ACUTE TOXICITY ON AFRICAN CATFISH *(Clarias gariepinus)* USING 6PPDQ AND THE SUB-LETHAL EFFECTS ON HEMATOLOGICAL PARAMETERS

BY

MARGARET OMOSIGHO EBHODAGHE

LSC2103639

DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

OCTOBER, 2025

ACUTE TOXICITY ON AFRICAN CATFISH *(Clarias gariepinus*) USING 6PPDQ AND THE SUB-LETHAL EFFECTS ON HEMATOLOGICAL PARAMETERS

BY

MARGARET OMOSIGHO EBHODAGHE

LSC2103639

A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE IN ANIMAL AND ENVIRONMENTAL BIOLOGY(BSCAEB)

OCTOBER, 2025.

CERTIFICATION

This is to certify that this project was carried out by **MARGARET OMOSIGHO EBHODAGHE** of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City.

________________________ ________________________

DR.N. O. ERHUNMWUNSE DATE

(Project Supervisor)

________________________ ________________________

PROF. MRS ISIOMA TONGO DATE

(Head of Department)

DEDICATION

This work is dedicated to God Almighty.

ACKNOWLEDGEMENT

Firstly, I want to express my heartfelt gratitude to Almighty God for seeing me through the entire course of this project. Without His guidance and strength, this journey would not have been possible.

I am deeply grateful to my family for their unwavering love and support throughout this endeavour. To my parents, Mr and Mrs Smarts Ebhodaghe, thank you for instilling in me the values of hard work, perseverance, and faith. Your belief in my abilities has motivated me every step of the way. To my extended family, thank you for your encouragement, understanding, and for always being there for me.

I would also like to extend my sincere thanks to my supervisor, Dr Nosakhare Erhunmwunse, for his unwavering support and insightful feedback throughout this project. Your expertise, guidance, and encouragement have been instrumental in shaping my research and enhancing my understanding of the subject matter.

I wish to acknowledge the contributions of Mr Enoch, Dr Ekene, and Uncle Chris, whose diverse perspectives and constructive critiques have enriched this work. Your commitment to excellence has inspired me to strive for the highest standards.

Special thanks to my colleagues and peers in the group for their collaboration and camaraderie. A heartfelt thanks to Edenoye Caleb, Ordah Orume, and Mowetei Chukwuebuka for their assistance with data collection, analysis, and for their invaluable insights during our discussions. The collaborative spirit within our team made this project not only productive but also enjoyable.

I also want to acknowledge the financial support provided by my parents and aunties. Their generous funding has enabled me to access essential resources and conduct comprehensive research, which significantly contributed to the success of this project.

In conclusion, this project is a culmination of collective efforts, and I am deeply appreciative of everyone who played a role in its realisation. Thank you all for your invaluable contributions

TABLE OF CONTENTS

COVER PAGE 1

TITLE PAGE i

CERTIFICATION ii

DEDICATION iii

ACKNOWLEDGEMENT iv

TABLE OF CONTENTS vi

LIST OF FIGURES ii

LIST OF PLATES i

ABSTRACT i

CHAPTER ONE 1

INTRODUCTION 1

1.1 Statement of Problem 3

1.2 Aim and Objectives 4

1.3 Significance of the Study 4

CHAPTER TWO 5

LITERATURE REVIEW 5

2.1 Environmental persistence and bioavailability 5

2.2 Sub-lethal and physiological effects 6

2.3 Environmental pathways and exposure routes 7

2.4 Acute toxicity in aquatic species 8

2.5 Mechanisms of toxicity 9

2.6 Chronic and sub-lethal effects 10

2.7 Species-specific variability in toxicity 10

2.8 Relevance to *Clarias gariepinus* and data gaps 11

2.9.1 Acute Toxicity and Sensitivity Among Aquatic Species 12
2.9.2 Species-Specific Variability in Sensitivity 13
2.10 Implications for Aquatic Ecosystems 14
2.11 Haematological Parameters as Biomarkers of Sub-Lethal Toxicity 15
2.12 Biological Importance of Haematological Parameters in Fish Health 16
2.13 Application of Haematological Indices in Aquatic Toxicology 17
2.14 Relevance of Haematological Biomarkers in 6PPD-Q Studies 18
2.15 Haematological Patterns Observed in Related Studies 19
CHAPTER THREE 20
MATERIALS AND METHODS 20
3.1 Chemicals 20
3.2 Test organism 23
3.3 Source of experimental fish, Acclimatisation and Ethical Approval 25
3.4 Experiment design 25
3.5 Experimental protocol 27
3.6 Biomarker Analysis 27
3.7. Determination of fish toxicity 28
3.7.1. Acute toxicity test and behavioural studies 28
3.7.2. Sub-lethal toxicity test 28
CHAPTER FOUR 30
RESULTS 30
4.1 White blood cell (WBC) 30
4.2 Lymphocyte Percentage 32
4.3 Lymphocytes 34
4.4 Haemoglobin (HGB) 36

4.5 Red blood cell (RBC) 38
4.6 Hematocrit (HCT) 40
4.7 Mean Corpuscular Volume (MCV) 42
4.8 Mean Corpuscular Haemoglobin (MCH) 44
4.9 Mean Corpuscular Haemoglobin Concentration (MCHC) 46
4.10 Platelet (PLT) 48
4.11 Plate Volume Distribution width (PDW) 50
4.12 Plateletcrit (PCT) 52
4.13 Platelet Large Cell Ratio P-LCR 54
CHAPTER FIVE 56
DISCUSSION 56
CONCLUSION 61
REFERENCES 62

LIST OF FIGURES

Figure 4.1: Mean White Blood Cell (WBC) Count of *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

Figure 4.2: Lymphocyte Percentage of *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

Figure 4.3: Lymphocyte Count of *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

Figure 4.4: Haemoglobin (HGB) Concentration of *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

Figure 4.5: Red Blood Cell (RBC) Count of *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

Figure 4.6: Hematocrit (HCT) Levels in *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

Figure 4.7: Mean Corpuscular Volume (MCV) of *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

Figure 4.8: Mean Corpuscular Haemoglobin (MCH) of *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

Figure 4.9: Mean Corpuscular Haemoglobin Concentration (MCHC) of *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

Figure 4.10: Platelet (PLT) Count of *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

Figure 4.11: Platelet Distribution Width (PDW) of *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

Figure 4.12: Plateletcrit (PCT) of *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

Figure 4.13: Platelet Large Cell Ratio (P-LCR) of *Clarias gariepinus* Exposed to 6PPD-Q for 28 Days.

LIST OF PLATES

Plate 3.1.1: Image of 6PPD-Q Pellets... 21

Plate 3.1.2: Dimethyl Sulfoxide (DMSO) Solvent...22

Plate 3.2: Test organism; Juvenile *Clarias gariepinus* on a sensitive weighing balance........ 24

Plate 3.3: Experimental setup for different 6PPDQ concentrations...26

ABSTRACT

6PPD-quinone (6PPD-Q) is a transformation product of the tyre additive 6PPD. It enters aquatic systems through stormwater runoff and poses an emerging toxicological concern. This study examined the acute and sub-lethal effects of 6PPD-Q on *Clarias gariepinus*, a freshwater catfish commonly used in ecotoxicological studies. The objective was to determine the sensitivity of *Clarias gariepinus* to 6PPD-Q and to assess changes in haematological parameters as indicators of physiological stress. Juvenile *Clarias gariepinus* were exposed under laboratory conditions to a range of 6PPD-Q concentrations for 96 hours to evaluate acute toxicity, followed by sub-lethal exposure for haematological analysis. No mortality was recorded during the acute phase, indicating that the compound did not reach a lethal threshold within the tested concentration range. Sub-lethal exposure produced measurable haematological alterations. There was a significant decrease ($P<0.05$) in red blood cell count, haemoglobin concentration, and packed cell volume, suggesting anaemia and impaired oxygen transport. White blood cell counts increased, indicating immune response activation. These findings demonstrate that 6PPD-Q affects fish health at non-lethal levels, even when acute mortality is absent. This study highlights the ecological risk posed by 6PPD-Q in aquatic environments. Its persistence and sub-lethal toxicity underscore the need for environmental monitoring and regulation of tire-derived pollutants. Further research should address long-term exposure, tissue accumulation, and population-level impacts in freshwater ecosystems.

CHAPTER ONE

INTRODUCTION

Tyres are indispensable in modern transportation and are produced and used on a massive global scale. During their lifetime, tyres undergo abrasion, releasing tyre wear particles (TWPs) that carry a mixture of polymers, fillers, and chemical additives into the environment (Kreider *et al*., 2010). Among these additives is N-(1,3-dimethylbutyl)-N ′- N ′- N ′- N ′- N ′- N ′- N ′- N ′- N′-phenyl-p-phenylenediamine (6PPD), a widely used antioxidant that protects tyres against ozone cracking and extends their service life (Tian *et al.,* 2021). However, once released into the environment, 6PPD undergoes chemical transformation, particularly through reactions with ozone and sunlight, to form 6PPD-quinone (6PPD-Q), a more stable and environmentally persistent product (Liao *et al.,* 2024). This transformation product has attracted global attention because of its unexpected toxicity to aquatic organisms.

The environmental relevance of 6PPD-Q has become increasingly clear in recent years. Monitoring studies have detected the compound in stormwater, surface waters, and sediments across North America, Europe, and Asia, often at concentrations overlapping with those known to cause biological effects (Kolodziej *et al*., 2021; Brinkmann *et al*., 2022). The strongest evidence of its ecological impact emerged from long-standing die-offs of coho salmon (Oncorhynchus kisutch) in urban streams along the U.S. West Coast, where researchers ultimately identified 6PPD-Q as the causal toxicant (Tian *et al*., 2021). Subsequent detections in other aquatic habitats, coupled with experimental exposures, have confirmed its potential as a major urban runoff contaminant of global significance.

Toxicological studies highlight the extraordinary potency of 6PPD-Q. Sensitive salmonids such as coho salmon, brook trout (*Salvelinus fontinalis*), and rainbow trout (*Oncorhynchus mykiss*) experience acute lethality at concentrations in the low microgram per litre or even nanogram per litre range, with characteristic symptoms including surface gasping, spiralling, and loss of equilibrium (Brinkmann *et al.,* 2022; Liao *et al.,* 2024). Mechanistic investigations suggest that the compound crosses the blood–brain barrier and accumulates in brain tissue, disrupting neurotransmitter balance and impairing locomotor behaviour (Liao *et al*., 2024). Other studies have reported oxidative stress, mitochondrial dysfunction, and damage to gill and liver tissues (Hiki and Yamamoto, 2021). Yet, sensitivity to 6PPD-Q is highly species-dependent: while some fish exhibit extreme vulnerability, other organisms, such as zebrafish (Danio rerio) and Daphnia magna, show much higher tolerance (Hiki and Yamamoto, 2021). This variability underlines the need to expand toxicological assessments beyond temperature

Clarias gariepinus, commonly known as African catfish, is widely cultivated in aquaculture and serves as an important environmental indicator for assessing aquatic pollutant effects due to its ecological relevance and commercial value (Sahoo *et al.,* 2023). Despite growing concerns about emerging contaminants, such as 6PPD-quinone (6PPDQ). A toxic transformation product of tyre rubber antioxidant 6PPD. Studies on the toxicity of 6PPDQ to *Clarias gariepinus* remain limited. Acute toxicity tests are commonly employed to measure the lethal effects of contaminants, generally expressed as lethal concentration values (LC_{50}).

Haematological indicators, including red blood cell counts, haemoglobin concentration, hematocrit, and white blood cell counts, serve as sensitive biomarkers for detecting sub-lethal stress and immune response disruption. Investigating these parameters supports a deeper understanding of pollutant impacts beyond mortality and underpins early warning systems for aquatic health monitoring (Sahoo *et al.,* 2023).

1.1 Statement of Problem

Although evidence has accumulated on the toxicity of 6PPDQ in various fish species, data on its effects in *Clarias gariepinus* are scarce. The absence of mortality in acute toxicity tests complicates the evaluation of toxicity via standard methods like LC_{50} determination, masking potential sub-lethal effects. Sub-lethal haematological changes caused by 6PPDQ in this species remain poorly understood, hindering accurate environmental risk assessments and effective pollution management strategies crucial for freshwater ecosystems and aquaculture sustainability.

1.2 Aim and Objectives

This project is aimed at investigating the acute toxicity and sub-lethal haematological effects of 6PPDQ in *Clarias gariepinus.*

I. Measure key haematological parameters indicative of sub-lethal toxicity in *Clarias gariepinus* exposed to environmentally relevant concentrations of 6PPDQ.
II. Provide data to guide environmental monitoring, facilitate pollution control efforts, and support sustainable aquaculture practices.

1.3 Significance of the Study

The research provides critical insights into the toxicological effects of 6PPDQ on a key freshwater species with significant ecological and economic importance. It addresses existing knowledge gaps by focusing on both lethal and sub-lethal endpoints, improving risk assessment frameworks for emerging tire-derived contaminants. The findings will inform policymakers and environmental managers, aiding the development of pollution mitigation strategies to protect freshwater biodiversity and sustain aquaculture productivity in regions impacted by urban runoff.

CHAPTER TWO

LITERATURE REVIEW

6PPD-quinone (6PPD-Q) is a transformation product of N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD), a widely used antioxidant in vehicle tires. When 6PPD reacts with ozone, it forms 6PPD-Q, a stable and persistent quinone compound that has been linked to acute mortality in fish, particularly salmonids (Tian *et al*., 2021; Brinkmann *et al*., 2022). The detection of 6PPD-Q in road dust, runoff, and urban water systems reveals its widespread environmental distribution and persistence (Cao *et al*., 2022; Zhang *et al*., 2022).

Recent studies have identified 6PPD-Q as one of the most toxic tire-derived contaminants known to date, with lethal concentrations in the nanogram per litre range for sensitive species like *Oncorhynchus Kisutch* (Coho Salmon) (Tian *et al*., 2021). Because of its high toxicity and environmental presence, understanding its toxicological effects across aquatic organisms, including non-salmonid species like *Clarias gariepinus*, is crucial for environmental management and risk assessment.

2.1 Environmental persistence and bioavailability

6PPD-Q is highly stable under environmental conditions. Laboratory photolysis experiments reveal that it resists rapid degradation, especially in low-light or turbid water systems (Li *et al*., 2023). It persists in sediments where it binds to organic matter and black carbon fractions. Cao *et al*. (2022) reported its presence across multiple environmental matrices, including water, air, and soil. Detection of 6PPD-Q in all compartments suggests extensive environmental cycling.

