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Effects of insecticide, lesenta on some haematological parameters of common carp (*Cyprinus carpio* L.)

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ABSTRACT

The purpose of this study was to determine the effect of the insecticide lesenta on several haematological markers of common carp (*Cyprinus carpio* L.). A sublethal dose of 0.12, 0.25, and 0.5 µg/L was administered on the seventh, fourteenth, and twenty-first days of exposure, respectively. The RBC count in group IV fishes (0.5 µg/L lesenta treated) reduced from 6.83±0.53 to 44.00±0.82 ($x10^6$ mm^{-3}) after 21 days of exposure, and this decrease is statistically significant ($p<0.01$) when compared to control fishes. The higher WBC count in group IV (0.5 µg/L lesenta) treated fish at 21 days of exposure ranged from 4.45±0.16 to 7.70±0.60, which is statistically significant (p0.001) when compared to the control. The decrease in Hb level of treated fish may indicate a decrease in Hb synthesis as well as a decrease in oxygen carrying capacity, which could be caused by lesenta interfering with the haem or globin production pathway. This study concluded that acute exposure to lesenta resulted in a significant decrease ($p<0.05$) in RBC, WBC, and Hb content levels for the fish compared to the control groups. The decrease in values obtained for hematological parameters of treated fish in this study indicated that the physiological activities of the treated fish were impacted.

Key words: *Cyprinus carpio*, Lesenta, Insecticide, Haematology, RBC, WBC, Hb.

INTRODUCTION

Chemical pesticides are now essential for ensuring the quality and production of agricultural goods (Oerke, 2004) as well as controlling domestic pests including termites, fire ants, and mosquitoes (Elliott and Barnes, 1963). Pesticides, on the other hand, have detrimental effects on non-target organisms (Clasen et al, 2012), which may be advantageous. Chemical pesticides are projected to have a greater impact on aquatic environments than terrestrial environments since they eventually flow into surface water (Pritchard, 1993). Environmental contamination is obviously connected to an unbalanced environment in the emerging countries.

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Pesticide contamination has been found in both biotic and abiotic components of the environment. The haphazard application of various pesticides frequently results in significant damage to non-target organisms. With the rise of industry, agriculture, and population growth over the last century, environmental degradation caused by pesticide use has become a growing problem (Syed, et al., 2012).

Several researchers have looked on the haematological changes caused by pesticide toxicity in fish (Kumar and Nelson, 1997; Tilak, et al., 2003). Pesticides are used indiscriminately in modern agricultural methods, which frequently end up in the aquatic environment. Pesticide usage in the field has the ability to alter the aquatic medium, changing aquatic fauna and flora tolerance limits and posing a threat to the ecosystem (Adedeji and Okocha, 2012). Water contamination has emerged as one of the century's most pressing issues. The primary concerns to preserving our future are water quality management, water pollution control, and environmental protection.

Lesenta insecticide is a combination of two modes of chemistry (Fipronil 40% + Imidacloprid 40%) that provides

dual action against pests and is indicated for soil insects like white grub in sugarcane. It can be used as a soil drench at any time (Gregorc et al, 2018). When used during the planting process, Spray setts in furrows well and cover with dirt. Soon after therapy, you may discover that you are no longer eating. It is almost compatible with other pesticides. In comparison to helpful insects, it's a lot safer. Because of the dual mode of action and the low cost, there is a lower possibility of resistance. In India and other countries of the world, imidacloprid is one of the most extensively used insecticides (Arfat et al, 2014).

Hematological analysis is commonly used to determine the physiological status of animals that is known to be affected by various environmental factors, and it is also used as a guide in the diagnosis of many diseases in both animals and humans (Solomon and Okomoda, 2012). The potential effects of these commonly used pesticide lesenta on the economically and ecologically significant Cyprinus carpio have never been reported in the peer-reviewed literature. As a result, determining the pesticides' negative impacts on this particular species was vital. As a result, the goal of this work was to estimate the LC50 value of lesenta and demonstrate the effect of sublethal concentrations of this widely used pesticide in India on selected hematological parameters in *Cyprinus carpio.*

MATERIALS AND METHODS

Specimen collection

Healthy common carp, Cyprinus carpio (length 08.40±1.14 to 10.50±1.56 cm and weight 12.14±0.45 to 15.54±0.48 gm), of both sexes, were obtained from a local market in Adilabad, Telangana, and utilized as test animals. The identification of the fish was done using established keys and literature. The fish were disinfected for two minutes with a 0.01 percent KmNO4 solution before being placed to a 45 litre glass aquarium to acclimate for 15 days in laboratory conditions before being used.

