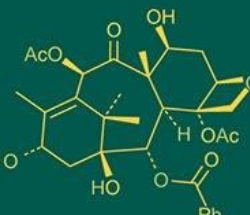
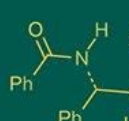


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Evaluation of hematological parameters in freshwater fish species, with special emphasis on *Catla catla* exposed to chlorpyrifos

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Abstract

The present investigation evaluated the toxicological effects of the organophosphate pesticide chlorpyrifos on freshwater fish, with particular emphasis on hematological alterations in *Catla catla* under controlled laboratory conditions. The experiment was conducted during 2024-25 at Bilaspur, Chhattisgarh, following a Completely Randomized Design (CRD). Healthy juvenile fish were exposed to sublethal concentrations of chlorpyrifos (4.0, 8.0, 16.0, 32.0 and 64.0 ppb) along with a control, using static-renewal bioassay techniques for a chronic duration of 60 days. Water quality parameters were maintained within optimal ranges throughout the exposure period. Hematological parameters including hemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and erythrocyte sedimentation rate (ESR) were analyzed using standard methods. The results revealed marked dose-dependent hematological disturbances in *Catla catla* following prolonged chlorpyrifos exposure. Hemoglobin content significantly declined from 3.25 ± 0.03 g/L in the control to 1.90 ± 0.03 g/L at the highest concentration (64.0 ppb), indicating the onset of anemia. A similar decreasing trend was observed in TEC and PCV, reflecting erythrocyte destruction and impaired hematopoiesis. In contrast, TLC increased progressively with rising pesticide concentration, suggesting activation of immune and stress responses. MCV values showed a significant increase, indicating macrocytosis and possible compensatory mechanisms to anemia, whereas MCH remained relatively stable across treatments. ESR exhibited a pronounced increase at higher concentrations, signifying inflammatory and pathological conditions induced by chlorpyrifos toxicity. Overall, the study demonstrates that chronic exposure to sublethal concentrations of chlorpyrifos induces significant hematological impairments in *Catla catla*, even at environmentally relevant levels. These alterations highlight the sensitivity of hematological parameters as reliable biomarkers for pesticide-induced stress and underscore the potential ecological risks of chlorpyrifos contamination in freshwater ecosystems.

Keywords: Chlorpyrifos, *Catla catla*, hematological biomarkers, sublethal toxicity, organophosphate pesticide, freshwater fish, environmental stress, aquatic pollution

1. Introduction

The intensification of agricultural practices to meet the increasing global demand for food has led to a substantial rise in the use of chemical pesticides, many of which ultimately enter non-target ecosystems, particularly freshwater bodies. Among these agrochemicals, organophosphate pesticides continue to be widely used in developing countries because of their high efficacy against insect pests and relatively low persistence compared to organochlorines. However, repeated applications, surface runoff, leaching, spray drift and irrigation return flows facilitate the transport of these compounds into rivers, reservoirs, ponds and dams, where they pose serious risks to aquatic organisms (Alengebawy *et al.* 2021; Deb & Das, 2013) [5, 14].

Chlorpyrifos is a broad-spectrum organophosphate insecticide extensively applied in agriculture to control a wide range of crop pests. Its primary toxic mode of action involves inhibition of acetylcholinesterase, leading to disruption of nerve impulse transmission. Although chlorpyrifos is intended for terrestrial use, numerous studies have documented its frequent detection in surface waters and sediments, particularly in regions dominated by intensive agriculture (Abrantes *et al.* 2010; Hossain *et al.* 2022) [3, 15]. Even at sublethal and environmentally relevant concentrations, chlorpyrifos has been shown to induce

physiological, biochemical, hematological and histopathological alterations in fish, thereby compromising growth, immunity and survival (Majumder & Kaviraj, 2019; Deb & Das, 2013) [18, 14].

Fish are widely recognized as sensitive bio-indicators of aquatic pollution because they occupy higher trophic levels, integrate contaminant exposure over time and respond to toxic stress at multiple levels of biological organization. Hematological parameters such as hemoglobin content, erythrocyte and leucocyte counts, packed cell volume and erythrocyte indices are particularly valuable biomarkers, as they reflect both the health status of fish and the functional integrity of hematopoietic tissues (Blaxhall & Daisley, 1973; Barton *et al.* 2002) [9, 8]. Alterations in these parameters often occur earlier than overt signs of toxicity or mortality, making them reliable early-warning indicators of pesticide stress.

