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Effects of Erythrosine Red Exposure on Hepatic Antioxidant-Related Biomarkers and Hematological Responses in Juvenile *Clarias Gariepinus*

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ABSTRACT

Erythrosine red is a synthetic xanthene food colourant whose biological effects have attracted toxicological attention. This study evaluated the effects of erythrosine red on selected hepatic antioxidant-related biomarkers and hematological responses in juvenile *Clarias gariepinus* after 21 days of waterborne exposure. Fifty juvenile fish were allocated to five groups of ten fish: a negative control maintained in dechlorinated tap water, a positive control exposed to benzene at 0.05 mL/L, and three erythrosine groups exposed to 1.16, 1.74, or 2.32 mg/L. Fish were fasted overnight before sampling. Liver homogenates were analysed for superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA), while hematological parameters were assessed from EDTA-anticoagulated blood. Data were analysed by one-way analysis of variance followed by Dunnett's post-hoc test at $p < 0.05$. SOD activity was significantly lower at 1.74 and 2.32 mg/L, and CAT activity was significantly lower at 2.32 mg/L. GSH was not significantly altered, whereas MDA was significantly lower at 2.32 mg/L. At 1.74 mg/L, haemoglobin concentration decreased significantly. At 2.32 mg/L, RBC count, haemoglobin, packed cell volume, and mean corpuscular volume decreased, mean corpuscular haemoglobin increased, lymphocyte percentage increased, and heterophil percentage and platelet count decreased. The findings demonstrate concentration-specific alterations in selected hepatic antioxidant-related and hematological biomarkers, but do not establish generalized oxidative damage, direct reactive oxygen species generation, or a specific hematological disease.

Keywords: Erythrosine red, *Clarias gariepinus*, antioxidant biomarkers, liver homogenate, hematology, synthetic food colourant, aquatic toxicology

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1. INTRODUCTION

Food colour is an important sensory attribute that influences product recognition, consumer preference, and the perceived quality of foods. Natural pigments may be altered or lost during processing, storage, heat treatment, and exposure to light. Colour additives are therefore used in food and pharmaceutical manufacturing to restore colour lost during processing, improve product uniformity, and enhance visual appeal. Synthetic colourants are particularly attractive because they generally provide intense coloration, relative stability, ease of application, and comparatively low production costs. However, their widespread use has also increased scientific interest in their possible biological effects under different exposure conditions.

Erythrosine red, also known as erythrosine B, is a synthetic xanthene colourant used in selected food, pharmaceutical, and related applications. Experimental evidence indicates that erythrosine can alter biochemical and physiological processes, although the nature and magnitude of the response depend on the organism, exposure route, concentration, duration, tissue, and endpoint examined. In Wistar rats, Singh and Chadha (2025) reported erythrosine-associated alterations in antioxidant enzyme activities together with lipid-peroxidation-related, DNA-damage, and histopathological endpoints. Their study provides direct evidence that erythrosine can affect biological processes in a mammalian experimental model. Nevertheless, those findings cannot be transferred directly to fish because the organism, exposure route, tissue responses, and measured endpoints differ from those examined in aquatic studies.

Fish are widely used in aquatic toxicology because their physiological responses can provide information about the biological effects of waterborne chemical exposure. Biochemical biomarkers are particularly useful for detecting alterations that may occur before overt behavioural or structural effects become evident. Superoxide dismutase, catalase, reduced glutathione, and malondialdehyde are frequently assessed together because they represent different components of antioxidant defence and redox-related processes. SOD and CAT are enzymatic components of antioxidant defence, GSH is an important non-enzymatic cellular antioxidant, and MDA is commonly measured as a product associated with lipid oxidation. Changes in these biomarkers must, however, be interpreted collectively because an alteration in one parameter does not independently demonstrate generalized oxidative damage.

Evidence from synthetic food-colourant and chemical-exposure studies supports the relevance of examining these endpoints in fish. Wu et al. (2021) reported that tartrazine exposure in crucian carp (*Carassius auratus*) altered antioxidant-related responses, including reductions in antioxidant enzyme activities and an increase in MDA. In *Clarias gariepinus*, Hamed et al. (2024) reported alterations in antioxidant-defence and related biomarkers following pyrogallol exposure. Together, these studies demonstrate that antioxidant-related biomarkers in fish can respond to synthetic colourants and other chemical stressors. However, tartrazine, pyrogallol, and erythrosine are chemically distinct substances, and their effects should not be assumed to arise through an identical biological mechanism.

Hematological assessment provides an additional approach for evaluating physiological responses in exposed fish. Red blood cell count, haemoglobin concentration, packed cell volume, erythrocyte indices, total and differential white blood cell counts, and platelet-related measurements may change in response to chemical exposure, handling conditions, nutritional status, water quality, or other environmental factors. These parameters can therefore complement biochemical measurements when evaluating the effects of a contaminant.

