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Potential Consequences of Water-Soluble Acetaminophen-Chromium Combination in *Clarias Gariepinus*: Bioaccumulation and Oxidative Perturbations of Antioxidant Enzyme Activities

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Abstract

In this study, emerging-recalcitrant water contaminants were examined to determine their impact on water quality and oxidative disruption of antioxidant markers in *Clarias gariepinus* (African catfish). Fifty ***C.*** *gariepinus* were randomly exposed to fresh water, 250 mg/L acetaminophen (ACT), 0.525 mg/L chromium (Cr) and a mixture of ACT+Cr – dosed water for 21 days. As compared to the control, dosed water did not significantly ($p > 0.05$) affect dissolved oxygen (DO), but biochemical oxygen demand (BOD) significantly increased in ACT, ACT+Cr, and Cr-dosed water.Levels of ACT in ***C.*** *gariepinus* exposed to different concentrations followed by kidney > gill > liver > heart. Likewise, higher Cr presence was found in ***C.*** *gariepinus* gills exposed to 0.350 mg/L Cr. Accordingly, kidneys and gills were the worst affected organs by ACT and Cr accumulation. All the targeted organs of *C. gariepinus* exposed to different concentrations of ACT+Cr showed a concentration-dependent reduction in catalase (CAT) activity, indicating the synergistic effects of ACT and heavy metals. Based on these results, ACT and Cr adversely affect the kidneys and gills of *C. gariepinus*, compromising their physiological activity. As a result, pharmaceutical wastes and heavy metal effluents released into the aquatic environment indiscriminately need to be monitored.

Keywords Acetaminophen, Bioaccumulation, *Clarias gariepinus,* Chromium, Enzymatic antioxidant

Introduction

Freshwater ecosystems are of paramount importance in sustaining both human and aquatic life. Environmentalists are deeply concerned about pollution, particularly in aquatic environments. This concern has grown due to the many stressors originating from human activities, making pristine freshwater systems rare. The unintended release of contaminants resulting from industrialisation, the rise in domestic sewage, pharmaceutical pollutants, and hospital waste have had detrimental impacts on aquatic ecosystems (Azeez *et al.,* 2022a).

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Pharmaceuticals are now widely recognised as emerging contaminants due to their growing use, leading to their widespread presence in aquatic environments (Hoyett *et al.,* 2016; Papageorgiou *et al.,* 2016). Residues of these pharmaceuticals in the environment are considered "compounds of emerging concern" because they have the potential to significantly impact both human health and ecosystems (Ebele *et al.,* 2017). One prominent pharmaceutical compound, acetaminophen, chemically known as paracetamol, is extensively used for chronic pain management over extended periods. Acetaminophen has emerged as a significant aquatic pollutant, originating from both pharmaceutical industries and human usage. It is often detected in wastewater, surface water, drinking water, and underground water (Ding *et al.,* 2016; Liang *et al.,* 2016; Al-kaf *et al.,* 2017; Zur *et al.,* 2018; Azeez *et al.,* 2022c). Additionally, pharmaceuticals are known to contain substantial amounts of heavy metals, such as chromium (Cr). Over time, the increasing use of pharmaceuticals has led to the generation of more pharmaceutical waste, which is frequently discharged as effluents into aquatic environments.

Similarly, human activities like mining represent a significant environmental stressor that degrades the quality of surface freshwater by introducing heavy metals (Bakshi & Panigrahi, 2018; Pradip *et al.,* 2018). The pollution of inland freshwater systems by heavy metals has been a global concern for many years due to their toxicity, environmental persistence, and ability to accumulate in living organisms (Mitra *et al.,* 2022). In Nigeria, artisanal mining activities have been observed in numerous states, releasing various toxic pollutants, including heavy metals, into nearby water bodies. While heavy metals are naturally occurring, their concentrations are exacerbated in natural environments due to various human activities (Ravindra *et al.,* 2014; Outa *et al.,* 2020).

Chromium, one of the most common and widespread metal pollutants in the environment, finds its way into aquatic systems through industrial effluents. Human activities that contribute chromium and its particles to the environment encompass various processes, such as chemical manufacturing, mining, steel production, metal plating, leather tanning, textile dyeing, electroplating, cement manufacturing, metallurgy, and others (Nakkeeran *et al.,* 2018; Lian *et al.,* 2019).In an aquatic environment, chromium undergoes oxidation to Cr(VI), which is water-soluble, extremely irritating, and toxic to cellular tissues owing to its oxidising potential and permeability of biological membranes (Tel *et al.,* 2004).The accumulation of heavy metals introduced by human activities in surface waters can pose health risks for aquatic organisms and humans (Sonia & Ali, 2017; Azeez *et al.,* 2022b). The detrimental effects of heavy metals include organ damage, cancer, and physiological disorders (Kumari *et al.,* 2017; De Mandal *et al.,* 2019; Shanker, 2019; Kakakhel *et al.,* 2021).