Indian major carps play a crucial role in inland fisheries and aquaculture, contributing significantly to food security and rural livelihoods. Among them, *Catla catla* is an economically important and widely cultured freshwater species in India. Due to its ecological relevance, sensitivity to pollutants and commercial importance, *C. catla* has been extensively used in toxicological studies to assess the impacts of pesticides and other contaminants (Abhijith *et al.* 2012; Kavitha *et al.* 2010) [2, 16]. However, region-specific data on the hematological effects of chronic sublethal chlorpyrifos exposure remain limited, particularly for freshwater systems associated with reservoirs and dams used for fisheries and irrigation.

In this context, the present study was undertaken to evaluate the hematological responses of freshwater fish species, with special emphasis on *Catla catla*, following chronic exposure to sublethal concentrations of chlorpyrifos under controlled laboratory conditions. By integrating multiple hematological endpoints, the study aims to provide insight into the physiological stress and hematopoietic disruptions induced by chlorpyrifos, thereby contributing to ecological risk assessment and supporting the use of hematological biomarkers in monitoring pesticide contamination in freshwater ecosystems.

2. Methodology

The present investigation was conducted during 2024-25 in a controlled laboratory facility at Bilaspur, Chhattisgarh, under the supervision of the School of Sciences, MATS University, Raipur, following a Completely Randomized Design (CRD). The study aimed to evaluate the toxicological effects of the organophosphate pesticide chlorpyrifos on selected freshwater fish species, with special emphasis on hematological responses. Chlorpyrifos (technical grade, ~95% purity) was procured from a certified supplier and stock solutions were prepared using analytical-grade methanol.

Healthy juvenile *Catla catla* were acclimatized under laboratory conditions and exposed to sublethal concentrations of chlorpyrifos (4.0, 8.0, 16.0, 32.0 and 64.0 ppb) along with a control for 60 days using a static-renewal bioassay. Fish were maintained in FRP tanks with dechlorinated water under controlled conditions (temperature 26 ± 2 °C, pH 7.2-7.6, dissolved oxygen > 6 mg L⁻¹, photoperiod 12L:12D) and fed a commercial pellet diet (~30% protein) at 3% body weight per day. At the end of exposure, fish were anesthetized with MS-222 and blood

samples were collected by cardiac puncture using heparinized syringes. Hemoglobin content, total erythrocyte count, total leucocyte count and packed cell volume were estimated following standard hematological methods, while mean corpuscular volume, mean corpuscular hemoglobin and erythrocyte sedimentation rate were calculated using established formulae. Data were expressed as mean $\pm$ SD and analyzed statistically using one-way ANOVA under a Completely Randomized Design, followed by standard error at $p \leq 0.05$ to determine significant treatment effects.

3. Results

The hematological data of *Catla catla* exposed to different sublethal concentrations of chlorpyrifos for 60 days are presented in Table 1 and Fig 1-7. A clear dose-dependent alteration was observed in most hematological indices, indicating the physiological stress and hematopoietic disruption caused by prolonged pesticide exposure.

3.1 Hemoglobin Content (Hb, g/L)

The hemoglobin content ranged from 3.25 ± 0.03 g/L in the control (T₁) to a minimum of 1.90 ± 0.03 g/L in the highest concentration (T₆, 64.0 ppb), with a mean value of 2.54 ± 0.04 g/L. The decline in Hb levels with increasing chlorpyrifos concentration indicates anemic conditions possibly due to the destruction of erythrocytes or inhibition of erythropoiesis under pesticide stress. Reduced Hb may also reflect lowered oxygen-carrying capacity, impacting metabolic efficiency.

3.2 Total Erythrocyte Count (TEC, $\times 10^6/\text{mm}^3$)

The total erythrocyte count decreased progressively from $3.57 \pm 0.16 \times 10^6/\text{mm}^3$ in the control to $2.35 \pm 0.01 \times 10^6/\text{mm}^3$ in T₆, with a mean of $2.97 \pm 0.14 \times 10^6/\text{mm}^3$. The reduction in erythrocyte count suggests destruction or lysis of red blood cells and suppression of hematopoietic activity due to toxic interference of chlorpyrifos with hematological tissues.

