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RESEARCH ARTICLE

Evaluation of Gonadotoxicity of Waste Burnt Tyres Residues on *Clarias gariepinus* Juveniles

Sulaiman Yusuf[1], Audu Bala Sambo[2], Wade John Wokton[2]

[1]*Department of Zoology, Nasarawa State University Keffi, Nasarawa State Nigeria.*

[2]*Department of Zoology, Faculty of Natural Sciences, University of Jos, Nigeria.*

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Corresponding Author: Sulaiman Yusuf, Department of Zoology, Nasarawa State University Keffi, Nasarawa State Nigeria.

Abstract

Waste tyres are intentionally burned for various purposes, releasing toxic substances that ultimately find their way into aquatic environments. This study examined the sublethal effects of water-soluble fractions (WSFs) of waste burnt tyre residue (WBTR) on the gonads of *Clarias gariepinus* juveniles. Juveniles of *C. gariepinus* were exposed to sublethal concentrations (0.00, 0.23, 0.46, 0.94, 1.87, and 3.74 g/L) of WSF derived from the 11.2 g/L median lethal concentration over two months. Antioxidant activities, as well as the histological and histomorphometric features of testes and ovaries, were analyzed using standard protocols. The average antioxidant activity of superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT) were found to be higher in the testes and ovaries of catfish exposed to the highest WSF concentration (3.74 g/L) compared to the control. Glutathione peroxidase (GPx) showed a similar trend, while the highest levels of reduced glutathione (GSH) and GPx were observed in the ovaries of control fish. Significant differences ($P < 0.05$) in CAT and SOD activity were noted in the ovaries and testes of exposed fish compared to controls. The histo-architecture of the testes and ovaries in control fish displayed normal structures, while exposure to WSF led to dose-dependent depletion of seminiferous luminal contents in the testes and mild nuclear atrophy in the ovaries, with the highest severity observed in fish exposed to the highest concentrations. Histomorphometric analysis of the ovaries showed significant differences ($P < 0.05$) in mean values for Maturing Follicular Diameter (MGD) and Matured Follicular Diameter (MFD) in fish exposed to varying WSF concentrations compared to controls. In the testes, there was no significant difference ($P > 0.05$) in seminiferous luminal diameter (SLD), but a significant difference ($P < 0.05$) was found in seminiferous tubular diameter (STD) in fish exposed to WSF compared to controls. In conclusion, the study confirmed that the WSF of WBTR affects antioxidant levels, histology, and histomorphometry of the reproductive organs of *C. gariepinus* juveniles.

Keywords: Waste Burnt Tyres, Sublethal-Toxicity, Testes and Ovaries, Antioxidants, Histology, Histomorphometry, African Catfish.

1. Introduction

Automobiles play a vital role in infrastructure, facilitating the transportation of people, goods, and services (Stanley et al., 2021). Increased patronage of the automotive industry has led to a rise in car part sales (Juma et al., 2006). However, in emerging African nations like Nigeria, the unregulated importation of vehicles and spare parts, including tyres, poses significant environmental concerns due to improper disposal of used parts like waste tyres (Stanley et al., 2021). Nigeria faces a large accumulation of waste tyres (Bala & Malachy, 2020), which are often burned to extract iron and steel for various uses. This process releases toxic substances such as carbon black, zinc oxide, wax, sulfur, and heavy metals like cadmium (Cd), chromium (Cr), iron (Fe), lead (Pb), and zinc (Zn) into the atmosphere and aquatic environments

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(Aya & Nwite, 2016). High concentrations of heavy metals in biota negatively impact the ecological health of aquatic species, potentially leading to population declines (Luo, Gao, & Wang, 2014). Heavy metals have also been linked to fish deformities that harm their welfare, growth, and reproductive systems, both in natural populations and laboratory studies (Sfakianakis et al., 2015).

The reproductive systems of teleost fish are particularly sensitive to environmental pollution, making them valuable indicators for aquatic biomonitoring (Sulochana et al., 2021). Fish are often chosen for ecotoxicological research due to their adaptability to environme ntal conditions, especially in captivity under extreme water quality (Okoro et al., 2019; Iheanacho et al., 2020). Heavy metal pollution has detrimental effects on the reproductive tissues of fish, reducing the fertility of their testes and ovaries (Rayees et al., 2023). It is well-established that alterations in antioxidant defence mechanisms and the generation of reactive oxygen species (ROS) are linked to pollutant toxicity (Livingstone, 2001). Several enzymatic and non-enzymatic antioxidants, including reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), are involved in detoxification and maintaining cellular homeostasis (Rayees et al., 2023). These enzymes serve as biomarkers for stress in fish exposed to toxicants, with SOD converting superoxide radicals into hydrogen peroxide (H_2O_2) and oxygen (O_2), while CAT detoxifies H_2O_2 into water and oxygen (Livingstone, 2023). Fish tissues possess antioxidant defense systems, such as CAT, SOD, and GPx, to protect against oxidative stress caused by heavy metals (Basha & Rani, 2003). These enzymes act as a first line of defence against oxygen-derived free radicals, and their activity is used to diagnose potential contamination in aquatic organisms (Ahmad et al., 2000). Pollutants in the water can lead to oxidative stress in fish, compromising reproduction and increasing susceptibility to infections (Padmini, Geetha, & Rani, 2009).

