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Effect of Chlorpyrifos on the Red Blood Cells Morphology of Common Carp (*Cyprinus Carpio*): A Hematological Study

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ABSTRACT

Chlorpyrifos, a widely applied insecticide, enters waterways through runoff, affecting a wide range of aquatic species. The environmental impact of chlorpyrifos (CPF), a commonly used organophosphate pesticide, on aquatic organisms remains a significant concern due to its toxicity and persistence in ecosystems. This study evaluates the effects of CPF on the morphology of red blood cells (RBCs) in the common carp (Cyprinus carpio) to better understand its hematological toxicity. Common Carp were exposed to sublethal concentrations of CPF (0, 10, 20, and 30 µL) for duration of seven days. Blood samples were collected and examined for alterations in RBC morphology. Control specimens showed minimal morphological changes which were due to natural conditions, while CPF exposure induced a concentration-dependent increase in cellular abnormalities, including double nucleated cells, nuclear displacement, cell deformation, and fragmentation. The highest CPF concentration (30 µL) resulted in severe RBC damage, including nuclear pyknosis, loss of cell integrity, and necrosis. These findings suggest that CPF exposure leads to significant erythrocyte damage, which may compromise overall fish health and survival. The results underscore the need for stringent environmental monitoring and regulation of pesticide use to mitigate its detrimental effects on aquatic life.

INTRODUCTION

There has been growing alarm that natural aquatic ecosystems are facing devastating ecological effects (Ullah et al., 2023) due to the widespread use and dumping of chemicals such as pesticides, which can contribute to short-term behavioral disparity to long-term genetic abnormalities in the aquatic populations. Currently, different types of pesticides such as organochlorines, organophosphates, carbonates, synthetic pyrethroids, and other natural products are broadly applied to control agricultural pests (Tiwari, 2014). Chlorpyrifos (CPF), IUPAC name for Chlorpyrifos is O, O-diethyl-O-(3,5,6-trichlor-2pyridyl) phosphorothioate, an organophosphate introduced in 1965, is widely used against various agricultural pests. It is the second largest selling organophosphate and has been found to be more toxic to fish than organochlorines compounds. Its environmental persistence raises concerns, with detection in global water bodies contributing to pollution and affecting non-target aquatic organisms (Samsum et al., 2005; Cui et al., 2023). In aquatic ecosystems, CPF concentrations can range from nanogram per liter (ng/L) to microgram per liter (µg/L), depending on the level of contamination and agricultural runoff (e.g.,

concentrations up-to 50 µg/L has been detected in some water bodies) (Kegley et al., 2015). These concentrations have been shown to affect fish species, even at low levels, resulting in significant toxicity and behavioral changes. Extensive studies have documented both acute and chronic toxic effects of CPF on different fish species (Anita et al., 2016).

Significant changes in histopathology, hematology, biochemical parameters, and the immune profile of fish act as important biomarkers in toxicological studies particularly pesticides toxicity (Vali et al., 2022). Fish blood cells are generally similar in function to their mammalian and are found in all other tissues and organs throughout the body (Ilyas et al., 2023). Blood carries various kinds of substances (gases, water, minerals, nutrients, hormones, immune effectors, toxins, microbial structures, or waste products), so its study can give complete report about fish physiology and physical condition. Changes in blood-based indicators like metabolite concentrations, hormonal profiles, and transcript abundances can reflect systemic reactions to variations or disturbances in homeostasis that can alert scientists and veterinarians (as well as fish farmers), who monitor the physical status of an individual fish or an entire population. Blood examinations on fish have been carried out for decades (Shen et al., 2018).

Moreover, chlorpyrifos (CPF) exposure during development leads to neurobehavioral dysfunction in zebrafish, even at low does that do not cause acute toxicity. Research shows that early exposure results in persistent deficits in swimming behaviors and discriminate learning, indicating critical molecular impact (Levin et al., 2004; Linney et al., 2003). Additionally, CPF induces long-term alteration in sensory motor and stress responses, alongside decreased brain activity (Sledge et al., 2011; Eddins et al., 2010). Behavioral studies demonstrate that CPF exposure can trigger hostile behavior and erratic swimming patterns in fish, reflecting significant nervous system effects (Ali et al., 2009).

