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Study on the Toxic Effects of Deltamethrin on Hematological Parameters of a Fresh Water Fish, Common Carp (*Cyprinus carpio* L.)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Fish health is adversely affected by pesticide contamination, which is a significant cause of pollution in freshwater ecosystems. One helpful technique for assessing the general health of different fish species is the analysis of blood parameters. Present study investigated the detrimental effects of deltamethrin on different hematological parameters of a fresh water fish, common carp (*Cyprinus carpio*). An experiment with a 28-day exposure to 1/20th and 1/10th of the 96-hour LC_{50} value (0.114µl/L) was conducted in order to investigate the effects of sub-lethal concentrations. On the 7th, 14th, 21st, and 28th day of exposure, several hematological parameters like Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Hemoglobin (Hb), Hematocrit/Packed Cell Volume (Hct/PCV),

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Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were analyzed in comparison to control. After the exposure of different concentrations of deltamethrin significant decline (P*<0.05 and P**<0.01) in Hb, TEC, PCV and MCHC was noted as compared to control. On the contrary TLC, MCV and MCH showed increasing trend after the treatment of pesticide. This study showed that deltamethrin considerably altered the hematological markers of fish, even at sub-lethal concentrations.

Keywords: Fish; common carp; hematology; pyrethroid; deltamethrin.

1. INTRODUCTION

Freshwater habitats are facing an increasing number of environmental problems as a result of growing industry, urbanization, and agricultural activities. Chemicals such as, pesticides, halogenated polycyclic hydrocarbons, food additives, and agrochemicals were introduced as a result of industrialization and technical growth [1]. The effects of these compounds on the environment are now a global issue. Toxicologists see pesticides as a necessary evil, and like many discoveries or technological advances, people have been quick to take benefit of them but incredibly slow to recognize and deal with their effects [2]. These frequently long-lasting effects may have adverse consequences for humans and the environment. Each type of pesticide has varying levels of toxicity to fish due to differences in chemical characteristics; insecticides are usually the most harmful [3]. Contamination of freshwater ecosystems poses one of the biggest risks to aquatic life since water sources are the intended endpoint of a complex interaction between xenobiotics and agrochemicals [4].

Pyrethroid insecticides are widely utilized pesticides which are used to treat ectoparasitic infestation in farm animals and to control various agricultural insect pests. Due to its limited soil persistence and minimal toxicity to animals and birds, pyrethroid insecticides are becoming more and more widely used in agriculture. But the improper disposal and seepage of these chemicals from agricultural runoff contaminates aquatic environments, exposing fish and other aquatic species to dangerous conditions. Deltamethrin (DM) is one of the most commonly used synthetic pyrethroids world wide [5]. It is one of the fourth generation synthetic pyrethroids and among the most effective pyrethroid preparation [6]. Like other pesticides, DM has the potential to contaminate aquatic life when it gets into water systems. The fish's vulnerability to aqueous pyrethroid exposures may be explained by the lipophilic nature of pyrethroids, which results in a high rate of gill absorption. According to Haya [7], the enzyme system responsible for hydrolyzing pyrethroids appears to be absent in fish. It seriously damages aquatic life and even jeopardizes human safety at every stage of the food chain.

According to Dawood *et al*. [8], fish are susceptible to insecticides through various means, including skin absorption, gill uptake during respiration, and most importantly consuming polluted water or food. Because fish are extremely sensitive to changes in their habitat, they can help assess the risks related to new chemical contamination in aquatic ecosystems [9,10]. When fish are exposed to xenobiotics, their hematological changes might vary depending on the toxic agent, its concentration, the length of time the fish are exposed, the surrounding environment, and intrinsic characteristics like the type, age, and size of the fish [11]. Fish may react differently hematologically to toxic exposures according to the species and life stage and how sensitive they are to environmental influences. Various authors have studied hematological markers of different fish species as potential indicators of pesticide toxicity [12,13]. One of the most widely cultivated freshwater fish species worldwide is the common carp (*Cyprinus carpio*). Because of its relative resistance to water pollution, including pesticides, which is essential for the identification of biological indicators in lab and field investigations, it is regarded as an excellent model organism for eco-toxicological research [14].

