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Research Article

Haematological and Parasitological Biomarkers of Sub-Lethal Manganese Stress in African Catfish *Clarias gariepinus*: Implications for Fish Health and Freshwater Monitoring

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ABSTRACT
The African catfish, *Clarias gariepinus*, is a major freshwater aquaculture species in Africa due to its fast growth, environmental tolerance, and high market value. Manganese (Mn) enters aquatic systems through agricultural runoff, industrial discharge, and geogenic sources and, at elevated levels, can induce oxidative stress, tissue damage, and immunosuppression in fish. This study assessed haematological and parasitological responses of *C. gariepinus* to sub-lethal Mn exposure. Forty-eight sub-adult *C. gariepinus* (mean length 200 ± 0.9 mm; weight 500 ± 1.5 g) were obtained from a fish farm in Ondo State, Nigeria. Following 14 days of acclimation in dechlorinated water (pH 7.7 ± 0.2; 27 ± 1°C), fish were exposed for 144 h to Mn concentrations of 6.48 mg/L (T1) and 8.64 mg/L (T2) in 60-L glass aquaria, with a Mn-free control. Blood was collected at 24-h intervals for haematological analysis, while gills and skin were examined for parasites. Mn exposure significantly increased packed cell volume, haemoglobin, RBC and WBC counts, indicating compensatory erythropoiesis and immune activation, but significantly decreased erythrocyte sedimentation rate and lymphocyte count, suggesting immunosuppression. MCV, MCH, MCHC and neutrophils were unaffected, and no significant differences occurred between T1 and T2. *Trichodina* sp. prevalence and mean intensity increased with Mn concentration, from 0% in the control to 70% at the highest dose. Short-term Mn exposure alters haematological parameters and increases susceptibility to parasitic infection in *C. gariepinus*. These biomarkers may facilitate early detection of Mn contamination and fish health decline in tropical aquaculture systems.

Keywords: Biomarker; *Clarias gariepinus*; Haematology; Manganese; Parasitology; Sub-lethal toxicity

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INTRODUCTION

Clarias gariepinus, universally refers to as an African catfish, is a commercially important freshwater species extensively cultured across Africa. Its importance lies in its fast growth, ability to endure poor water quality, resistance to diseases, and high nutritional and economic value. It contributes considerably to food security and livelihoods for fish farmers.

Manganese (Mn) is an essential trace element required for enzymatic and metabolic functions, but becomes neurotoxic and haematotoxic at elevated concentrations (Li *et al*., 2022). Anthropogenic inputs from steel manufacturing, alloy production, mining, and fossil fuel combustion have increased Mn loading in surface waters, groundwater, and sediments (Zhang and Wang, 2021; Faraday *et al*., 2026). In humans, chronic Mn exposure is linked to neurological disorders including Parkinsonism, as well as respiratory and hepatic dysfunction (Chen *et al*., 2023).

In aquatic systems, fish are useful sentinels because they bioaccumulate metals and exhibit measurable physiological responses (Abdel-Tawwab and Wafeek, 2020). Haematological indices are widely used as early biomarkers of stress because blood integrates responses across organ systems and reflects exposure to pollutants before gross pathology appears (Velisek *et al*., 2021). Immunosuppression caused by metals can also increase fish vulnerability to parasitic infections, which are a major constraint in aquaculture (Adeyemi *et al*., 2022).

Previous studies report that metals such as Cu, Cd, Zn and Cr alter RBC, WBC and immune cell profiles in teleosts, though responses are species- and dose-dependent (Saha and Kaviraj, 2020; Kumar *et al*., 2024).

Data on Mn effects in tropical African freshwater species remain limited. *Clarias gariepinus* is an economically important and hardy catfish widely used in aquaculture and ecotoxicology in West Africa. This study therefore aimed to evaluate temporal changes in haematological parameters of *C. gariepinus* exposed to sub-lethal Mn for 144 hours, and to assess the potential link to fish health and parasitological risk.

MATERIALS AND METHODS

Experimental Site and Aquarium Size

The experiment was conducted at the Haematology and Parasitology Laboratory, Department of Biological Sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria. One hundred and twenty juvenile *Clarias gariepinus* were stocked in eight 60-L glass aquaria measuring 60 cm x 30 cm x 35 cm. Each aquarium contained 40 L of dechlorinated tap water and was equipped with continuous aeration.

Experimental fish and acclimation

Forty-eight sub-adult *C. gariepinus* (mean length 200 ± 0.9 mm; mean weight 500 ± 1.5 g) were obtained from a local fish farm in Ondo State, Nigeria. Fish were transported in oxygenated bags and distributed into eight glass aquaria containing dechlorinated freshwater (pH 7.7 ± 0.2; temperature 27 ± 1°C). They were acclimated for 14 days and fed commercial floating pellets daily. Water was partially renewed every 48 hours. All procedures followed institutional animal care guidelines.