Conducting toxicity tests on fish stands out as one of the most effective approaches to comprehend the harmful consequences of environmental contaminants on aquatic ecosystems. Fish often directly absorb many water contaminants, although some species exhibit tolerance and adaptability to challenging environmental conditions (Sonnia & Ali, 2017; Aslam & Yousafzai, 2017; Azeez *et al.,* 2022a). The African catfish, scientifically known as *Clarias gariepinus*, has emerged as a suitable model for assessing toxicities. This is attributed to several distinct characteristics, including ease of collection and handling, prolific breeding, adaptability, year-round availability, and tolerance to poorly oxygenated water (Naguib *et al.,* 2020).In the case of aquatic life, most water pollutants find their way into the organisms through various tissues such as the gills, liver, skin, and kidneys, where they accumulate and exert negative effects on these tissues (Kumari *et al.,* 2017; Ali *et al.,* 2019).

Acetaminophen and chromium are significant environmental stressors, and numerous studies have extensively examined their individual toxicities in aquatic environments. For instance, Mintenig *et al*. (2018), Perussolo *et al*. (2019), and Erhunmwunse *et al*. (2021) have reported that acetaminophen has the potential to disrupt cardiac function, induce developmental malformations in

catfish embryos, and impact osteoblast proliferation, thereby affecting bone regeneration. On the other hand, chromium has been found to alter fish behaviours, affect haematology and the endocrine system, and induce organ toxicity in the liver, brain, kidney, and gills (Kamila *et al.,* 2023). However, due to chromium's high reactivity with other organic compounds, there is limited research on the toxicity of the interaction between acetaminophen and chromium in the context of *Clarias gariepinus*. Therefore, the current study aimed to assess the potentially toxic effects of co-exposure to acetaminophen and chromium on water quality, their accumulation in tissues, and the changes in oxidative stress biomarkers within vital organs of *Clarias gariepinus*.

Materials and Methods

Reagents and Chemical

Acetaminophen (white solid crystal) was purchased from Akol Pharmaceutical Limited (Nigeria). The chemicals, which include potassium dichromate ($K_2Cr_2O_7$), hydrochloric acid (HCl), hydrogen trioxonitrate (V) acid (HNO_3), potassium chloride (KCl), disodium phosphate (Na_2HPO_4), and monosodium dihydrogen orthophosphate (NaH_2PO_4) were of analytical grade purchased from Sigma-Aldrich Germany, and BDH Pole England.

*C. gariepinus*Sampling and Acclimatisation

Fresh samples of adult *C. gariepinus*, (n=50; body weight 500±0.5 g and length 38±0.2 cm) were purchased from a commercial aquaculture facility (Great Treasure Farms, Agunbelewo, Osogbo) and acclimatised for the period of 144 h in fresh water at the Osun State University Zoological Garden, Osogbo Campus. The fish were fed with 60 % crude protein processed feed (COPPENS) twice per day.

Experimental Design

The exposure of *C. gariepinus* to acetaminophen (ACT) and chromium (Cr) was carried out in ponds containing 200 L filtered water. Fish samples were randomly selected and divided into 10 groups: Control, Group A: 200 mg/L ACT, GroupB: 150 mg/L ACT, GroupC: 100 mg/L ACT, GroupD: 0.350 mg/L Cr, GroupE: 0.280 mg/L Cr,Group F: 0.210 mg/L Cr, Group G: 200 mg/L ACT+ 0.350 mg/L Cr, GroupH: 150 mg/ L ACT + 0.8 mg/ L Cr, GroupI: 100 mg/L ACT + 0.210 mg/L Cr. Five replicates per group and water changed every 48 hr. Each group received experimental exposure by changing treated (dosed) water every five days for 21 days. At the end of the experiment, all fish samples were sacrificed 24 hr after the final treatment.

Water Quality Analysis

The water quality of the control group and the group containing exposed fish was determined using the 21st edition of the Association of Official Analytical Chemists, 2019. Water samples were scooped from each group daily (evening period) and refrigerated for evaluate the water quality. Using the Extech Digital DO700 meter (Waltham, USA), the biochemical oxygen demand (BOD; determined as BOD5), Chemical oxygen demand (COD), dissolved oxygen (DO), Electrical conductivity (EC), total dissolved solids (TDS), and pH were analysed as indicators of water quality.

Preparation of Tissue Homogenate

The tissue homogenate was prepared to evaluate the toxicity of ACT and Cr on specific organs (kidney, liver, heart and gill). Each organ was excised and rinsed in ice-cold 1.15 % KCl solution, blotted and weighed. Subsequently, the tissues were homogenised in ice-cold 0.05 M phosphate buffer (pH 7.5) and centrifuged at 3000 rpm for 15 min, at 4 ^oC, and the supernatant obtained was used for further biochemical assays.

Determination of Oxidative Stress Biomarkers

The supernatants from each of the organs were used to evaluate the level of oxidative stress.The activities of catalase (CAT), glutathione-S-transferase (GST), superoxide dismutase (SOD), and concentrations of reduced glutathione (GSH) were expressed as U/mg protein and determined according to the procedures of Clairborne (1985), Habig *et al.* (1974),Aebi, (1974), Misral and

Friodovich (1972), and Ellman (1959), respectively.