Because blood is essential for maintaining fish equilibrium and other key functions, using hematological approaches to evaluate the detrimental consequences of chemicals in aquatic species have acquired significance in recent years. Blood indices are quick-acting, susceptible indicators of various environmental effects, such as hazardous agent contamination of water. Blood parameters are indicators of a broad spectrum of physiological changes, both beneficial and harmful. As trustworthy biomarkers of an organism's health, they offer a wealth of knowledge on several physiological processes. The significance of hematological investigations in fish rests in the potential for the blood to identify abnormalities within the fish's body long before any illness signs or the impact of unfavorable environmental conditions emerge externally [15]. Since most fish species lack well-defined reference hematological values, assessing the hematotoxicity of a particular chemical or contaminated environment necessitates comparing the results with those obtained simultaneously in similar fish species residing under controlled conditions. Blood collection is a simple and comparatively non-invasive procedure. According to Adewumi *et al*. [16] any physiological variations would be apparent in the values of one or more hematological markers of aquatic animals when the water quality is affected by pollution.

Hematocrit (Ht), hemoglobin concentration (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) are some of the characteristics related to red blood cells. The percentage or quantity of each of the following leukocyte types is determined using total leukocyte count (WBC or TLC) and, occasionally, differential leukocyte count (DLC) [17]. According to Ahmed *et al*. [18], variations in surrounding environment and the fish's dynamic internal environment are the factors that cause the hematological parameters in fish to fluctuate.Toxic chemicals frequently impact leukocyte count, and like red blood parameters, a rise or reduction may be seen. MCV of a given blood sample gives information on the average size of the red blood cells. The average weight of hemoglobin in each red blood cell (RBC) within a specific blood volume is represented by the MCH. The concentration of hemoglobin and the size of the cell both affect MCH. The average hemoglobin content in each red blood cell within a specific blood volume is represented by the MCHC. The primary goal of current study is to assess the detrimental effects of synthetic pyrethroid deltamethrin on the hematological markers of common carp (*C. carpio*), a freshwater fish.

2. MATERIALS AND METHODS

2.1 Test Chemical

Deltamethrin (11%EC formulation) is a broad spectrum contact insecticide which is used against various pests like bollworms, sucking insects, caterpillars, leaf rollers, fruit borers and thrips. It was purchased from the local market Shimla, Himachal Pradesh. The total quantity of DM that needed to be added was calculated after determining the capacity of each tank.

2.2 Experimental Fish

Healthy specimens of common carp (*C. carpio*) with an average body weight (55.71 ± 5.32 g) and average length (11.45 ± 1.15cm) were obtained from Deoli Fish Farm Ghagas, Bilaspur, Himachal Pradesh. Fish were transported to Animal house facility of Department of Biosciences, Himachal Pradesh University, Summerhill, Shimla. For 15 days, the fish were acclimatized to the lab environment. During the acclimatization period, they were given commercial pellets at a rate of 3% body weight twice a day.

2.3 Experimental Setup

During the experiment, the water parameters (pH = 7.23 ± 0.51, dissolved oxygen = 7.86±0.26 mg/L, water temperature = 25±2 °C, photoperiod = 10±2:14±2, light: dark) were all within the suggested limits. The necessary concentrations (10% and 5% of the 96-hour LC50) of DM (11%EC) for the current study were produced on the basis of 96hrs LC_{50} value (96hrs LC_{50} value observed was 0.114µl/L). Fish were fed 3% of their body weight every day. Fish were exposed to two concentrations of DM for duration of 28 days to study the detrimental effects of this pyrethroid on hematology of common carp. Concentrations that were selected for thepresent study were 0.005µl/L/T1 ($1/20^{th}$ of 96hrs LC_{50} value) and 0.011µl/L/T2 ($1/10^{th}$ of 96hrs LC_{50} value). Following exposure to DM for 7, 14, 21 and 28 days, blood samples were taken from the caudal veins of five fish per group, averaging 0.5–0.8 milliliters per fish. Blood was collected in tubes containing 0.5M EDTA as anticoagulant for a complete cell count picture.

2.4 Estimation of Hematological Parameters

2.4.1 Estimation of Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC)

Red blood cells (RBCs) and white blood cells (WBCs) were counted using Neubauer's improved hemocytometer, with Hayem's and Turk's solutions being used as dilution fluids, respectively. RBC count was expressed as

10^6/mm^3 and WBC count was expressed as 10^3/mm^3.