Studies have shown increased activity of SOD, CAT, and GPx in the gonads of *Oncorhynchus mykiss* from uncontaminated sites, with reduced activity in fish from polluted areas (Rayees et al., 2023). In *C. gariepinus* from Lake Maryout, Egypt, a significant reduction ($P < 0.05$) in these antioxidant enzyme activities was observed in gonads collected from contaminated regions compared to relatively unpolluted areas (Heba & Mohamed, 2019). Histological analysis of *O. mykiss* ovaries from a relatively uncontaminated hatchery showed normal structure, while those from a contaminated site exhibited nuclear disorganization, cytoplasmic vacuolation, and abnormalities in oocyte structure (Rayees et al., 2023).

Other studies reported various testicular and ovarian alterations in fish exposed to different pollutants. For example, Mayank et al. (2015) observed discontinuous connective tissue, atrophic spermatocytes, and degeneration in *Heteropneustes fossilis* testes exposed to lead nitrate for 21 days. Similarly, Claramma and Radhakrishnan (2016) noted disorganization in *C. batrachus* testes exposed to chromium, and Murugananthkumar et al. (2016) documented testicular changes in *C. batrachus* after exposure to copper nanoparticles and $CuSO_4$. Ovarian changes, such as thickened membranes, non-bursting atresia, and reduced oocyte numbers, were seen in *O. niloticus* and *Garra gotyla* from polluted environments (Hala et al., 2018; Sharma et al., 2017). In *C. gariepinus,* exposure to antifouling paints, thiobencarb, and paraquat dichloride led to ovarian degeneration and atretic follicles (Ochuwa et al., 2017; Elias et al., 2020; Oladunjoye et al., 2021). Testicular histomorphometric measurements, such as seminiferous tubular and luminal diameters, have also been used to assess reproductive changes in catfish exposed to various toxicants (Okoye et al., 2017; Sulaiman et al., 2022).

Finally, studies have demonstrated that the water-soluble fractions of waste burnt tyres induce toxic effects on the liver and gills of African catfish, with altered antioxidant enzyme activities and behavioral changes, such as reduced feeding and hypoactivity (Bala & Malachy, 2020; Stanley et al., 2021, 2023). This study is the first to investigate the sublethal effects of water-soluble fractions of waste burnt tyres on catfish gonads over 58 days, examining antioxidant alterations, histological, and histomorphometric changes.

2. Methodology

2.1 Collection and Acclimation of Experimental Fish (*Clarias gariepinus*)

Healthy, mixed-sex juveniles of *C. gariepinus* (mean weight: 46.90 ± 0.3.44g; mean total length: 14.54 ± 0.36 cm) were obtained from Catfish Expert Global Venture Farm, located in Zarmaganda, Jos, Plateau State, Nigeria, and transported to the Aquaculture Laboratory at the Hydrobiology and Fisheries Unit, Department of Zoology, University of Jos, Nigeria.

The fish were placed into six plastic tanks, each with a 35L capacity and filled with 20L of borehole water. They were allowed to acclimatize to laboratory conditions for one week. During this period, the water in the holding tanks was replaced daily at 8:00 AM, and the fish were fed to satiation with a commercial diet (Coppens®) twice daily at 8:00 AM and 5:00 PM. To maintain tank cleanliness, three-quarters of the water was siphoned daily to remove leftover food and faecal matter, which was then replaced with fresh water. Additionally, nets were placed over the tanks to prevent the fish from jumping out.

2.2 Preparation of Water-Soluble Fractions of Waste Burnt Tyre Residues

Waste tyre particles were collected from Ring Road, Jos North Local Government Area, Plateau State, Nigeria, where numerous tyres are burned daily to tenderize rocks. The collected particles were sorted into steel, iron, and residue (black rubber) components. The waste tyre residue was then ground into powder using a mortar and pestle, and sieved through a 40µm mesh (ASTM D40µm) to obtain fine particles. These fine particles were macerated in distilled water for 48 hours, and the filtrate was used as the water-soluble fraction (WSF) of waste burnt tyre residues (WBTRs), following the method of Bala & Malachy (2020).

2.3 Sublethal Concentrations of Water-Soluble Fractions of Waste Burnt Tyres Residues and Test Procedure

A serial dilution of the LC50 (11.22 g/L) determined by Bala and Malachy (2020) was used to prepare sublethal concentrations at 1/3rd, 1/6th, 1/12th, 1/24th, and 1/48th of 11.22 g/L, following OECD guidelines (1992) and the methods of Bala, Kabiru, and Ofojekwu (2014). This resulted in five sublethal test concentrations: 3.74, 1.87, 0.93, 0.47, and 0.23 g/L, along with a control (0.00 g/L). The renewable bioassay was conducted over two months (58 days). Test fish were fed to satiation twice daily, at 8:00 AM and 5:00 PM, using a commercial diet (Coppens®). The photoperiod was set to natural light conditions, with 12 hours of light and 12 hours of darkness.