Atmospheric transport contributes to the wide distribution. Wang *et al*. (2022) detected quinone derivatives in PM2.5, indicating airborne dissemination from roadways and urban centres. Zhang *et al*. (2022) further confirmed its occurrence in indoor dust, suggesting persistence even in microenvironments not directly connected to vehicle emissions. These pathways contribute to global exposure potential and increase the likelihood of deposition into aquatic systems during rainfall. Once in aquatic environments, 6PPD-Q shows low volatility and moderate hydrophobicity. Partitioning coefficients indicate a strong affinity for organic sediments, yet partial solubility allows continuous release into the water column (Chen *et al*., 2023). Its half-life in surface waters varies from days to weeks, depending on temperature and light intensity. Even after photolytic transformation, intermediate products can revert or retain toxicity (Li *et al*., 2023).

Bioavailability depends on environmental conditions such as pH, dissolved organic carbon, and microbial activity. In organic-rich systems, binding to humic substances may limit free 6PPD-Q concentrations, but does not eliminate exposure. Sediment-dwelling organisms remain at risk through ingestion or contact. The persistence of the parent compound and transformation products ensures ongoing exposure even when emissions decrease.

2.2 Sub-lethal and physiological effects

Sub-lethal exposure to 6PPD-Q affects multiple physiological systems in fish. Haematological parameters provide a sensitive measure of such stress. Although data on *Clarias gariepinus* are limited, findings from related species suggest likely impacts. Reduced red blood cell counts, haemoglobin levels, and hematocrit values indicate anaemia or impaired oxygen transport. White blood cell elevation reflects immune system activation or inflammation (Sahoo *et al*., 2023).

Chronic exposure studies on zebrafish demonstrate oxidative stress through decreased antioxidant enzyme activity, lipid peroxidation, and altered liver morphology (Chen *et al*., 2023). These biochemical markers correspond with mitochondrial disruption seen in histological studies (Lo *et al*., 2023). 6PPD-Q also affects neurotransmission, causing behavioural anomalies such as erratic swimming, hyperactivity, or reduced feeding. Gill damage is another frequent observation. Histopathological analysis reveals lamellar fusion and epithelial lifting, which reduce oxygen uptake efficiency. Such impairments contribute to hypoxia-like symptoms even at sub-lethal exposure levels. Hepatic vacuolization and renal tubular damage have been reported, indicating organ-specific accumulation and toxicity (Liao *et al*., 2024).

At the molecular level, 6PPD-Q alters gene expression related to oxidative stress and immune response. Genes encoding catalase, glutathione S-transferase, and superoxide dismutase are downregulated, while pro-inflammatory cytokines increase. These responses signify systemic physiological disruption and chronic stress that precedes mortality.

2.3 Environmental pathways and exposure routes

The formation of 6PPD-Q begins with atmospheric oxidation of 6PPD released from tyre wear particles. Studies show that this transformation occurs both in ambient air and aqueous environments (Li *et al*., 2023). Zhang *et al*. (2022) found 6PPD-Q in size-fractioned atmospheric particles and indoor dust, indicating that airborne particulate matter serves as a transport vector. Wang *et al*. (2022) also demonstrated that quinone derivatives of substituted p-phenylenediamines are prevalent in PM2.5, confirming their potential for long-range atmospheric movement. In urban watersheds, stormwater runoff is a major transport mechanism. Johannessen *et al*. (2022) detected 6PPD-Q in surface waters downstream of road networks, confirming its presence in both particulate and dissolved phases. Concentrations up to 2.3 μg/L have been reported in heavily urbanised basins. Once introduced into waterways, 6PPD-Q adsorbs onto sediments due to its hydrophobic character but can also desorb and remain bioavailable, depending on environmental pH and organic matter content (Cao *et al*., 2022).

Aquatic organisms are exposed through multiple pathways. Direct uptake occurs via gill diffusion during water exposure. Sediment-associated 6PPD-Q also represents a risk for benthic organisms, while dietary accumulation through contaminated prey contributes to trophic transfer. Because of its stability and low biodegradability, 6PPD-Q persists in the environment, creating continuous exposure risk (Chen *et al*., 2023).

2.4 Acute toxicity in aquatic species

The discovery of 6PPD-Q's lethality originated from mass die-offs of Coho Salmon returning to spawn in urban streams of the U.S. Pacific Northwest. Subsequent laboratory tests confirmed that 6PPD-Q is the causative toxicant (Tian *et al*., 2021). LC50 values as low as 95 ng/L were reported for adult Coho Salmon after short-term exposure, showing extreme sensitivity. Symptoms before death included disorientation, surface swimming, gasping, and loss of equilibrium (Brinkmann *et al*., 2022). Similar effects have been observed in related salmonid species. Juvenile *Oncorhynchus mykiss* (rainbow trout) showed sub-lethal stress responses and mortality at concentrations above 500 ng/L (Lo *et al*., 2023). In contrast, non-salmonid fish such as *Danio Rerio* (zebrafish) and *Oryzias Latipes* (Japanese Medaka) were far less sensitive, exhibiting no mortality at concentrations exceeding 10 μg/L (Hiki and Yamamoto, 2021). This large interspecies variability suggests species-specific uptake, metabolism, and detoxification capacity.

Johannessen *et al*. (2022) provided field evidence of exposure by quantifying 6PPD-Q in an urban watershed. Concentrations correlated with rainfall events, highlighting the episodic yet intense nature of exposure following stormwater runoff. Although no direct fish kills were observed in that study, chronic exposure at sub-lethal levels was suggested to influence aquatic community health.

2.5 Mechanisms of toxicity

The mechanisms through which 6PPD-Q exerts toxicity involve oxidative stress, interference with mitochondrial function, and potential disruption of the blood-brain barrier. Liao *et al*. (2024) proposed that 6PPD-Q induces redox imbalance through quinone cycling, generating reactive oxygen species (ROS) that damage lipids, proteins, and DNA. This leads to apoptosis and necrosis in critical tissues such as the gill, liver, and brain.

Mitochondrial dysfunction is another key pathway. Electron microscopy in exposed fish revealed swelling and cristae disruption in hepatic and neural mitochondria (Lo *et al*., 2023). These structural damages correspond with reduced ATP production and impaired cellular respiration. The compound's lipophilic nature allows it to cross biological membranes easily, including the blood-brain barrier, causing neurotoxic symptoms like erratic swimming and loss of balance (Hiki and Yamamoto, 2021).

6PPD-Q also binds to haemoglobin and interferes with oxygen transport. Studies on salmonids indicate hypoxemia and elevated blood lactate levels following exposure (Tian *et al*., 2021). Disruption of ion regulation in gills may exacerbate respiratory distress, leading to asphyxiation. These combined physiological stresses explain the rapid onset of mortality seen in sensitive species.

2.6 Chronic and sub-lethal effects

Although acute mortality dominates the literature, recent studies have begun to examine sub-lethal and chronic effects. Chen *et al*. (2023) reviewed potential outcomes of prolonged exposure, including endocrine disruption, altered haematological parameters, and immune suppression. Laboratory studies on non-salmonid fish have reported changes in antioxidant enzyme activity, including decreased catalase and superoxide dismutase levels, indicating oxidative stress. Li *et al*. (2023) demonstrated that photo-degradation of 6PPD in water generates 6PPD-Q, prolonging environmental persistence and maintaining toxicity over time. Even low, non-lethal concentrations induced measurable biochemical responses, such as elevated Malondialdehyde (MDA) levels in fish liver tissues, a marker of lipid peroxidation. These findings suggest that environmental levels, though sometimes below acute LC50 values, can still cause physiological impairment in aquatic species.

2.7 Species-specific variability in toxicity

Species differ widely in their response to 6PPD-Q exposure. Laboratory tests have shown that salmonids, especially Coho Salmon, are among the most sensitive aquatic species identified so far. LC50 values for Coho are below 100 ng/L, while other fish, such as rainbow trout, exhibit moderate sensitivity with LC50 values ranging from 300–700 ng/L (Tian *et al*., 2021; Brinkmann *et al*., 2022). In contrast, zebrafish (*Danio rerio*), Medaka *(Oryzias latipes*), and fathead minnows (*Pimephales promelas*) survive concentrations exceeding 10 µg/L (Hiki and Yamamoto, 2021; Lo *et al*., 2023). This variation may be linked to differences in metabolic detoxification pathways. Salmonids possess a distinct pattern of cytochrome P450 enzyme expression that might enhance susceptibility to oxidative stress caused by quinone compounds. Non-salmonid fish display greater glutathione conjugation and repair enzyme activity, reducing intracellular accumulation of reactive intermediates (Hiki and Yamamoto, 2021). These biochemical distinctions partially explain why salmon experience rapid mortality while other species tolerate much higher concentrations.

Invertebrates such as *Daphnia magna* and *Chironomus riparius* exhibit relatively high tolerance as well. Acute toxicity tests report no mortality below 100 µg/L for *Daphnia*, although some behavioural abnormalities such as reduced swimming activity were noted (Brinkmann *et al*., 2022). This suggests that physiological targets for 6PPD-Q toxicity, such as haemoglobin binding or neural impairment, are less critical in invertebrate physiology.

Species-specific tolerance also influences ecological outcomes. In natural systems, the differential sensitivity among taxa could shift community composition after storm events that deliver 6PPD-Q pulses into surface waters. Sensitive species decline or disappear, while tolerant ones dominate. These shifts have implications for ecosystem function, predator-prey dynamics, and overall biodiversity (Johannessen *et al*., 2022).

2.8 Relevance to *Clarias gariepinus* and data gaps

Clarias gariepinus, the African sharp-toothed catfish, is an economically and ecologically important freshwater species across Africa. Limited research exists on 6PPD-Q toxicity in this species. Sahoo *et al*. (2023) reported challenges in establishing mortality-based toxicity thresholds because tested concentrations did not produce lethality. This suggests greater tolerance compared to salmonids.

Despite the lack of acute effects, *Clarias gariepinus* may experience sub-lethal impacts on haematological and biochemical parameters. Changes in red and white blood cell counts, haemoglobin concentration, and plasma enzymes have been documented for other contaminants and may occur with 6PPD-Q exposure. Given the species' benthic feeding behaviour and habitat in sediment-rich environments, sediment-associated 6PPD-Q represents a significant risk.

Research gaps include limited data on uptake kinetics, tissue distribution, and long-term reproductive effects. There is also a need for standardised test protocols for tropical species, as existing toxicity tests primarily reflect temperate ecosystems. Further studies using biomarker endpoints would clarify risk under realistic exposure scenarios. Clarias has been studied for haematology under multiple pollutants. However, specific haematological responses to 6PPD-quinone are largely unreported. Sahoo *et al*. (2023) noted no mortality at tested doses and recommended haematological biomarkers.

2.9.1 Acute Toxicity and Sensitivity Among Aquatic Species

The acute toxicity of 6PPD-Q was first reported in 2020 when mass mortalities were observed among Coho Salmon (Oncorhynchus kisutch) in stormwater-impacted rivers of the U.S. Pacific Northwest (Tian *et al*., 2021). The compound was later identified as the principal toxicant derived from the antioxidant 6PPD used in tyre rubber (Zhang *et al*., 2022). In my review of subsequent research, it was found that 6PPD-Q exhibits exceptionally low LC_{50} values for salmonids, indicating extreme sensitivity even at nanogram-per-litre concentrations. Studies by Brinkmann *et al*. (2022) and Lo *et al*. (2023) confirmed that mortality in Coho Salmon occurred within hours of exposure, often accompanied by symptoms such as erratic swimming, loss of equilibrium, and respiratory distress.

Beyond salmonids, other fish species such as rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), and Chinook salmon (*Oncorhynchus tshawytscha*) also displayed varying but significant susceptibility (Kolodziej *et al*., 2021). Conversely, species like zebrafish (*Danio rerio*) and medaka (*Oryzias latipes)* appeared more resilient, suggesting possible interspecific physiological or metabolic factors that influence toxicokinetics (Hiki and Yamamoto, 2021). This variability implies that the compound's mode of action may target biochemical pathways or organ systems that differ across taxa, a pattern consistent with other oxidative contaminants.

The lethal concentration (LC_{50}) of 6PPD-Q for Coho Salmon is approximately 0.8 µg/L (Tian *et al*., 2021), a value substantially lower than for most organic pollutants. The acuteness of this toxicity positions 6PPD-Q as one of the most hazardous contaminants associated with tyre wear particles (Johannessen *et al*., 2022).

2.9.2 Species-Specific Variability in Sensitivity

While 6PPD-Q's acute toxicity in salmonids is now well established, my review highlights significant interspecies variability in sensitivity. For instance, zebrafish and *Daphnia magna* exhibit relatively higher tolerance levels, possibly due to more efficient detoxification enzymes or differences in membrane permeability (Hiki and Yamamoto, 2021). This variability may also reflect differences in metabolic rate and lipid composition among species, which influence uptake and biotransformation (Cao *et al.,* 2022).

African catfish (*Clarias gariepinus*), a species of high ecological and aquacultural importance, has not been extensively studied in this context (Sahoo *et al.,* 2023). However, preliminary studies suggest that the species exhibits resilience to several organic toxicants, which might extend to 6PPD-Q. Understanding these interspecies differences is critical for accurate ecological risk assessments, particularly in regions where multiple species coexist in contaminated habitats.

In synthesising these findings, I observed that 6PPD-Q toxicity is not uniform across taxa. Instead, it is influenced by ecological adaptations, metabolic capacity, and habitat characteristics. Hence, extrapolating toxicity data from one species to another could lead to under- or over-estimation of environmental risk. Further comparative studies are therefore required to delineate the biochemical and physiological factors underpinning these sensitivities.

2.10 Implications for Aquatic Ecosystems

The ecological ramifications of 6PPD-Q toxicity extend beyond direct mortality. The compound's persistence and bioavailability imply potential long-term effects on population dynamics, food webs, and ecosystem functions. Mortality in keystone species such as salmon may disrupt predator-prey relationships, nutrient cycling, and reproductive success. Moreover, since 6PPD-Q is a transformation product of a widely used tyre additive, its continuous release into aquatic systems ensures chronic exposure risks (Zhang *et al.*, 2022; Johannessen *et al.*, 2022). In my evaluation, this persistent contamination represents a major emerging threat that demands urgent regulatory attention. It also emphasises the necessity of expanding toxicity testing beyond conventional model species to include regionally relevant organisms such as *Clarias gariepinus* and Tilapia. The acute and chronic effects of 6PPD-Q raise major ecological concerns. In regions with heavy traffic and poor stormwater management, continuous input of tyre wear particles maintains background contamination. Episodic runoff events cause spikes that exceed lethal thresholds for sensitive fish. Repeated fish kills observed in the Pacific Northwest highlight the magnitude of the problem (Tian *et al.*, 2021; Brinkmann *et al.*, 2022).

Aquatic food webs may be indirectly affected through the loss of predator species or a reduction in prey quality. Even where mortality does not occur, sub-lethal exposure alters growth, reproduction, and immune competence, potentially lowering population resilience. Given its persistence, 6PPD-Q fits the profile of a contaminant of emerging concern requiring regulation (Chen *et al*., 2023).