2.4.2 Estimation of Hemoglobin (Hb)

Hb concentration in the blood was estimated by cyanmethaemoglobin method by Blaxhall and Daisley [19]. By adding Potassium Ferricyanide (KCN), Hb is changed into cyanmethaemoglobin. The color change was measured in a spectrophotometer at 540 nm against reagent blank.

2.4.3 Estimation of Hematocrit (Hct) value

Hematocrit value was estimated according to microhematocrit method by Blaxhall and Daisley [19]. Using a Pasteur pipette, the blood was poured into the hematocrit tube until it reached mark 100. The tube was then centrifuged for 30 minutes at 3000 rpm. Packed cell volume (PCV), expressed as a percentage of the total blood column drawn in the hematocrit tube was determined by measuring the height of the RBCs packed at the bottom of the tube.

2.4.4 Determination of Mean Corpuscular Volume (MCV)

The following formula was used to determine MCV, which was then represented in femtoliters (fL).

MCV = Hematocrit (%) x10/RBCcount

2.4.5 Determination of Mean Corpuscular Hemoglobin (MCH)

The following formula was used to determine MCH, which was then represented in picograms (pg).

MCH = Hemoglobin (g/dL) x 10/RBC count

2.4.6 Determination of Mean Corpuscular Hemoglobin Concentration (MCHC)

The following formula was used to calculate MCHC, which was then represented as a gram percent (g%).

MCHC = Hemoglobin (g/dL) x 100/Hematocrit (%)

2.5 Statistical Analysis

Data was presented as mean ± SEM. Comparison amongst groups was performed using one way analysis of variance (ANOVA) test followed by post-hoc Tukey HSD test to assess the significance of difference between different groups. Statistical significance was set at p*<0.05 and p**<0.01, n=3.

3. RESULTS AND DISCUSSION

The current study showed several alterations in the hematological indices (TEC, TLC, Hb, Hct/PCV, MCV, MCH, and MCHC) of common carp (*C. carpio*) following exposure to varying concentrations of DM. (Table 1). From the data, it was observed that TEC, Hb, Hct/PCV and MCHC declined noticeably in treated fish in contrast to fish in control conditions. This significant decline in Hct, TEC and Hb signify the presence of erythropenia-related anemia. The anemia might result from a decrease in hemosynthesis and erythropoiesis as well as a rise in the rate at which erythrocytes are destroyed in hemopoietic organs. Fish subjected to different concentrations of deltamethrin had lower RBC counts in comparison to control fish, potentially as a result of hematopoietic system failure. The increased rate of erythrocyte breakdown in hematopoietic organs and suppression of erythropoiesis resulted in a decrease in red blood cell count [20]. Reduced red blood cells (RBCs) can also result from the development of hypoxic conditions during exposure, which might eventually cause RBC destruction, or from the deprivation of Hb content in the cellular content [21]. Significant decrease in Hb levels might result from harmful effects of this pesticide on this molecule's production. By altering the normal processing of the enzymes associated with the production of Hb, these toxins may prevent the usual pathway from occurring. Reduced cell size following intoxication may be the cause of the drop in PCV. So we can say that the fish was anemic, based on the decline in packed cell volume (PCV) or Hct (%) observed in current study. Following exposure to deltamethrin, anemic condition may be brought on by gill injury or/and by poor osmoregulation [22].

Significant increase in MCV, MCH and total amount of WBC was noticed in exposed fish after the treatment of deltamethrin in comparison to control. Given that MCH and MCHC originate from Hb and RBC, changes in these parameters would also affect MCH and MCHC. Fish raise their levels of MCV and MCH (as seen in current investigation) to combat the hypoxic conditions in the highly toxic medium [23]. Enhanced WBC count throughout the deltamethrin exposure

Table 1. Changes in various hematological parameters of common carp after the treatment of different concentrations (T1 and T2) of deltamethrin for a period of 28 days (mean±SEM, p*<0.05 and p<0.01)**