3. Experimental Design

The sublethal toxicity experiment involved six rectangular glass tanks (40x25x23 cm) and 120 mixed-sex *C. gariepinus* juveniles, with a mean weight of 47.95 ± 3.44 g and a mean length of 15.54 ± 0.36 cm, arranged in a randomized block design. Each of the six tanks was filled with 10L of borehole water, and five tanks were inoculated with varying concentrations of the water-soluble fractions (WSF) of waste burnt tyre residues (WBTRs). Ten *C. gariepinus* juveniles were introduced into each tank (Bala & Malachy, 2020). Two additional tanks, serving as controls (0.00 g/L), were also stocked with 10 juveniles but were not exposed to the test material. The experimental setup was replicated in duplicate.

3.1 Assay for Biomarkers of Oxidative Stress of Gonads of *Clarias gariepinus* Juveniles Exposed to Sub Lethal Concentrations of Water-Soluble Fractions of Waste Burnt Tyre Residues

Biomarkers of oxidative stress, including superoxide dismutase (SOD), malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), and glutathione peroxidase (GPx), were assayed in *C. gariepinus* juveniles exposed to 58 days of sublethal concentrations (SLCs) of water-soluble fractions (WSFRs) of waste burnt tyre residues (WBTRs) using the following procedures:

3.2 Preparation of Tissue Samples

After the experiment, *C. gariepinus* juveniles from both the treatment and control groups were sacrificed, and their liver and gills were carefully excised and washed in chilled phosphate-buffered saline. Tissue homogenates were prepared using chilled phosphate buffer (0.1 M, pH 7.4), and the homogenized samples were centrifuged at 9,000 rpm for 20 minutes at 4°C (Charity et al., 2018). The resulting supernatant was collected and used for subsequent biochemical

3.3 Determination of Superoxide Dismutase (SOD)

3.3.1 Superoxide Dismutase (SOD) Activity Analysis

SOD activity was analyzed following the method by Kakkar, Das, & Viswanathan (1984).

Principle: SOD inhibits the reduction rate of cytochrome C by 50% in a coupled system using xanthine and xanthine oxidase at pH 7.8, 25°C, in a 3.0 mL reaction volume, competing for the superoxide radical.

Procedure: The assay mixture contained 0.1 mL of tissue homogenate, 1.2 mL of sodium pyrophosphate buffer (52 mM, pH 8.3), 0.15 mL of PMS (0.186 mM), 0.35 mL of NBT (0.3 mM), 1.0 mL of distilled water, and 0.25 mL of NADH (0.75 mM). The reaction was initiated by the addition of NADH, followed by incubation at 30°C for 90 seconds. The reaction was halted by adding 1.0 mL of glacial acetic acid. The mixture was stirred and shaken with 4.0 mL of

n-butanol. After centrifuging for 5 minutes at 5,000 rpm, the butanol layer was collected, and the colour intensity of chromogen in this layer was measured at 560 nm.SOD activity was expressed as 50% inhibition of nitro blue tetrazolium redu ction/min/mg protein. Tris-HCl buffer (0.025 M, pH 7.4) was used as the control in place of the tissue homogenate.

3.3.2 Determination of Malondialdehyde (MDA)

Principle: The assay is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA), forming an MDA-TBA2 complex that strongly absorbs at 532 nm. Butylated hydroxytoluene (BHT) and EDTA are added to the sample and reaction mixture to prevent lipid oxidation during processing (Botsoglou, 1994).

Procedure: The samples and MDA standards were reacted with TBA.After incubation, the absorbance was measured spectrophotometrically at 532 nm.MDA concentrations were calculated by comparing the sample results to the MDA standard curve.

3.3.3 Determination of Reduced Glutathione (GSH)

The reduced glutathione content was determined using the method of Ellman (1959). Principle: Reduced glutathione reacts with alloxan to produce a substance that absorbs maximally at 305 nm.

Procedure: 1.0 mL of tissue homogenate in Tris-HCl buffer (25 mM, pH 7.4) was added to 1.0 mL of 10% TCA and mixed vigorously.The mixture was centrifuged at 5,000 rpm for 5 minutes.The assay mixture included 1.0 mL of the supernatant, 0.5 mL of Ellman's reagent (0.02% DTNB in 1% trisodium citrate), and 3.0 mL of phosphate buffer (200 mM, pH 8.0).The yellow colour produced was measured at 412 nm using the molar extinction coefficient of 1.36 × 10^4 M^-1 cm^-1 for calculations.

3.3.4 Catalase Activity (CAT) Assay

Catalase activity was assayed colourimetrically using the method by Sinha (1972).Principle Catalase

$$2H_2O \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Catalase (CAT) Activity Assay

In this method, the breakdown of hydrogen peroxide (H_2O_2) is monitored spectrophotometrically at 240 nm. One unit of catalase decomposes one mole of hydrogen peroxide per minute at 25°C and pH 7.0 under the given conditions.

Procedure: The reaction mixture (1.5 mL) consisted of:1.0 mL of phosphate buffer (10 mM, pH 7.0), and 0.1 mL of tissue homogenate (e.g., liver or gills). The reaction was initiated by adding 0.4 mL of H_2O_2 (2000 mM) to the mixture.The reaction mixture was incubated at room temperature for 3 minutes. The reaction was terminated by adding 2.0 mL of dichromate-acetic acid reagent (a 1:3 mixture of 5% potassium dichromate and glacial acetic acid). The mixture was further incubated at 100°C for 2 minutes. After the incubation, the absorbance of the mixture was measured at 620 nm.