3.3 Total Leucocyte Count (TLC, $\times 10^6/\text{mm}^3$)

In contrast, the total leucocyte count showed a progressive increase from $6.71 \pm 0.38 \times 10^6/\text{mm}^3$ (control) to a maximum of $9.38 \pm 0.26 \times 10^6/\text{mm}^3$ (T₆), with a mean of $7.96 \pm 0.33 \times 10^6/\text{mm}^3$. This leukocytosis indicates an immune response to pesticide-induced stress and possible tissue damage, reflecting activation of defense mechanisms to counter toxic effects.

3.4 Packed Cell Volume (PCV, %)

The packed cell volume showed a decreasing trend, ranging from $44.94 \pm 3.48\%$ in the control to $30.93 \pm 1.39\%$ in the highest treatment (T₆), with an overall mean of $36.54 \pm 1.75\%$. The marked reduction in PCV at higher concentrations indicates hemodilution or erythrocyte destruction due to pesticide toxicity, which impairs blood oxygen transport and viscosity balance.

3.5 Mean Corpuscular Volume (MCV, %)

MCV values increased from $1.42 \pm 0.04\%$ in the control to $2.92 \pm 0.15\%$ in T₆, with an average of $2.09 \pm 0.08\%$. The rise in MCV suggests the formation of larger but fewer red blood cells, possibly as a compensatory response to anemia, reflecting macrocytosis caused by erythrocyte swelling under toxic stress conditions.

3.6 Mean Corpuscular Hemoglobin (MCH, %)

MCH showed minimal variation among treatments, ranging between $8.23 \pm 0.29\%$ (T_5) and $8.76 \pm 0.30\%$ (T_4), with a mean of $8.48 \pm 0.24\%$. This parameter remained nearly constant, indicating that while erythrocyte size and number changed, the hemoglobin concentration per cell remained relatively stable, suggesting partial maintenance of erythrocyte hemoglobin content despite chlorpyrifos exposure.

3.7 Erythrocyte Sedimentation Rate (ESR, 1st hr)

The ESR increased significantly from 2.71 ± 0.17 mm/hr in the control to 7.36 ± 0.18 mm/hr at the highest concentration (T_6), with an overall mean of 5.28 ± 0.25 mm/hr. The elevated ESR values indicate inflammatory or pathological conditions, possibly due to alterations in plasma protein composition and cellular damage resulting from chlorpyrifos stress.

4. Discussion

Hematological parameters are widely recognized as sensitive biomarkers for assessing physiological stress and toxicological impacts in fish, as they reflect the functional status of hematopoietic tissues and the overall health of the organism (Blaxhall & Daisley, 1973; Barton *et al.* 2002) [9, 8]. In the present investigation, *Catla catla* exposed to increasing sub-lethal concentrations of chlorpyrifos for 60 days exhibited pronounced, dose-dependent alterations in key hematological indices, clearly indicating pesticide-induced stress and hematopoietic dysfunction.

The significant reduction in hemoglobin (Hb) content observed with increasing chlorpyrifos concentration suggests the induction of anemia in treated fish. Similar declines in Hb have been reported in *Catla catla* and other teleosts exposed to organophosphate pesticides, including methyl parathion and chlorpyrifos (Abhijith *et al.* 2012; Lakshman *et al.* 2013) [2, 17]. The decrease in Hb may be attributed to erythrocyte destruction, inhibition of hemoglobin synthesis, or impaired iron metabolism caused by pesticide-induced oxidative stress. Reduced hemoglobin levels ultimately compromise oxygen transport efficiency, leading to impaired aerobic metabolism and reduced physiological performance (Barton, 2002) [7].

A parallel decline in total erythrocyte count (TEC) further supports the occurrence of pesticide-induced anemia. The reduction in erythrocyte numbers may result from hemolysis, suppression of erythropoiesis in hematopoietic organs such as kidney and spleen, or damage to erythrocyte membranes due to lipid peroxidation (Dautremepuits *et al.* 2004; Deb & Das, 2013) [13, 14]. Similar reductions in TEC have been documented in *Labeo rohita*, *Oreochromis mossambicus* and *Catla catla* following exposure to organophosphate and pyrethroid pesticides, confirming the hematotoxic nature of these compounds (Ali & Rani, 2009; Abhijith *et al.* 2012) [2].