Nevertheless, hematological findings require careful interpretation. Routine blood measurements can identify alterations in circulating-cell parameters, but they do not independently establish the mechanism responsible for those changes. Witeska et al. (2023) emphasized that hematological and hematopoietic analyses are valuable tools in fish toxicology while also noting that their interpretation may be influenced by species, age, environmental conditions, sampling procedures, and analytical methods. Consequently, routine hematological findings should not, without additional evidence, be interpreted as proof of bone-marrow injury, immune failure, leukemia, or a defined blood disease.

The African catfish, *C. gariepinus*, is an economically important freshwater species that is widely cultured and frequently used in toxicological investigations. Its relative hardiness, accessibility, and measurable biochemical and hematological responses make it suitable for controlled exposure studies. Although evidence from other synthetic colourants indicates that biochemical and hematological parameters can respond to dye exposure, information on erythrosine-associated hepatic antioxidant-related and hematological responses in juvenile *C. gariepinus* remains limited.

Comparisons across the available literature also demonstrate the importance of chemical and biological context. Singh and Chadha (2025) provide direct erythrosine evidence, but their study involved mammalian oral exposure. Wu et al. (2021) provide direct fish and food-colourant evidence, but the tested colourant was tartrazine rather than erythrosine. Hamed et al. (2024) provide same-species evidence of chemical-responsive antioxidant biomarkers, but the test substance was pyrogallol. Witeska et al. (2023) provide a broader framework for interpreting fish hematology but do not examine erythrosine specifically. These studies collectively support the biological plausibility of measuring antioxidant-related and hematological responses while also showing that the results of one chemical or experimental model cannot be transferred uncritically to another.

Accordingly, the present study investigated the effects of erythrosine red exposure on selected hepatic antioxidant-related biomarkers and hematological parameters in juvenile *C. gariepinus* following a 21-day exposure period. The biochemical endpoints evaluated were SOD, CAT, GSH, and MDA in liver homogenate, while the hematological assessment included erythrocyte indices, total and differential leukocyte-related parameters, and platelet counts. The study was designed to determine whether erythrosine exposure produced measurable concentration-specific alterations in these physiological indicators under the experimental conditions.

2. MATERIALS AND METHODS

2.1. Experimental Fish and Acclimatization

Fifty juvenile *Clarias gariepinus*, with a mean body weight of 27.23 ± 2.68 g and a mean total length of 14.03 ± 1.10 cm, were obtained from a commercial fish farm in Obinze, Imo State, Nigeria. The fish were acclimatized for two weeks in white plastic aquaria at the Department of Fisheries and Aquaculture Technology, Federal University of Technology Owerri.

During acclimatization, the recorded laboratory conditions were approximately 25.5 °C, pH 7.2, and a 12 h light:12 h dark photoperiod. Fish were fed commercial feed pellets.

2.2. Ethical Approval

The experimental procedures were conducted in accordance with the Guidelines for Use of Fishes in Research (Jenkins et al., 2014), and ethical approval was obtained from the Animal Care and Use in Research Ethics Committee of the Federal University of Technology Owerri.

2.3. Test Chemical and Experimental Design

Erythrosine red powder of 99% purity was obtained from Sigma-Aldrich. The dye was dissolved in water to prepare the nominal exposure concentrations. Fifty fish were allocated to five experimental groups containing ten fish each: a negative control, a benzene positive control, and three erythrosine exposure groups.

Table 1. Experimental Design for the 21-Day Exposure of Juvenile *Clarias Gariepinus*.

Group	Treatment	Exposure concentration
I	Negative control	Dechlorinated tap water
II	Positive control	Benzene, 0.05 mL/L
III	Erythrosine red	1.16 mg/L
IV	Erythrosine red	1.74 mg/L
V	Erythrosine red	2.32 mg/L

Each group contained ten fish. The erythrosine concentrations were nominal.

2.4. Exposure Protocol

The exposure period lasted 21 days and used a semi-static renewal system. Exposure media were renewed every 48 h to reduce the accumulation of metabolic waste and residual feed and to maintain the nominal treatment conditions. Dechlorinated tap water served as the negative control, and benzene at 0.05 mL/L served as the positive control.

2.5. Sample Collection

At the end of the exposure period, fish were fasted overnight before sampling. Sampling was conducted at approximately the same time of day to reduce variation associated with feeding status and diurnal timing.

Blood was collected from fish in the control and exposed groups and transferred into ethylenediaminetetraacetic acid (EDTA) tubes for hematological analysis.

Liver tissues were collected for biochemical analysis. SOD, CAT, GSH, and MDA were measured in liver homogenates.

2.6. Preparation of Liver Homogenate and Total Protein Determination

Liver tissues were homogenized in ice-cold phosphate buffer at pH 7.4. The homogenates were centrifuged at 1,000 rpm for 15 min at 4 °C, and the resulting supernatants were stored at -20 °C until analysis.