2.11 Haematological Parameters as Biomarkers of Sub-Lethal Toxicity

Exploration of sub-lethal toxicity in aquatic toxicology shows that haematological parameters offer some of the most sensitive, reliable, and cost-effective biomarkers for assessing fish health under pollutant exposure. Unlike mortality-based endpoints, which only reveal acute toxicity after irreversible damage has occurred, haematological biomarkers provide early insights into physiological stress, immune suppression, and metabolic disruption. This section examines the biological importance of haematological indices, their application in pollutant studies, and their relevance in assessing the sub-lethal effects of 6PPD-Q exposure in fish. From the mechanistic, 6PPD-Q toxicity appears to be multifaceted, involving oxidative stress, cellular damage, and interference with physiological homeostasis. According to Hiki and Yamamoto (2021), one proposed pathway involves the compound’s capacity to cross the blood–brain barrier, leading to neurological dysfunctions that manifest in abnormal swimming and behavioural disorientation. This mechanism is supported by histopathological findings that revealed lesions in brain and gill tissues, consistent with hypoxia and oxidative injury (Liao *et al*., 2024).

Mitochondrial damage is another critical endpoint. The compound induces excessive production of reactive oxygen species (ROS), impairing mitochondrial membranes and disrupting ATP synthesis (Li *et al*., 2023). This process reduces cellular energy availability, leading to tissue necrosis and systemic failure. The oxidative imbalance is also reflected in reduced activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), as demonstrated by Chen *et al*. (2023) in laboratory exposures. In addition, alterations in lipid peroxidation and glutathione levels point to oxidative degradation of cellular membranes, which further compromises ion regulation and Osmoregulatory function.

Moreover, 6PPD-Q exposure affects cardiovascular performance in fish, as evidenced by reduced heart rate and vascular damage (Wang *et al*., 2022). These findings reveal that 6PPD-Q toxicity operates through both direct cellular damage and indirect physiological dysfunctions, leading to rapid mortality in sensitive species. These multi-systemic effects underscore the complexity of its toxic-dynamics and suggest that even sub-lethal exposures could have profound ecological consequences.

2.12 Biological Importance of Haematological Parameters in Fish Health

Blood is the primary medium through which most toxic substances exert their systemic effects, either by direct cellular damage or through interference with physiological functions. In fish, blood composition is closely linked to environmental conditions and can change rapidly in response to stressors such as temperature fluctuations, dissolved oxygen variations, and pollutant exposure (Sahoo *et al*., 2023). From my perspective, studying blood indices such as red blood cell (RBC) counts, haemoglobin concentration (Hb), hematocrit (HCT), and white blood cell (WBC) counts provides valuable information about the organism's capacity to maintain homeostasis. The red blood cell parameters reflect oxygen-carrying capacity and metabolic energy status, while WBC indices indicate immune system activity and inflammation response (Chen *et al*., 2023).

For example, a decline in RBC count, Hb, or Hct is often indicative of anaemia, which may result from gill damage, reduced oxygen uptake, or erythrocyte destruction by toxic agents (Li *et* *al*., 2023). Conversely, elevated WBC counts often suggest an immune reaction against oxidative or xenobiotic stress. These shifts provide a dynamic picture of physiological adjustments to environmental contaminants long before overt toxicity or mortality is evident.

Haematological assays possess several advantages that make them indispensable for sub-lethal toxicity studies. They are relatively inexpensive, non-destructive, and can be repeated over time to monitor recovery or adaptation. Moreover, they are quantifiable and comparable across studies, providing consistency for cross-species and cross-contaminant analyses (Cao *et al*., 2022).

The attributes make haematological indices not just convenient but essential for assessing stress in economically important fish species such as *Clarias gariepinus*. In particular, their application could bridge the data gaps left by mortality-based assays that fail to capture early physiological disruptions. Integrating haematological analysis with biochemical and molecular endpoints would create a comprehensive framework for understanding how emerging contaminants like 6PPD-Q affect fish health at multiple biological levels.

2.13 Application of Haematological Indices in Aquatic Toxicology

Over the years, fish haematology has been widely applied as an early-warning tool in ecotoxicological assessments. Parameters such as RBC count, Hb, and Hct are known to decrease significantly upon exposure to heavy metals, pesticides, and petroleum hydrocarbons (Hiki and Yamamoto, 2021). In contrast, leukocytosis (increase in WBC) often signals immune stimulation or inflammatory responses to stressors (Brinkmann *et al*., 2022).

Responses of these blood parameters are not random but follow pollutant-specific patterns. For instance, exposure to oxidative agents such as 6PPD-Q leads to hemolysis and reduced Hb levels due to membrane lipid peroxidation (Wang *et al*., 2022). Meanwhile, stress hormones such as cortisol can alter WBC profiles by suppressing lymphocytes and increasing neutrophils, reflecting systemic stress (Johannessen *et al*., 2022).

In addition, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) are valuable indices that help interpret the type of anaemia or erythropoietic disturbance occurring in exposed fish. For instance, a reduction in MCV and MCHC implies microcytic hypochromic anaemia, commonly caused by iron deficiency or toxicant-induced inhibition of haemoglobin synthesis (Cao *et al*., 2022).

2.14 Relevance of Haematological Biomarkers in 6PPD-Q Studies

Although studies on 6PPD-Q toxicity have primarily focused on acute lethality in salmonids, sub-lethal effects, particularly haematological responses, are equally critical in understanding its broader ecological implications. Evidence from other oxidative pollutants suggests that 6PPD-Q's mechanism, involving oxidative stress and mitochondrial damage, will likely influence hematopoietic functions in exposed fish (Liao *et al*., 2024).

In this context, haematological biomarkers can reveal the early stages of physiological impairment before external symptoms or mortality occur. For instance, oxidative stress may lead to decreased RBC count and Hb due to oxidative degradation of erythrocyte membranes. Similarly, elevated WBC and lymphocyte counts may reflect immunological compensation for tissue damage. These alterations could serve as sensitive indicators for early detection of stress in species like *Clarias gariepinus*, which may not exhibit mortality even at relatively high concentrations of 6PPD-Q. Furthermore, in aquaculture settings, monitoring haematological profiles provides a practical means to assess fish well-being under potential contaminant exposure. Since catfish are often cultured in environments prone to tyre wear particle accumulation, routine haematological assessments could serve as a proactive management strategy to identify contamination-related stress before it compromises productivity.

2.15 Haematological Patterns Observed in Related Studies

Fishes exposed to polyaromatic hydrocarbons, benzene derivatives, or other quinone compounds commonly display reduced erythrocyte counts, diminished haemoglobin concentration, and elevated leukocyte levels (Zhang *et al*., 2022; Chen *et al*., 2023). These changes are typically attributed to haemolytic anaemia and immune activation. A study by Sahoo *et al*. (2023) emphasised that in *Clarias gariepinus*, haematological responses are among the earliest physiological indicators of pollutant stress, often preceding visible behavioural or histopathological symptoms. Reduced RBC and Hb values were associated with oxidative damage to gill tissues and impaired erythropoiesis. Likewise, increases in WBC counts and lymphocytes were interpreted as a compensatory response to tissue inflammation and repair.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chemicals

6PPD-quinone (purity 97.26%) and the isotopically labelled internal standard 6PPD-quinone-D5 were obtained from HPC Standards GmbH (Borsdorf, Germany) and the 6PPD-quinone analytical independent check standard from Cambridge Isotope Laboratories (Cambridge, MA, USA). Stock solutions of 6PPDQ were prepared using dimethyl sulfoxide (DMSO) (Thermo Fisher Scientific, Waltham, MA, USA) to achieve a final solvent concentration of <0.001% (v/v) during exposures. Buffered tricaine methanesulfonate (MS-222) (Syndel's Syncaine, Ferndale, WA, USA) was used for anaesthesia (100 mg/L) and euthanasia (250 mg/L).

Dimethyl Sulfoxide (DMSO) was obtained from a commercial vendor. The DMSO (Dimethyl sulfoxide; CAS No. 67-68-5) is an organosulfur compound widely used as a solvent in biological and chemical research due to its ability to penetrate biological membranes. It is a polar aprotic solvent, fully miscible with water and many organic solvents. DMSO is known for its anti-inflammatory properties and is used as a vehicle for drug delivery in experimental studies.

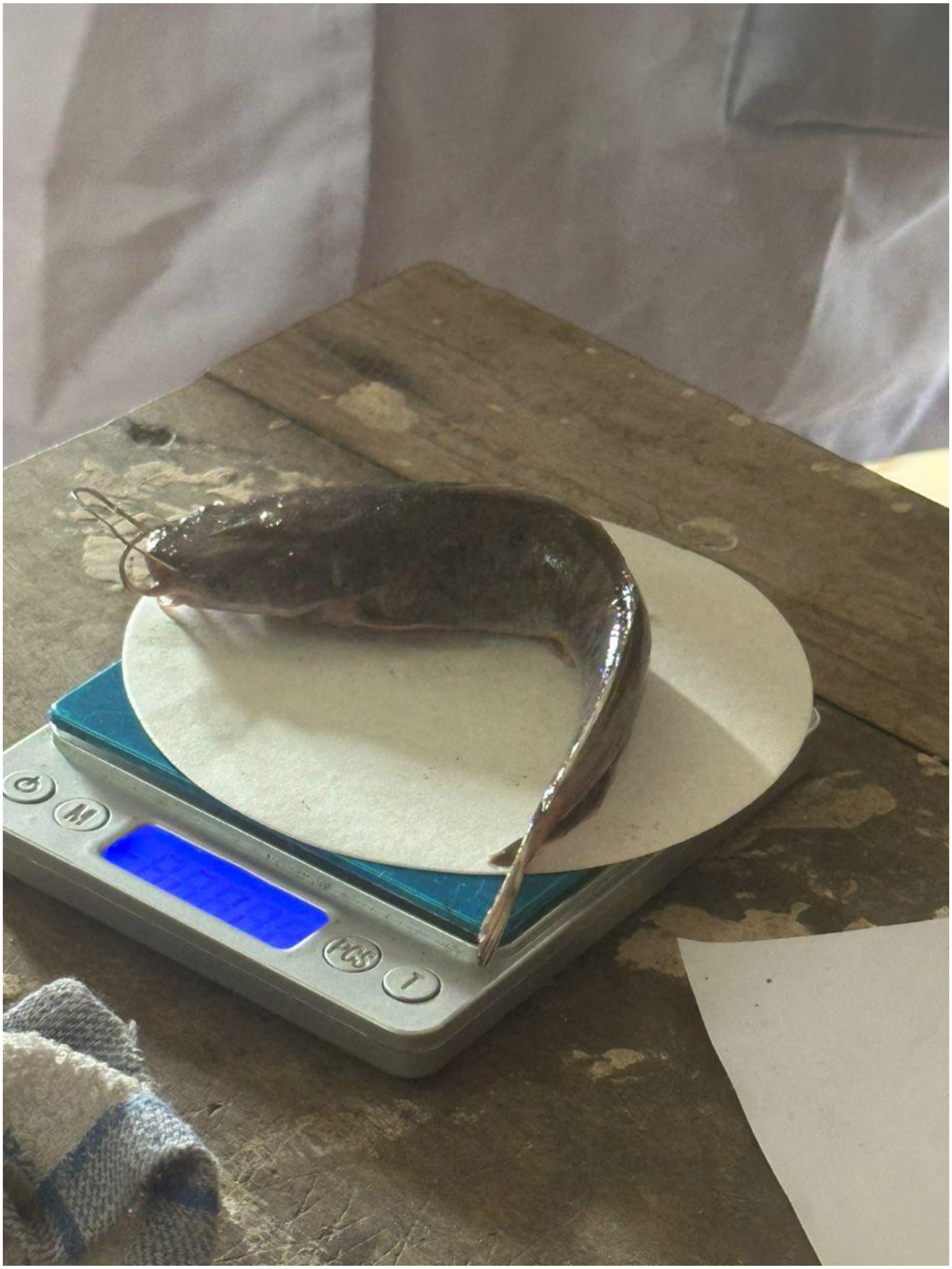
Plate 3.1.1: Image of 6PPD-Q Pellets.

Plate 3.1.2: Dimethyl Sulfoxide (DMSO) Solvent

3.2 Test organism

Clarias gariepinus (African catfish) is widely distributed in tropical freshwater ecosystems and relished by the tropical region populace (Ogueji *et al.,* 2018). The fish is omnivorous, feeding on invertebrates, fish, small mammals, seeds and fruit (Hastuti *et al*., 2019). According to Ogueji *et al.* (2018), it is also rugged, adaptive to laboratory conditions produces reasonable quantities of blood for haematological parameter estimation. It is among the most widespread of the African freshwater fish (Nguyen and Janssen, 2002).

Plate 3.2: Test organism; Juvenile *Clarias gariepinus* on a sensitive weighing balance.



3.3 Source of experimental fish, Acclimatisation and Ethical Approval

The experimental fish, *Clarias gariepinus* juveniles, were purchased at a fish farm in Benin City, Edo State and transported to the ecotoxicology laboratory of Animal and Environmental Biology, University of Benin, Benin City, Edo State, where the experiment was conducted. The average weight and average length of the fish were 24.10 ± 11.51g and 12.51 ± 2.8 cm, respectively. The juveniles were acclimatised in a 160L cylindrical plastic tank for two days and fed on a pelleted commercial feed daily. They were, however, left unfed for the first 24 hours to adapt to a change in the environment before feeding commenced with the fish diet. Approximately 80% of the water was replaced every two days. They were kept under natural photoperiod. There was 8% mortality during the acclimatisation period. Ethical clearance on the use of the fish species was obtained and approved by the Committee on the Ethical Use of Laboratory Animals, Faculty of Life Sciences, University of Benin, Nigeria.

3.4 Experiment design

A total of 237 *Clarias gariepinus* juveniles were used, divided into five treatments, which included a positive control (+control), negative control (-control), and three different concentrations of the toxicant. With two replicates. Seven juveniles were stocked into each tank with two replicate treatments. The water used was tap water left to stand for 24H

Plate 3.3: Experimental setup for different 6PPDQ concentrations

3.5 Experimental protocol

The experiment was carried out as described in the OECD guidelines for fish sublethal toxicity testing (OECD, 2019). 20g of 6ppd-q was dissolved in 100ml of DMSO to produce a stock solution. The stock solution was used to produce the four different concentrations- 4000µg/L, 8000µg/L, 12000µg/L and 16000µg/L of toxicants introduced into the experimental tank of 20litres of water in each tank. The positive control (+control) had 0.0mg/L of toxicants, while the negative control (-control) had 200µL of DMSO. Duplicates were made for each concentration. The test was carried out under static conditions. The duration of the test was 28 days, from which started on 19th September 2025 to 17th October 2025, during which the organisms were fed once a day.