In contrast to erythrocytic parameters, total leucocyte count (TLC) showed a marked increase with rising chlorpyrifos

concentrations. This leukocytosis reflects activation of the immune system in response to pesticide-induced tissue damage and physiological stress. Increased TLC has been widely interpreted as a defensive or adaptive response, enhancing the organism's ability to counteract toxic injury and infection (Akinrotimi *et al.* 2009; Cericato *et al.* 2004) [4, 10]. Similar findings have been reported in fish exposed to chlorpyrifos and acephate, where elevated leucocyte counts were associated with stress-mediated immunological activation (Kulkarni & Bhilave, 2015; Hossain *et al.* 2022) [15].

The observed reduction in packed cell volume (PCV) further corroborates the anemic condition induced by chlorpyrifos exposure. Decreased PCV values are indicative of reduced erythrocyte mass or hemodilution and have been consistently reported in pesticide-exposed fish species (Abdallah & Abdallah, 2008; Lakshman *et al.* 2013) [1, 17]. Lower PCV values impair blood viscosity and oxygen transport capacity, thereby reducing endurance and growth potential in fish. Interestingly, mean corpuscular volume (MCV) increased significantly with pesticide concentration, indicating macrocytosis. This enlargement of erythrocytes may represent a compensatory response to anemia, where fewer but larger erythrocytes are produced to partially offset reduced oxygen-carrying capacity (Dacie & Lewis, 1991) [11]. Similar increases in MCV have been reported in fish exposed to organophosphate pesticides and heavy metals, reflecting erythrocyte swelling and membrane damage under toxic stress (Akinrotimi *et al.* 2009; Das *et al.* 2004) [4, 12].

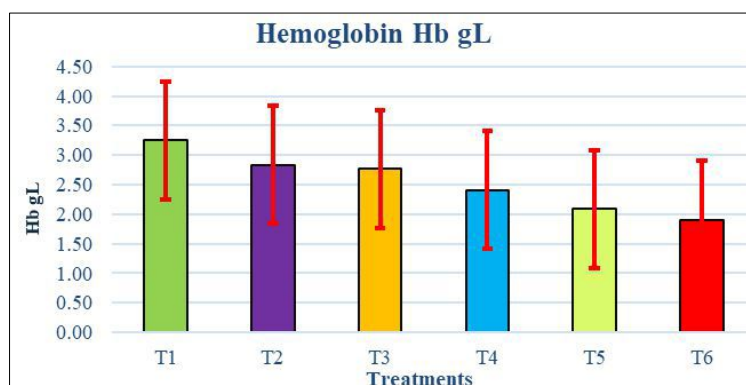
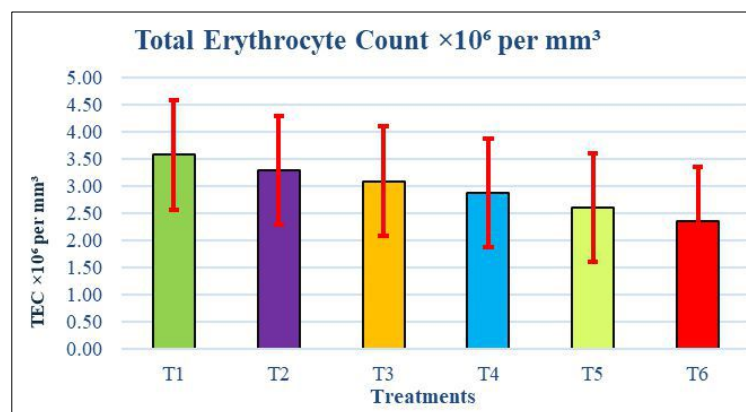
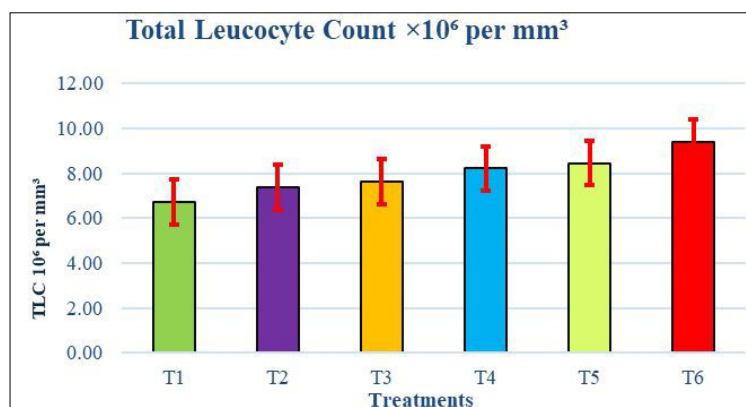
Mean corpuscular hemoglobin (MCH) remained relatively stable across treatments, suggesting that despite reductions in erythrocyte number and Hb concentration, the hemoglobin content per individual erythrocyte was maintained. This stability may indicate partial physiological compensation aimed at sustaining oxygen transport at the cellular level during prolonged exposure (Blaxhall & Daisley, 1973) [9].