Total protein content was determined by the Biuret reaction described by Gornall et al. (1949). Biochemical results were normalized to protein content. SOD, GSH, and MDA were expressed as unit/mg protein, whereas CAT activity was expressed as µmol H_2O_2 consumed/min/mg protein.

2.7. Determination of Reduced Glutathione

Reduced glutathione was determined using a modified DTNB sulfhydryl assay based on Ellman (1959).

A 100 µL aliquot of liver homogenate was mixed with 500 µL of 10% trichloroacetic acid and centrifuged at 4,000 rpm for 5 min. A 300 µL aliquot of the supernatant was mixed with 600 µL of 1 M Tris-HCl buffer at pH 8.9 and 40 µL of 0.1 M 5,5′-dithiobis(2-nitrobenzoic acid) prepared in ethanol. After 20 min, absorbance was measured at 412 nm against a reagent blank. A glutathione calibration curve was used, and values were normalized to protein content and expressed as unit/mg protein.

2.8. Determination of Malondialdehyde

Malondialdehyde was measured using the thiobarbituric acid-reactive procedure attributed to Gutteridge and Wilkins (1982). One millilitre of glacial acetic acid and 1 mL of 1% thiobarbituric acid prepared in 20% sodium hydroxide were added to 0.1 mL of liver homogenate. The mixture was heated in a boiling-water bath for 15 min, cooled, and centrifuged. Absorbance of the supernatant was measured at 532 nm.

MDA concentration was calculated using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}, normalized to protein content, and expressed as unit/mg protein.

2.9. Determination of Superoxide Dismutase Activity

Superoxide dismutase activity was determined by inhibition of epinephrine auto-oxidation, with modification of the method of Misra and Fridovich (1972). A 0.1 mL aliquot of liver homogenate was placed in a cuvette, followed by 1 mL of bicarbonate buffer at pH 10.2 and 120 µL of epinephrine. Absorbance was measured at 480 nm after 30 s and 3 min. A blank containing no sample was analysed under the same conditions.

The recorded rate was calculated as R = (absorbance at 3 min - absorbance at 30 s)/2. Percentage inhibition was calculated as [(Rblank - Rtest)/Rblank] × 100, and enzyme activity was calculated from the percentage inhibition and dilution factor. Values were normalized to protein and reported as unit/mg protein.

2.10. Determination of Catalase Activity

Catalase activity was determined using the residual hydrogen peroxide-permanganate procedure described by Cohen et al. (1970).

A 0.1 mL aliquot of liver homogenate was mixed with 1 mL of 30 mM H_2O_2 prepared in phosphate buffer at pH 7.4. After 3 min, the reaction was stopped with 0.2 mL of 6 M H_2SO_4. Thereafter, 1.4 mL of 0.01 M $KMnO_4$ was added, and absorbance was measured at 480 nm against distilled water. Activity was expressed as µmol H_2O_2 consumed/min/mg protein.

2.11. Hematological Analysis

EDTA-anticoagulated blood was analysed using a Mindray BC-2800 automated hematology analyser. The recorded parameters were packed cell volume, haemoglobin concentration, red blood cell count, total white blood cell count, leukocyte differential percentages, and platelet count. Mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration were also reported. Fish hematological terminology and index interpretation followed the routine framework described by Blaxhall and Daisley (1973).

2.12. Statistical Analysis

Data were analysed using GraphPad Prism version 5.0 and are presented as mean ± standard deviation. Statistical comparisons were performed using one-way analysis of variance followed by Dunnett's post-hoc multiple-comparison test. Statistical significance was accepted at $p < 0.05$.

3. RESULTS

3.1. Effects of Erythrosine Red on Hepatic Antioxidant-Related Biomarkers

The effects of erythrosine red exposure on hepatic CAT, GSH, MDA, and SOD are presented in Table 2. The response differed among biomarkers and exposure concentrations.

SOD activity was significantly lower in fish exposed to 1.74 and 2.32 mg/L erythrosine than in the negative-control group ($p < 0.05$). Mean SOD activity decreased from 0.1692 ± 0.04 unit/mg protein in the negative control to 0.0731 ± 0.01 and 0.0748 ± 0.02 unit/mg protein at 1.74 and 2.32 mg/L, respectively. SOD activity at 1.16 mg/L did not differ significantly from the negative control.

CAT activity was significantly lower at 2.32 mg/L than in the negative-control group ($p < 0.05$), with mean values of 93.13 ± 0.04 and 121.00 ± 23.62 µmol H_2O_2 consumed/min/mg protein, respectively. CAT activities at 1.16 and 1.74 mg/L were not significantly different from the negative-control value.