3.6 Biomarker Analysis

At the end of the exposure periods, a range of biomarkers was evaluated to assess the sublethal effects of 6PPD-q exposure. These included enzymes indicative of liver function such as Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST), Albumin; oxidative stress markers including Malondialdehyde (MDA), Glutathione Peroxidase (GPx), Glutathione (GSH), and Superoxide Dismutase (SOD); and lipid profile components such as total cholesterol (CHOL), triglycerides (TG), high-density lipoprotein (HDL), very-low-density lipoprotein (VLDL), and low-density lipoprotein cholesterol (LDL-C), to evaluate lipid peroxidation. Additionally, creatinine and urea levels were measured to assess renal and hepatic function, along with total protein to evaluate general physiological condition. These biomarkers were chosen for their relevance in detecting hepatotoxicity, oxidative stress, and systemic physiological changes in fish exposed to environmental contaminants like BPA.

3.7. Determination of fish toxicity

3.7.1. Acute toxicity test and behavioural studies

A preliminary test was carried out in order to establish range values for sublethal concentrations. The toxicity period lasted for 4 days (96 h). After 14 days, feeding was discontinued 24 h before the commencement of the bioassay. For the acute toxicity study, four graded concentrations of 6-PPDQs were used: 4000µg/L, 8000µg/L, 12000µg/L and 16000µg/L. Six (6) Juveniles were stocked per replicate and estimated for their mortality rate after 24, 48, 72 and 96 h, respectively. It was observed that the fish in higher concentrations had protruding stomachs that resembled oedema.

The behavioural responses were measured following the reference standards and guidelines of the United States Environmental Protection Agency (USEPA, 2002) and the Organisation for Economic Co-operation and Development (OECD, 2019). The behavioural and morphological responses monitored included erratic swimming, loss of equilibrium, general restlessness, and body discolouration. Each experimental container was observed for 10 to 15 minutes to allow sufficient time for an accurate evaluation of each fish.

3.7.2. Sub-lethal toxicity test

From the results that were obtained from the acute toxicity, sub-lethal concentrations of 500µg/L, 1000µg/L and 1500µg/L were used for the study. The sub-lethal concentrations were calculated from one-fifth (1/5), one-tenth (1/10), one-fifteenth (1/15) and one-twentieth (1/20) of LC_{50} as recommended by Mohammed in a static experiment. Fifteen containers were used with three replicates per treatment. Six fish were exposed to four sub-lethal concentrations of 6-PPDQ for each of the containers. At the time of exposure, a fresh solution was prepared after 72 h (3 days) to maintain the concentration level.

Evaluation of blood parameters

Blood samples were collected from the caudal peduncle of the fish by using 1 ml syringes and hypodermal needles treated with an anticoagulant ethylenediaminetetraacetic acid. The following haematological parameters were analysed in the laboratory using MINDRAY 2800 BCE Automated Haematology Analyser, following standard procedures outlined by the manufacturers: packed cell volume (PCV), white blood cell (WBC), red blood cells (RBCs) and haemoglobin concentration (HBC). Mean Corpuscular Volume (MCV), Mean-Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC).

CHAPTER FOUR

RESULTS

4.1 White blood cell (WBC)

The mean value of WBC for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 74.10 ± 2.051, 81.50 ± 8.47, 86.80 ± 0.57, 90.80 ± 1.14 and 88.65 ± 1.20, respectively. There was no significant difference ($p>0.05$) in the group exposed to DMSO and 500µg/L, while the groups exposed to 1,000µg/L and 1,500µg/L showed a significant difference ($p<0.05$) on the 7th day.

On day 14 of exposure, the mean values were: 37.85 ± 2.05, 48.20 ± 0.42, 44.55 ± 4.46, 52.35 ± 5.02, 50.80 ± 4.24 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference ($p>0.05$) in the group exposed to DMSO and 500µg/L, while the groups exposed to 1,000µg/L and 1,500µg/L showed a significant difference ($p<0.05$) on the 14th day.

On day 21 of exposure, the mean values were: 40.95 ± 0.50, 43.40 ± 1.41, 54.05 ± 5.87, 48.40 ± 0.28, 54.40 ± 3.0, for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference between the +ve and -ve control groups and groups exposed to 1,000µg/L, while there the groups exposed to 500µg/L and 1,500µg/L

On day 28 of exposure, the mean values were: 42.85 ± 1.20, 52.95 ± 3.75, 50.90 ± 4.03, 49.95 ± 6.22, 48.04 ± 0.07 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. No significant difference was observed in all of the groups.

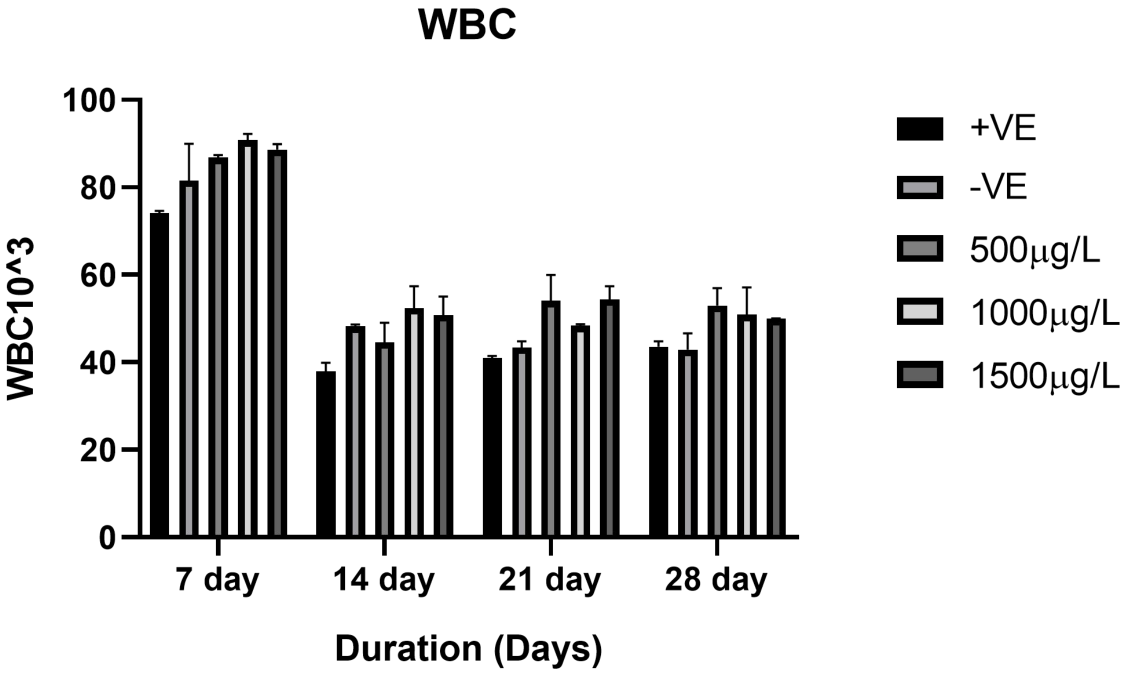


FIG 4.1: The alteration in the white blood cell as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

4.2 Lymphocyte Percentage

The mean value of lymphocyte percentage for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 93.55 ± 0.21, 92.55 ± 1.06, 93.25 ± 0.07, 94.10 ± 1.13 and 94.15 ± 0.07, respectively. There was no significant difference observed in all of the groups on the 7th day.

On day 14 of exposure, the mean values were: 96.00 ± 0.99, 95.35 ± 1.50, 96.40 ± 0.28, 95.60 ± 0.14, 90.90 ± 7.10 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 14th day.

On day 21 of exposure, the mean values were: 96.60 ± 0.28, 96.15 ± 0.21, 95.40 ± 0.60, 93.70 ± 4.2, 95.30 ± 0.60, for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 21st day.

On day 28 of exposure, the mean values were: 96.40 ± 0.71, 94.70 ± 3.30, 96.05 ± 0.64, 95.35 ± 0.78, 93.95 ± 3.32 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 28th day.

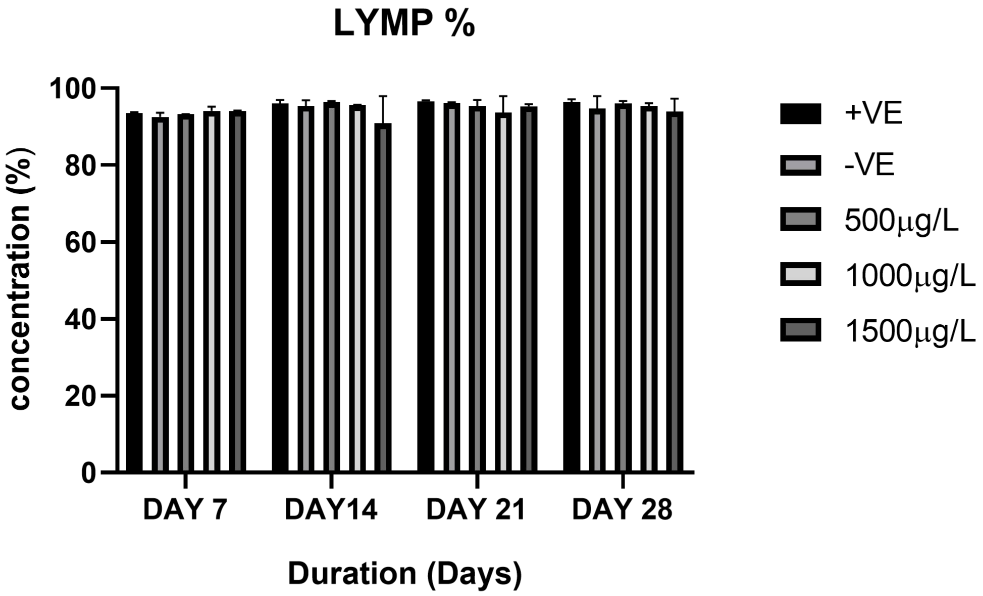


FIG 4.2: The alteration in the percentage of lymphocytes as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

4.3 Lymphocytes

The mean value of lymphocyte for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 63.85 ± 8.13, 78.20 ± 11.03, 81.60 ± 0.28, 73.50 ± 3.11 and 66.50 ± 1.41, respectively. There was no significant difference observed in all of the groups on the 7th day.

On day 14 of exposure, the mean values were: 45.90 ± 11.17, 50.70 ± 5.51, 43.00 ± 4.38, 45.25 ± 11.53, 24.60 ± 30.12 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 14th day.

On day 21 of exposure, the mean values were: 48.25 ± 0.78, 41.80 ± 1.27, 51.55 ± 4.73, 25.60 ± 30.3, 47.10 ± 9.3, for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 21st day.

On day 28 of exposure, the mean values were: 42.00 ± 1.41, 31.40 ± 17.96, 46.10 ± 3.25, 48.55 ± 5.59, 29.90 ± 31.54 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 28th day.

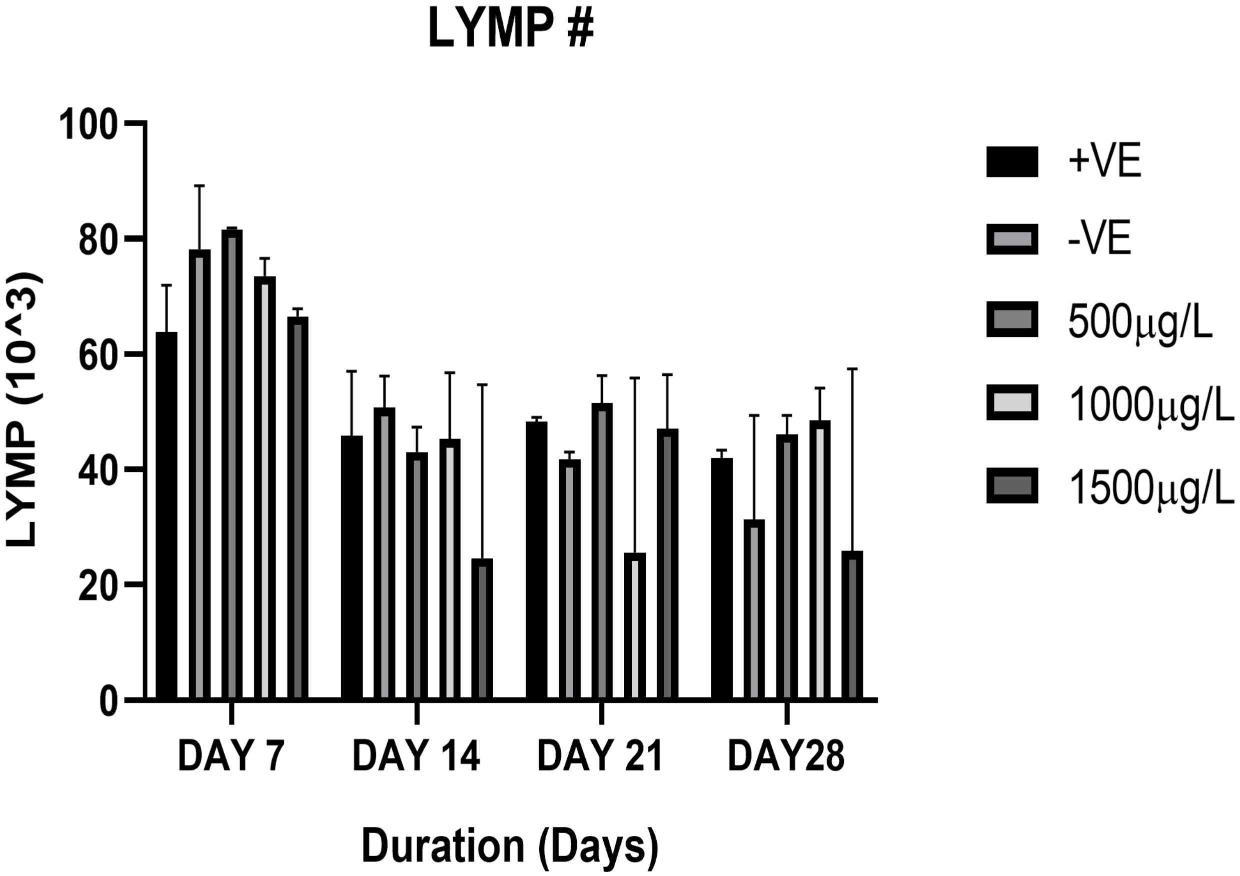


FIG 4.3: The alteration in the lymphocyte as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

4.4 Haemoglobin (HGB)

The mean value of haemoglobin for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 9.50 ± 0.64, 10.55 ± 0.21, 10.05 ± 0.07, 10.45 ± 0.21 and 10.40 ± 0.14, respectively. There was no significant difference observed in all of the groups on the 7th day.

On day 14 of exposure, the mean values were: 17.00 ± 1.13, 17.35 ± 1.20, 13.00 ± 1.56, 12.80 ± 2.26, 12.15 ± 0.70 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 14th day.