Experimental design and Mn exposure

Manganese was administered as manganese sulphate ($MnSO_4 \cdot H_2O$). Based on USEPA (2021) guidelines for sub-lethal testing, two treatments were used: T1 = 6.76 mg/L and T2 = 8.45 mg/L, each with three replicates. Two aquaria served as controls. Exposure lasted 144 hours under static-renewal conditions.

Water Quality Monitoring

Water quality parameters including temperature (°C), pH, dissolved oxygen (DO) (mg/L), and manganese concentration (mg/L) were measured weekly. Temperature and pH were measured using a digital pH meter. Dissolved oxygen (DO) was determined using a dissolved oxygen meter. Manganese concentration was analyzed using Atomic Absorption Spectrophotometry (AAS).

Blood collection and haematological analysis

At 24-hour intervals, two fish per tank were randomly sampled. Blood (2 mL) was drawn from the caudal/ventral aorta using a 5 mL syringe with a 21G needle and transferred into EDTA tubes (Blaxhall and Daisley, 1973). Parameters assessed: Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC), White Blood Cell (WBC), Erythrocyte Sedimentation Rate (ESR), differential WBC, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) following standard fish haematology protocols (Adeyemi *et al*., 2022).

Parasitological Examination

Gill arches, skin mucus, and fins were examined for ectoparasites under a light microscope. Parasites were identified to genus level following Kabata (1985). Prevalence and mean intensity were calculated according to Bush *et al*. (1997).

Statistical analysis

Data were expressed as mean ± SE. One-way ANOVA followed by Tukey's test was used to compare treatments and control at $p < 0.05$ using SPSS v26.

RESULTS

Temperature, pH, and dissolved oxygen (DO) were within the acceptable range for *Clarias gariepinus* across all treatments. Manganese concentration increased significantly ($p<0.05$) with increasing treatment levels.

Mn exposure induced time-dependent changes in haematological parameters (Fig. 1).

From 24 h to 144 h, PCV, Hb, RBC, WBC and Neutrophil counts increased significantly relative to control. Peak WBC occurred at 72 h, suggesting acute immune activation. Conversely, ESR and lymphocyte counts decreased consistently throughout exposure (Fig. 1a-f).

MCV and MCH showed a transient increase at 24 h, declined between 48–120 h, and rose again at 144 h. MCHC remained relatively stable with minor fluctuations toward control values (Fig. 1g-j).

No significant difference was observed between T1 and T2 for any parameter, indicating a threshold response within the tested range.

Malondialdehyde (MDA) increased significantly from 2.10 ± 0.18 nmol/mL in control to 5.67 ± 0.32 nmol/mL at 10.0 mg/L. Histopathological examination revealed epithelial hyperplasia and lamellar fusion in gills, and hepatocellular vacuolation in the liver at higher concentrations.

The prevalence of *Trichodina* sp. and mean intensity increased significantly ($p<0.05$) with increasing manganese concentration.

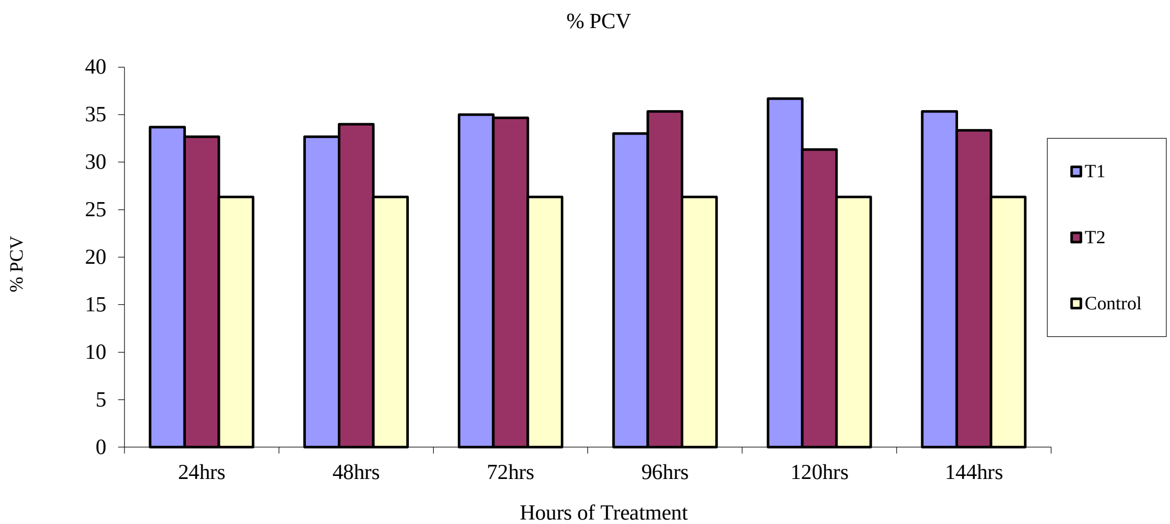

Fig.1a: Temporal dynamics of key haematological indices in *Clarias gariepinus* following 144h exposure to manganese
(a) PCV, (b) Hb, (c) RBC, (d) WBC, (e) Lymphocytes, (f) ESR, (g) MCV, (h) MCH, (i) MCHC, (j) Neutrophil rophil
PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cell; WBC: White Blood Cell; ESR: Erythrocyte Sedimentation Rate; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration.