Hematological Parameters	Treated Groups	Exposure period				
		Control	7th day	14th day	21st day	28th day
TEC	T1	3.02±0.06	2.86±0.07	2.61±0.11	2.47±0.09	2.21±0.07**
($X 10^6/mm^3$)	T2	2.98±0.11	2.63±0.06*	2.31±0.09*	1.81±0.10*	1.52±0.06**
Hb	T1	9.32±0.17	8.86±0.19	8.37±0.14*	8.02±0.21**	7.63±0.18**
(g/dL)	T2	9.46±0.20	8.48±0.19	7.93±0.18*	6.42±0.20*	5.91±0.26**
Hct (%)	T1	36.03±0.51	34.64±0.36	33.11±0.46**	31.92±0.40**	30.39±0.34**
	T2	36.21±0.46	33.92±0.52*	31.97±0.48**	29.63±0.34**	28.72±0.43**
TLC	T1	13.68±0.23	14.29±0.16	14.83±0.19*	15.21±0.17**	15.86±0.25**
($X 10^3/mm^3$)	T2	13.64±0.19	14.88±0.21**	15.62±0.21**	16.83±0.17**	17.42±0.19**
MCH	T1	30.45±0.14	31.02±0.17	31.86±0.19**	32.35±0.12**	34.32±0.18**
(pg)	T2	30.74±0.12	32.34±0.17**	34.42±0.18**	35.74±0.13**	38.98±0.11**
MCV	T1	119.61±0.93	122.21±0.97	127.91±1.13**	130.33±0.89**	136.98±0.86**
(fL)	T2	120.41±0.86	129.87±1.15*	139.49±1.03**	164.80±0.97**	189.89±0.95**
MCHC	T1	25.86±0.12	25.37±0.11	25.06±0.11*	24.88±0.14**	24.19±0.16**
(g%)	T2	25.97±0.11	25.09±0.14**	24.18±0.09**	22.46±0.11**	20.97±0.10**

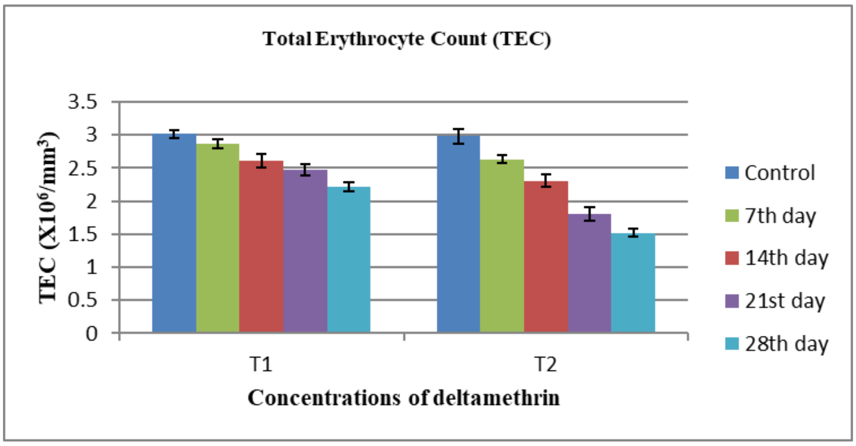

Fig. 1. Changes in the total amount of RBC (cells x $10^6/mm^3$) of common carp treated with different concentrations of DM for a period of 28 days. Values are represented as mean±SEM

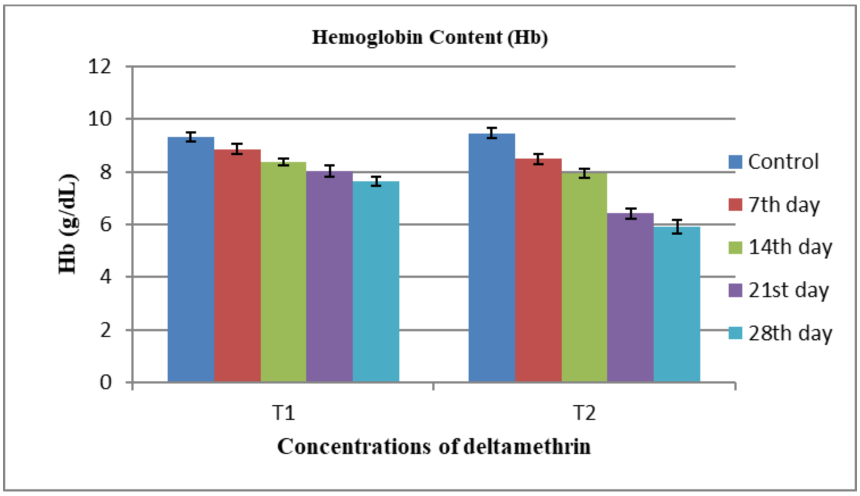

Fig. 2. Changes in the Hemoglobin (g/dL) of common carp treated with different concentrations of DM for a period of 28 days. Values are represented as mean±SEM