3.3.5 Determination of Glutathione Peroxidase (GPx)

The activity of (GPx) was assayed as described by Paglia and Valentine (1967) with little modifications according to Lawrence and Burke (1978). The mixture of the reaction contained 0.2mM nicotinamide adenine dinucleotide phosphate, 50mM potassium phosphate buffer (pH 8.3), 1mM sodium azide, 1mM EDTA, and 1 U/mL glutathione reductase. The reaction was initiated with the addition of 1.5 mM cumene hydroperoxide. The enzyme activity was estimated from the rate of oxidation of NADPH. The reagents were mixed and the absorbance was measured at 340 nm. Enzyme activity was expressed in mmol/ minute/ milligram protein.

3.4 Procedure for Histological Examination of Organs of *Clarias gariepinus* Juveniles Exposed to Sub Lethal Concentrations of Water-Soluble Fractions of Waste Burnt Tyres Residues

The routine paraffin wax method and haematoxylin-eosin staining techniques of tissue processing described by Drury and Wallington (1967) and Avwioro (2011) were adopted for the examination of testes and ovaries of *C. gariepinus* juveniles exposed WSF of WBTRs. The harvested organs *were* fixed in 10% formalin for 3 days, cut into thin slices of 5 x 2 x 1mm thick and then processed with the SPIN Tissue Processor (STP) 120 (Thermoscientific model). The tissues were buffered in 10% formalin before passing through the following levels of hydrocarbons for two hours each: 70%, 80%, 90% and 95% Alcohol; Absolute Alcohol I, II, & III; Xylene I & II; Paraffin Wax Oven I & II.

Tissues were embedded in molten paraffin wax using embedding moulds. The tissues were embedded using embedding cassettes on a tissue Tek Embedding Centre (SLEE MPS/P2), and cooled rapidly on the cooling component. Tissues were sectioned using a rotary microtome (MICROM HM340E Thermoscientific) set at 4 micromes, picked on slides and ready for staining.

Haematoxylin and eosin staining technique was used for the staining of the tissues. Tissue sections were dewaxed and hydrated by passing through two changes of xylene and through descending levels of alcohol (100%, 80%, 70%) for three min each and then into water before staining in Harris' haematoxylin solution for 5 min and washed in running water. They were differentiated in 1% Acid alcohol and then washed thoroughly in water, blued in Scott's tap water substitute for 5 min and rinsed briefly in distilled water. Each tissue was then counterstained in 1% aqueous eosin for 2 min and then washed in water, dehydrated in descending grades of alcohol before clearing in xylene and mounted in Destrene, Plasticiser and Xylene (DPX). Sections were then placed in slide carriers and placed in a 40°C oven to dry overnight. Each tissue was read microscopically.

3.5 Histomorphemetrics Measurements of Organs of *Clarias gariepinus* Juveniles Exposed to Sub Lethal Concentrations of Water-Soluble Fractions of Waste Burnt Tyres Residues

Testicular histomorphemetric measurments like seminiferous epithelial height (SEH), seminiferous luminal diameter (SLD) and seminiferous tabular diameter (STD) as well as ovarian histomorphometric measurements such as maturing follicular diameter (MGD), immature follicular diameter (IMD) and matured follicular diameter were determined by using Motic image plus 2.0 (Motic Asia, Hong Kong) software (Bala & Malachy 2020).

4. Statistical Analyses

Statistical analyses were conducted using IBM SPSS (version 20) software. Data were evaluated using one-way analysis of variance (ANOVA). Significance was determined at a 0.05 probability level, with $P<0.05$ considered statistically significant. Treatment means were compared using Tukey's multiple comparisons test. Results are presented as mean ± standard error (±SE).

5. Results

5.1 Antioxidantsof Gonads of *Clarias gariepinus* Exposed to sub-lethal Concentrations of Water-soluble Fractions of Waste Burnt tyres Residues

As presented in Table 1, the mean malondialdehyde (MDA) and reduced glutathione (GSH) activities were higher in the testes of catfish exposed to the highest concentration (3.74 g/L) of WSF of WBTRs (4.75±0.50 mmol/mg protein and 4.81±0.21 µg/ml, respectively) compared to the control (1.81±0.07 mmol/mg protein and 8.35±0.32 µg/ml). Similar to the activities of superoxide dismutase (SOD) and catalase (CAT), glutathione peroxidase (GPx) activity was also significantly higher in the testes of catfish exposed to the highest concentrations of WSF of WBTRs compared to the control, with a significant difference ($P<0.05$) recorded in CAT activity.

In Table 2, the mean MDA activity in the ovaries of catfish exposed to the highest concentration of WSF of WBTRs was significantly higher (4.31±0.00 mmol/mg protein) than that of the control (1.92±0.04 mmol/mg protein). Similarly, the highest activities of SOD and CAT (18.62±0.30 µmole SOD/min and 5.17±0.17 µmole H_2O_2/min/mg protein) were observed in the ovaries of catfish exposed to the highest concentration of WSF of WBTRs, while the lowest values (9.83±0.23 µmole SOD/min and 5.06±0.00 µmole H_2O_2/min/mg protein) were recorded in the control group. A significant difference ($P<0.05$) in SOD activity was observed compared to the control. The mean highest activity of GSH and GPx (8.81±0.08 µg/ml and 3.16±0.15 µmol/min/mg protein) were recorded in the ovaries of catfish from the control, while the lowest values (9.83±0.23 µg/ml and 5.06±0.00 µmol/min/mg protein) were found in those exposed to the highest concentration of WSF of WBTRs. A significant difference ($P<0.05$) in CAT activity was observed compared to the control.