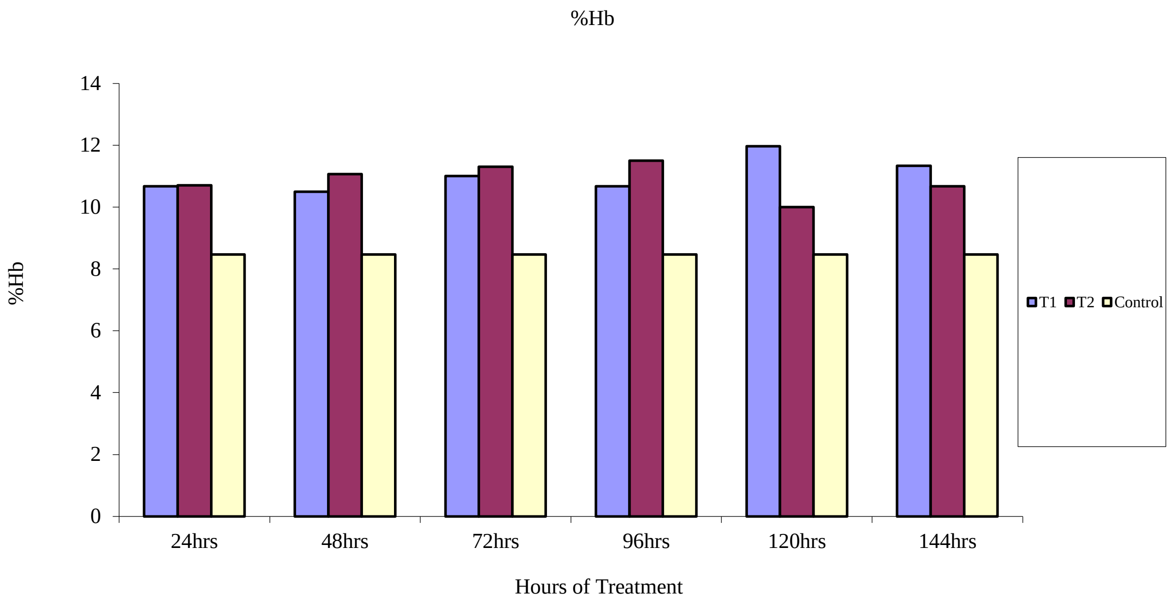

Fig.1b: Temporal dynamics of key haematological indices in *Clarias gariepinus* following 144h exposure to manganese
(a) PCV, (b) Hb, (c) RBC, (d) WBC, (e) Lymphocytes, (f) ESR, (g) MCV, (h) MCH, (i) MCHC, (j) Neutrophil
PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cell; WBC: White Blood Cell; ESR: Erythrocyte Sedimentation Rate; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration.