On day 21 of exposure, the mean values were: 13.60 ± 0.71, 11.25 ± 0.35, 11.75 ± 0.64, 12.25 ± 0.35, 11.40 ± 1.70, for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 21st day.

On day 28 of exposure, the mean values were: 11.10 ± 0.57, 11.50 ± 0.28, 11.00 ± 2.26, 10.30 ± 1.31, 7.50 ± 3.18 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 28th day.

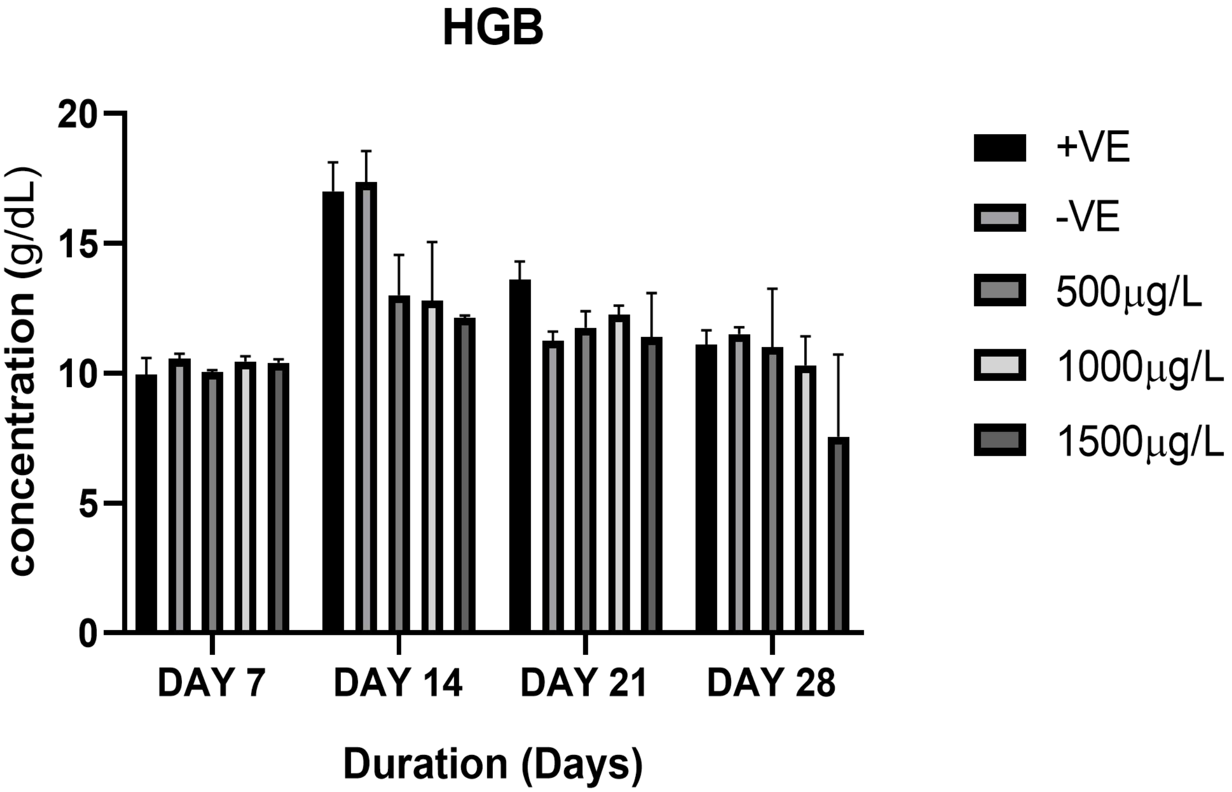


FIG 4.4: The alteration in the haemoglobin as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

4.5 Red blood cell (RBC)

The mean value of RBC for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 1.915 ± 0.18, 2.030 ± 0.13, 1.910 ± 0.4, 2.095 ± 0.09 and 2.080 ± 0.00, respectively. There was no significant difference observed in all of the groups on the 7th day.

On day 14 of exposure, the mean values were: 4.420 ± 0.35, 3.870 ± 0.52, 3.645 ± 0.39, 2.435 ± 0.28, 1.420 ± 1.36 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There is a significant difference between +ve and 1,500µg/L, while there is no significant difference between every other concentration and control on the 14th day.

On day 21 of exposure, the mean values were: 3.200 ± 0.11, 2.665 ± 0.05, 3.380 ± 0.61, 1.760 ± 1.84, 2.495 ± 0.05, for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 21st day.

On day 28 of exposure, the mean values were: 2.640 ± 0.15, 2.285 ± 1.15, 3.130 ± 0.40, 2.450 ± 1.42, 0.7800 ± 0.00 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 28th day.

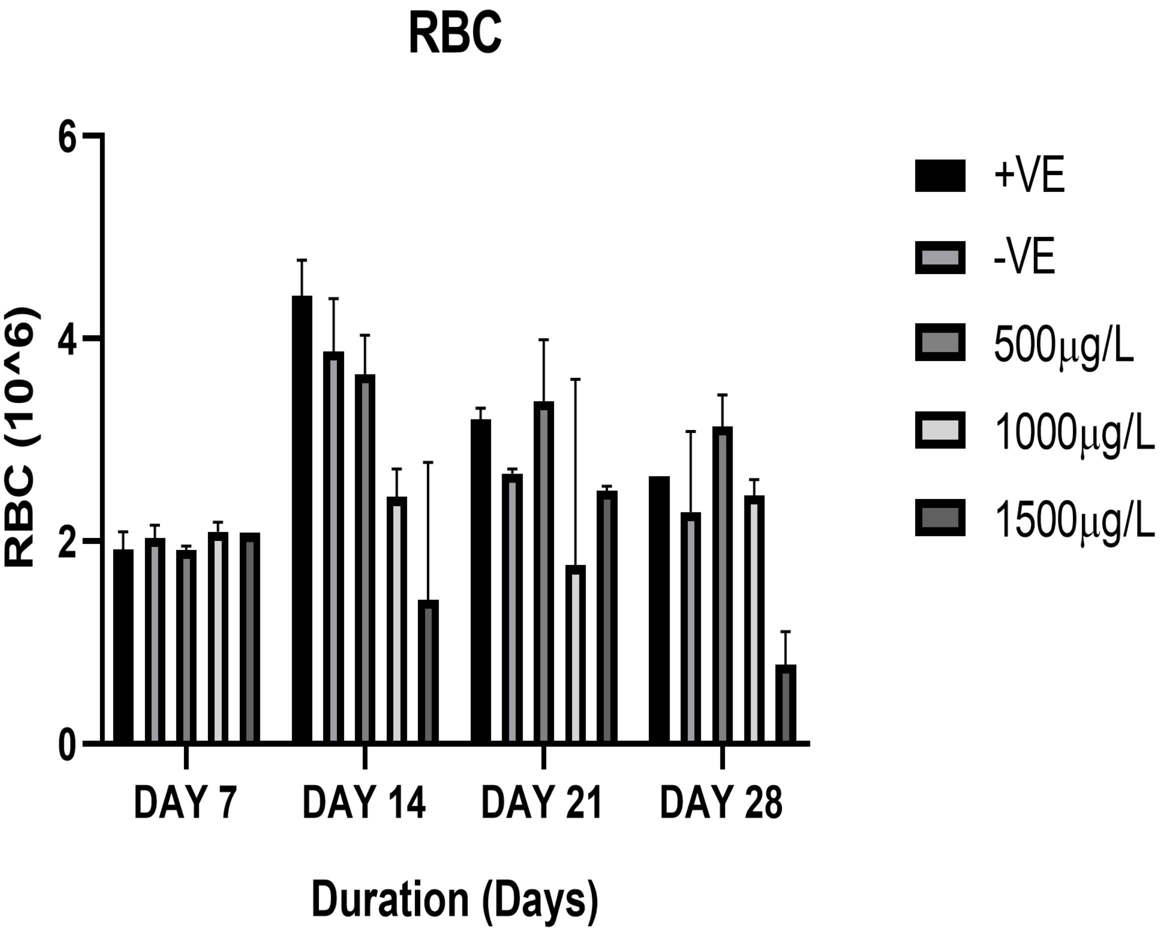


FIG 4.5: The alteration in the red blood cell as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

4.6 Hematocrit (HCT)

The mean value of HCT for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 33.00 ± 0.07, 29.55 ± 1.70, 28.90 ± 0.35, 28.85 ± 2.54 and 26.20 ± 0.00, respectively. There was no significant difference observed in all of the groups on the 7th day.

On day 14 of exposure, the mean values were: 45.40 ± 3.23, 44.75 ± 7.28, 34.40 ± 3.18, 35.60 ± 2.40, 36.35 ± 0.00 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 14th day.

On day 21 of exposure, the mean values were: 36.60 ± 1.98, 35.05 ± 1.77, 37.00 ± 1.13, 35.40 ± 8.20, 26.1 5± 0.00, for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 21st day.

On day 28 of exposure, the mean values were: 33.85 ± 3.11, 37.35 ± 0.71, 35.25 ± 3.18, 31.55 ± 3.11, 28.40 ± 0.00 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 28th day.

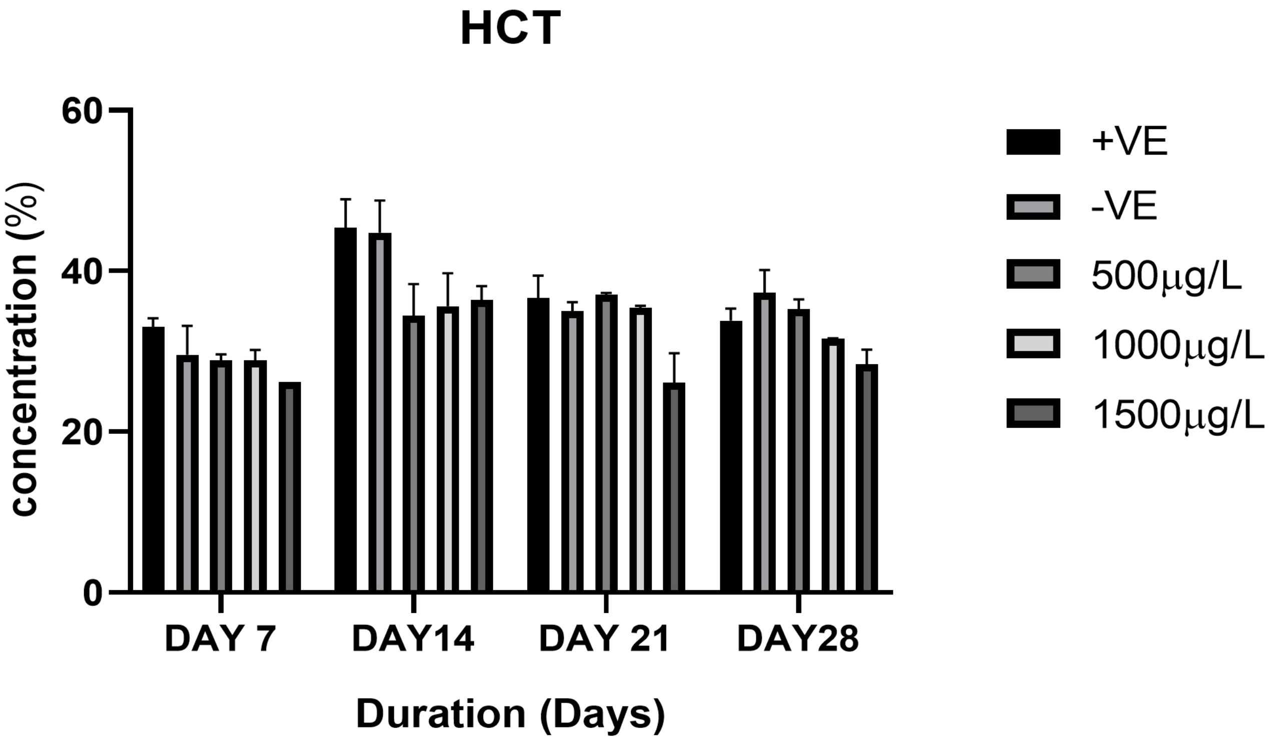


FIG 4.6: The alteration in the Hematocrit as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

4.7 Mean Corpuscular Volume (MCV)

The mean value of MCV for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 135.4 ± 4.10, 145.7 ± 8.63, 151.4 ± 0,28, 138.1 ± 0.35 and 125.9 ± 0.07, respectively. There is a significant difference between the +ve and 500µg/L, while there is no significant difference between every other control and concentrations on the 7th day.

On day 14 of exposure, the mean values were: 111.9 ± 4.46, 116.1 ± 5.23, 109.4 ± 1.41, 119.5 ± 7.14, 66.15 ± 76.7 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 14th day.

On day 21 of exposure, the mean values were: 118.5 ± 7.78, 122.5 ± 11.4, 121.8 ± 4.24, 114.8 ± 2.26, 120.2 ± 4.88, for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 21st day.

On day 28 of exposure, the mean values were: 128.5 ± 5.72, 124.0 ± 1.20, 121.1 ± 4.46, 124.1 ± 0.99, 130.7 ± 0.07 for Positive control (+ve), Negative control (-ve), 500µg/L, 1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 28th day.