GSH was not significantly altered at any erythrosine concentration. Mean values were 775.20 ± 148.9 unit/mg protein in the negative control, 789.25 ± 101.3 unit/mg protein at 1.16 mg/L, 792.70 ± 192.1 unit/mg protein at 1.74 mg/L, and 803.41 ± 179.8 unit/mg protein at 2.32 mg/L.

MDA was significantly lower in fish exposed to 2.32 mg/L than in the negative-control group ($p < 0.05$). Mean MDA decreased from 16.37 ± 1.27 unit/mg protein in the negative control to 10.26 ± 1.18 unit/mg protein at 2.32 mg/L. The numerical reductions at 1.16 and 1.74 mg/L were not statistically significant.

Table 2. Effects of Erythrosine Red Exposure on Hepatic Antioxidant-Related Biomarkers In Jivenile *Clarias Gariepinus* After 21 Days.

Exposure group	CAT (µmol H_2O_2 consumed/min/mg protein)	GSH (unit/mg protein)	MDA (unit/mg protein)	SOD (unit/mg protein)
Negative control	121.00 ± 23.62	775.20 ± 148.9	16.37 ± 1.27	0.1692 ± 0.04
Erythrosine, 1.16 mg/L	108.10 ± 18.93	789.25 ± 101.3	14.72 ± 1.05	0.1652 ± 0.05
Erythrosine, 1.74 mg/L	110.42 ± 13.39	792.70 ± 192.1	13.83 ± 1.10	0.0731 ± 0.01*
Erythrosine, 2.32 mg/L	93.13 ± 0.04*	803.41 ± 179.8	10.26 ± 1.18*	0.0748 ± 0.02*

Values are mean ± standard deviation; experimental group size = 10 fish. *Significantly different from the negative control at $p < 0.05$ following one-way ANOVA and Dunnett's post-hoc test.

3.2. Effects of Erythrosine Red on Red Blood Cell Count and Erythrocyte Indices

The effects of erythrosine red exposure on MCV, RBC count, haemoglobin concentration, PCV, MCHC, and MCH are presented in Table 3.

Exposure to 1.16 mg/L erythrosine did not produce a statistically significant change in any measured erythrocyte-related parameter compared with the negative-control group.

At 1.74 mg/L, haemoglobin concentration was significantly lower than the negative-control value ($p < 0.05$), decreasing from 12.79 ± 0.41 to 10.57 ± 0.91 g/dL. MCV, RBC count, PCV, MCHC, and MCH were not significantly altered.

At 2.32 mg/L, MCV, RBC count, haemoglobin concentration, and PCV were significantly lower than their respective negative-control values ($p < 0.05$). MCV decreased from 93.90 ± 2.10 to 91.38 ± 1.65 fL, RBC count from 4.02 ± 0.05 to 3.00 ± 0.25 × 10^6/µL, haemoglobin from 12.79 ± 0.41 to 10.03 ± 0.50 g/dL, and PCV from 36.72 ± 1.65% to 31.62 ± 0.05%.

MCH was significantly higher at 2.32 mg/L, increasing from 33.02 ± 0.22 to 37.59 ± 0.91 pg ($p < 0.05$). MCHC decreased numerically from 35.05 ± 0.91% to 33.08 ± 0.22%, but the change was not marked as statistically significant.

The benzene positive-control group had significantly lower MCV, RBC count, haemoglobin concentration, PCV, MCHC, and MCH than the negative-control group ($p < 0.05$).

Table 3. Effects of Erythrosine Red Exposure on Red Blood Cell Count and Erythrocyte Indices in Juvenile *Clarias Gariepinus* After 21 Days.

Exposure group	MCV (fL)	RBC (×10⁶/µL)	Hb (g/dL)	PCV (%)	MCHC (%)	MCH (pg)
Negative control	93.90 ± 2.10	4.02 ± 0.05	12.79 ± 0.41	36.72 ± 1.65	35.05 ± 0.91	33.02 ± 0.22
Erythrosine, 1.16 mg/L	92.48 ± 1.37	4.04 ± 0.39	12.08 ± 0.53	36.80 ± 0.39	35.10 ± 0.20	35.22 ± 0.61
Erythrosine, 1.74 mg/L	92.24 ± 0.71	3.86 ± 0.50	10.57 ± 0.91*	32.51 ± 0.28	34.72 ± 0.04	35.62 ± 0.06
Erythrosine, 2.32 mg/L	91.38 ± 1.65*	3.00 ± 0.25*	10.03 ± 0.50*	31.62 ± 0.05*	33.08 ± 0.22	37.59 ± 0.91*
Positive control	90.37 ± 1.22*	2.97 ± 0.10*	9.21 ± 0.30*	30.00 ± 1.02*	31.23 ± 0.18*	30.42 ± 0.10*

Values are mean ± standard deviation; experimental group size = 10 fish. Negative control: dechlorinated tap water. Positive control: benzene, 0.05 mL/L. *Significantly different from the negative control at $p < 0.05$.