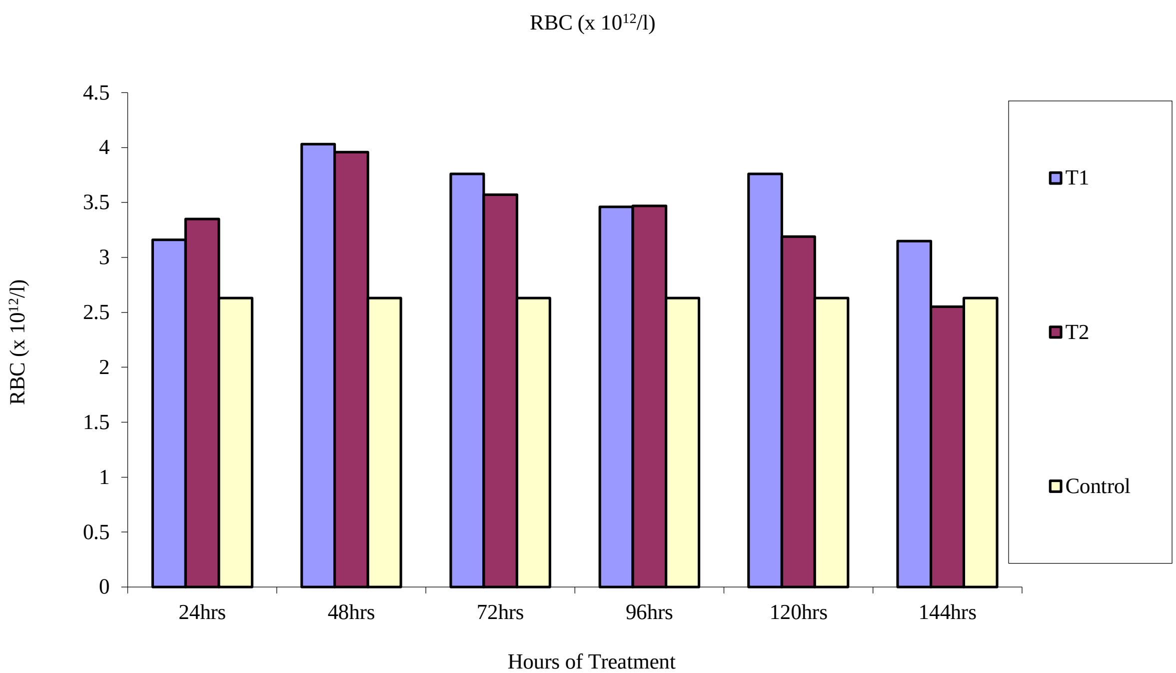

Fig.1c: Temporal dynamics of key haematological indices in *Clarias gariepinus* following 144h exposure to manganese
(a) PCV, (b) Hb, (c) RBC, (d) WBC, (e) Lymphocytes, (f) ESR, (g) MCV, (h) MCH, (i) MCHC, (j) Neutrophil
PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cell; WBC: White Blood Cell; ESR: Erythrocyte Sedimentation Rate; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration.

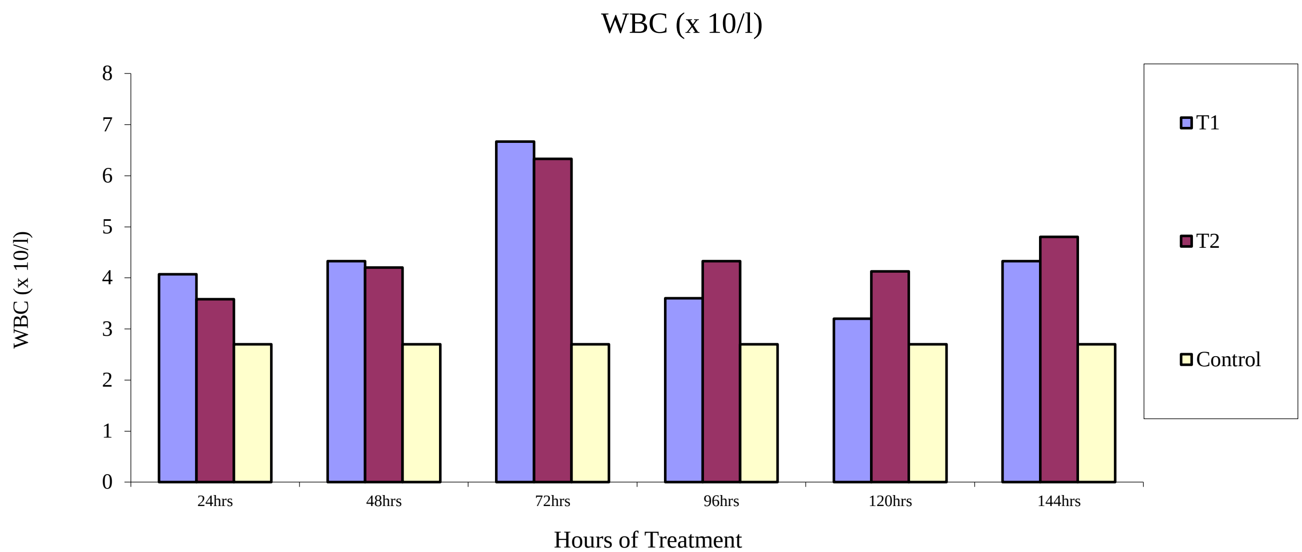

Fig.1d: Temporal dynamics of key haematological indices in *Clarias gariepinus* following 144h exposure to manganese
(a) PCV, (b) Hb, (c) RBC, (d) WBC, (e) Lymphocytes, (f) ESR, (g) MCV, (h) MCH, (i) MCHC, (j) Neutrophil
PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cell; WBC: White Blood Cell; ESR: Erythrocyte Sedimentation Rate; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration.