Fish were housed in varied concentrations of lesenta in 5 litre capacity plastic tubs after acclimation. The LC50 concentration was determined using the Probit Analysis LC50 determination method (Finney, 1971). The WHO (2004) and FAO (2004) suggested formula for calculating fish mortality (2002). The insecticide lesenta, a commercial grade insecticide, was chosen for the experiment and was bought from a local market in Adilabad, Telangana.

LC_{50} determination

The toxicity of lesenta on C. carpio was tested and the LC50 was obtained using probit analysis according to the Organisation for Economic Co-operation and Development (OECD) guidelines for chemical testing (OECD 203 "Fish, acute toxicity test," 1992 and 2010). A group of ten common carp fishes were treated to six concentrations of lesenta for 96 hours to determine lesenta LC50 values: 0.5, 1.0, 2.0, 4, 8, and 1.6 µg/L. In addition, an untreated control group was maintained. Fish mortality was found after 24, 48, 72 and 96 h. According to Iqbal et al., LC50 values were computed (2005). To provide a consistent presentation of the toxicity data, the concentration response curve was linearized by logarithmic transformation of concentrations (log+2). The 96h LC50 with 95 percent confidence limits and slope function were calculated after the concentration response curve was linearized by logarithmic transformation of concentrations (log+2).

Experimental Design

Fish (n=24) were gathered and randomly placed into four glass aquariums after being fasted for 24 hours. Each treatment group had three tanks, each containing six fish and 25L of test fluid. For this experiment, a sub-lethal concentration was used. Four groups of fish have been established. Group I fishes were given no treatment, while group II fishes were given lesenta 0.12 µg/L for 21 days. Group III fishes were given 0.25 µg/L lesenta for 21 days, while Group IV fishes were given 0.5 µg/L lesenta for 21 days. With the exception of group I, the remaining three groups of fish were exposed to their respective sub-lethal concentrations of lesenta (0.12, 0.25, and 0.5 µg/L) for 21 days. The control group, Group I, was kept. All of the groups were fed the same sort of food and had identical living conditions. Fish specimens were sedated with tricaine methane sulfonate at the end of each exposure period (7th, 14th, and 21st days) to allow blood sampling.

Blood sample collection

Blood was collected from each fish by conducting a heart puncture on the 7th, 14th, and 21st days of exposure at the end of each experiment. Using a hematology analyzer, a portion of the blood was utilized to evaluate hematological parameters such as hemoglobin (Hb), red blood cells (RBC), and white blood cells (RBC).

Statistical analysis

Each blood parameter was analyzed three times, and the results were statistically analyzed using the students' t' test for significance. Haematological values were averaged and standard deviations were calculated. The mean + standard error of the mean is used to express all data (SEM). The normality of the data for haematological parameters was evaluated before a two-way analysis of variance (ANOVA) was used to discover if there were any significant differences between them. Statistical significance was considered as $p<0.05$ in all statistical analyses performed in Microsoft Excel.

RESULTS AND DISCUSSION

Determination of LC50

Figure 1 shows the median lethal concentrations (LC50) of lesenta for C. carpio after 24, 48, 72, and 96 hours. Tables also provide the probit numerical values together with their

Figure-1. Effect of lesenta in fish *Cyprinus carpio* with reference to RBC (x10^6 mm^{-3})