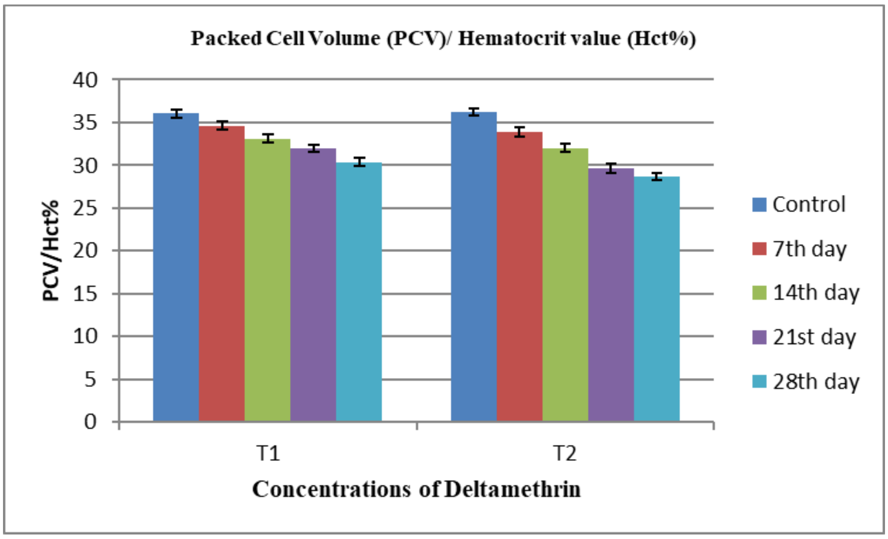

Fig. 3. Changes in the Hematocrit (%)/Packed Cell Volume of common carp treated with different concentrations of DM for a period of 28 days. Values are represented as mean±SEM

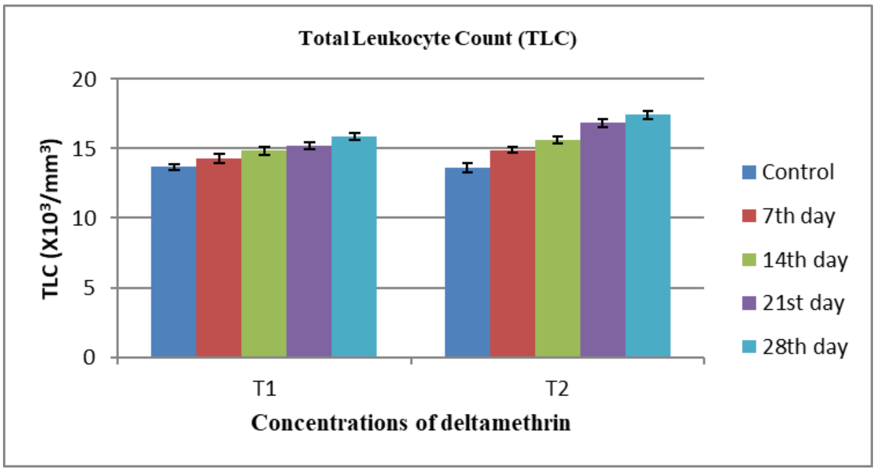

Fig. 4. Changes in the total amount of WBC (cells x $10^3/mm^3$) of common carp treated with different concentrations of DM for a period of 28 days. Values are represented as mean±SEM

