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$, while the control recorded 26.89 $\pm$ 7.90 $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$.

Catalase (CAT) activity increased markedly following exposure to the plant extract. The lowest CAT activity (38.86 $\pm$ 8.70 $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$) was recorded in the control treatment, whereas the highest activity (70.00 $\pm$ 0.93 $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$) occurred at 2.00 g L⁻¹. Fish exposed to 1.00 and 3.00 g L⁻¹ recorded CAT activities of 39.23 $\pm$ 0.22 and 61.70 $\pm$ 1.88 $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$, respectively.

Malondialdehyde (MDA) concentrations generally decreased in the exposed fish relative to the control. The highest MDA concentration (1.47 $\pm$ 0.24 mmol mg protein⁻¹) was recorded in the control group, whereas the lowest value (0.82 $\pm$ 0.29 mmol mg protein⁻¹) was observed at 1.00 g L⁻¹. Fish exposed to 2.00 and 3.00 g L⁻¹ recorded MDA concentrations of 1.03 $\pm$ 0.08 and 1.36 $\pm$ 0.09 mmol mg protein⁻¹, respectively.

Reduced glutathione (GSH) concentrations exhibited only slight variation among treatments. The lowest GSH concentration (2.13 $\pm$ 0.12 $\mu\text{g mL}^{-1}$) was recorded in the control group, whereas values of 2.25 $\pm$ 0.25, 2.25 $\pm$ 0.00 and 2.25 $\pm$ 0.00 $\mu\text{g mL}^{-1}$ were obtained at 1.00, 2.00 and 3.00 g L⁻¹, respectively, indicating a slight increase in GSH following exposure to the crude bark extract.

Table 4: Mean Testes Antioxidants Biomarkers of *Clarias gariepinus* Juveniles Exposed to Sub Lethal Concentrations of *A. gummifera* crude bark extract

Conc. (g/L)	SOD unit=($\mu\text{mole SOD/min}$)	GPX ($\mu\text{mole/min/mgprotien}$)	Cat. ($\mu\text{mole/min/mgprotein}$)	MDA (mm/mg/protein)	GSH ($\mu\text{g/ml}$)
0.00	17.99 $\pm$ 0.60	26.89 $\pm$ 7.90	38.86 $\pm$ 8.70	1.47 $\pm$ 0.24	2.13 $\pm$ 0.12
1.00	18.10 $\pm$ 0.70	32.51 $\pm$ 4.70	39.23 $\pm$ 0.22	0.82 $\pm$ 0.29	2.25 $\pm$ 0.25
2.00	18.89 $\pm$ 0.29	23.04 $\pm$ 0.05	70.00 $\pm$ 0.93	1.03 $\pm$ 0.08	2.25 $\pm$ 0.00
3.00	35.32 $\pm$ 16.93	29.33 $\pm$ 3.14	61.70 $\pm$ 1.88	1.36 $\pm$ 0.09	2.25 $\pm$ 0.00

SOD= Superoxide Dismutase

GPX= Glutathione Peroxidase

Cat=Catalase

Conc=Concentrations

MDA=Malondialdehyde

GSH= Reduced Glutathione

 $\pm$ =mean standard error

3.5 Ovarian Antioxidant Biomarkers of *Clarias gariepinus* Juveniles Exposed to Sublethal Concentrations of *Albizia gummifera* Crude Bark Extract

The antioxidant responses of the ovaries of *Clarias gariepinus* juveniles exposed to sublethal concentrations of *A. gummifera* crude bark extract are presented in Table 5.

Superoxide dismutase (SOD) activity varied only slightly among the treatment groups. The lowest mean SOD activity ($17.39 \pm 0.11 \mu\text{mol SOD min}^{-1}$) was recorded at 2.00 g L^{-1} , while the highest value ($18.80 \pm 0.12 \mu\text{mol SOD min}^{-1}$) was observed in fish exposed to the highest concentration (3.00 g L^{-1}). The control and 1.00 g L^{-1} treatments recorded SOD activities of 17.48 ± 0.14 and $17.74 \pm 0.02 \mu\text{mol SOD min}^{-1}$, respectively.