The significant elevation in erythrocyte sedimentation rate (ESR) with increasing chlorpyrifos concentration is indicative of inflammatory and pathological conditions. Elevated ESR values are commonly associated with alterations in plasma protein composition, tissue injury and systemic inflammation (Barton *et al.* 2002) [7]. Similar increases in ESR have been reported in fish exposed to toxicants, reflecting chronic stress and cellular damage (Dautremepuits *et al.* 2004; Hossain *et al.* 2022) [13, 15].

Overall, the present findings clearly demonstrate that chronic sub-lethal exposure to chlorpyrifos induces severe hematological disturbances in *Catla catla*, characterized by anemia, leukocytosis, macrocytosis and inflammatory responses. These alterations are consistent with previous studies on organophosphate toxicity and confirm the usefulness of hematological parameters as early warning indicators of pesticide-induced stress in freshwater fish. The observed hematological impairments may ultimately reduce growth, survival and fitness of fish populations in contaminated aquatic ecosystems.

Table 1: Evaluation of Hematological Parameters in Freshwater on Hematology Parameters of *Catla catla*.

Spp.	Tr. No.	Treatment Details	Hemoglobin contain HB (g/l)	Total erythrocyte count ($\times 10^6/\text{mm}^3$)	Total leucocyte count ($\times 10^6/\text{mm}^3$)	Packed cell volume (%)	Mean corpuscular volume (%)	Mean corpuscular hemoglobin (%)	Erythrocyte sedimental rate (1 st hr)
<i>Catla catla</i>	T ₁	Control	3.25 $\pm$ 0.03	3.57 $\pm$ 0.16	6.71 $\pm$ 0.38	44.94 $\pm$ 3.48	1.42 $\pm$ 0.04	8.38 $\pm$ 0.38	2.71 $\pm$ 0.17
	T ₂	LC ₅₀ (4.0 ppb)	2.83 $\pm$ 0.03	3.29 $\pm$ 0.29	7.36 $\pm$ 0.41	40.18 $\pm$ 2.21	1.69 $\pm$ 0.07	8.37 $\pm$ 0.14	3.95 $\pm$ 0.30
	T ₃	LC ₅₀ (8.0 ppb)	2.76 $\pm$ 0.02	3.09 $\pm$ 0.17	7.62 $\pm$ 0.45	36.16 $\pm$ 1.62	1.93 $\pm$ 0.08	8.51 $\pm$ 0.22	5.12 $\pm$ 0.14
	T ₄	LC ₅₀ (16.0 ppb)	2.41 $\pm$ 0.09	2.88 $\pm$ 0.12	8.21 $\pm$ 0.27	35.03 $\pm$ 1.18	2.29 $\pm$ 0.07	8.76 $\pm$ 0.30	5.72 $\pm$ 0.27
	T ₅	LC ₅₀ (32.0 ppb)	2.08 $\pm$ 0.04	2.61 $\pm$ 0.12	8.45 $\pm$ 0.18	31.98 $\pm$ 0.65	2.30 $\pm$ 0.09	8.23 $\pm$ 0.29	6.78 $\pm$ 0.41
	T ₆	LC ₅₀ (64.0 ppb)	1.90 $\pm$ 0.03	2.35 $\pm$ 0.01	9.38 $\pm$ 0.26	30.93 $\pm$ 1.39	2.92 $\pm$ 0.15	8.60 $\pm$ 0.14	7.36 $\pm$ 0.18
		Mean $\pm$ SD	2.54 $\pm$ 0.04	2.97 $\pm$ 0.14	7.96 $\pm$ 0.33	36.54 $\pm$ 1.75	2.09 $\pm$ 0.08	8.48 $\pm$ 0.24	5.28 $\pm$ 0.25
		Variance	2.54	2.97	7.96	36.54	2.09	8.48	5.28
		SE	0.02	0.08	0.19	1.01	0.05	0.14	0.14
		Sig/NS	Highly Significant	Highly Significant	Highly Significant	Highly Significant	Highly Significant	Non-significant	Highly Significant