3.3. Effects of erythrosine red on total white blood cell count, leukocyte differentials, and platelet count

The effects of erythrosine red exposure on monocytes, lymphocytes, total WBC count, heterophils, platelets, and eosinophils are presented in Table 4.

Exposure to 1.16 or 1.74 mg/L erythrosine did not produce a statistically significant change in any measured leukocyte-related parameter or platelet count compared with the negative-control group.

At 2.32 mg/L, lymphocyte percentage was significantly higher than in the negative-control group ($p < 0.05$), increasing from 45.28 ± 0.51% to 53.00 ± 0.01%. In contrast, heterophil percentage decreased significantly from 47.19 ± 0.27% to 43.73 ± 1.21%, and platelet count decreased significantly from 335,201 ± 22,121 to 295,800 ± 12,326.

Monocyte percentage, total WBC count, and eosinophil percentage were not significantly altered at 2.32 mg/L. The positive-control group showed a significantly higher lymphocyte percentage and significantly lower heterophil percentage and platelet count than the negative-control group. Monocyte percentage, total WBC count, and eosinophil percentage in the positive-control group were not marked as statistically significant.

Table 4. Effects of Erythrosine Red Exposure on Total White Blood Cell Count, Leukocyte Differentials, And Platelet Count In Juvenile *Clarias Gariepinus* After 21 Days.

Exposure group	Mono. (%)	Lymph. (%)	WBC (×10³/µL)	Hetero. (%)	Platelet count	Eosin. (%)
Negative control	4.01 ± 0.21	45.28 ± 0.51	24653 ± 1874	47.19 ± 0.27	335201 ± 22121	4.97 ± 0.01
Erythrosine, 1.16 mg/L	4.20 ± 0.03	44.53 ± 0.73	24365 ± 1021	47.20 ± 0.87	333800 ± 15732	4.60 ± 0.03
Erythrosine, 1.74 mg/L	3.97 ± 0.18	46.27 ± 0.34	23221 ± 1930	46.40 ± 0.78	313000 ± 11032	4.00 ± 0.37

Erythrosine, 2.32 mg/L	3.20 ± 0.66	53.00 ± 0.01*	23456 ± 1382	43.73 ± 1.21*	29580 0 ± 12326*	3.94 ± 0.04
Positive control	2.42 ± 0.06	54.01 ± 1.40*	24300 ± 1252	38.00 ± 1.20*	25768 0 ± 15058*	3.63 ± 0.35

Values are mean ± standard deviation; experimental group size = 10 fish. Negative control: dechlorinated tap water. Positive control: benzene, 0.05 mL/L. *Significantly different from the negative control at $p < 0.05$. Mono., monocytes; Lymph., lymphocytes; Hetero., heterophils; Eosin., eosinophils.

4. DISCUSSION

The present study demonstrated that erythrosine red exposure altered selected hepatic antioxidant-related and hematological biomarkers in juvenile *Clarias gariepinus* following 21 days of exposure. The biochemical response was not uniform across the measured endpoints. SOD activity decreased significantly at 1.74 and 2.32 mg/L, whereas CAT activity decreased significantly only at 2.32 mg/L. GSH was not significantly altered at any erythrosine concentration, while MDA decreased significantly at 2.32 mg/L. These findings indicate a biomarker-specific alteration in hepatic antioxidant regulation rather than unequivocal evidence of generalized oxidative damage.

SOD and CAT are functionally related components of enzymatic antioxidant defence. SOD participates in the conversion of superoxide radicals to hydrogen peroxide, while CAT contributes to hydrogen peroxide decomposition. Reduced activities of these enzymes may reflect altered antioxidant regulation, enzyme utilization, synthesis, turnover, or inhibition during chemical exposure. However, these possible explanations remain inferential because the present study did not directly measure superoxide, hydrogen peroxide, other reactive oxygen species, antioxidant-gene expression, or enzyme-protein abundance. The observed reductions in SOD and CAT should therefore not be presented as direct proof that erythrosine generated excess reactive oxygen species.

The reductions in SOD and CAT observed in the present study are broadly consistent with reports that synthetic colourants and other chemical stressors can alter antioxidant-related biomarkers across aquatic and mammalian models. Wu et al. (2021) reported reduced SOD, CAT, and glutathione peroxidase activities in tartrazine-exposed crucian carp, while Hamed et al. (2024) documented altered antioxidant-defence responses in pyrogallol-exposed *C. gariepinus*. Gao et al. (2011) also reported reductions in antioxidant-enzyme activities following tartrazine exposure in mice and rats. Together, these studies demonstrate that antioxidant enzymes may respond to synthetic-colourant or chemical exposure. Nevertheless, tartrazine, pyrogallol, and erythrosine are chemically distinct substances, and differences in species, exposure route, concentration, duration, tissue, and analytical endpoint prevent the findings from being interpreted as evidence of an identical mechanism.