In previous studies on *Channa punctatus*, exposure to chlorpyrifos (CPF) resulted in a concentration-dependent increase in DNA single-strand breaks, followed by a time-dependent decrease in damage attributed to DNA repair mechanisms (Banu et al., 2001). Although a decrease in DNA damage was noted across various CPF concentrations, the relationship was non-linear, potentially indicating either DNA repair, loss of heavily damaged cells, or both (Rank et al., 2003). This study aimed to evaluate the impact of chlorpyrifos (CPF) on the morphology of red blood cells (RBCs) in common carp (*Cyprinus carpio*). By examining potential alterations in RBC structure, the research sought to shed light on the hematological effects of CPF exposure in aquatic species, offering insights into how pesticide contamination may affect fish health. The study compared the RBCs of CPF-exposed fish to those of a control group, emphasizing the broader implications of CPF exposure on the physiological well-being of freshwater organisms.

MATERIALS AND METHODS

Collection and acclimatization of the experimental Animal

Common carp (*Cyprinus carpio*) were collected from Sardaryab on February 26, 2023, using a casting net, with assistance from an experienced colleague. 30 carp were caught, placed in water tank, and transported to the laboratory of department of zoology at University of Peshawar in a healthy, live condition. Fish identification followed standard keys based on characteristics including body color (greyish to bronze), dorsal fin anatomy (3-4spines and 17-23 soft rays), anal fin anatomy (2-3 spines and 5-6 soft rays), and vertebrae count (36-37). On February 27, the fish were placed in water tanks, and a twice-daily feeding schedule was initiated (Morning and Evening) to ensure proper acclimation. The fish were maintained under laboratory conditions for seven days, with consistent food supply, aeration, and temperature regulation. Daily cleanliness of the tanks was also observed to support optimal conditions.

Experimental design

A total of 30 fish were included in this study, housed across four tanks with different conditions (Table 1):

Control Group: contained seven fish, maintained under normal conditions without chlorpyrifos exposure.

Experimental Group 1: contained seven fish, exposed to 10 µL of chlorpyrifos.

Experimental Group 2: contained eight fish, exposed to 20 µL of chlorpyrifos.

Experimental Group 3: contained eight fish, exposed to 30 µL of chlorpyrifos.

After the acclimation period, chlorpyrifos was introduced to experimental groups using a pipette. As chlorpyrifos has high toxicity, a minimal dosage was carefully selected to assess its physiological effects on fish.

Table 1
Dosages for Different Treatment Units

Groups	Number of Fish	Dose of Chlorpyrifos (µL)
Control group	7	0 µL (no exposure)
(Experimental Group 1)	7	10 µL
(Experimental Group 2)	8	20 µL
(Experimental Group 3)	8	30 µL

Collection of Blood Sample

After a seven-day exposure period, blood samples (2 mL per fish) were drawn from the caudal vein using a sterile 3 mL syringe with a 23-gauge needle. Some fish experienced mortality due to chlorpyrifos absorption, while others survived. Blood samples were drawn immediately transferred to EDTA tubes to prevent clotting. Blood smears were prepared by placing a drop of blood on slides and spreading it to create a thin smear, which was air- dried for thirty minutes. Ethanol was then applied to harden the blood smears. Eight slides were prepared in total, with two slides allocated per experimental group. These slides were analyzed under a compound fluorescent microscope in the Department of Biotechnology at University of Peshawar. Fluorescent microscopy settings were optimized to observe cellular responses, assessing morphological changes possibly induced by chlorpyrifos exposure.