**Fig 1:** To study the hemoglobin contain HB (g/l) of *Catla catla*.**Fig 2:** To study the total erythrocyte count ($\times 10^6/\text{mm}^3$) of *Catla catla*.**Fig 3:** To study the total leucocyte count ($\times 10^6/\text{mm}^3$) of *Catla catla*.

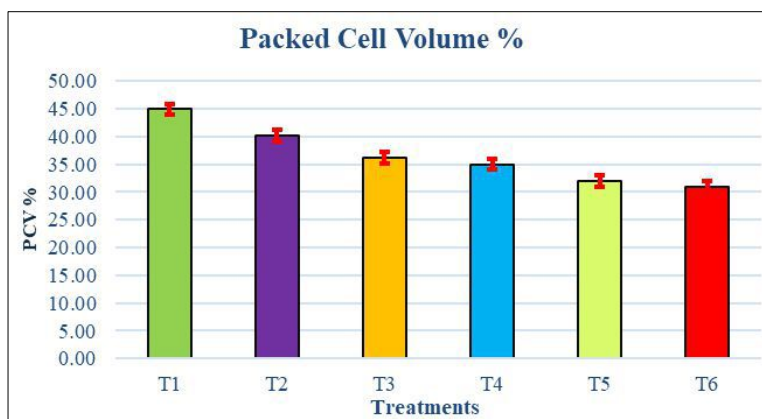


Fig 4: To study the packed cell volume (%) of *Catla catla*.

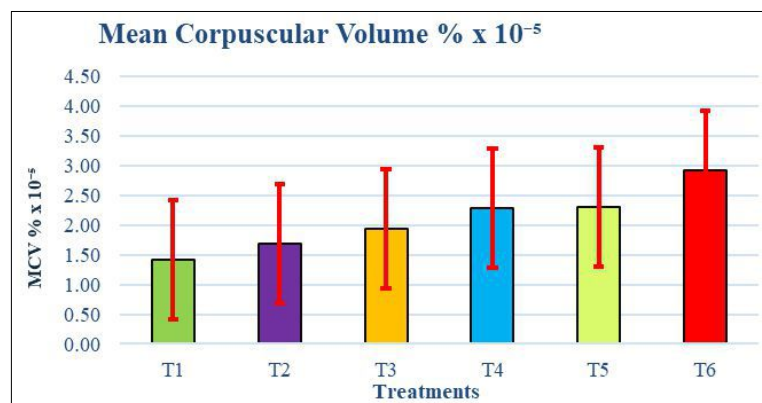


Fig 5: To study the mean corpuscular volume (%) of *Catla catla*.