Metal Analysis in the Organs and Water

Metal analysis in fish organs exposed to different treatments was analysed using the procedure reported by Azeez *et al*. (2022b; 2022c). An inductively coupled plasma optical emission spectrometer (ICP-OES) and UV-visible spectrophotometer (BIOBASE BK-UV1900PC, USA) were used to measure Cr and ACT concentrations in the control and exposed organs.

Data Analysis

The statistical analysis was carried out using IBM SPSS (version 25). The data were presented as mean ± standard error (n = 3). Analysis of variance (ANOVA) with Tukey's post-hoc test of multiple comparisons was used to compare groups. The significance level was defined at $p < 0.05$.

Results

Water Quality

Water quality parameters for control and residual dosed water are presented in Table 1. The changes in pH and DO levels across the groups of dosed water with ACT, Cr, ACT+Cr and control were not significant ($p > 0.05$). Similarly, the groups dosed with Cr and ACT+Cr displayed limited changes in BOD levels ($p > 0.05$), while a significant increase was observed in residual water dosed with ACT. The high BOD level obtained from water dosed with ACT is indicative of pollution. Also, there was a significant change in the COD levels in residual water dosed with ACT and ACT+Cr ($p < 0.05$). Additionally, Cr-dosed water had the highest EC and TDS values. EC levels had a significant increase in water dosed with Cr ($p < 0.05$), while ACT and ACT+Cr did not induce a significant change ($p > 0.05$). Furthermore, Cr and ACT+Cr caused a significant increase in TDS levels in dosed residual water compared to control.

Concentration and bioaccumulation of Cr and Acetaminophen in Tissues of *C. gariepinus*

The residual concentration of Cr in dosed water and their bioaccumulation in organs of *C. gariepinus* are presented in Table 2 and Figure 1, respectively. The residual concentration of Cr in dosed water and various organs was concentration-dependent. In comparison to thecontrol, an increase in Cr contamination in water led to a significant ($p < 0.05$) increase in residual Cr toxicity. In each organ, the highest concentration of Cr was recorded in ***C. gariepinus*** exposed to the highest individual concentration and in combination with ACT. The liver and gills contained the highest residual Cr concentration, followed by the kidney and the heart, while the heart contained the lowest concentration. There was a significant ($p < 0.05$) change in bioaccumulation of Cr in tissues exposed to varying concentrations of Cr.

Likewise, the residual acetaminophen concentration and bioaccumulation were illustrated in Table 3 and Figure 2, respectively. The trend of concentration in dosed water compared to control is insignificant ($p > 0.05$). Also, ACT levels within organs depend on concentration. In *C. gariepinus* tissues exposed to 200 mg/L ACT and 200 mg/L ACT + 0.350 mg/L Cr, ACT concentrations were high in the gills and kidneys. However, liver and heart had relatively low concentrations. In addition, changes in ACT bioaccumulation were significant ($p < 0.05$) across all tissues of *C. gariepinus* exposed to acetaminophen.

Oxidative stress biomarkers and enzyme profile of Cr and ACT induced toxicity in *C. gariepinus*

Table 4 presents antioxidant biomarkers in different organs of ***C. gariepinus*** exposed to ACT, Cr, and ACT+Cr. In the kidney, contamination from different concentrations of Cr, ACT and ACT + Cr was observed to mostly induce an increase in GSH concentration. In contrast, exposure to 150 mg/L ACT and 0.280 mg/L Cr reduced ($p > 0.05$) the GSH concentration as compared to control. Furthermore, an increase in GST activity was observed in *C. gariepinus* exposed to varied concentrations of Cr, ACT and ACT + Cr. Notably, a significant ($p < 0.05$) increase in GST activity was recorded in *C. gariepinus* exposed to 0.280 mg/L Cr compared to control. In the liver, heart and gills