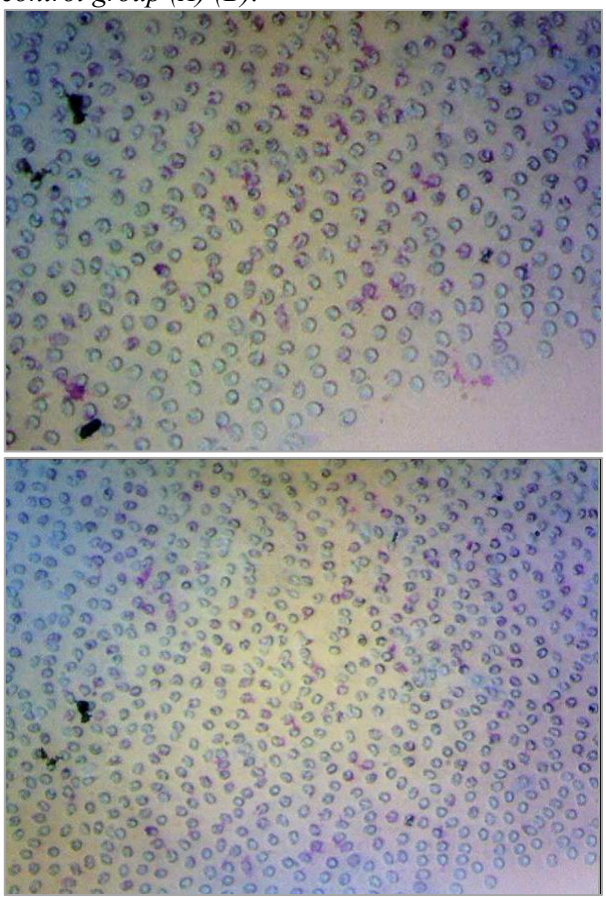
RESULTS

The experimental work was performed which included the determination or the effects of chlorpyrifos on the morphology of the common carps red blood cells of common carp.

Control Group

Control group showed no significant morphological changes were observed in the red blood cells (RBCs). Minor damage was noted in some cells, likely due to natural factors such as cell lifespan or immune activity, rather than chemical exposure. In one slide with approximately 459 cells, 370 were single-nucleated, 25 double-nucleated, 11 exhibited nuclear shifts, 30 had irregular nuclei, and 23 were deformed. A second slide analysis of 509 cells revealed similar results, with 432 single-nucleated, 28 double-nucleated, 11 showing nuclear shifts, 22 irregular nuclei, and 18 deformed cells. These observations suggest that these morphological changes were due to natural factors rather than chlorpyrifos exposure.

Figure 1
Fluorescence microscopy of red blood cells of control group (A) (B).



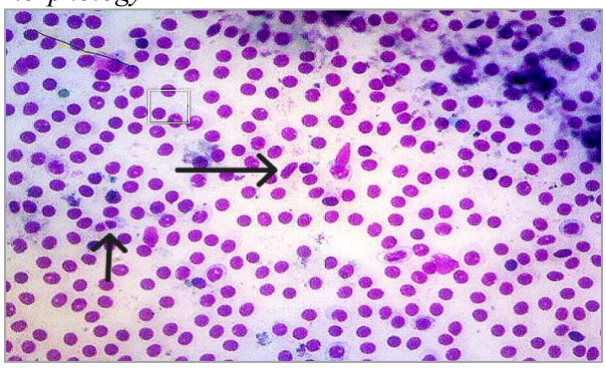
Experimental Group 1: Low Chlorpyrifos Exposure

The effects on red blood cells (RBCs) in Experimental Group 1, which was exposed to low levels of chlorpyrifos (CPF), were examined using 784 RBCs. The morphological distribution was as follows: There were 546 single nucleated cells, 51 double nucleated cells, 85 nuclear shift cells, 50

irregular nucleated cells, and 63 malformed cells. The low CPF concentration (10 µL) resulted in mild morphological changes in RBCs. The number of deformed and abnormal cells increased somewhat as compared to the predicted baseline, but the general distribution of abnormalities remained largely consistent. Furthermore, the appearance of debris on the slides, presumably owing to contamination, indicates that minor fluctuations in the effects of CPF may occur between observations. Despite these small differences, the data suggest that low-level CPF exposure results in relatively minimal morphological changes in RBCs (Fig. 2).

Figure 2

Red Blood Cells of Group1 showing abnormal morphology

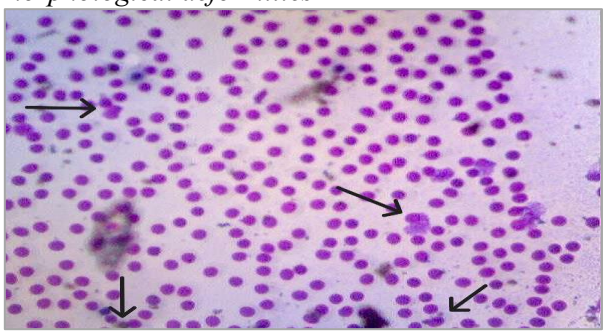


Experimental Group 2: Moderate Chlorpyrifos Exposure

In Experimental Group 2, 775 red blood cells (RBCs) were analyzed after exposure to a greater quantity of chlorpyrifos (20 µL). The morphological distribution found on both slides was as follows: There were 524 single nucleated cells, 35 double nucleated cells, 98 nuclear shift cells, 53 irregular nucleated cells, and 64 deformed or injured cells. Some cells seemed clogged and had debris, most likely owing to contamination. The study found that, while the frequency of double nucleated cells remained relatively low, nuclear shifts, irregular nuclei, and malformed cells increased significantly. These findings show that intermediate chlorpyrifos exposure resulted in more dramatic morphological alterations in RBCs than the low-exposure group, indicating that this concentration has a bigger influence on RBC morphology (Fig.3).