Table 1. *Mean Testis Antioxidants Biomarkers of Clarias gariepinus Juveniles Exposed 58days Sub Lethal Concentrations of Water-Soluble Fractions of Waste Burnt Tyres Residues*

Conc. (g/L)	MDA (mmol/mg protein)	GSH (GSH=µg/ml)	SOD unit= (µmole SOD/ min	CAT (µmoleH_2O_2/i/ mg protein)	GPx (µmol/min/ mgprotie)
0.00	1.81±0.07^{a}	8.35±0.32^{a}	10.60±0.55^{a}	4.33±0.08)^{a}	14.16±0.05^{a}
0.23	2.20±0.20^{a}	7.48±0.05^{ab}	12.15±0.03^{a}	6.45±0.56^{b}	15.51±0.51^{ab}
0.46	2.95±0.06^{a}	6.43±0.36^{bc}	12.54±0.48^{a}	6.69±0.19^{b}	15.53±0.52^{ab}
0.94	2.25±0.25^{ab}	6.62±0.39^{cd}	12.59±0.58^{a}	7.34±0.33^{b}	16.73±0.27^{ab}
1.87	3.36±0.35^{ab}	5.55±0.35^{cd}	11.88±0.55^{a}	8.16±0.10^{bc}	18.75±0.28^{c}
3.74	4.75±0.50^{b}	4.81±0.21^{e}	16.15±0.45^{b}	9.44±0.32^{c}	24.45±1.44^{d}

LPO= Lipid peroxidation,GSH= Reduced glutanione, SOD= Superoxide dismutase, CAT=Catalase,GPX= Gulathione peroxidase

Table 2. *Mean Ovaries Antioxidants Biomarkers of Clarias gariepinus Juveniles Exposed 58days Sub Lethal Concentrations of Water-Soluble Fractions of Waste Burnt Tyres Residues*

Con. g/L	MDA mmol/mg	GSH µg/ml	SOD µmole SOD/min	CAT µmoleH_2O_2/min/mg	GPx µmol/min/mgprotie
0.00	1.92±0.04[a]	8.81±0.08 [a]	9.83±0.23 [a]	5.06±0.00[a]	13.38±0.05 [a]
0.23	2.25±0.25[a]	7.11±0.01 [bc]	11.08±0.02 [b]	5.93±0.06 [b]	14.26±0.25[b]
0.46	2.23±0.03[a]	6.95±0.06 [c]	12.43±0.01 [c]	4.55±0.05[d]	15.00±0.54[d]
0.94	2.41±0.00 [ab]	5.94±0.05 [d]	14.24±0.16[d]	5.38±0.05 [a]	13.06±2.39[a]
1.87	3.55±0.46[cd]	4.49±0.03 [e]	17.20±0.20 [e]	7.08±0.07 [c]	11.82±0.18[c]
3.74	4.31±0.00 [d]	3.16±0.15 [f]	18.62±0.30 [f]	5.17±0.17[a]	11.30±0.29[c]

Values with asterisks (*) in the same column indicate significant difference (P<0.05) compared with the control

LPO= Lipid peroxidation, RGx= Reduced glutanione, SOP= Superoxide dismutase, CAT=Catalase, GPX=Gulathione peroxidase

5.2 Histopathology of Gonada of *Clarias gariepinus* Juveniles Exposed to 58 days Sublethal Concentrations of Water-Soluble Fractions of Waste Burnt Tyres Residues

The histo-architecture of the testes in the control *C. gariepinus* displayed normal, numerous tubular structures housing spermatogonia (indicated by short black arrows), spermatozoa (indicated by white arrows), and distinct intertubular septae (indicated by short white arrows). Interstitial cells were present within the interstitium between the tubules, outlined by an oval shape (Plate 1). However, exposure to graded concentrations of WSF of WBTRs for 58 days resulted in a dose-dependent depletion of seminiferous luminal contents (Plate 1b-E), with the most severe effects observed in fish exposed to the highest concentration (3.74 g/L) of WSF of WBTRs (Plate 1F).

The histo-architecture of the ovary in *C. gariepinus* showed normal morphology, characterized by a thick ring of ooplasm (indicated by white arrowheads) covered by an intact follicular epithelium (indicated by black arrowheads). Nuclei (indicated by white stars) were surrounded by nucleoli located at their periphery. In contrast, the ovaries of catfish exposed to WSF of WBTRs maintained normal morphology but exhibited mild nuclear atrophy (indicated by white stars).