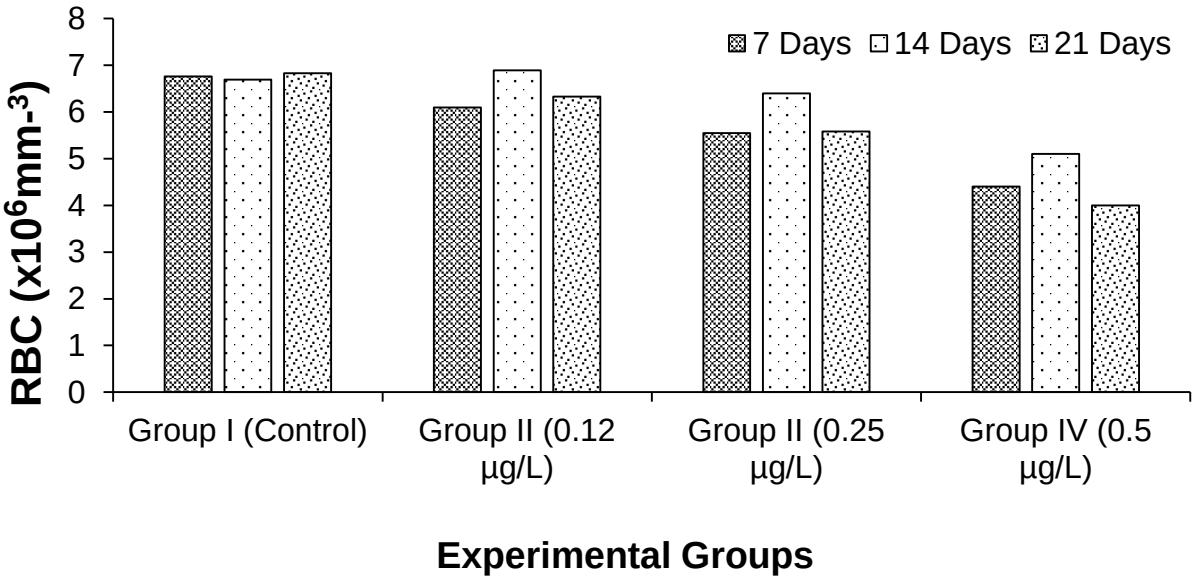

Figure-2. Effect of lesenta in fish *Cyprinus carpio* with reference to WBC (x10^6 mm^{-3})

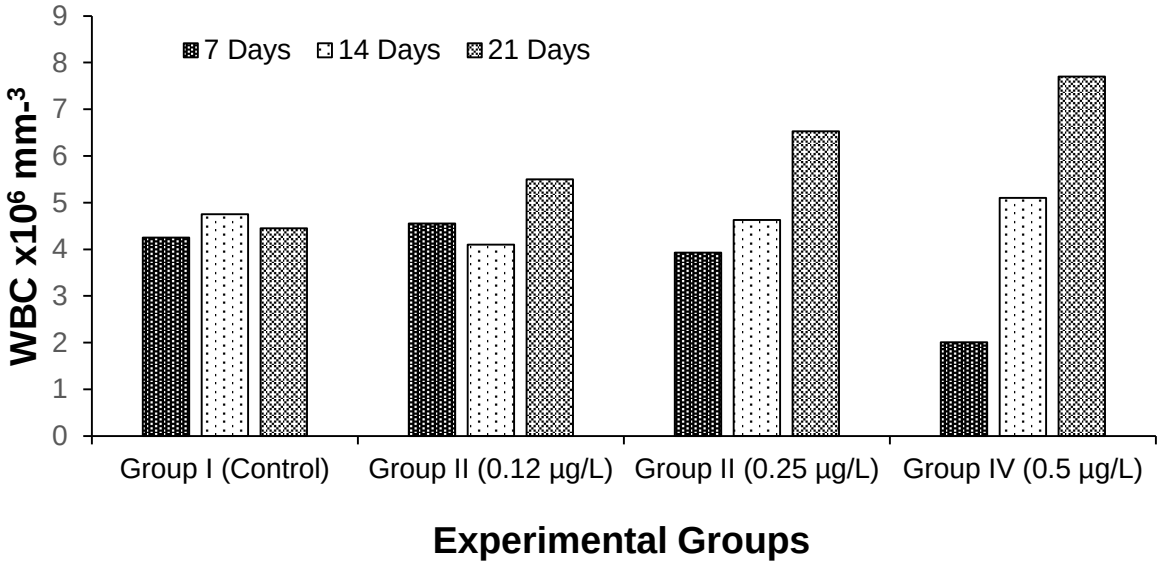

Table-1. Effect of lesenta in fish *Cyprinus carpio* with reference to Hb (g/dL) (Values are mean ± SE); *p = < 0.05

Tissue	Day of Exposure	Experimental Groups			
		Group I (Control)	Group II (0.12 µg/L)	Group III (0.25 µg/L)	Group IV (0.5 µg/L)
Hb (g/dL)	7	11.46±0.63	10.20±0.43	9.55±0.16*	7.3+0.24*
	14	11.20±0.13	9.59±1.7	8.20±0.64	7.11+0.28*
	21	11.45±0.33	8.13±0.44	7.24±0.55*	6.20+0.43*

95 percent confidence ranges. Lesenta has a 96-hour LC50 of 6.12 µg/L. Fish were subjected to a concentration of 0.5 g/L of lesenta according to the 96-hour LC50 of lesenta in *C. carpio*. The LC50 of lesenta was chosen as the lethal quantity to evaluate the haematological modifications of the fish, *Cyprinus carpio*, because the effect of an insecticide on fish becomes consistent after 96 hours of exposure. Furthermore, acute toxicity studies have important limitations, such as the possibility of test animals adapting to the imposed toxicity. Sub-lethal investigations are required because unique changes involving a sequence of events in the responses of test animals may occur at sub-lethal concentrations. For further research, 1/10th of the 96h LC50 (<0.5 g/L) was chosen as the sub lethal concentration of lesenta.