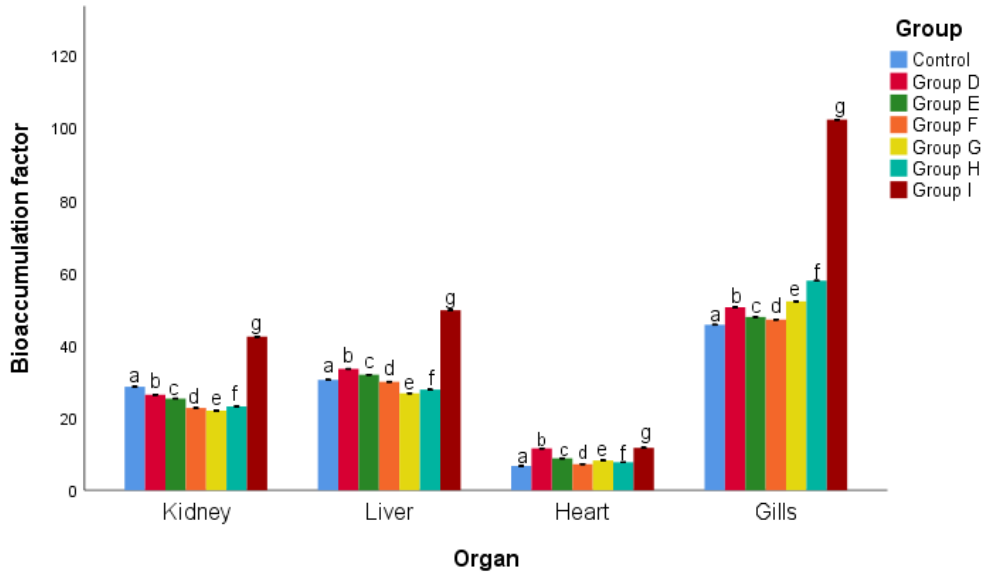

Figure 1. Bioaccumulation of Chromium in vital organs of *C. gariepinus*

Results are expressed in mean±SE (n = 3). The different superscript indicates significant difference at $p < 0.05$ down the group. Group D: 0.350 mg/L Cr, Group E: 0.280 mg/L Cr, Group F: 0.210 mg/L Cr, Group G: 200 mg/L ACT + 0.350 mg/L Cr, Group H: 150 mg/ L ACT + 0.8 mg/ L Cr, Group I: 100 mg/L ACT + 0.210 mg/L Cr

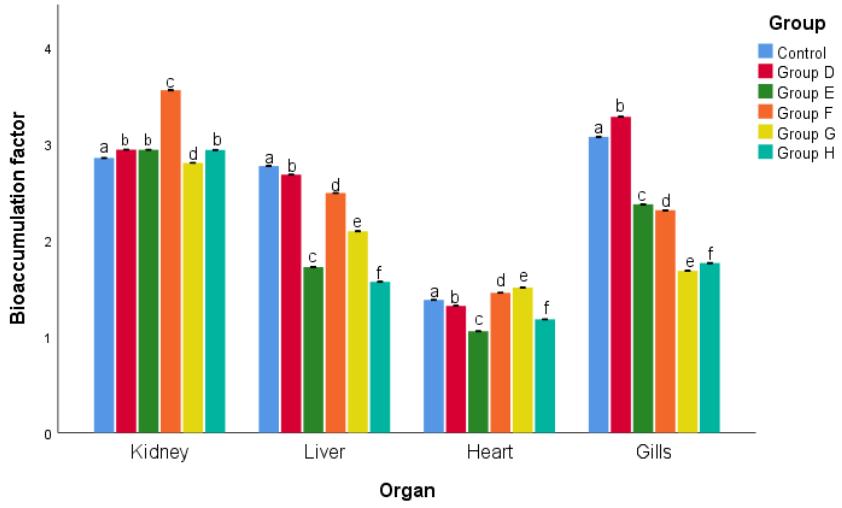

Figure 2. Bioaccumulation of Acetaminophen in vital organs of *C. gariepinus*

Results are expressed in mean±SE (n = 3). Different superscript indicates significant difference at $p < 0.05$ down the group. Group D: 0.350 mg/L Cr, Group E: 0.280 mg/L Cr, Group F: 0.210 mg/L Cr, Group G: 200 mg/L ACT + 0.350 mg/L Cr, Group H: 150 mg/ L ACT + 0.8 mg/ L Cr, Group I: 100 mg/L ACT + 0.210 mg/L Cr

Table 1. Physicochemical parameters of raw and dosed water samples

SAMPLE	DO (mg/L)	BOD_5 (mg/L)	COD (mg/L)	pH	EC (μScm^{-1})	TDS (mg/L)
Control	5.13 ± 0.01^{a}	25.22 ± 0.61^{a}	41.63 ± 1.27^{a}	7.62 ± 0.01^{b}	165.23 ± 0.28^{a}	82.83 ± 0.38^{a}
ACT	5.01 ± 0.05^{a}	44.50 ± 6.56^{b}	65.07 ± 5.80^{b}	8.03 ± 0.01^{b}	$185.23\pm17.97^{a,b}$	93.27 ± 9.24^{a}
Cr	5.02 ± 0.06^{a}	$34.55\pm1.13^{a,b}$	$52.45\pm2.69^{a,b}$	8.00 ± 0.08^{b}	243.33 ± 9.87^{b}	119.73 ± 2.70^{b}
ACT+Cr	5.08 ± 0.02^{a}	$38.37\pm2.51^{a,b}$	61.10 ± 2.08^{b}	8.15 ± 0.10^{b}	$222.70\pm24.16^{a,b}$	111.57 ± 12.07^{b}
NESREA/WHO	≥4.5	<40	NA	6.5-8.5	≤1500	500-1000

Results are expressed in mean±SE(n = 3).DO – dissolved oxygen, BOD – biochemical oxygen demand, COD – chemical oxygen demand, EC – electrical conductivity, TDS – total dissolved solids, NESREA- National Environmental Standards and Regulations Enforcement Agency (2011), WHO – World Health Organization (WHO, 2011). NA- not available. Data with different superscripts differ significantly ($p<0.05$) down the group.