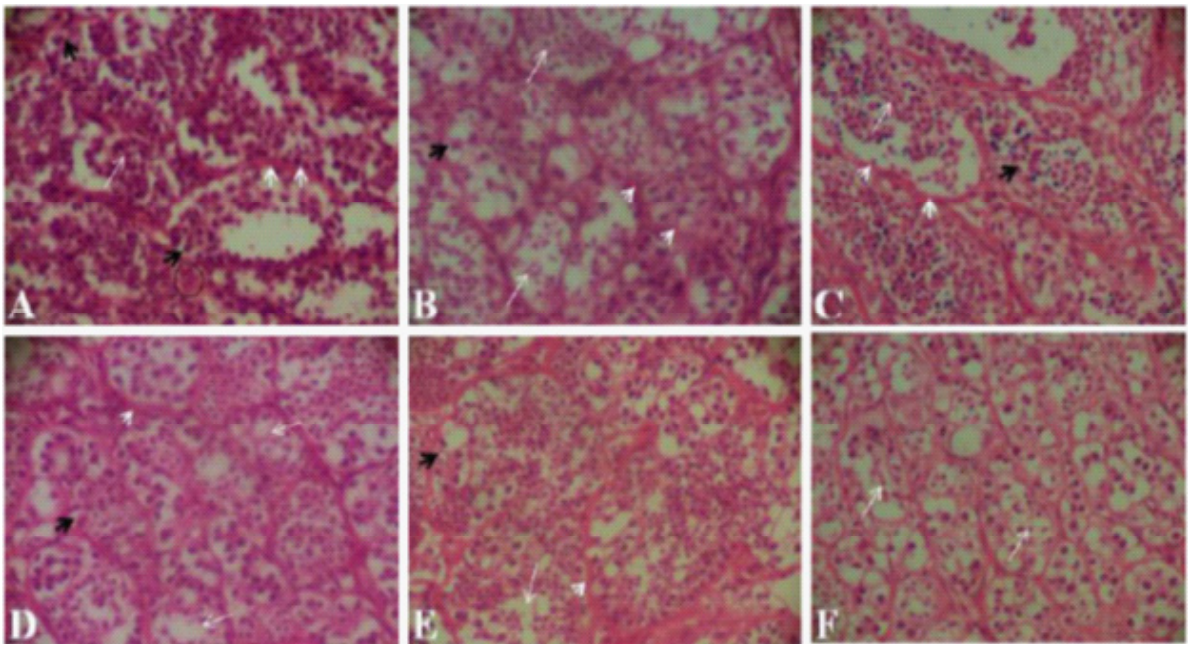

Figure 1. *Photomicrographs of the testis of Clariasgariepinus exposed to 58 days of sub-lethal concentrations of water-soluble fractions of waste burnt tyres. H&E: X400.A. Control (0.00 mg/L): The testis bears normal numerous tubular structures housing spermatogonia (short black arrow), spermatozoa (white arrow) and distinct intertubularseptae (short white arrow). Also, interstitial cells are present within the interstitium between the tubules (oval outline). B. 0.23 g/L, C. 0.47 g/L, D. 0.94 g/L & E. 1.87 g/L: No visible lesion in the spermatogonia (short black arrow) and tubular septa (short white arrow) except for a moderate reduction in seminiferous luminal content (white arrow). F. 3.74 mg/L: Severe depletion of seminiferous luminal content (white arrow)*