Effect on haematolological parameters

There were no significant changes in hematocrit levels between lesenta exposed and control fishes (Figure-1). Red blood cell (RBC) counts in fishes that had 7 and 14 days exposure to lesenta were not significantly different, compared to control fishes, but there was a significant, $P < 0.01$ reduction in RBC count in fishes that received 0.5 µg/L lesenta for 21 days (Figure-1).

The RBC count in group IV fishes (0.5 µg/L lesenta treated) at 21 day of exposure, was decreased from 6.83±0.53to 44.00±0.82 ($x10^6$ mm^{-3}) and it is statistically significant ($p<0.01$), and also this decrease is statistically significant to compare to control fishes.

Additionally, white blood cell (WBC) count was increased at all concentrations of lesenta treatment fishes. WBC counts in fishes that had 7 and 14 days exposure to lesenta were not significantly different, compared to control fishes, but there was significant, $P < 0.01$, elevation in WBC counts in the fishes that were exposed to lesenta for 21 days (Figure-2).

The elevated WBC count in group IV (0.5 µg/L lesenta) treated fish at 21 day of exposure was from 4.45±0.16 to 7.70+0.60 which is statistically significant ($p<0.001$), it also statistically significant ($p<0.001$) when compared to control.

When lesenta was exposed to fish for 7 to 21 days, the Hb content decreased from 7.3±0.24 to 6.20±0.43 at a concentration of 0.5 µg/L, compared to control. To control fishes, the reduction values are statistically significant ($p<0.05$). The decrease in Hb level in treated fish could indicate a decrease in Hb synthesis as well as a decrease in oxygen carrying capacity, which could be caused by lesenta interfering with the haem or globin production pathway. On acute exposure to lesenta, the fish showed a significant drop ($p<0.05$) in RBC, WBC, and Hb content when compared to the control groups. The decrease in values obtained for hematological parameters of treated fish in this study indicated that the treated fish's physiological activities were harmed.

Because unfavorable changes in the ambient environment are the first to affect the blood, biochemical profiles of blood can provide crucial information about the internal environment of the organism. Changes in serum enzyme activities, metabolites, and ions levels in pesticide-exposed fish were shown to be the biochemical manifestation of toxicants' toxic effects, according to the current study. The observed increases/decreases in all serum biochemical parameters of fish-treated insecticides could be a result of toxicant-induced target tissue damage and dysfunction (gill, liver, and brain), and these parameters could be used as quick and sensitive indicators of toxicant impact on aquatic organisms and, ultimately, the ecosystem.

Hematological indicators are critical criteria for determining the physiological status of fish. Intrinsic and external influences can alter major hematological parameters in fish, such as red blood cell counts, white blood cell counts, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Chemical contaminants can cause either an increase or a drop in hematological levels in fish. On general metabolism and physiological status of fishes, detailed information can be collected.

The count of red and white blood cells is a very steady index, and fish try to keep it within specified physiological limitations by employing a variety of physiological compensatory processes. A fall in the erythrocyte count or percent hematocrit suggests that the treated fish's condition is deteriorating and that anemia is developing. The lower red blood cell count could be related to reduced red blood cell formation or decreased hemoglobin synthesis. Decrease in red blood cells may be attributed to oxygen carrying capacity of the blood which may be due to the inhibition of erythropoiesis, hemosynthesis and increase in the rate of erythrocyte destruction.

The concentration of hemoglobin represents an organism's oxygen supply. The current study's lower hemoglobin concentration could be attributable to a lower red blood cell count. Hemoglobin levels that are much lower could indicate severe anemia caused by erythrocyte breakdown (Kori-Siakpere et al., 2009). Dorucu and Girgin found a reduction in hemoglobin in carp after cypermethrin poisoning (2001). Long-term reductions in hemoglobin content are harmful to oxygen delivery, and any blood dyscrasia and erythrocyte degeneration could be considered unhealthy in fish exposed to toxicants. The inhibition of aerobic glycolysis reduces de novo hemoglobin synthesis to a normal level. Decrease in the hemoglobin levels may limit oxygen availability to various tissues resulting in sluggish metabolic rate and reduced energy generation (Ahmad et al., 1995). (Ahmad et al., 1995).

CONCLUSION

The hematological changes seen in the current study, as well as the initial time of exposure in the fish's organs after exposure to lesenta toxicity, could constitute a defense strategy. On prolonged exposure, the accumulation of lesenta in the fish's organs produced severe hematological changes and organ structure deterioration. These

alterations may have an impact on the C. carpio's capacity to survive in their native habitat. This should be considered when using it for pest management in agricultural fields near natural freshwater reservoirs.

Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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