Singh and Chadha (2025) provide more directly relevant chemical evidence because their investigation examined erythrosine rather than another colourant. In Wistar rats, erythrosine exposure reduced SOD and CAT activities at the highest tested dose and was accompanied by increased hydrogen peroxide, lipid-peroxidation-related endpoints, DNA damage, and liver and kidney histopathological changes. The reductions in SOD and CAT are broadly comparable with the enzymatic responses observed in the present study. However, the wider response patterns differed substantially. Hydrogen peroxide and DNA damage were not assessed in the present fish experiment, histopathology was not assessed in the present study, GSH was not significantly altered, and MDA decreased rather than increased.

The mammalian study therefore supports the conclusion that erythrosine can affect antioxidant-related processes, but it does not demonstrate that identical mechanisms or tissue effects occurred in juvenile *C. gariepinus*.

The MDA response is particularly important because it differs from the pattern reported in several comparative studies. Increased MDA or other lipid-peroxidation-related endpoints were reported following tartrazine exposure by Wu et al. (2021) and Gao et al. (2011) and following erythrosine exposure in rats by Singh and Chadha (2025). In the present study, however, MDA decreased numerically across the erythrosine-exposed groups and was significantly lower at 2.32 mg/L than in the negative control. This finding does not support a conclusion of increased lipid peroxidation under the present experimental conditions.

The decreased MDA concentration could potentially reflect reduced formation of thiobarbituric acid-reactive products, altered lipid metabolism, adaptive regulation, changes in the removal or turnover of oxidation products, or biological and analytical variability. These possibilities remain hypotheses because no additional lipid-oxidation marker, lipid-composition analysis, antioxidant-gene expression measurement, or pathway-specific assessment was performed. The most defensible interpretation is therefore that MDA decreased significantly at the highest erythrosine concentration, while the biological basis of this response remains unresolved.

The absence of a significant GSH response further distinguishes the present findings from a uniform antioxidant-depletion pattern. Mean GSH values increased slightly across the erythrosine groups, but none of the differences was statistically significant. The present results therefore provide no evidence of GSH depletion. When the unchanged GSH and decreased MDA findings are considered together with the reductions in SOD and CAT, the biochemical response appears to have involved selected enzymatic antioxidant components rather than simultaneous depletion of enzymatic and non-enzymatic defences accompanied by increased lipid oxidation.

The available literature collectively indicates that antioxidant-related responses to synthetic colourants are context-dependent. Reductions in antioxidant-enzyme activities have been observed across erythrosine, tartrazine, and other chemical-exposure models, but the accompanying GSH, MDA, molecular, and tissue responses have varied (Gao et al., 2011; Hamed et al., 2024; Singh and Chadha, 2025; Wu et al., 2021). These differences support the biological responsiveness of antioxidant biomarkers while cautioning against treating all synthetic colourants as though they produce a common toxicodynamic pattern. Accordingly, the present findings are most appropriately described as alterations in selected hepatic antioxidant-related biomarkers.

The concentration pattern must also be interpreted cautiously. At 1.16 mg/L, none of the measured biochemical parameters differed significantly from the negative control. At 1.74 mg/L, SOD activity was significantly reduced, while CAT, GSH, and MDA were not significantly altered. At 2.32 mg/L, SOD, CAT, and MDA were significantly reduced, whereas GSH remained unchanged. Although a larger number of biochemical endpoints was altered at the highest concentration, a formal concentration-response trend analysis was not conducted. The response should therefore be described as concentration-specific rather than uniformly dose-dependent.

Erythrosine exposure also altered selected erythrocyte-related parameters. No significant erythrocyte-related changes were observed at 1.16 mg/L. At 1.74 mg/L, haemoglobin concentration decreased significantly, while the other red-cell parameters were not significantly altered. At 2.32 mg/L, RBC count, haemoglobin concentration, PCV, and MCV decreased significantly. In contrast, MCH increased significantly, while MCHC was not significantly different from the negative-control value.

RBC count, haemoglobin concentration, and PCV provide related information about the circulating erythrocyte population and the oxygen-carrying characteristics of blood. Their concurrent reductions at 2.32 mg/L indicate that the highest erythrosine concentration affected erythrocyte-related physiology. The significant reduction in haemoglobin at 1.74 mg/L, in the absence of significant changes in most other erythrocyte indices, suggests that haemoglobin was among the more responsive blood parameters in the experiment. However, oxygen transport, oxygen saturation, erythrocyte production, erythrocyte lifespan, haemolysis, and erythropoietic activity were not directly assessed. The results therefore identify altered hematological measurements but do not establish the biological process responsible for them.