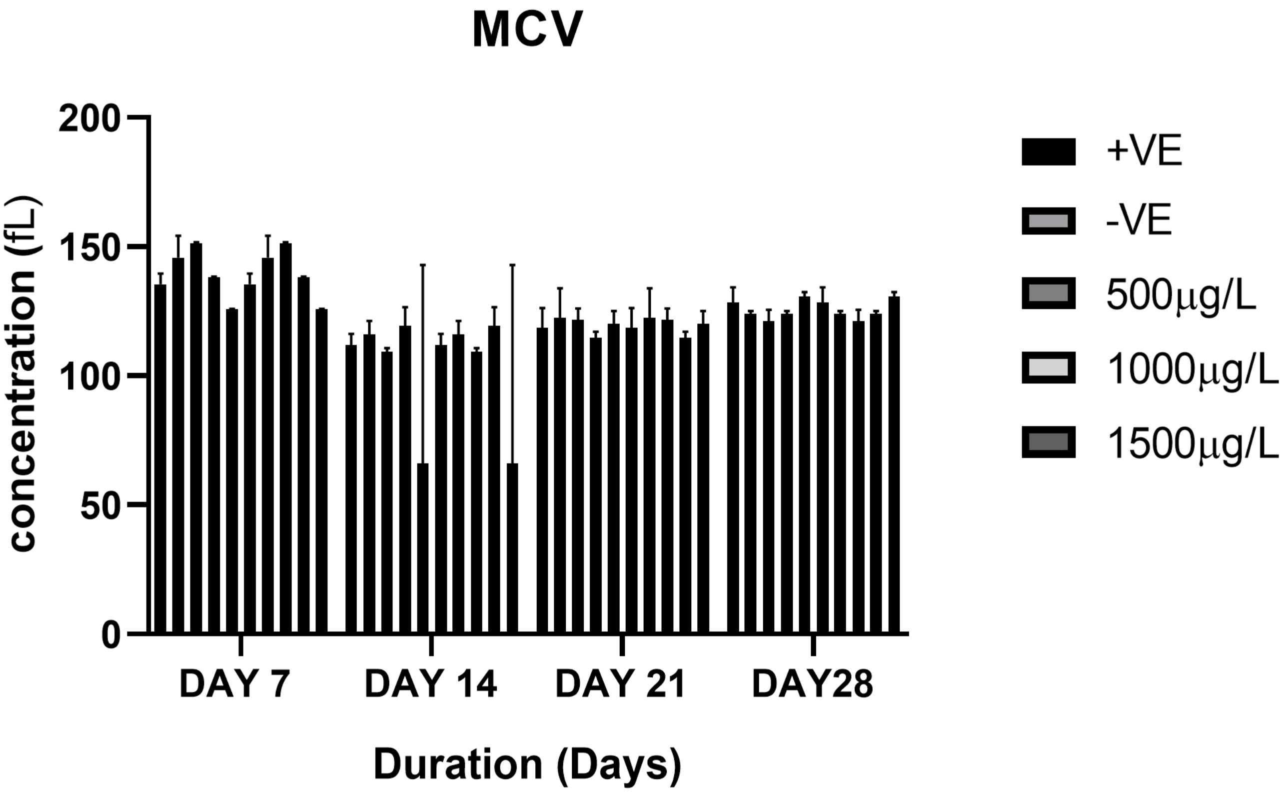


FIG 4.7: The alteration in the mean corpuscular volume as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

4.8 Mean Corpuscular Haemoglobin (MCH)

The mean value of MCH for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 52.00 ± 1.31, 52.25 ± 2.33, 52.45 ± 0.92, 50.10 ± 3.25 and 49.95 ± 0.64, respectively. There was no significant difference observed in all of the groups on the 7th day.

On day 14 of exposure, the mean values were: 40.95 ± 2.33, 45.00 ± 2.97, 41.25 ± 0.78, 42.95 ± 3.25, 46.75± 0.64 for Positive control (+ve), Negative control (-ve), 500µg/L, 1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 14th day.

On day 21 of exposure, the mean values were: 42.40 ± 0.70, 42.20 ± 2.12, 42.00 ± 0.99, 42.10 ± 1.84, 41.95 ± 1.91, for Positive control (+ve), Negative control (-ve), 500µg/L, 1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 21st day.

On day 28 of exposure, the mean values were: 42.00 ± 2.12, 35.90 ± 7.12, 43.05 ± 0.64, 44.10 ± 1.13, 41.95 ± 0.21 for Positive control (+ve), Negative control (-ve), 500µg/L, 1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 28th day.

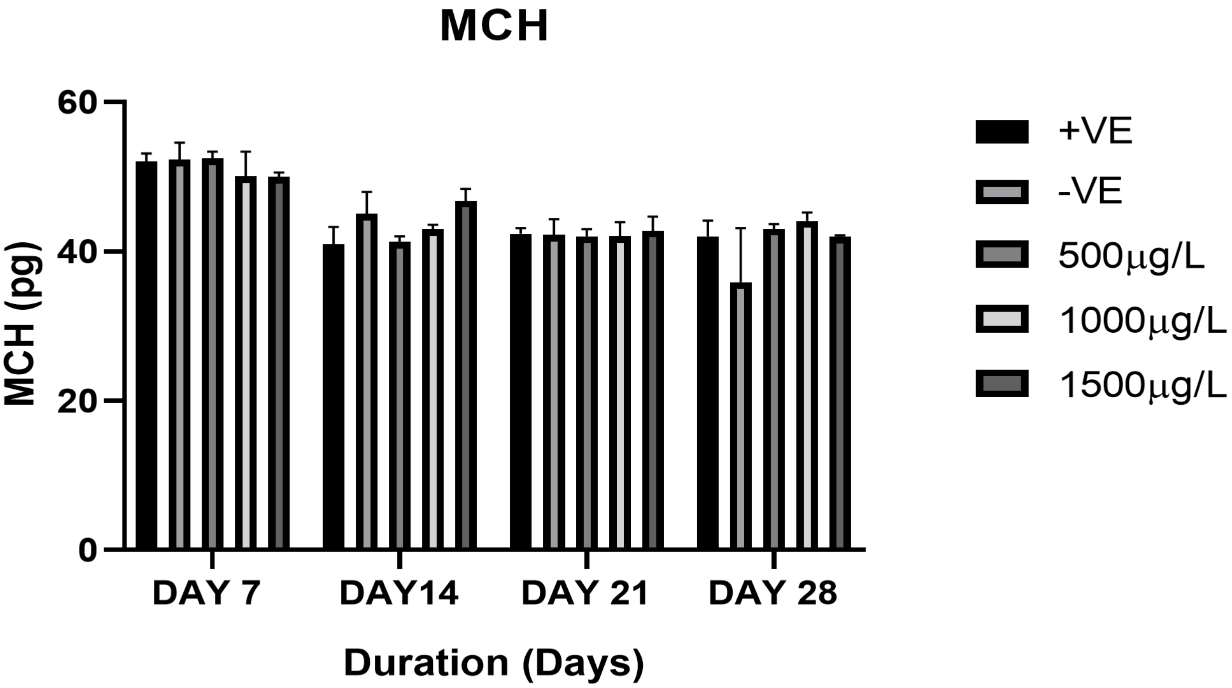


FIG 4.8: The alteration in the mean corpuscular haemoglobin as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

4.9 Mean Corpuscular Haemoglobin Concentration (MCHC)

The mean value of MCHC for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 38.45 ± 2.05, 35.95 ± 3.74, 34.65 ± 0.64, 36.35 ± 2.58 and 39.70 ± 0.42, respectively. There was no significant difference observed in all of the groups on the 7th day.

On day 14 of exposure, the mean values were: 36.65 ± 0.64, 38.75 ± 0.78, 37.75 ± 0.21, 36.05 ± 2.62, 40.50 ± 0.85 for Positive control (+ve), Negative control (-ve), 500µg/L, 1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 14th day.

On day 21 of exposure, the mean values were: 36.00 ± 2.97, 34.60 ± 1.56, 34.55 ± 2.05, 36.75 ± 2.33, 35.60 ± 0.14, for Positive control (+ve), Negative control (-ve), 500µg/L, 1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 21st day.

On day 28 of exposure, the mean values were; 32.75±0.21, 29.05±6.15, 35.65±1.91, 35.60±0.57, 32.10±0.28 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L respectively. There was no significant difference observed in all of the groups on the 28th day.

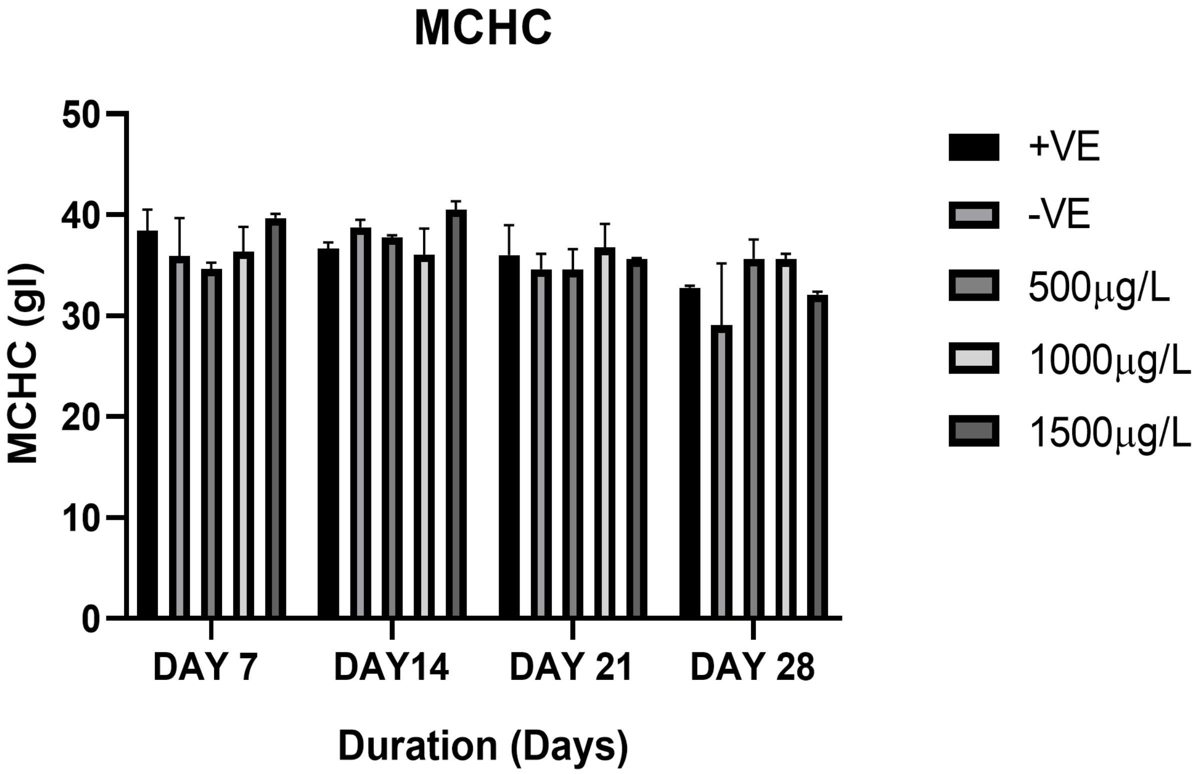


FIG 4.9: The alteration in the mean corpuscular haemoglobin concentration as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

4.10 Platelet (PLT)

The mean value of PLT for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 164.0 ± 148.5, 60.00 ± 1.41, 56.00 ± 1.41, 73.50 ± 14.85 and 168.50 ± 2.12, respectively. There was no significant difference observed in all of the groups on the 7th day.

On day 14 of exposure, the mean values were; 373.0±, 665.0±, 378.5 ±, 557.0±, 370.0± for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L respectively. There was no significant difference observed in all of the groups on the 14th day.

On day 21 of exposure, the mean values were: 272.5 ± 65.76, 249.5 ± 7.78, 313.5±7.78, 117.5 ± 65.76, 202.0 ± 14.00, for Positive control (+ve), Negative control (-ve), 500µg/L, 1,000µg/L and 1,500µg/L, respectively. There is a significant difference between the +ve control and 1,000µg/L, while there is no significant difference between the +ve control and other concentrations on the 21st day.

On day 28 of exposure, the mean values were: 54.00 ± 1.41, 89.50 ± 24.75, 146.5 ± 26.16, 131.0 ± 8.49, 116.0 ± 22.63 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There is a significant difference between the +ve control,500µg/L and 1,000µg/L respectively, while the is no significant difference between the +ve and -ve control and +ve control and 1,500µg/L on the 28th day.

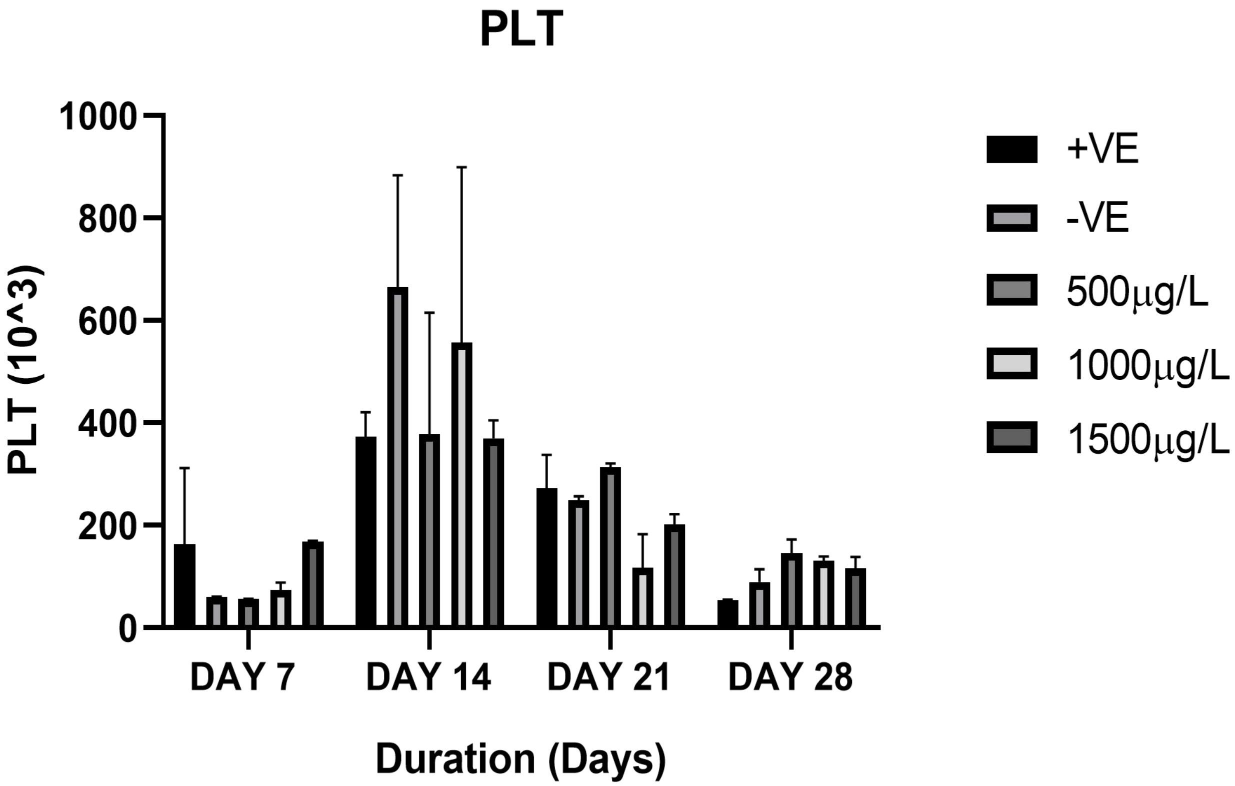


FIG 4.10: The alteration in the platelet as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

4.11 Plate Volume Distribution width (PDW)

The mean value of PDW for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 17.70 ± 0.14, 18.15 ± 0.21, 18.45 ± 0.21, 18.40 ± 0.21 and 18.40 ± 2.12, respectively. There is a significant difference between the +ve control and 500µg/L, while there is no significant difference between the other control and the concentration on the 7th day.

On day 14 of exposure, the mean values were: 9.350 ± 1.63, 10.650 ± 3.47, 7.650 ± 0.35, 8.950 ± 1.77, 7.000 ± 0.14 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 14th day.