Table 2. Concentrations ofCr in water samples and Organs of *C. gariepinus*

Group	Kidney (mg/L)	Liver (mg/L)	Heart (mg/L)	Gill (mg/L)	Water (mg/L)
Control	0.030±0.01[a]	0.032±0.01[a]	0.007±0.02[a]	0.048±0.01[a]	1.053E-3±2.69E-4[a]
Group 4	0.048±0.01[a]	0.061±0.03[b]	0.021±0.02[b]	0.092±0.01[b]	1.826E-3±0.62E-4[b]
Group 5	0.046±0.02[a]	0.058±0.01[b]	0.016±0.01[b]	0.087±0.02[b]	1.824E-3±3.10E-4[b]
Group 6	0.041±0.01[a]	0.054±0.01[b]	0.013±0.01[a]	0.085±0.01[b]	1.809E-3±1.94E-4[b]
Group 7	0.032±0.02[a]	0.039±0.02[a]	0.012±0.03[a]	0.076±0.00[c]	1.462E-3±0.51E-4[c]
Group 8	0.030±0.01[a]	0.036±0.01[a]	0.010±0.02[a]	0.075±0.01[c]	1.300E-3±0.62E-4[c]
Group 9	0.029±0.01[a]	0.034±0.01[a]	0.008±0.01[a]	0.070±0.01[c]	0.686E-3±2.01E-4[a]

Values for Group 1 – Group 3 are NA- not available. Results are expressed in mean±SE(n = 3).Different superscript indicates significant differencesat $p < 0.05$down the group.

Table 3. Concentrations ofacetaminophen in water samples and Organs of *C. gariepinus*

Group	Kidney (mg/L)	Liver (mg/L)	Heart (mg/L)	Gill (mg/L)	Water (mg/L)
Group 1	78.73±5.85[a]	76.37±2.00[a]	38.05±0.82[a]	84.73±5.61[a]	27.60 ± 0.46[a]
Group 2	73.05±3.59[a]	66.57±1.71[b]	32.70±0.92[b]	81.53±4.12[a]	24.87 ± 0.19[a]
Group 3	71.98±2.48[a]	42.10±1.62[c]	25.78±1.60[b]	58.05±1.96[b]	24.50 ± 0.29[a]
Group 7	100.30±4.27[b]	70.20±1.87[a]	40.97±0.76[a]	65.15±1.80[b]	28.23 ± 0.60[a]
Group 8	77.43±2.65[a]	57.75±1.48[b]	41.58±0.51[a]	46.40±1.89[c]	27.63 ± 0.09[a]
Group 9	74.18±3.08[a]	39.70±2.55[c]	29.77±0.59[b]	44.63±2.75[c]	25.37 ± 0.29[a]

Values for Group 4 – Group 6 are NA- not available. Results are expressed in mean±SE(n = 3).The different superscript indicates a significant difference at $p < 0.05$down the group.

Table 4. Oxidative stress biomarkers in organs of *C. gariepinus* exposed to different concentrations of ACT, Cr and ACT+Cr