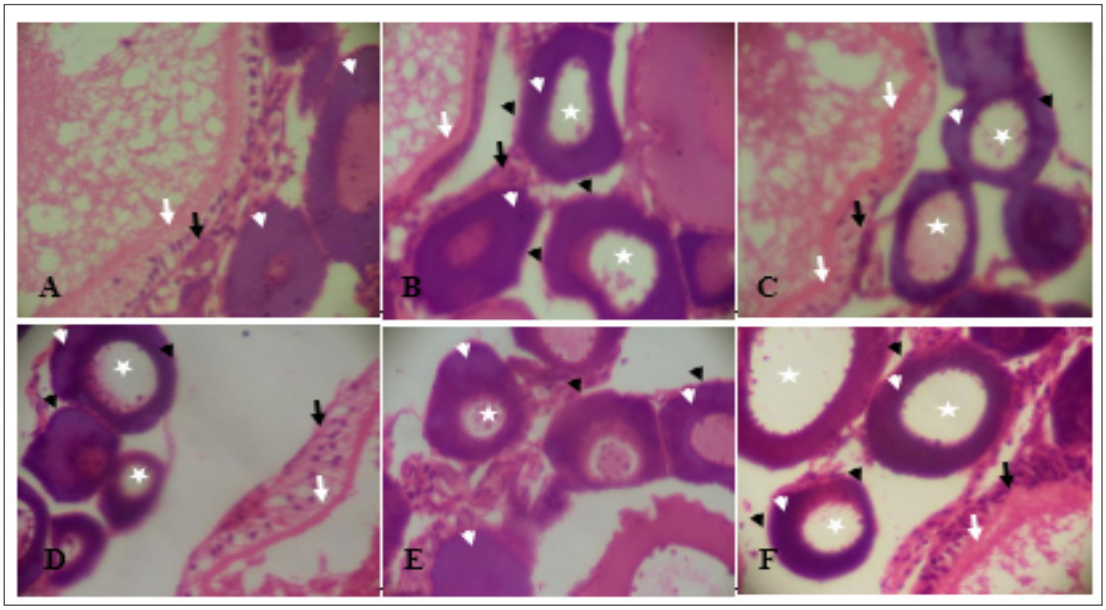

Figure 2. *Ovaryof Clarias gariepinus Exposed to Sublethal Concentrations of Water-Soluble Fractions of Waste Burnt Tyres for 58 Days, Showing Different Morphological Presentations at Different Stages of Growth. H&E X400 (A).Control (0.00 mg:/L): The Ovary Bears Normal Morphology as Presented by a Thick Ring of Ooplasm (white arrowheads) Covered by an Intact Follicular Epithelium (black arrowheads). Nuclei(white stars) are surrounded by the nuclei which Appear at their Periphery. White arrows= Vitellin Envelope, Black arrows= Cortical Alveoli.(B) 0.23 g/L, (D), 0.94 g/L, E) 1.87 and (F) 3.74 g/L displayed Normal Morphology with Mild Nuclei Atrophy (white stars).*

5.3 Testicular Histomorphometry of *Clarias gariepinus* Juveniles Exposed to Sub Lethal Concentrations of Water-Soluble Fractions of Waste Burnt Tyres Residues

The results of testicular histomorphometry (SEH, SLD, and STD) in *C. gariepinus* juveniles exposed to 58 days of sublethal concentrations of WSF of WBTRs are shown in Table 5. The highest mean seminiferous epithelial height (SEH) of 112.35±16.38 µm was recorded in the control group, while the lowest mean SEH of 41.90±4.71 µm was observed in the group exposed to the highest concentration (3.74 g/L). The highest mean seminiferous luminal diameter (SLD) of 469.03±24.60 µm was also found in the control group, with the lowest mean value of 152.43±7.97 µm in the group exposed to 3.74 g/L of WSF of WBTRs. No significant difference ($P > 0.05$) was observed in SLD between the control and the exposed groups. However, there was a noticeable decrease in the mean seminiferous tubule diameter (STD) in the juveniles exposed to sublethal concentrations of WSF of WBTRs compared to the control group. A significant difference ($P < 0.05$) was found in the STD of the testis in fish exposed to WSF of WBTRs compared to the control

Table 3. *Mean Testicular Histomorphometry of Clarias gariepinus Juveniles Exposed to Sub Lethal Concentrations of Water-Soluble Fractions of Waste Burnt Tyres Residues*

Con.(g/L)	SHE (µm)	SLD (µm)	STD (µm)
0.00	112.35±16.38 ^{a}	469.03±24.60 ^{c}	534.50±45.31 ^{c}
0.23	78.73±6.44 ^{b}	300.00±23.57 ^{b}	439.41±11.98 ^{a}
0.47	90.01±4.49^{b}	467.76±64.28 ^{bc}	479.38±24.63 ^{a}
0.94	86.31±4.40^{b}	278.52±20.26 ^{ab}	508.06±26.89 ^{a}
1.87	58.35±4.10 ^{bc}	294.33±21.55 ^{b}	483.52±31.94 ^{b}
3.74	41.90±4.71^{c}	152.43±7.97^{a}	504.40±63.35 ^{a}

SHE=Seminiferous Epithelial Height SLD=Seminiferous Luminal Diameter STD=Seminiferous Tabular Diameter

5.4 Ovarian Histomorphometry of *Clarias gariepinus* Juveniles Exposed to Sublethal Concentrations of Water-Soluble Fractions of Waste Burnt Tyres Residues

The results of ovarian histomorphometry (MGD, IFD, and MFD) in *C. gariepinus* juveniles exposed to 58 days of sublethal concentrations (SLCs) of WSFs of WBTRs are shown in Table 6. The highest mean value of maturing follicular diameter (MGD) was 662.90±56.02 µm in the control group, while the lowest mean value of 367.67±13.69 µm was recorded in the 0.94 g/L concentration. A significant difference

($P < 0.05$) in MGD was found between the control and the exposed groups. The highest mean immature follicular diameter (IFD) of 366.07±18.40 µm was recorded in the group exposed to 0.23 g/L concentration, whereas the lowest mean value of 1698.62±62.94 µm was observed in the 3.74 g/L group. No significant difference ($P > 0.05$) in IFD was found between the control and the exposed groups. The highest mean matured follicular diameter (MFD) of 3060.11±121.10 µm was recorded in the control group. A significant difference ($P < 0.05$) in MFD was observed between the control and the exposed groups.

Table 4. *Mean Ovarian Histomorphometry of Clarias gariepinus Juveniles Exposed to 58 Days Sublethal Concentrations of Water-Soluble Fractions of Waste Burnt Tyres Residues*

Conc. (g/L)	MGD (µm)	IFD (µm)	MFD (µm)
0.00	662.90±56.02 [a]	345.28±19.14 [a]	3060.11±121.10 [b]
0.23	471.06±29.66 [b]	366.07±18.40 [a]	2475.55±54.60 [a]
0.47	494.67±18.46 [bc]	336.57±18.07 [a]	1905.50±146.79 [a]
0.94	367.29±13.69 [c]	362.30±14.37 [a]	1698.62±62.94 [a]
1.87	455.47±25.02 [bc]	353.03±38.89 [a]	1715.74±114.72 [a]
3.74	484.99±14.08 [bc]	278.43±12.44 [a]	2387.02±37.95 [a]