Comparative evidence indicates that hematological parameters may respond to synthetic-colourant exposure, although the observed patterns vary among colourants and experimental models. Khan et al. (2019) reported alterations in RBC count, haemoglobin concentration, haematocrit, MCH, and selected biochemical parameters in male Wistar rats exposed to Orange Red. Hayat et al. (2026) reported hematological alterations following short-term exposure of rats to tartrazine and Sunset Yellow, while Mehedi et al. (2013) documented hematological and biochemical changes in tartrazine-exposed Swiss mice. These studies collectively support the general sensitivity of blood-related endpoints to some synthetic colourants. However, Orange Red, tartrazine, and Sunset Yellow are not erythrosine, and mammalian oral-exposure experiments are not directly equivalent to waterborne exposure in fish. These sources therefore provide comparative toxicological context rather than direct evidence of the mechanism responsible for the present erythrosine-associated findings.

The erythrocyte-index response was mixed rather than uniformly reduced. MCV decreased significantly at 2.32 mg/L, but MCH increased significantly and MCHC was not significantly altered. The increase in MCH indicates that the calculated mean quantity of haemoglobin per erythrocyte was higher at 2.32 mg/L despite the reductions in total RBC count and haemoglobin concentration. Because MCH is calculated from the relationship between haemoglobin concentration and RBC count, its increase may partly reflect the relative changes in those two parameters. It should not be interpreted independently as proof of increased haemoglobin synthesis or a specific erythrocyte disorder.

The significant reduction in MCV indicates a lower calculated mean erythrocyte volume at 2.32 mg/L, but the combined hematological pattern is insufficient to classify a specific form of anaemia. Such classification would require validated species- and life-stage-specific reference intervals, repeated blood measurements, erythrocyte morphology, immature erythrocyte assessment, iron-related indices, and information on hematopoietic tissues.

Witeska et al. (2023) emphasized that hematological and hematopoietic measurements are valuable in fish toxicology but are influenced by species, age, environmental conditions, nutrition, handling, sampling procedures, and analytical methods. Routine hematological findings can reveal altered physiological status, but they cannot independently identify a specific hematopoietic mechanism or disease process. Accordingly, the reductions in RBC count, haemoglobin concentration, PCV, and MCV observed in the present study indicate erythrocyte-related alterations but do not demonstrate bone-marrow suppression, destruction of hematopoietic tissues, haemolysis, or a defined clinical blood disorder.

The benzene positive-control group showed significant reductions in MCV, RBC count, haemoglobin concentration, PCV, MCHC, and MCH compared with the negative control. This response was broader than that observed in the erythrosine groups, particularly because MCH decreased in the benzene group but increased at 2.32 mg/L erythrosine, while MCHC was not significantly altered by erythrosine.

The positive-control findings provide a reference condition showing that substantial hematological changes were detectable within the experimental system. Nevertheless, similarities or differences between benzene and erythrosine cannot establish whether they acted through the same or different mechanisms because mechanistic endpoints were not measured.

The leukocyte-related response to erythrosine was also selective. At 1.16 and 1.74 mg/L, none of the measured leukocyte-related parameters or the reported platelet count differed significantly from the negative control. At 2.32 mg/L, lymphocyte percentage increased significantly, whereas heterophil percentage and platelet count decreased significantly. Total WBC count, monocyte percentage, and eosinophil percentage were not significantly altered.

The lymphocyte and heterophil findings represent changes in relative leukocyte percentages. Because total WBC count was not significantly altered and absolute lymphocyte and heterophil counts were not reported, the results do not establish an absolute increase in circulating lymphocyte number or an absolute reduction in circulating heterophil number. Changes in the percentage represented by one leukocyte category may also influence the relative percentage represented by another category.

Comparative synthetic-colourant studies demonstrate that leukocyte-related parameters may respond to dye exposure, but the direction and magnitude of the responses are not consistent across studies. Hayat et al. (2026) reported hematological alterations following tartrazine and Sunset Yellow exposure, while Mehedi et al. (2013) reported reduced WBC counts in tartrazine-exposed mice. In contrast, total WBC count was not significantly altered in the present erythrosine-exposed fish. Differences in the tested colourant, species, exposure route, duration, concentration, and hematological method may have contributed to these contrasting responses. The comparison reinforces the need to avoid transferring hematological findings directly from one colourant or organism to another.

Routine leukocyte measurements can indicate alterations in circulating-cell distribution but do not independently establish immune activation, immune suppression, inflammatory disease, infection, or failure of hematopoietic tissues. The present study did not assess cytokines, immunoglobulins, phagocytic activity, lymphoid-tissue structure, pathogen resistance, or absolute differential leukocyte counts. In accordance with the interpretative framework described by Witeska et al. (2023), the increased lymphocyte percentage and decreased heterophil percentage should therefore be described as alterations in relative leukocyte distribution rather than evidence of a specific immune mechanism.

The absence of a significant total WBC response is also important. It indicates that erythrosine exposure did not produce a statistically detectable overall increase or reduction in the reported circulating WBC count. The findings consequently do not support claims of leukopenia, generalized leukocyte depletion, or leukemia. No leukemia testing was performed, and routine total and differential WBC measurements cannot establish such a diagnosis.