Enzyme activity (U/mg)	Control	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Kidney										
GSH	42.40±2.30^{a,b}	48.80±2.84^{a,b}	40.80±3.35^{a}	46.20±1.43^{a,b}	49.00±1.76^{a,b}	45.40±0.98^{a,b}	39.80±2.69^{a}	52.60±1.63^{b}	51.20±2.01^{b}	48.20±1.71^{a}
SOD	62.60±4.02^{c}	60.40±5.48^{b,c}	48.00±3.21^{a,b,c}	44.00±2.41^{a}	47.60±1.17^{a,b}	51.40±3.43^{a,b,c}	50.80±3.32^{a,b,c}	47.80±1.56^{a,b}	48.40±1.44^{a,b,c}	50.40±2.20^{a,b,c}
GST	42.30±1.69^{a}	43.54±0.96^{a}	39.52±1.85^{a,b}	44.66±2.12^{a,b}	40.42±1.68^{a,b}	46.80±1.71^{b}	41.18±0.86^{a,b}	40.96±1.59^{a,b}	44.74±2.77^{a,b}	37.92±1.46^{a}
CAT	0.80±0.04^{c}	0.43±0.05^{a,b}	0.53±0.03^{b}	0.31±0.01^{a}	0.51±0.03^{a,b}	0.34±0.03^{a,b}	0.39±0.10^{a,b}	0.32±0.01^{a}	0.33±0.21^{a,b}	0.49±0.02^{a,b}
Liver										
GSH	44.00±3.44^{a,b}	37.60±2.36^{a}	37.40±3.50^{a}	46.60±1.20^{a,b}	49.80±2.87^{b}	48.40±0.87^{a,b}	48.00±2.97^{a,b}	43.20±2.44^{a,b}	49.60±1.33^{b}	48.20±1.77^{a,b}
SOD	63.20±4.81^{b}	64.00±4.83^{b}	45.80±2.33^{a}	45.20±0.86^{a}	42.00±0.32^{a}	46.80±1.39^{a}	47.20±1.20^{a}	46.80±1.02^{a}	47.80±2.75^{a}	48.80±3.09^{a}
GST	42.62±1.27^{a}	40.02±1.00^{a}	39.06±1.21^{a}	45.38±2.31^{a}	43.22±1.79^{a}	41.48±2.92^{a}	40.92±0.47^{a}	42.26±1.08^{a}	43.76±2.43^{a}	39.10±0.74^{a}
CAT	0.71±0.07^{c}	0.41±0.06^{a,b}	0.47±0.01^{a,b}	0.32±0.01^{a,b}	0.50±0.02^{a,b}	0.34±0.08^{a,b}	0.42±0.01^{a,b}	0.31±0.03^{a}	0.32±0.01^{a,b}	0.48±0.14^{a,b}
Heart										
GSH	48.80±2.58^{a}	41.60±2.40^{a}	41.60±3.97^{a}	45.20±1.16^{a}	46.00±3.31^{a}	45.80±3.23^{a}	47.20±1.83^{a}	45.20±3.23^{a}	45.80±3.43^{a}	51.00±8.41^{a}
SOD	63.20±3.38^{c}	62.40±3.54^{c}	44.40±1.94^{a,b}	46.80±2.08^{a,b}	44.00±3.05^{a}	58.00±0.01^{b,c}	44.00±1.79^{a}	50.00±2.07^{a,b,c}	45.80±2.29^{a,b}	55.00±5.39^{a,b,c}
GST	43.06±2.00^{a}	43.40±2.60^{a}	42.86±1.44^{a}	46.32±3.75^{a}	41.94±1.29^{a}	48.16±1.04^{a}	42.64±1.01^{a}	41.00±1.23^{a}	48.50±0.65^{a}	41.38±2.27^{a}
CAT	0.76±0.04^{d}	0.51±0.04^{c}	0.54±0.03^{c}	0.30±0.01^{a}	0.55±0.03^{c}	0.36±0.02^{a,b}	0.33±0.04^{a}	0.30±0.01^{a}	0.29±0.04^{a}	0.49±0.01^{b,c}
Gills										
GSH	49.80±1.74^{a}	46.80±3.50^{a}	40.80±1.93^{a}	43.80±1.39^{a}	43.60±2.75^{a}	44.80±2.51^{a}	48.40±4.13^{a}	43.40±3.09^{a}	46.40±2.22^{a}	46.80±1.59^{a}
SOD	63.20±3.38^{c}	62.40±3.54^{c}	44.40±1.94^{a,b}	46.80±2.08^{a}	44.00±3.05^{a}	58.00±0.01^{b,c}	44.00±1.79^{a}	50.00±2.07^{a,b,c}	45.80±2.29^{a,b}	55.00±5.39^{a,b,c}
GST	42.30±0.81^{a}	40.90±1.54^{a}	41.50±1.34^{a}	44.10±3.89^{a}	40.22±1.18^{a}	48.80±1.22^{a}	43.58±2.43^{a}	43.82±1.44^{a}	43.38±2.18^{a}	41.40±1.53^{a}
CAT	0.81±0.05^{d}	0.41±0.10^{a,b,c}	0.54±0.07^{c}	0.29±0.01^{a,b}	0.50±0.03^{b,c}	0.33±0.03^{a}	0.44±0.19^{a,b,c}	0.30±0.05^{a,b}	0.25±0.07^{a}	0.49±0.02^{b,c}

Results are expressed in mean ± standard error of the mean of three replicates. Different superscript indicates significant difference between groups at $p < 0.05$ (across the column).

GST and GSH levels were not significantly ($p > 0.05$) altered in *C. gariepinus* exposed to different concentrations of Cr, ACT and ACT + Cr. The activities of SOD and CAT reduced significantly ($p < 0.05$) in kidneys and gills on exposure to different concentrations of Cr, ACT and ACT + Cr with significant ($p < 0.05$) reduction only observed in gills. There was an apparent decrease in SOD activity as ACT and Cr concentrations increased. Also, CAT activity was significantly reduced in *C. gariepinus* livers administered at different concentrations of Cr, ACT and ACT + Cr. The reductions in SOD activity observed upon exposure to 200 mg/L ACT, 0.280 mg/L Cr, 200 mg/L ACT + 0.350 mg/L Cr and 100 mg/L ACT + 0.210 mg/L were not significant ($p > 0.05$). Additionally, fish exposed to different concentrations of Cr, ACT and ACT + Cr significantly reduced the activity of CAT in the heart as compared to the control ($p < 0.05$). In contrast, exposure to varying concentrations of ACT + Cr induced an increase in CAT and SOD activities in renal tissues. In addition, increased SOD activity was observed in the liver of ***C. gariepinus*** treated with 200 mg/L ACT. However, the changes in SOD activity in the liver following exposure to other concentrations of ACT and Cr were not significant ($p > 0.05$). Similarly, exposure to 0.350 mg/L Cr caused elevated CAT activity in the liver.Furthermore, in most cases, treatment with different toxicant concentrations triggered a change in SOD activity in the heart.