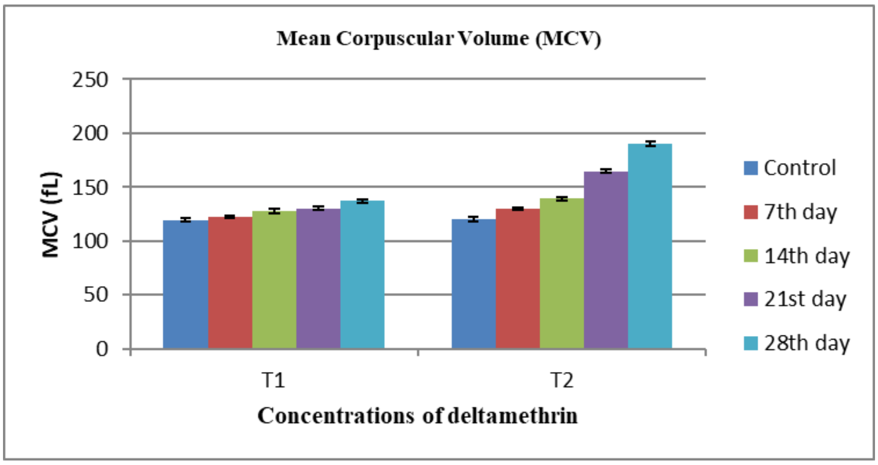

Fig. 5. Changes in the MCV (fL) of common carptreated with different concentrations of DM for a period of 28 days. Values are represented as mean±SEM

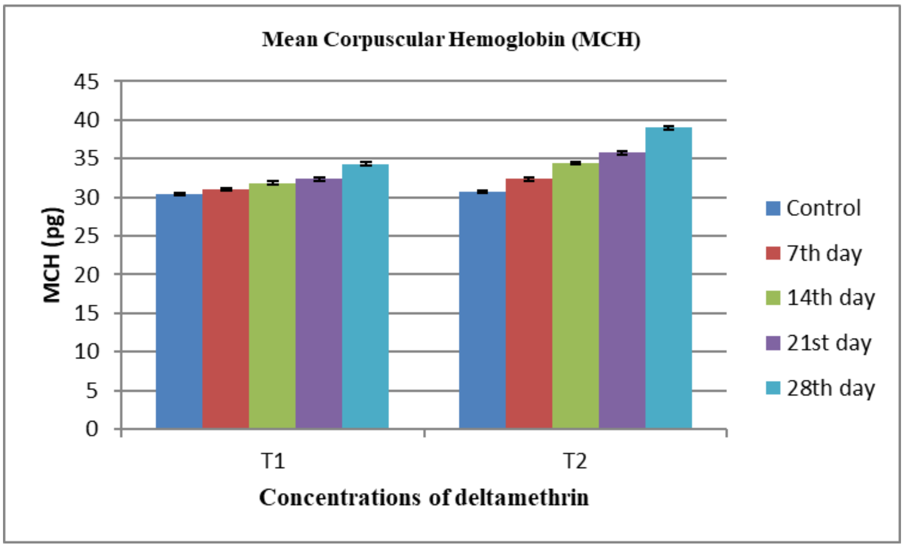

Fig. 6. Changes in the MCH (pg) of common carp treated with different concentrations of DM for a period of 28 days. Values are represented as mean±SEM

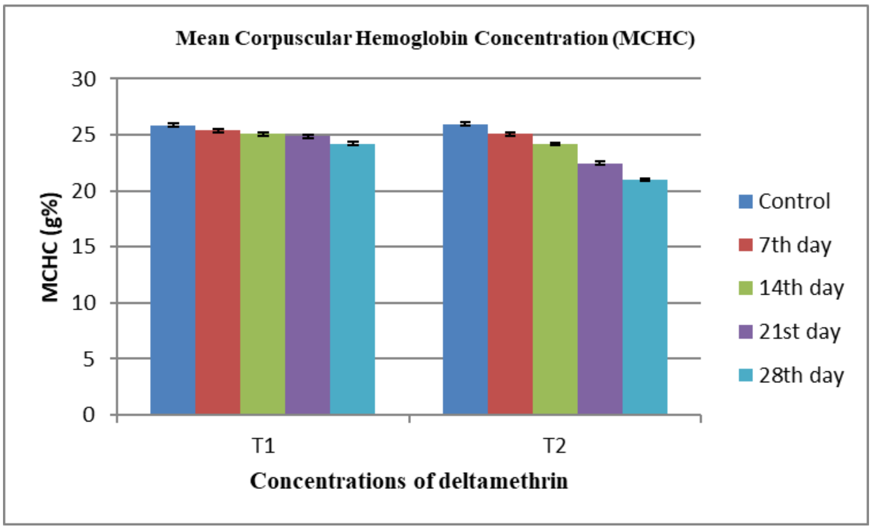

Fig. 7. Changes in the MCHC (g%) of common carp treated with different concentrations of DM for a period of 28 days. Values are represented as mean±SEM