Figure 3

Red Blood Cells of Group2 showing morphological deformities

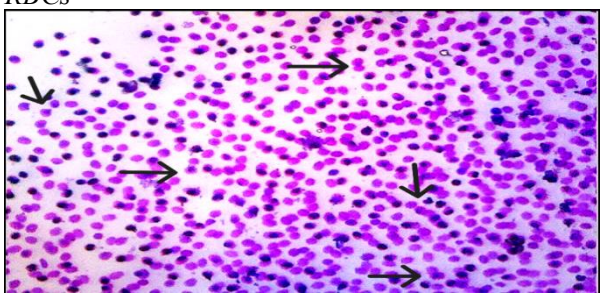


Experimental Group 3: High Chlorpyrifos Exposure

In Experimental Group 3, which received greater amounts of chlorpyrifos, 1,189 red blood cells (RBCs) were analyzed over two slides, resulting in significant cellular damage. The morphological distribution was as follows: There are 293 single nucleated cells, 461 double nucleated cells, 173 nuclear shift cells, 55 irregular nucleated cells, and 196 deformed cells. Notably, a large percentage of RBCs revealed total loss of nuclear integrity, with 160 cells devoid of nuclei, indicating rupture or necrosis. The considerable rise in double nucleated cells, nuclear shift cells, and deformed cells, together with the loss of nuclear integrity, showed severe cellular stress, abnormal cell division, and extensive damage as a result of the high chlorpyrifos exposure. These data indicated the severe impact of increased CPF concentrations on RBC shape, which results in cell death and disintegration (Fig.4).

Figure 4

Red Blood Cells of Group3 showing abnormal RBCs



The counts and percentages of various cellular abnormalities found in the three experimental groups exposed to varied chlorpyrifos doses follow unique tendencies. In Group 1, the most common

abnormality was single nucleated cells, which accounted for 83.01% of the total cells analysed, followed by double nucleated cells (14.46%) and malformed cells. In Group 2, the percentage of single nucleated cells declined to 64.13%, while double nucleated cells and nuclear shift cells increased, accounting for 4.50% and 16.39%, respectively. Group 3 had a considerable shift, with single nucleated cells accounting for just 28.69% and a large rise in double nucleated cells (38.95%) and nuclear shift cells (16.52%), indicating serious cellular damage. Irregular nucleated cells and malformed cells were found in all groups, but were more noticeable in Groups 1 and 2, with Group 3 having a reduced prevalence of these abnormalities. Overall, the findings show a clear pattern of increasing cellular abnormalities as chlorpyrifos concentrations rise, with Group 3 having the most abnormalities. Single nucleated cells accounted for the majority (56.88%) of the total count across all groups (Table 3).

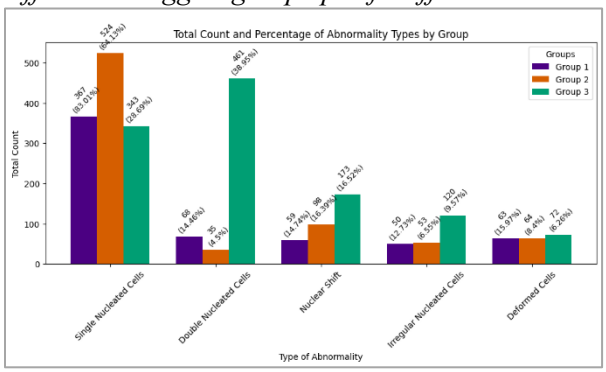
Table 3

Counts and percentages of cellular abnormalities observed in the Experimental groups

Type of Abnormality	Group 1 (Total Count & %age)	Group 2 (Total Count & %age)	Group 3 (Total Count & %age)	Total Count & %age
Single Nucleated Cells	367 (83.01%)	524 (64.13%)	343 (28.69%)	1,234 (56.88%)
Double Nucleated Cells	68 (14.46%)	35 (4.50%)	461 (38.95%)	564 (10.58%)
Nuclear Shift	59 (14.74%)	98 (16.39%)	173 (16.52%)	330 (9.91%)
Irregular Nucleated Cells	50 (12.73%)	53 (6.55%)	120 (9.57%)	223 (7.34%)
Deformed Cells	63 (15.97%)	64 (8.40%)	72 (6.26%)	199 (7.43%)
Total	814	775	1,169	2,758

Figure 5

shows cell abnormalities across three groups. "Single Nucleated Cells" are most common in Group 2 (64.1%), "Nuclear Shift" and "Irregular Nucleated Cells" are notable in Group 3, and "Deformed Cells" are highest in Group 1. These differences suggest group-specific effects.