MGD=Maturing Follicular Diameter MD=Immature Follicular Diameter MFD=Matured Follicular Diameter

6. Discussion

The production of reactive oxygen species (ROS) has been elevated by various toxicants in aquatic environments, disrupting the prooxidant/antioxidant balance in fish cells (Burak et al., 2016). Cytotoxic ROS attacks critical macromolecules in cells, potentially leading to tissue damage (Livingstone et al., 2003). Malondialdehyde (MDA) is a well-known marker of lipid peroxidation and oxidative stress (Tabrez & Ahmad, 2009). Lipid peroxidation (LPO) occurs when ROS degrade lipids in cell membranes, leading to toxic by-products that can be measured by assessing MDA levels (Tabrez & Ahmad, 2009). Our results show increased MDA levels in the testes and ovaries of *C. gariepinus* exposed to sublethal concentrations (SLCs) of WSFs from WBTRs compared to the control group, indicating oxidative stress in the gonads of the exposed fish. A similar increase in MDA was reported in the ovary of *Heteropneustes fossilis* exposed to fluoride (Yadav et al., 2015). Ekaete (2014) also reported elevated MDA levels in fish from polluted areas near the sawmill industry in Lagos Lagoon. These findings align with previous research, such as that by Kaptaner (2015), who observed high MDA levels in the ovaries of *Chalcalburnus tarichi* from a polluted area in Lake Van. The increased MDA and ROS levels resulted in the depletion of GSH in the gonads of the exposed catfish. GSH, an endogenous antioxidant, protects cells from damage by ROS and peroxides (Pompella et al., 2003). In addition to scavenging free radicals directly, GSH also acts as a substrate for GPx (Yadav et al., 2015). The reduction in GSH levels could be due to its direct conjugation with electrophiles generated by SLCs of WSFs from WBTRs. A decrease in GSH levels has also been reported in the gonads of *H. fossilis* exposed to fluoride (Yadav et al., 2015). The enzymes SOD, CAT, and GPx play essential roles in the antioxidant defense system by neutralizing ROS and preventing cellular damage (Ighodaro & Akintoye, 2018). SOD catalyzes the conversion of ROS into H_2O, while CAT breaks down H_2O_2 into water and oxygen, thus protecting cells from ROS-induced damage (Yadav et al., 2015). In this study, the levels of SOD and CAT in the gonads of catfish increased significantly with higher concentrations of WSFs from WBTRs. Similar increases in SOD and CAT levels have been reported in the gonads of *H. fossilis* exposed to fluoride (Yadav et al., 2015). Dabas et al. (2012) also observed similar results in *Channa punctatus* exposed to cadmium.

Increased levels of toxicants in aquatic environments intensify stress in fish species, leading to tissue alterations (Hussain et al., 2021). Histopathological changes in fish tissues have long been used as biomarkers of pollutant exposure (Greenfield et al., 2008), providing direct evidence of the harmful effects of pollutants in fish species (Fricke et al., 2012). Exposure of *C. gariepinus* to SLCs of WSFs from WBTRs for 58 days led to a dose-dependent depletion of seminiferous luminal contents, with the most severe damage occurring in fish exposed to the highest concentration (3.74 g/L). Murugananthkumar et al. (2016) reported similar findings, including disruption of the basal lamina and enlarged spermatocytes in the testes of *C. batrachus* exposed to copper for 21 days, supporting these results. However, Mayank et al. (2015) observed different histopathological changes,

including discontinuous interlobular connective tissue and atrophied spermatocytes, in *H. fossilis* exposed to lead nitrate for 21 days, which do not align with the present findings. Claramma and Radhakrishnan (2016) also reported seminiferous tubule distortion and other cellular changes in *Clarias batrachus* exposed to chromium for 45 days.

Histopathological studies of ovary tissues in various fish species exposed to toxicants are well-documented. Sharma et al. (2017) found reduced numbers of oocytes and follicular degeneration in *Garra gotyla* exposed to manganese for 9 weeks. In contrast, juvenile *C. gariepinus* exposed to antifouling paints for 28 days showed varying degrees of follicle degeneration. Exposure to different SLCs of paraquat dichloride led to the appearance of spent follicles, while no significant lesions were observed in the control group (Oladunjoye et al., 2021). However, in our study, the histopathology of *C. gariepinus* ovaries exposed to WSFs from WBTRs exhibited mild nuclear atrophy, which differs from the findings of the studies mentioned above.

The testicular (SEH, STD, and SLD) and ovarian (MGD, IFD, and MFD) morphometric measurements in the exposed catfish decreased with increasing toxicant concentrations, which supports the histopathological findings. The observed changes in gonadal morphometry could be attributed to the increased permeability of membranes to heavy metals present in burnt tyres (Bala & Malachy, 2020). Sulaiman et al. (2022) reported an increase in gonadal morphometry in *C. gariepinus* exposed to *Vernonia amygdalina,* which contrasts with the findings of this study.

In conclusion, this study confirms that WSFs from WBTRs affect the antioxidants, histology, and histomorphometry of *C. gariepinus* juveniles' reproductive organs. This is the first report assessing the antioxidant, histological, and histomorphometric changes in the gonads of *C. gariepinus* exposed to WBTRs, offering deeper insight into the impact of burning tyres in aquatic environments on fish reproduction.

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