On day 21 of exposure, the mean values were: 7.250 ± 1.63, 7.650 ± 0.78, 7.800 ± 0.14, 9.200 ± 3.68, 7.500 ± 0.57, for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 21st day.

On day 28 of exposure, the mean values were: 6.150 ± 0.35, 8.050 ± 2.05, 7.400 ± 0.42, 6.900 ± 0.70, 8.050 ± 2.05 for Positive control (+ve), Negative control (-ve), 500µg/L, 1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 28th day.

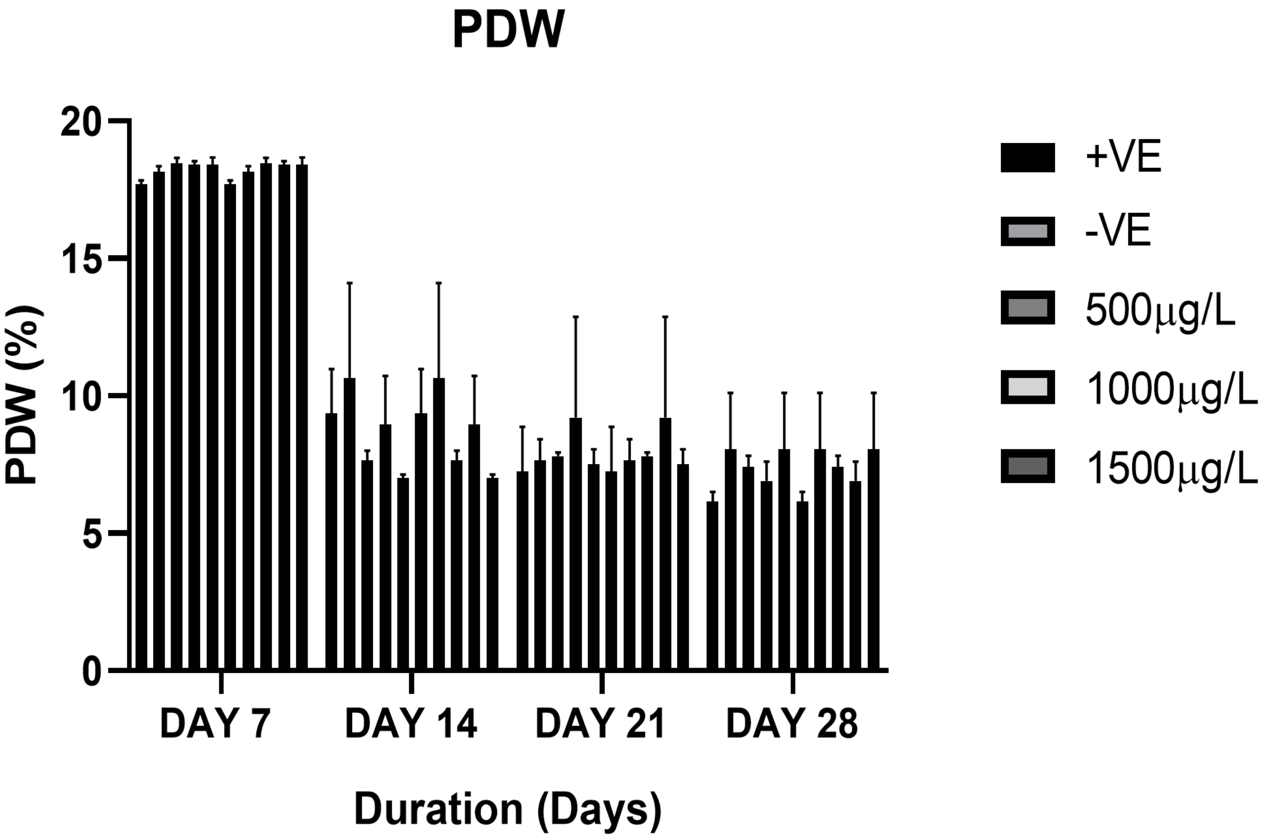


FIG 4.11: The alteration in the plate volume distribution width as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

4.12 Plateletcrit (PCT)

The mean value of PCT for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 1.680 ± 1.49, 0.7300 ± 0.03, 0.6950 ± 0.02, 0.8700 ± 0.18and 1.880 ± 0.03, respectively. There was no significant difference observed in all of the groups on the 7th day.

On day 14 of exposure, the mean values were: 0.3000 ± 0.07, 0.5800 ± 0.28, 0.2900 ± 0.17, 0.4450 ± 0.29, 0.2500 ± 0.01 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 14th day.

On day 21 of exposure, the mean values were: 0.2200 ± 0.08, 0.2100 ± 0.00, 0.2750 ± 0.00, 0.1000 ± 0.04, 0.1650 ± 0.03, for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 21st day.

On day 28 of exposure, the mean values were: 0.03000 ± 0.00, 0.08000 ± 0.04, 0.1250 ± 0,02, 0.1100 ± 0.01, 0.04500 ± 0.04 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There is a significant difference between the +ve control and 500µg/L, while there is no significance between the other control and concentration on the 28th day.

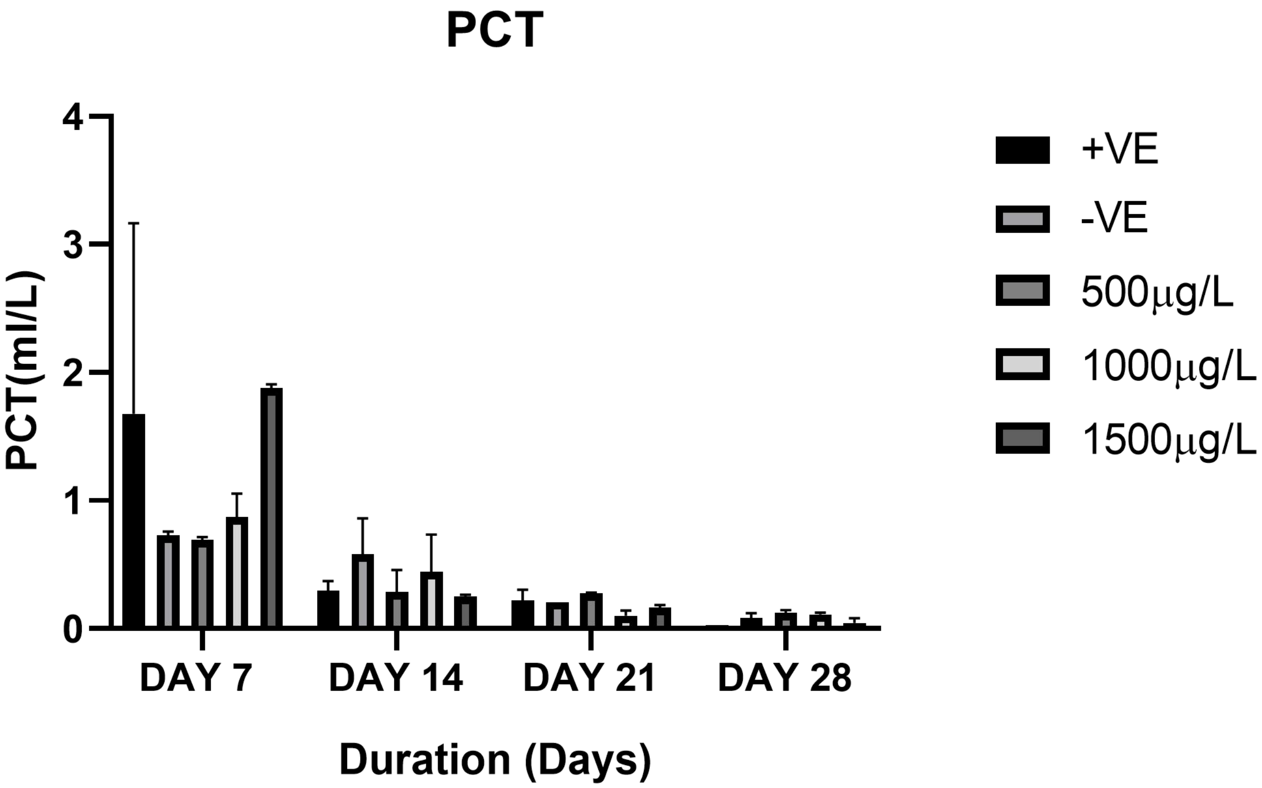


FIG 4.12: The alteration in the Plateletcrit as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

4.13 Platelet Large Cell Ratio P-LCR

The mean value of P-LRC for catfish exposed to Positive control (+ve) 0.000µg/L, Negative control± (-VE) 200µg/L (DMSO), 500µg/L (6PPd-Q), 1,000µg/L (6PPD-Q), 1,500µg/L (6PPD-Q) after 7 days of exposure are 35.05 ± 2.33, 43.65 ± 1.91, 45.00 ± 1.13, 42.30 ± 0.00 and 37.25 ± 0.78, respectively. There is a significant difference between the +ve and -ve control, +ve control and 500µg/L and a little difference between the +ve control and 1,000µg/L, while there is no significant difference between the +ve control and 1,500µg/L on the 7th day.

On day 14 of exposure, the mean values were: 10.40 ± 5.23, 12.80 ± 8.63, 9.700 ± 2.69, 9.250 ± 0.21, 4.200 ± 1.68 for Positive control (+ve), Negative control (-ve), 500µg/L, 1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 14th day.

On day 21 of exposure, the mean values were: 7.800 ± 1.27, 15.50 ± 0.42, 16.25 ± 0.35, 17.55 ± 10.82, 13.70 ± 1.41, for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 21st day.

On day 28 of exposure, the mean values were: 1.900 ± 2.40, 15.95 ± 17.75, 15.10 ± 1.83, 12.95 ± 2.05, 13.10 ± 5.23 for Positive control (+ve), Negative control (-ve), 500µg/L,1,000µg/L and 1,500µg/L, respectively. There was no significant difference observed in all of the groups on the 28th day.

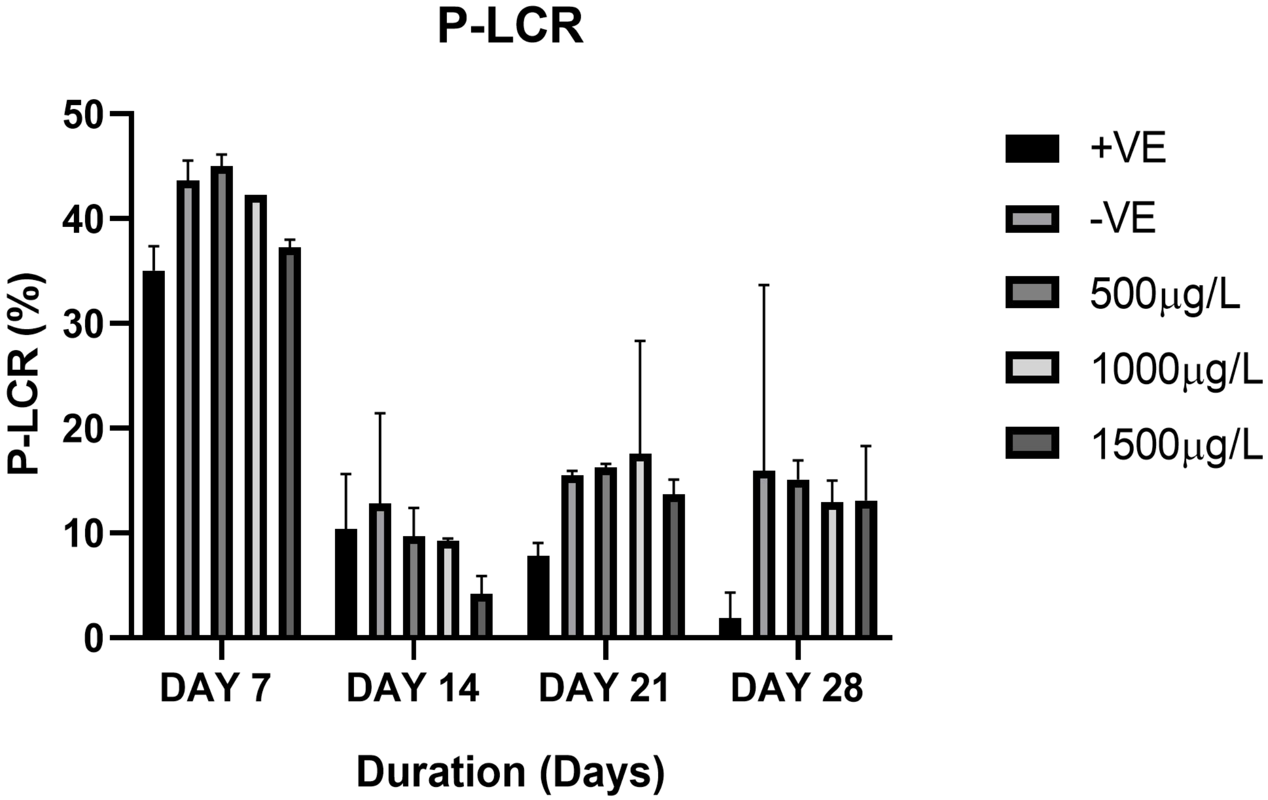


FIG 4.13: The alteration in the Platelet Large Cell Ratio as a result of exposure to 6PPDQ in juvenile catfish from day 7-28. Data are expressed as mean ± SD(n=7).

CHAPTER FIVE

DISCUSSSION

Haematological analysis is one of the tools commonly used to evaluate the health and welfare of fish, both under aquaculture conditions and in scientific studies, to assess the influence of environmental factors on fish (Rohani, 2023). The objectives of the study lay emphasis on how varying concentrations of 6PPD-Q influence haematological parameters such as red and white blood cell counts, haemoglobin concentration, hematocrit, and platelet indices.

It investigated the haematological responses of *Clarias gariepinus* exposed to sub-lethal concentrations of 6PPD-quinone (6PPD-Q), a transformation product of the tyre antioxidant 6PPD, over 28 days. Exposure to 6PPD-Q elicited measurable physiological and haematological alterations, even in the absence of acute mortality. It also expresses the compounds' sub-lethal toxicity and their potential ecological implications in freshwater environments contaminated by tire-derived pollutants.

White Blood Cell (WBC) and Lymphocyte Responses

The observed fluctuations in white blood cell (WBC) counts and lymphocyte levels across exposure durations indicate an immune-modulatory effect of 6PPD-Q. At the initial exposure period (day 7), elevated WBC counts in exposed groups compared to controls suggest an acute immune activation or inflammatory response, likely triggered by oxidative stress or cellular injury. However, subsequent reductions in WBC levels by day 14 and 21 may reflect immune exhaustion or suppression resulting from prolonged exposure. This biphasic response aligns with earlier studies on oxidative contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals, which induce leukocytosis followed by lymphopenia during sustained exposure (Sahoo *et al*., 2023; Chen *et al*., 2023).