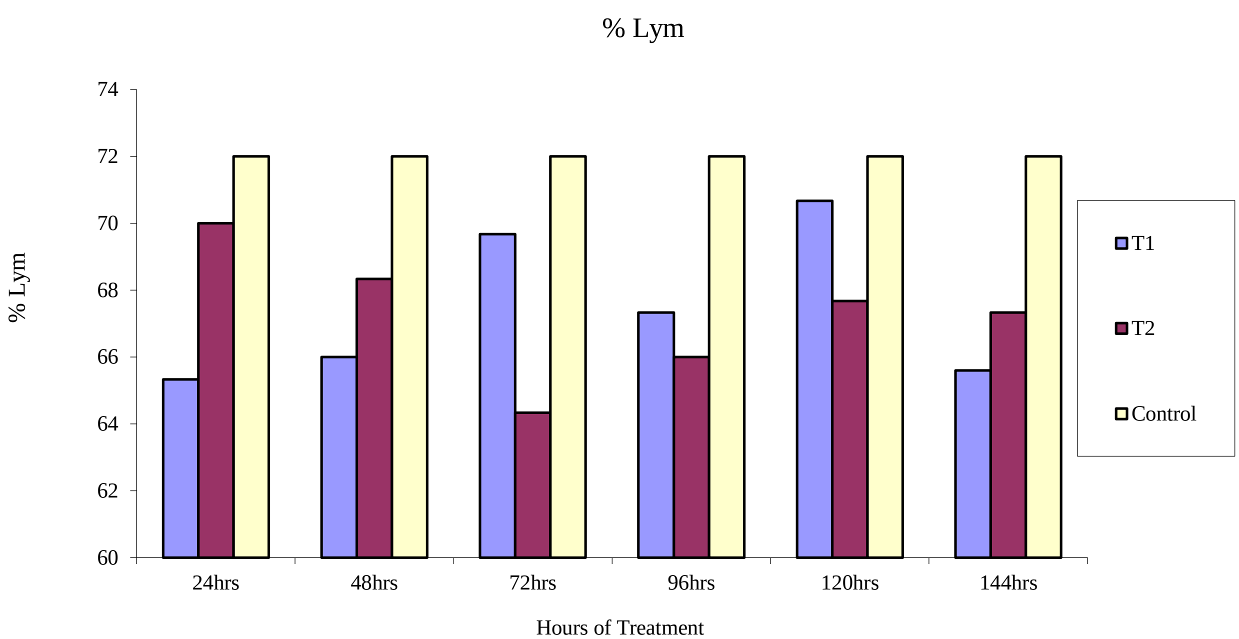

Fig.1e: Temporal dynamics of key haematological indices in *Clarias gariepinus* following 144h exposure to manganese.
(a) PCV, (b) Hb, (c) RBC, (d) WBC, (e) Lymphocytes, (f) ESR, (g) MCV, (h) MCH, (i) MCHC, (j) Neutrophil
PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cell; WBC: White Blood Cell; ESR: Erythrocyte Sedimentation Rate; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration.
PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cell; WBC: White Blood Cell; ESR: Erythrocyte Sedimentation Rate; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration.

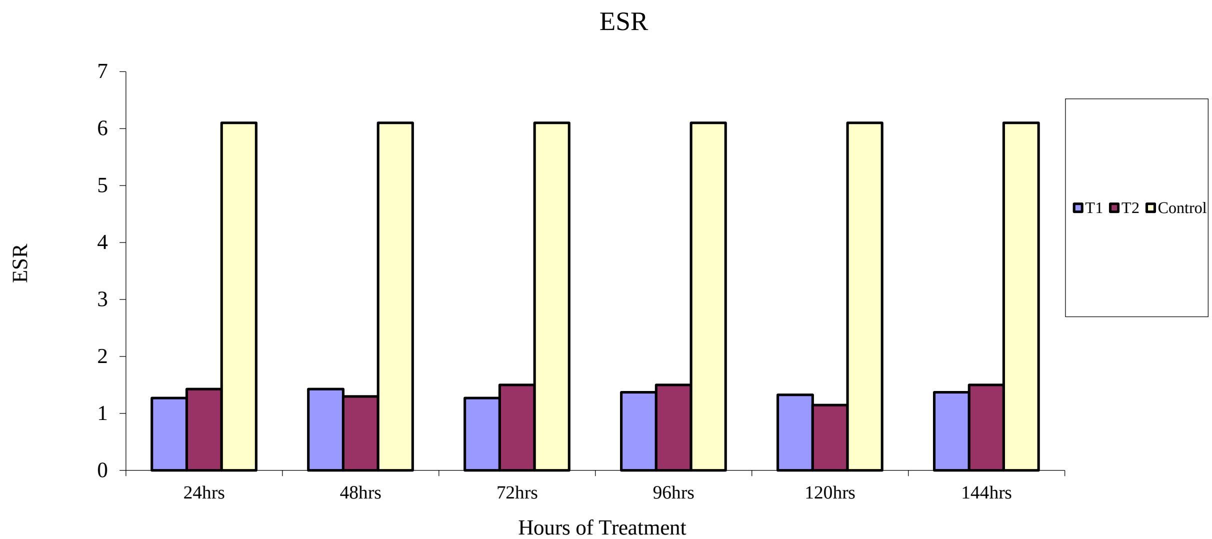

Fig.1f: Temporal dynamics of key haematological indices in *Clarias gariepinus* following 144h exposure to manganese.
(a) PCV, (b) Hb, (c) RBC, (d) WBC, (e) Lymphocytes, (f) ESR, (g) MCV, (h) MCH, (i) MCHC, (j) Neutrophil
PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cell; WBC: White Blood Cell; ESR: Erythrocyte Sedimentation Rate; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