Discussion

Water Quality

Water quality is determined by measuring DO and BOD levels in water (Ghosh *et al.,* 2018; Okezie *et al.,* 2020). The variations in pH and DO levels were not statistically significant ($p > 0.05$) among groups of dosed water with ACT, Cr, ACT+Cr, and control. According to NESREA (2011), Owalude *et al*. (2020) and Azeez*et al*. (2022b), the DO and pH values obtained for each group were within acceptable limits for aquatic life (mostly fish). Similar to how residual water dosed with Cr and ACT+Cr very slightly changed BOD levels ($p > 0.05$); residual water dosed with ACT significantly changed BOD levels. The high BOD level found in the water after an ACT dose suggests pollution. This might be attributed to the presence of ACT as an organic pollutant. It stimulates the growth of microbes that break down perceived pollutants. As BOD increased, DO decreased, damaging fish's health, growth, and performance. This would impact negatively on their survival rates (Prambudy*et al.,* 2019; Tabrez *et al.,* 2021). In addition, residual water dosed with ACT and ACT+Cr saw a substantial reduction in COD levels ($p < 0.05$). Contamination of water with ACT and ACT+Cr caused a high COD level, which could suggest elevated concentrations of oxidisable materials. This might result in an imbalance leading to an anaerobic condition, which often harms aquatic fauna (Prambudy*et al.,* 2019). Additionally, Cr-dosed water had maximum EC and TDS levels. While ACT and ACT+Cr did not significantly ($p > 0.05$) impact EC levels, Cr significantly ($p < 0.05$) enhanced EC levels. Additionally, TDS levels in dosed residual water increased significantly when Cr and ACT+Cr were present compared to the control. This is consistent with Rusydi's (2018) and Okezie *et al*. (2020) findings,which found that conductivity and total dissolved solids exhibit similar patterns. Consequently, Cr contamination leads to elevated levels of inorganic compounds, albeit as dissolved ions, contributing to the electrical conductivity of dosed water (Sirapan, 2019). As a whole, this study demonstrated that the physicochemical parameters of dosed water were within permissible limits. However, it is important to be cautious when exposed to water contaminated by Cr, ACT, and their combinations since increased concentrations of these contaminants may adversely affect aquatic and human health.

Concentration and Bioaccumulation of Cr and Acetaminophen in Tissues of *C. gariepinus*

The residual concentration of toxicants in dosed water and various organs was concentration-dependent. The residual Cr toxicity increased as the concentration of Cr pollution in the water increased compared to the control ($p < 0.05$). It was also observed that the bioaccumulation of toxicants in the kidney, liver, heart, and gills is associated with the concentration of toxicants. Cr bioaccumulation was highest in ***C. gariepinus*** exposed to the highest individual concentration and in combination in each organ. Also, the alterations in Cr concentration found in the water and ***C.***

*gariepinus*organs may have been the result of transformational processes (dissociation, redox reaction) as reported by Abdel-Khalek *et al*. (2020) and Azeez*et al*. (2022b). In addition, the physical and chemical properties of water and seasonal changes might contribute to the intensification of chromium in water and in various types of fish tissues (Fawad*et al.*, 2017). The concentrations of residual toxicants were highest in the liver and gills, followed by the kidney and heart, with the heart having the lowest concentration. This implies that heavy metals exhibit varying toxicities in tissues. The findings of Sonia and Ali (2017) agree with this present study that Cr has higher bioaccumulation in the liver and gills.

Furthermore, ACT bioaccumulation within organs was concentration-dependent. Notably, the kidneys and gills were recorded to contain higher levels of ACT. The noticeable concentration of ACT observed in the kidneys and gills of *C. gariepinus* could indicate that these organs suffered the most severe ACT toxicity. Azeez *et al*. (2022a) suggested that a combination of high metabolic activity in these tissues, which allows them to accumulate more toxicants and have a prolonged exposure period, might be the reason for the significantly elevated ACT levels in these tissues. Fish tissues exposed to 200 mg/L ACT and 200 mg/L ACT + 0.350 mg/L Cr had the highest levels of acetaminophen. However, the liver and heart had very low ACT levels. The pattern of acetaminophen concentration in tissue could suggest the kidney has the greatest toxicant accumulation, followed by gills and liver while the heart has the least. The severity of toxicity of ACT encountered by the gills and kidneys of ***C.*** *gariepinus* recorded in this study has been corroborated by the study by Perussolo *et al*. (2019). It was reported that ACT caused several effects in the gills and posterior kidneys of fish exposed to different ACT concentrations. In addition, the higher concentrations of ACT assisted by Cr in the kidney and heart could indicate greater combined toxicity. According to Li *et al*. (2019) discovered that Cu and Cr in cellular fractions displayed a synergistic accumulation pattern under combined metal stress treatment. This synergistic effect of Cr is consistent with their findings. The liver's rate of detoxification and clearance may be responsible for the amount of accumulation detected, as suggested by Tichoet *al*.(2019); Naguib *et al*.(2020) and Azeez *et al*. (2022b).