Glutathione peroxidase (GPx) activity was highest in the control group ($42.78 \pm 0.14 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$) and lowest at 1.00 g L^{-1} ($38.77 \pm 1.51 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$), and $4.38 \pm 0.37 \mu\text{g mL}^{-1}$, respectively.

Fish exposed to 2.00 and 3.00 g L^{-1} recorded GPx activities of 40.67 ± 2.69 and $39.63 \pm 3.70 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$, respectively.

Catalase (CAT) activity declined with increasing concentrations of the extract. The highest CAT activity ($48.88 \pm 5.06 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$) was observed at 1.00 g L^{-1} , whereas the lowest value ($25.86 \pm 3.05 \mu\text{mol min}^{-1} \text{ mg protein}^{-1}$) occurred at 3.00 g L^{-1} .

Malondialdehyde (MDA) concentrations increased slightly with increasing extract concentration. The lowest MDA value ($1.46 \pm 0.10 \text{ mmol mg protein}^{-1}$) was recorded at 1.00 g L^{-1} , while the highest concentration ($1.65 \pm 0.90 \text{ mmol mg protein}^{-1}$) occurred at 3.00 g L^{-1} . Reduced glutathione (GSH) showed relatively minor variation among treatments. The highest GSH concentration ($4.50 \pm 0.00 \mu\text{g mL}^{-1}$) was recorded at 1.00 g L^{-1} , whereas the lowest value ($3.87 \pm 0.63 \mu\text{g mL}^{-1}$) occurred at 2.00 g L^{-1} . The control and 3.00 g L^{-1} treatments recorded GSH concentrations of 3.88 ± 0.13

Table 5: Mean Ovaries Antioxidants Biomarkers of *Clarias gariepinus* Juveniles Exposed to Sub Lethal Concentrations of *A. gummifera* crude bark extract

Conc. (g/L)	SOD unit=($\mu\text{mole SOD/min}$)	GPX ($\mu\text{mole/min/mgprotien}$)	Cat. ($\mu\text{mole/min/mgprotein}$)	MDA (mm/mg/protein)	GSH ($\mu\text{g/ml}$)
0.00	17.48 $\pm$ 0.14	42.78 $\pm$ 0.14	43.39 $\pm$ 5.34	1.50 $\pm$ 0.04	3.88 $\pm$ 0.13
1.00	17.738 $\pm$ 0.02	38.77 $\pm$ 1.51	48.88 $\pm$ 5.06	1.46 $\pm$ 0.10	4.50 $\pm$ 0.00
2.00	17.39 $\pm$ 0.11	40.67 $\pm$ 2.69	38.33 $\pm$ 19.64	1.57 $\pm$ 0.09	3.87 $\pm$ 0.63
3.00	18.80 $\pm$ 0.12	39.63 $\pm$ 3.70	25.86 $\pm$ 3.05	1.65 $\pm$ 0.90	4.38 $\pm$ 0.37

SOD= Superoxide Dismutase

GPX=Glutathione Peroxidase

Cat=Catalase

Conc=Concentrations

MDA=Malondialdehyde GSH= Reduced Glutathione

Conclusion

This study demonstrated that sublethal exposure to aqueous crude bark extract of *Albizia gummifera* elicited measurable physiological and biochemical responses in *Clarias gariepinus* juveniles. Although most of the monitored water-quality parameters remained within acceptable limits, alkalinity was significantly affected, indicating that the plant extract can modify the chemical characteristics of the aquatic environment. These changes, however, were less pronounced than the biochemical alterations observed in the exposed fish.

Exposure to *A. gummifera* crude bark extract induced tissue-specific changes in oxidative stress biomarkers, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), and malondialdehyde (MDA) in the liver, gills, testes and ovaries. The differential responses among tissues indicate that the extract disrupted redox homeostasis and activated antioxidant defence mechanisms, with the liver exhibiting strong adaptive antioxidant responses, while the reproductive organs showed evidence of increased susceptibility to oxidative stress at higher exposure concentrations.

The findings demonstrate that oxidative stress biomarkers are more sensitive indicators of sublethal toxicity than routine physicochemical water-quality parameters. Consequently, the use of *A. gummifera* as a traditional piscicide may pose ecological risks to non-target fish species by inducing oxidative stress and potentially impairing normal physiological and reproductive functions, even at concentrations that do not cause immediate mortality.

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