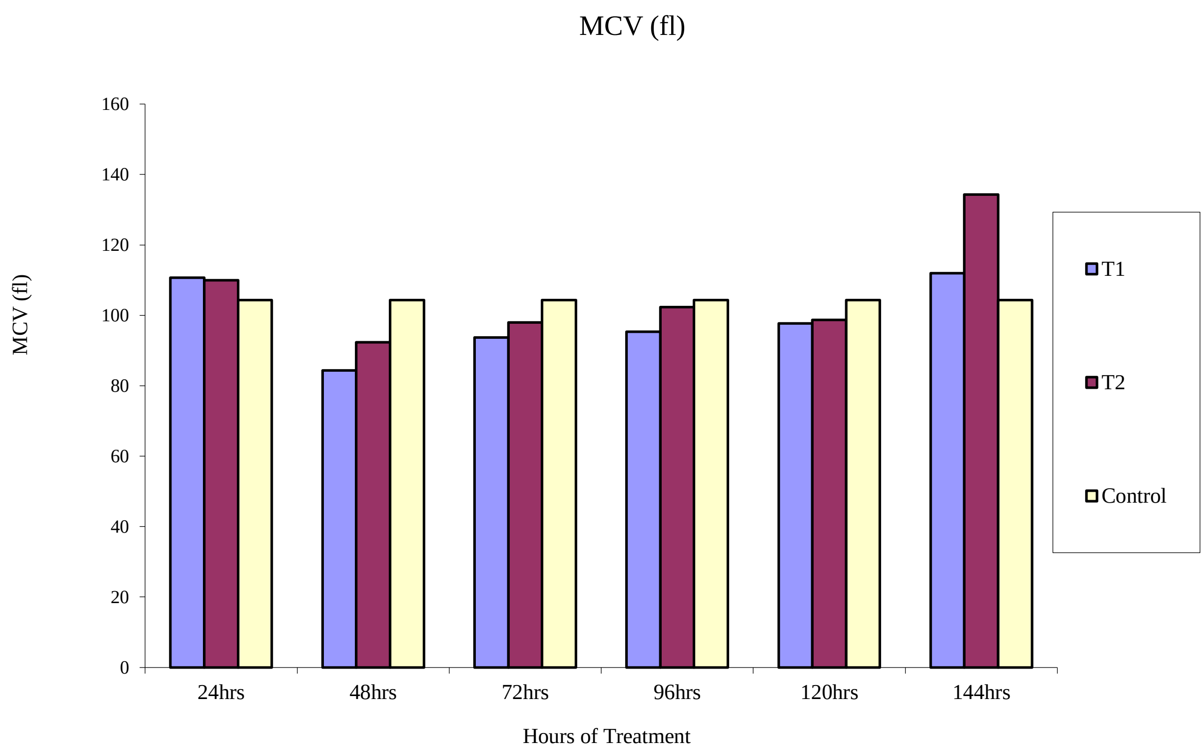

Fig.1g: Temporal dynamics of key haematological indices in *Clarias gariepinus* following 144h exposure to manganese
(a) PCV, (b) Hb, (c) RBC, (d) WBC, (e) Lymphocytes, (f) ESR, (g) MCV, (h) MCH, (i) MCHC, (j) Neutrophil
PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cell; WBC: White Blood Cell; ESR: Erythrocyte Sedimentation Rate; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

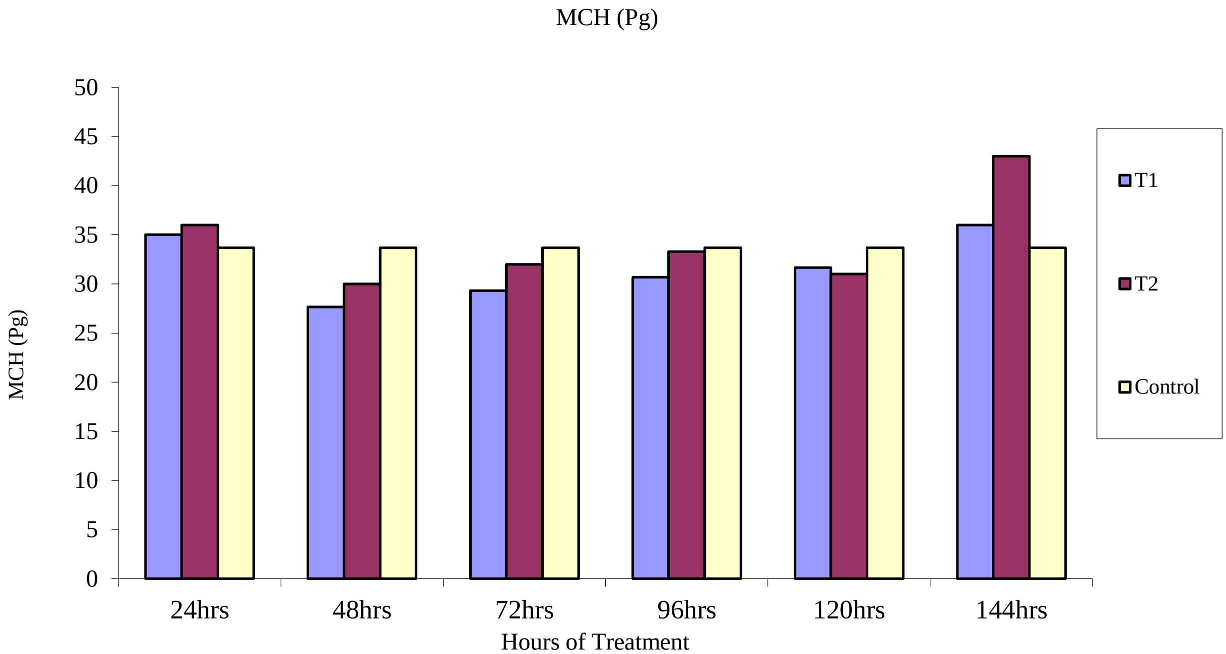

Fig.1h: Temporal dynamics of key haematological indices in *Clarias gariepinus* following 144h exposure to manganese
(a) PCV, (b) Hb, (c) RBC, (d) WBC, (e) Lymphocytes, (f) ESR, (g) MCV, (h) MCH, (i) MCHC, (j) Neutrophil
PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cell; WBC: White Blood Cell; ESR: Erythrocyte Sedimentation Rate; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration.