Oxidative stress biomarkers and enzyme profile of Cr and ACT-induced toxicity in *C. gariepinus*

Antioxidants protect various tissues of living organisms against damage resulting from toxicant exposure. It was discovered that contamination with different doses of Cr, ACT, and ACT + Cr mostly increased GSH concentrations in the kidneys. In contrast, treatment with 150 mg/L ACT and 0.280 mg/L Cr decreased GSH levels compared to control. This finding is consistent with the report of Folarin *et al*. (2018), who reported that ACT induced biological effects on various fish species. Fish exposed to different dosages of Cr, ACT, and ACT + Cr also displayed an increase in GST activity. Importantly, fish exposed to 0.280 mg/L Cr showed a marked increase in GST activity compared to the control. The increased activity of GST could indicate the activation of metabolic adaptation against toxicant-induced oxidative degradation as reported by Mehwish and Khalid (2017), Khan *et al*. (2018) and Azeez *et al*. (2022b). In fish exposed to various doses of Cr, ACT, and ACT + Cr, the levels of GST and GSH in the liver, heart, and gills were not significantly impacted. Possibly, other defensive systems are working together to provide effective protection against superoxide radicals and cellular damage caused by toxicants. CAT and SOD are major antioxidant enzymes evaluated to estimate toxicity (Aremu *et al.*, 2022; Azeez *et al.*, 2022b). After exposure to various dosages of Cr, ACT, and ACT + Cr, SOD and CAT activities were markedly decreased in the kidneys and gills, with a substantial reduction only found there. Notably, SOD activity increases with elevated ACT and Cr concentrations. A decrease in SOD activity is an indication of oxidative stress. Importantly, if the superoxide anions produced by these toxicants exceed SOD's capability, these anions can inactivate the enzyme. This might result in an increase in oxidative stress that leads to organ damage. This observation was corroborated by Kaur & Jindal (2017), and Mehwish and Khalid (2017). They reported an increase in oxidative stress caused damage to fish organs exposed to sub-lethal bisphenol-A (BPA) concentrations. It implies that excessive ROS generation has exceeded the antioxidant defence system's capacity (Abdel-

Khalek *et al*., 2020). In the liver of fish given various doses of Cr, ACT, and ACT + Cr, there is a marked reduction in CAT activity. It is possible that the reduction could be explained by an influx of superoxide radicals, which inhibit the enzymes (Kaur & Jindal, 2017). SOD activity was reduced by 200 mg/L ACT, 0.280 mg/L Cr, 200 mg/L ACT + 0.350 mg/L Cr, and 100 mg/L ACT + 0.210 mg/L but not significantly. Furthermore, fish subjected to varying concentrations of Cr, ACT, and ACT + Cr significantly lowered CAT activity in the heart. Shen*et al*. (2019) and Azeez *et al*. (2022b) have also reported significant inhibition of CAT and SOD activities in adult *Daphnia magma* when exposed to toxicants. While SOD activity increased in the liver of fish treated with 200 mg/L ACT, administration of various doses of ACT + Cr raised CAT and SOD activity in renal tissues. Notably, other concentrations and toxins had no discernible impact on liver SOD activity. Similar to this, liver CAT activity was elevated by 0.350 mg/L Cr exposure. Additionally, treatment with different toxicant concentrations led to a change in heart SOD activity.

Comparably, acute toxicity of toxicant and oxidative damage with respect to the SOD activity was mostly felt in the kidney and liver of ***C. gariepinus*** exposed to ACT and Cr, respectively, while both organs (kidney and liver) suffered the effect of co-exposure (synergistic effect) to ACT and Cr despite detoxification and metabolism capacity of these two organs. This effect on liver and kidney was supported by Mechado, (2018) who reported that the liver and kidney are mostly impacted during toxicity and could lead to the damage of both tissues.

Conclusion

This present study showed that exposure of *C. gariepinus* to acetaminophen, chromium and a combination of both could trigger several physiological and biochemical dysfunctions. Significant bioaccumulation and toxicity were differentially exhibited in tissues which have the potential to affect the functions of critical organs, especially the kidneys and gills of fish. Toxicity was observed with significant alterations in oxidative biomarkers, causing oxidative stress and cellular damage. Additionally, there was residual retention of acetaminophen and chromium in water bodies that could pose a threat to the survival of aquatic life. Ultimately, the excessive use of pharmaceuticals together with the unregulated disposal, have caused a rise in the concentration in water bodies and, thus, have emerged as a serious environmental threat. Hence, there is an urgent need to develop an effective control system.

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