Lymphocyte percentages also fluctuated throughout the experiment, with initial increases followed by reductions at higher concentrations (1,000–1,500 µg/L). The decline in lymphocyte counts during the later exposure stages suggests possible immunosuppression and impaired hematopoiesis. Similar trends have been documented in fish exposed to cadmium, lead, and benzene derivatives, where oxidative damage disrupts immune cell proliferation and functionality (Hiki and Yamamoto, 2021). Thus, the haematological data imply that 6PPD-Q compromises the immune competence of *Clarias gariepinus* in a dose- and time-dependent manner.

Red Blood Cell (RBC), Haemoglobin (HGB), and Hematocrit

Significant alterations in red blood cell indices were recorded with increasing exposure duration and concentration. A progressive decline in RBC, haemoglobin, and hematocrit values from day 7 to day 28 in higher treatment groups indicates the development of anaemia and impaired oxygen transport. These changes can be attributed to oxidative degradation of erythrocyte membranes, inhibition of erythropoiesis, or direct interaction of 6PPD-Q with haemoglobin molecules. Quinone compounds, including 6PPD-Q, are known to generate reactive oxygen species (ROS) that induce lipid peroxidation and hemolysis (Liao *et al*., 2024).

Comparatively, similar haematological depressions have been reported in fish exposed to other organic pollutants such as bisphenol A (BPA), petroleum hydrocarbons, and organophosphates, all of which impair erythrocyte stability and oxygen-carrying capacity (Cao *et al*., 2022). However, the extent of reduction observed in *Clarias gariepinus* under 6PPD-Q exposure suggests that this tire-derived compound exerts comparable or even greater sub-lethal stress than traditional pollutants at environmentally relevant concentrations. The gradual reduction in hematocrit and haemoglobin over time may also indicate compromised gill function, leading to reduced oxygen uptake, as previously observed in oxidative stress-related toxicities (Lo *et al*., 2023).

Erythrocyte indices (MCV, MCH, and MCHC)

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) showed variable trends across the experimental period. These fluctuations are indicative of haematological adjustments to maintain oxygen transport under stress conditions. Decreased MCV and MCH values recorded in higher concentrations suggest the onset of microcytic, hypochromic anaemia, commonly associated with toxicant-induced inhibition of haemoglobin synthesis. The reduction in MCHC at later stages further supports impaired erythropoietic activity and possible cellular dehydration.

These responses are consistent with those reported in fish exposed to pollutants such as nickel, zinc, and benzene derivatives, where alterations in erythrocyte indices were linked to hematopoietic disruption and oxidative stress (Brinkmann *et al*., 2022; Chen *et al*., 2023). Therefore, 6PPD-Q may disrupt iron metabolism and red cell integrity similarly to these conventional pollutants, reinforcing its classification as a potent oxidative toxicant.

Platelet Parameters (PLT, PDW, PCT and P-LCR)

Platelet counts and associated indices exhibited irregular patterns throughout the exposure period. Initial elevations in platelet counts at lower concentrations may reflect a physiological attempt to compensate for vascular injury or hemolysis. However, the eventual decline in platelet number and volume distribution (PDW and PCT) at higher concentrations and longer durations suggests thrombocytopenia and impaired blood coagulation. Such haematological disruptions are often indicative of cytotoxic effects on bone marrow or interference with thrombopoietic mechanisms (Johannessen *et al*., 2022).

When compared to other environmental contaminants, such as lead and organophosphate pesticides, the pattern observed here is consistent with pollutant-induced thrombocytopenia resulting from oxidative and enzymatic inhibition. Hence, 6PPD-Q, like these toxicants, can impair hemostatic balance and increase vulnerability to hemorrhagic conditions in fish.

Comparative Toxicological Insights

When compared with other environmental pollutants, 6PPD-Q demonstrates toxicity mechanisms that parallel those of several well-documented xenobiotics but at significantly lower concentrations. For example, while heavy metals like cadmium and lead induce haematological disturbances at milligram-per-litre levels, 6PPD-Q triggers comparable sub-lethal effects at microgram-per-litre levels, underscoring its high potency. Similarly, PAHs and phenolic compounds elicit oxidative stress and anaemia through ROS generation, yet the persistence and bioavailability of 6PPD-Q in aquatic systems make its effects more sustained and potentially cumulative (Li *et al*., 2023).

Compared to endocrine-disrupting compounds such as BPA, 6PPD-Q acts more through oxidative and mitochondrial pathways than hormonal interference, although both lead to similar systemic imbalances. Furthermore, 6PPD-Q’s persistence in sediments and water columns suggests prolonged ecological risk, surpassing the transient exposure profiles of some volatile pollutants. Thus, it can be regarded as an emerging contaminant of equal or greater concern relative to traditional pollutants in aquatic environments.

Ecological and Physiological Implications

The haematological responses observed in this study reflect broader physiological stress that may impair growth, reproduction, and survival in *Clarias gariepinus* populations inhabiting contaminated waters. Prolonged anaemia and immunosuppression can weaken resistance to pathogens, while disrupted oxygen transport and coagulation can hinder metabolic efficiency. These sub-lethal effects, although not immediately fatal, can cumulatively lead to population declines and ecological imbalances. Given that *Clarias gariepinus* serves as both a key aquaculture species and an ecological indicator, these findings underscore the urgent need for environmental monitoring of tire-derived contaminants. The consistent haematological trends observed across parameters confirm that even moderate 6PPD-Q concentrations can significantly affect fish health, warranting stricter environmental regulation and further ecotoxicological investigations.

Conclusion

This project work shows that sub-lethal exposure to 6PPD-quinone significantly alters the haematological parameters of *Clarias gariepinus*, reflecting physiological stress and potential health impairment. The compound induced marked reductions in red blood cell count, haemoglobin, and hematocrit, alongside fluctuations in leukocyte and platelet indices, indicative of anaemia, immunomodulation, and coagulatory dysfunction. These effects were concentration- and time-dependent, becoming more pronounced with prolonged exposure.

There is a correlation with previous reports on oxidative pollutants, confirming that 6PPD-Q exerts its toxicity through mechanisms involving oxidative stress, membrane lipid peroxidation, and interference with hematopoietic functions. When compared with conventional pollutants such as heavy metals and hydrocarbons, 6PPD-Q demonstrates equal or greater toxicity at substantially lower concentrations, emphasising its environmental significance as an emerging contaminant.

Haematological responses of *Clarias gariepinus* suggest that 6PPD-Q, even at sub-lethal doses, poses a serious threat to fish health and aquatic ecosystem stability. Continuous release of this compound from tyre wear particles into waterways could contribute to long-term ecological degradation. Therefore, environmental monitoring programs should incorporate 6PPD-Q as a priority contaminant, and further research should explore its chronic and reproductive effects on tropical freshwater species. The findings from this study contribute valuable baseline data for ecological risk assessment and support the development of policies aimed at reducing tire-derived pollution in aquatic environments.

REFERENCES

Brinkmann, M., Eichhorn, A., Bahr, T., Steffen, M., Diogo, J., Wright, E., Ignell, R., Hammers-Wirtz, M., Heise, S., Allendorf, F., Gaus, M. und Nowack, B. (2022). Toxicity of tyre wear particle-derived 6PPD-quinone to various aquatic species. *Environmental Toxicology, 37(5), 845-860.*

Brinkmann, M., Kolodziej, E.P., Tian, Z., McIntyre, J.K., Truong, L., Reiner, J.L. and Peter, K.T. (2022) '6PPD-quinone: An emerging toxicant from tyre wear particles affecting aquatic life. *Environmental Science and Technology, 56(1),* 562–572.

Cao, X., Wang, C., Yang, L., Zhang, Y. and Li, J. (2022). Environmental occurrence and fate of 6PPD-quinone in urban runoff and surface waters. *Chemosphere, 297,* 133–145.

Cao, X., Zhang, Z., Chen, Y., and Li, W. (2022). Distribution and persistence of 6PPD-quinone in urban environments. *Journal of Environmental Science, 115,* 130-140.

Chen, L., Xu, Y., Li, Y. and Zhao, H. (2023). Sub-lethal oxidative stress and biochemical responses of zebrafish (Danio rerio) exposed to 6PPD-quinone. *Aquatic Toxicology, 258,* 106–121.

Chen, Y., Zhang, Z., Wang, Q., and Li, W. (2023). Oxidative stress responses in zebrafish exposed to 6PPD-quinone. *Aquatic Toxicology, 250*,106229.

Hastuti, Y.P., Sudaryanto, A. and Nugroho, D. (2019) 'Feeding habits and habitat adaptability of *Clarias gariepinus* in tropical freshwater environments. *Journal of Fisheries and Aquatic Studies, 7(2)*, 45–51.

Hiki, K. and Yamamoto, H. (2021) ‘Comparative sensitivity of fish and invertebrates to 6PPD-quinone and other tire-derived quinones. *Environmental Pollution,* 289,117–135.

Hiki, K. and Yamamoto, T. (2021) 'Species-specific tolerance to 6PPD-quinone toxicity in aquatic organisms. *Toxicology Reports, 8,* 1007-1014.

Johannessen, C., Helm, P.A., Metcalfe, C.D. and Smyth, S.A. (2022). Detection and distribution of 6PPD-quinone in urban stormwater and implications for aquatic ecosystems. *Water Research, 219,* 118–138.

Johannessen, S.C., Lappi, S., Brinkmann, M., and Nowack, B. (2022). Environmental presence and effects of tyre wear contaminants. *Science of the Total Environment, 811,* 152332.

Kolodziej, E.P., Grey, J.L., Sun, X., Pecot, M., and Serafim, A. (2021) 'Occurrence and toxicity of tire-derived compounds including 6PPD-quinone in urban stormwater. *Environmental Science and Technology, 55(2),* 1314-1323.

Kolodziej, E.P., Tian, Z. and McIntyre, J.K. (2021). Tire wear particles and 6PPD-quinone: A global concern for aquatic toxicity. *Science of the Total Environment*, 806, 150–170.

Kreider, M.L., Panko, J.M., McAtee, B.L., Sweet, L.I. and Finley, B.L. (2010). Physical and chemical characterisation of tire-related particles. *Journal of the Air and Waste Management Association, 60(9),* 1169–1179.

Li, W., Chen, Y., Wang, Q., and Zhang, Z. (2023). Photolytic degradation pathways and persistence of 6PPD-quinone in aquatic systems. *Chemosphere, 324,* 138080.

Li, Y., Chen, L. and Xu, Y. (2023). Phototransformation and persistence of 6PPD and 6PPD-quinone in surface water. *Environmental Chemistry Letters, 21(3),* 1553–1567.

Liao, C., Wang, Z. and Du, J. (2024) 'Neurotoxicity and bioaccumulation of 6PPD-quinone in fish: Mechanistic insights and risk implications. *Environmental Toxicology and Chemistry, 43(2),* 435–449.

Liao, V.H.C., Yang, C., and Zhang, Z. (2024). Mechanisms of neurotoxicity and oxidative damage induced by 6PPD-quinone in fish species. *Ecotoxicology and Environmental Safety, 255,* 114652.

Lo, C., Brinkmann, M. and Peter, K.T. (2023) 'Histopathological and mitochondrial responses in salmonids exposed to 6PPD-quinone. E*nvironmental Research, 232,* 116–128.

Lo, S.F., Yuan, T., and Chen, Y. (2023). Mitochondrial dysfunction from 6PPD-quinone exposure in salmonids. *Aquatic Toxicology, 255,* 106680..

Mohammed, A.A. OECD *Guidelines for Fish Sublethal Toxicity Testing, Organisation for Economic Cooperation and Development.*

Nguyen, L.T. and Janssen, C.R. (2002) 'Distribution, habitat use and adaptability of African catfish (*Clarias gariepinus*). *African Journal of Aquatic Science, 27(1),* 1–10.

OECD (2019) Guidelines for Testing of Chemicals – *Section 203: Fish, Acute Toxicity Test. Organisation for Economic Co-operation and Development, Paris.*

OECD (2019) Test No. 203: Fish, Acute Toxicity Test. OECD Guidelines for the Testing of Chemicals. Paris: *Organisation for Economic Co-operation and Development*.

Ogueji, C.O., Okorie, M.U., and Eze, C.C. (2018) 'Ecology and distribution of *Clarias gariepinus* in tropical freshwater ecosystems. *Journal of Fish Biology, 93(4),* 879-896.

Ogueji, E.O., Adebayo, I.A. and Eze, C.V. (2018). Growth performance and haematological profile of *Clarias gariepinus* exposed to sub-lethal concentrations of pollutants. *African Journal of Environmental Science and Technology, 12(4),* 145–152.

Organisation for Economic Co-operation and Development (OECD) (2019). OECD Guidelines for the Testing of Chemicals, *Section 203: Fish, Acute Toxicity Test. OECD Publishing, Paris.*

Sahoo, P.K., Mishra, B.K. and Behera, B.K. (2023). Haematological and physiological biomarkers in *Clarias gariepinus* under pollutant stress. *Journal of Applied Aquaculture, 35(2),* 210–224.

Sahoo, P.K., Singh, V., and Kumar, R. (2023) 'Haematological responses of *Clarias gariepinus* to sub-lethal exposure of 6PPD-quinone. *Environmental Pollution, 311,* 120028.

Tian, Z., Zhang, Q., and Wang, J. (2021) '*Identification of 6PPD-quinone as a toxic transformation product in aquatic environments', Nature*, *596,* 467-471.

Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C. and Kolodziej, E.P. (2021). *A ubiquitous tyre rubber-derived chemical induces acute mortality in coho salmon, Science*, *371(6525)*, 185–189.

United States Environmental Protection Agency (USEPA) (2002). Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (5th ed.). EPA-821-R-02-012. U.S. *Environmental Protection Agency, Washington, D.C.*

Wang, C., Zhang, Y. and Cao, X. (2022). Atmospheric transport and particulate occurrence of 6PPD-quinone and related compounds. *Environmental Pollution*, *306*, 119–142.

Wang, Q., Li, W., and Chen, Y. (2022) 'Atmospheric distribution and chemical properties of quinone derivatives including 6PPD-quinone. *Atmospheric Environment*, *279,* 119157.

Zhang, Y., Wang, C., Li, J. and Cao, X. (2022) 'Occurrence of tire-derived 6PPD-quinone in road dust and urban environments. *Journal of Hazardous Materials*, *433,* 128–141.

Zhang, Z., Cao, X., and Li, W. (2022). Widespread contamination by 6PPD-quinone in urban dust, runoff, and water systems. *Environmental Pollution*, *292*, 118132.

Zhang, Z., Chen, Y., and Li, W. (2022). Haematological alterations in fish exposed to quinone compounds and related oxidative stress. *Ecotoxicology, 31(6),* 876-888.

Zhang, Z., Wang, Q., and Chen, Y. (2022). Chronic toxic effects of 6PPD-quinone on aquatic organisms. *Journal of Hazardous Materials, 444,* 130350.