The platelet count decreased significantly at 2.32 mg/L, indicating that this blood-cell parameter was responsive at the highest erythrosine concentration. However, coagulation time, bleeding time, thrombocyte morphology, aggregation, and coagulation-factor activity were not assessed. The result should therefore be interpreted as a reduction in platelet count rather than proof of impaired coagulation, bleeding tendency, or suppression of platelet production.

The benzene positive-control group showed increased lymphocyte percentage and reduced heterophil percentage and platelet count, which were directionally similar to the significant changes observed at 2.32 mg/L erythrosine. This similarity is descriptive only. It does not demonstrate that benzene and erythrosine altered leukocyte distribution or platelet-related measurements through the same biological pathway.

When the biochemical and hematological findings are considered together, erythrosine exposure produced a concentration-specific pattern of physiological biomarker alteration in juvenile *C. gariepinus*. At 1.16 mg/L, none of the reported biochemical or hematological parameters differed significantly from the negative control. At 1.74 mg/L, the significant responses were limited principally to reduced SOD activity and haemoglobin concentration. At 2.32 mg/L, a wider set of endpoints was altered, including SOD, CAT, MDA, RBC count, haemoglobin concentration, PCV, MCV, MCH, lymphocyte percentage, heterophil percentage, and platelet count.

Despite the larger number of significant findings at 2.32 mg/L, the direction of response was not uniform. The biochemical pattern included reduced SOD and CAT, unchanged GSH, and reduced MDA. The hematological pattern included reductions in several erythrocyte-related measurements, increased MCH, increased lymphocyte percentage, and reduced heterophil percentage and platelet count. These mixed findings do not support a single generalized description such as uniform oxidative damage, antioxidant depletion, immune suppression, or a defined hematological disease.

The literature synthesis indicates that synthetic colourants may affect antioxidant and hematological biomarkers across aquatic and mammalian models, but the specific response patterns vary substantially (Gao et al., 2011; Hayat et al., 2026; Khan et al., 2019; Mehedi et al., 2013; Singh and Chadha, 2025; Wu et al., 2021). Hamed et al. (2024) further demonstrate that antioxidant biomarkers in *C. gariepinus* are responsive to chemical exposure, while Witeska et al. (2023) provide the interpretative framework required for cautious evaluation of fish hematology. Collectively, these sources support the biological plausibility of the present biomarker changes but do not justify assuming that erythrosine, other synthetic dyes, and unrelated chemical stressors share an identical mechanism.

The present study therefore provides evidence that waterborne erythrosine exposure can alter selected hepatic antioxidant-related and hematological parameters in juvenile *C. gariepinus*. However, it does not identify the molecular or cellular pathways responsible for the observed changes. Mechanistic interpretation would require direct measurement of reactive oxygen species, antioxidant-gene and protein expression, additional lipid-oxidation endpoints, erythrocyte production and turnover, immune function, coagulation, and tissue structure.

Finally, the findings should not be extrapolated directly to humans or children. The experiment involved juvenile fish exposed through water under controlled laboratory conditions. Differences in species biology, exposure route, metabolism, concentration, and exposure duration prevent direct translation to human dietary exposure. The results are most appropriately interpreted within aquatic toxicology as evidence of concentration-specific hepatic antioxidant-related and hematological biomarker responsiveness in juvenile *C. gariepinus*.

5. CONCLUSIONS

Exposure of juvenile *Clarias gariepinus* to erythrosine red for 21 days produced concentration-specific alterations in selected hepatic antioxidant-related and hematological parameters. No statistically significant biochemical or hematological changes were detected at 1.16 mg/L. At 1.74 mg/L, SOD activity and haemoglobin concentration were significantly reduced.

At 2.32 mg/L, hepatic SOD and CAT activities were significantly reduced, GSH remained unchanged, and MDA was significantly lower than the negative-control value. The reduction in MDA does not support a conclusion of increased lipid peroxidation. The biochemical findings therefore indicate alterations in selected antioxidant-related biomarkers rather than uniform antioxidant depletion or generalized oxidative damage.

The highest erythrosine concentration also reduced RBC count, haemoglobin concentration, PCV, and MCV, while MCH increased and MCHC was not significantly altered. Lymphocyte percentage increased, whereas heterophil percentage and platelet count decreased. Total WBC count, monocyte percentage, and eosinophil percentage were not significantly altered.

Taken together, the findings demonstrate that waterborne erythrosine exposure can modify selected biochemical and hematological biomarkers in juvenile *C. gariepinus* under the experimental conditions. However, the responses do not establish direct reactive oxygen species generation, increased lipid peroxidation, immune suppression, bone-marrow toxicity, leukemia, histopathological injury, or a specific hematological disease.

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