period suggests that fish can acquire a defense mechanism to withstand the stress originated by any kind of toxicant. Fish immune defense is stimulated by leukocytosis when foreign chemicals are present [24]. Fish exposed to pesticides benefit from enhanced antibody production, which is correlated with higher WBC numbers [20]. The changes observed in present study were both time and dose dependent. Maximum change was observed on 28th day of exposure to 0.011µl/L (higher) concentration of deltamethrin.

Various researchers have investigated the detrimental outcomes of DM on hematological parameters of various fish species which are consistent with the results of present research. According to Jayaprakash and Shettu [25], the hematological markers of *Channa punctatus* (fresh water fish) showed significant alterations after the exposure of sub lethal concentrations of DM for a period of 45 days. After 45 days of exposure, TEC, Hb, PCV, MCH and MCHC values were dramatically lowered, while MCV values were significantly raised, in comparison with control. They observed that *C. punctatus* exposed to DM developed anemia, as evidenced by the anemia-causing substantial drop in Hb, TEC, and PCV levels. Similar reduction in TEC, PCV and Hb levels was observed in current findings indicating that anemic condition was developed in fish after the treatment of pesticide.

David et al. [26] investigated the effects of lethal and sub lethal concentrations of DM on selected hematological markers of *Cirrhinus mrigala*. After the exposure of DM they found that the TEC, Hb, and Hct values showed declining trend for both the concentrations of DM. Initial increase in WBC count followed by decrease in later days of exposure was observed. Non- significant changes in MCHC values were seen. MCV and MCH values of the fish that were subjected to the experiment exhibited a significant rise. The findings of this research are comparable to those found in the current research.

Srinivasa Rao et al. [27] conducted research to study the hematological alterations in fish, *Ctenopharyngodon idella*, after the exposure to DM 11% EC (Decis) and DM technical grade. When the fish was exposed different concentrations of both toxicants, reduction in RBC, Hct/PCV and Hb values was observed; in contrast WBC count and MCHC was increased. Increase in MCV and MCH values was seen at lethal dose of technical grade DM and similar trend was observed in case of both technical grade and 11 %EC DM (sub-lethal concentrations). On the contrary these values were decreased at lethal dose of 11% EC DM. Different changes observed in current findings are comparable to these results.

Various other researchers have found the similar results after the use of different pyrethroids on different fish species. Ghosh et al. [28] observed significant changes in hematological indices of *C. punctatus* after the exposure of another pyrethroid, cypermethrin (CYP) for a short period of time. According to them after the treatment of CYP for 14 days period, significant decrease in

TEC, Hb, PCV, MCH and MCV levels where as remarkable increase in TLC, and MCHC was noticed. In order to investigate the changes induced by CYP in hematological parameters of common carp, juvenile fish were exposed to various doses of CYP [29]. Decreasing trend was seen in total number of RBC, Hb and PCV at both concentrations (lethal and sub lethal). Total number of WBC and MCHC showed a tendency of increase at sub lethal concentrations and decrease at lethal dosages. Enhanced levels of MCV and MCH were seen at both the concentrations.

Some other researchers have also found similar results after the treatment of different fish species with different pesticides; *C. carpio* treated with monocrotophos [30], *C. mrigala* treated with Chlorfenapyr, Dimethoate and Acetamiprid [31] and *Barbonymus gonionotus* treated with sumithion [32].

4. CONCLUSION

In present investigation fish treated with sub-lethal doses of deltamethrin exhibited notable changes in the levels of several blood parameters of common carp. This highlights the necessity of stringent oversight and control over the use of these pesticides to protect the ecosystem and fish welfare. Humans must also use pesticides responsibly to protect aquatic life and the ecosystem. We can also conclude that in environmental bio-monitoring, the parameters examined in the current research can be employed successfully as prospective biomarkers of pesticide toxicity to different aquatic creatures including fish.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

The experiment was conducted as per the guidelines of the institutional animal ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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