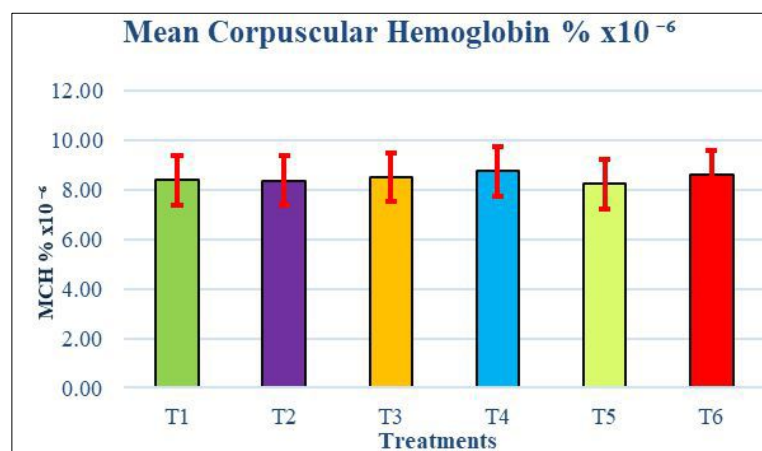


Fig 6: To study the mean corpuscular hemoglobin (%) of *Catla catla*.

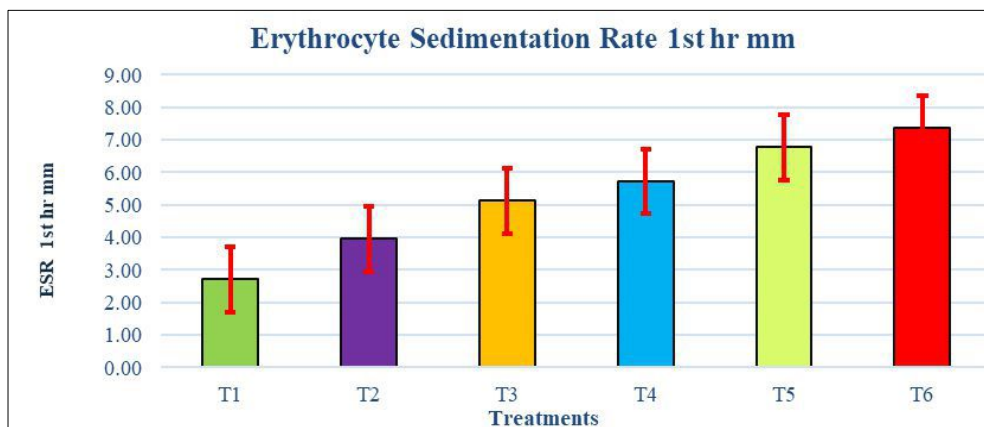


Fig 7: To study the erythrocyte sedimental rate (1st hr) of *Catla catla*.

5. Conclusion

The present study clearly demonstrates that chronic exposure to sublethal concentrations of the organophosphate pesticide chlorpyrifos induces significant hematological disturbances in *Catla catla*. A pronounced dose-dependent decline in hemoglobin content, total erythrocyte count and packed cell volume indicates the development of anemia and suppression of hematopoietic activity under prolonged pesticide stress. In contrast, the progressive increase in total leucocyte count reflects activation of immune defense mechanisms in response to toxic injury. Alterations in erythrocyte indices, particularly the elevation of mean corpuscular volume along with a relatively stable mean corpuscular hemoglobin, suggest compensatory physiological adjustments to maintain oxygen transport. The significant rise in erythrocyte sedimentation rate further confirms the onset of inflammatory and pathological conditions in exposed fish.

These findings highlight that even environmentally relevant sublethal concentrations of chlorpyrifos can adversely affect the physiological health of freshwater fish, posing a potential threat to aquatic biodiversity and fisheries sustainability. Hematological parameters proved to be sensitive and reliable biomarkers for early detection of pesticide-induced stress. The study emphasizes the need for judicious use of chlorpyrifos in agricultural practices and supports the implementation of stricter regulatory measures to prevent pesticide contamination of freshwater ecosystems.

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7. Conflict of Interest

The authors declare that there is no conflict of interest associated with this study

8. Future Suggestions

1. Long-term field-based studies should be conducted to evaluate the ecological impact of chlorpyrifos under natural environmental conditions.
2. Molecular and genetic biomarkers should be incorporated to better understand the mechanistic pathways of chlorpyrifos-induced toxicity in fish.
3. Comparative toxicity assessments involving multiple pesticides and their combined effects are recommended to simulate real agricultural runoff scenarios.
4. Recovery and depuration studies should be undertaken to assess the reversibility of hematological and biochemical alterations after pesticide withdrawal.

5. Development and promotion of eco-friendly pest management strategies are suggested to minimize pesticide contamination in freshwater ecosystems.

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