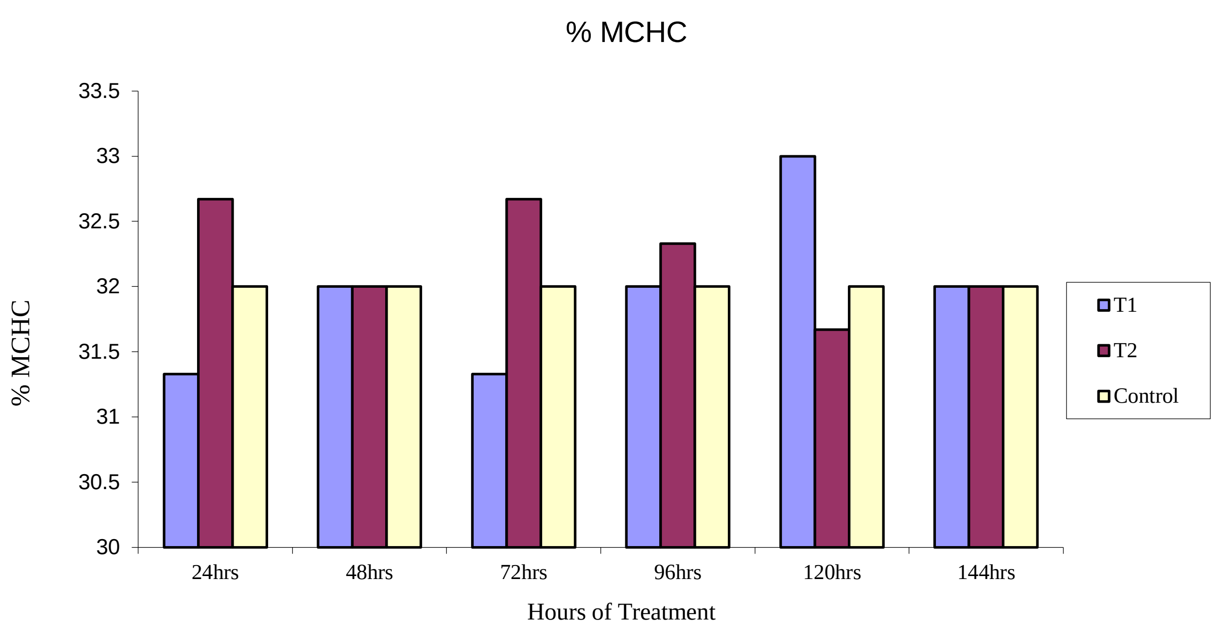

Fig.1i: Temporal dynamics of key haematological indices in *Clarias gariepinus* following 144h exposure to manganese
(a) PCV, (b) Hb, (c) RBC, (d) WBC, (e) Lymphocytes, (f) ESR, (g) MCV, (h) MCH, (i) MCHC, (j) Neutrophil
PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cell; WBC: White Blood Cell; ESR: Erythrocyte Sedimentation Rate; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