DISCUSSION

Fish blood, as a pathophysiological indicator of the entire organism, is crucial for diagnosing the structural and functional status of fish exposed to toxicants, with alterations in its physical and chemical properties offering valuable insights into their health and environmental conditions (Korkmaz et al., 2023). In the present study' exposure to chlorpyrifos, an organophosphate insecticide, resulted in observable behavioral and metabolic disruptions in *Cyprinus carpio* (Common Carp), particularly in groups exposed to higher concentrations. Similar findings by Hussain (2022) and Dembele et al. (2000) have demonstrated that organophosphates can induce abnormal behaviors, increased mortality, and metabolic dysfunctions in fish. Additionally, *Tilapia guineensis* was found to experience increased mortality rates at higher chlorpyrifos concentrations and longer exposure durations (Chindah et al., 2005), aligning with the mortality observed in the current study's high-exposure groups.

The destructive impact of chlorpyrifos on growth rates in species like *O. niloticus* (Majumder & Kaviraj, 2019; Yonar et al., 2012) consistent with our findings of reduced body biomass in chlorpyrifos-exposed carp. The decrease in body weight may result from reduced food intake or energy expenditure on stress response, as noted by Adel et al. (2017). Additionally, our study confirmed a significant reduction in red blood cell (RBC) count among the chlorpyrifos-exposed fish, likely due to inhibition of RBC production or

increased RBC destruction, mirroring findings in *Heteropneustes fossilis* where chlorpyrifos exposure led to anemia (Tahir et al., 2021). Muthusamy et al. (2018) attributed similar reductions in hemoglobin (Hb) content to toxicant inhibition of Hb synthesis, supporting the hematological disturbances observed in this study.

The study also observed severe cellular damage, with disturbed cell morphology and micronuclei formation in RBCs, highlighting chlorpyrifos's genotoxic effects. These morphological alterations, including the loss of nuclear structure, support findings by Ali et al. (2009) that exposure to chlorpyrifos and other organophosphates induce micronuclei in fish erythrocytes. (Anbumani & Mohankumar, 2011) further confirms that micronucleus induction in fish is both concentration- and time-dependent, correlating with our observation of higher genotoxic damage in the highest exposure group.

Our results underscore the need to consider alternative pest control chemicals with reduced toxicity or implement protective measures for groundwater to minimize chlorpyrifos contamination, as its bioaccumulation in aquatic ecosystems poses a significant threat not only to fish health but also to human health due to pesticide runoff. Chlorpyrifos's impact on fish behavior, cellular health, and overall physiology highlights its toxicological burden, indicating the importance of mitigating its environmental impact for both aquatic and human health safety.

CONCLUSION

The hematological study on the effects of chlorpyrifos on Common carp (*Cyprinus carpio*) reveals significant alterations in blood parameters, indicating a serious impact on the fish's health. The exposure to chlorpyrifos led to marked changes in behavioral activity, genotoxic effects, and enzymatic activities, culminating in severe damage to red blood cells (RBCs). Specifically, the RBCs in the third group, which received the highest concentration of pesticides, exhibited the most extensive cellular damage, including ruptured nuclei. These findings highlight the sensitivity of Common carp to chlorpyrifos and underscore the potential for serious implications for aquatic ecosystems and human health. Given the similarities in physiological processes between fish and humans, the observed hematological disturbances may serve as an indicator of broader ecological and health risks associated with pesticide exposure. Therefore, it is crucial to consider the hematological effects of pesticides like chlorpyrifos in assessing the overall health of aquatic organisms. Future research should focus on further elucidating these effects and exploring safer alternatives to mitigate the negative impacts on both aquatic life and human health. The need for stringent regulations on pesticide use and the promotion of environmentally friendly agricultural practices are imperative to protect not only fish populations but also the integrity of the ecosystems they inhabit.

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