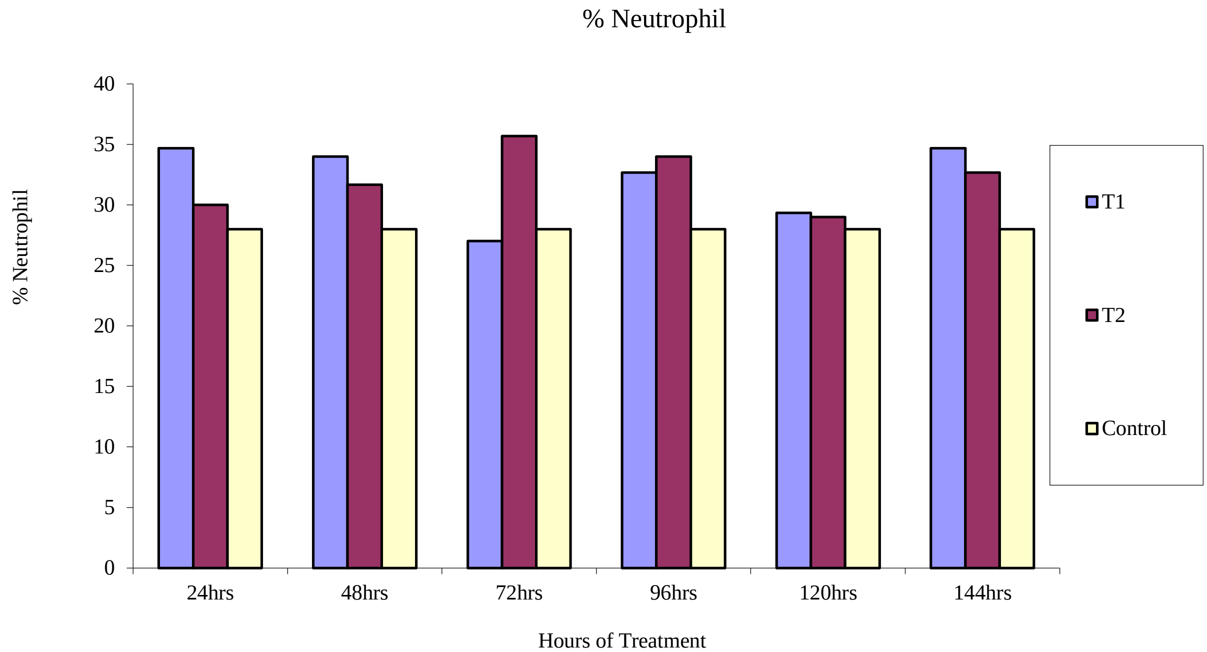

Fig.1j: Temporal dynamics of key haematological indices in *Clarias gariepinus* following 144hexposure to manganese
(a) PCV, (b) Hb, (c) RBC, (d) WBC, (e) Lymphocytes, (f) ESR, (g) MCV, (h) MCH, (i) MCHC, (j) Neutrophil
PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red Blood Cell; WBC: White Blood Cell; ESR: Erythrocyte Sedimentation Rate; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

DISCUSSION

The significant decrease in hemoglobin, packed cell volume (PCV), and red blood cell count observed in this study indicates anemia in *Clarias gariepinus* exposed to manganese. This agrees with Kumar *et al*. (2024) who reported similar reductions in *Labeo rohita*. The reduction may be due to inhibition of erythropoiesis and oxidative damage to erythrocytes caused by manganese.

The significant elevation in PCV, Hb and RBC aligns with compensatory erythropoiesis in response to metal-induced hypoxia and osmotic stress (Kumar *et al*., 2024). Similar increases have been reported in catfish and carp exposed to Cu and Cd (Saha and Kaviraj, 2020; Abdel-Tawwab and Wafeek, 2020).

The rise in WBC and Neutrophil reflects activation of the non-specific immune system to counteract metal stress, while the decline in lymphocytes suggests cortisol-mediated immunosuppression (Velisek *et al*., 2021). The observed lymphopenia and immunosuppression following Mn exposure may increase *C. gariepinus* vulnerability to parasitic infections. Previous studies have linked stress-induced declines in lymphocytes to higher prevalence of monogeneans and protozoans in farmed catfish (Adeyemi *et al*., 2022). Thus, haematological monitoring can serve as an early indicator of both toxicant exposure and potential parasitological risk.

The decrease in ESR may indicate RBC aggregation changes due to oxidative stress (Li *et al*., 2022). Stable MCV, MCH and MCHC suggest that despite increased RBC production, cell morphology was largely maintained during short-term exposure.

Lack of dose differentiation between T1 and T2 implies that even the lower concentration exceeded the threshold needed to elicit a haematological response in *C. gariepinus*. This supports the use of blood indices as sensitive early-warning tools for fish health (Chen *et al*., 2023).

The increase in malondialdehyde (MDA) suggests induction of lipid peroxidation and oxidative stress. This is consistent with Zhang *et al*. (2025) who reported elevated MDA in fish exposed to heavy metals. Oxidative stress can lead to cellular damage and impaired physiological function.

Histopathological lesions such as epithelial hyperplasia and lamellar fusion in gills, and vacuolation in the liver, indicate tissue damage due to manganese toxicity. These findings corroborate Saha and Kaviraj (2020) who reported similar lesions in catfish exposed to metals.

The significant increase in prevalence and mean intensity of *Trichodina* sp. suggests that manganese exposure induced immunosuppression in *Clarias gariepinus*. Pollutants are known to compromise fish immunity, making them more susceptible to parasitic infection. This is the first report linking manganese exposure to increased parasitism in *C. gariepinus*.

Overall, Mn disrupted homeostasis in *C. gariepinus* within 6 days, with implications for fish health and food safety given that fish are a dietary pathway for metals to humans (Zhang and Wang, 2021; Faraday *et al*., 2026).

CONCLUSION

Short-term exposure to sub-lethal manganese caused significant haematological alterations in *Clarias gariepinus*, characterized by erythrocytosis, leukocytosis and lymphopenia. These changes demonstrate the utility of blood parameters as biomarkers for Mn contamination and as predictors of fish health status, including susceptibility to parasitic infections, in tropical freshwater systems. Further studies integrating oxidative stress, gene expression, and direct parasitological screening will strengthen the mechanistic understanding of Mn toxicity in aquatic organisms.

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