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A Study on Biochemical Changes in Common Carp Cyprinus Carpio Exposed to Sub-Lethal Concentrations of Lesenta Insecticide

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***Abstract*---**Biochemical parameter investigations are important in toxicological evaluations because changes in these parameters show before clinical symptoms do. The purpose of this study was to see how Lesenta pesticide affected biochemical parameters in the freshwater teleost C. carpio. This study used 1/10th of the sub-lethal concentrations of lesenta (i.e., 1 mg/L) based on the toxicity study. Fish were treated to their respective sub-lethal concentrations of Lesenta and kept at these levels for the duration of the experiment. Fish were dissected after the control and experimental groups were completed to analyze biochemical changes in the liver, gills, and brain tissues. Changes in biochemical parameters such as carbohydrates, proteins, and lipids are crucial indicators of organ systems' sensitivity to pollution by affecting their function. The current study's findings revealed a considerable drop in protein content in the tissues examined. With increasing exposure time, the protein level in all of the treated tissue samples decreased. The present findings revealed a considerable reduction in carbohydrate content in all of the tissues examined.

***Keywords*---**biochemical, C. carpio, carbohydrate, lesenta, lipid, protein.

Introduction

Proteins are a vital component of animal cells' biological makeup. Being a component of the cell membrane and enzymes, it plays an important role in the process of interactions between intra and extracellular media (Ramalingam, et al., 2002). When compared to other animal protein sources, fish protein is less

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expensive. Fish is consumed by around 35% of the Indian population, with per capita consumption of 9.8 kg compared to the recommended requirement of 13 kg. In rural India, fisheries give employment and nourishment to large segments of the population, particularly the poor (CSO, 2011). The physiological status of an animal is frequently represented by the metabolic status of proteins, according to Sathyanarayana (2005). Proteins have a key role in the cell's architecture, which is the primary source of nitrogen metabolism. As a result, protein fraction decrease in liver, brain, and renal tissues could have been owing to breakdown and probable metabolic use.

Carbohydrates are a collection of compounds that include sugars, starches, celluloses, and other similar molecules. They are the primary elements of plant biomass, although glycogen, sugars, and their derivatives are only found in trace amounts in the animal body. Plants provide the majority of the carbohydrate in the diets of animals, including fish. Carbohydrate digestion is fairly good in common carp (Chow and Halver, 1980). As a readily available energy source, carbohydrate is only found in small amounts in the liver and muscle. Carbohydrate is not a better energy source for fish than protein or fat, and digested carbohydrate does free up protein for tissue growth.

Both fats and oils are referred to as lipids. Fat is a source of energy and aids in the absorption of fat-soluble vitamins. The lipid content of fish varies depending on the species of fish and the diet used. Fish has a lower fat content than red meat, ranging from 0.2 percent to 25%. Polyunsaturated lipids make up the majority of fish lipids, with tiny levels of saturated and monounsaturated lipids. Fish oil is essential for a baby's brain growth to be normal. Children require polyunsaturated fatty acids for optimum growth. Oily fish are high in omega 3 fatty acids, which help to avoid cardiovascular disease. This oily fish group includes carp, eel, and salmon.

Biochemical parameter investigations are important in toxicological evaluations because changes in these parameters show before clinical symptoms do (Patel, 2012). The purpose of this study was to see how Lesenta pesticide affected biochemical parameters in the freshwater teleost *C. carpio*. Imidacloprid $\pm$ Fipronil 80 WG (40 $\pm$ 40 percent w/w) are two active ingredients in Lesenta that have a dual mechanism of action. It has been shown to provide low-dose, high-effective controls against a long-term problem that farmers have been dealing with. Lesenta is an antagonist to the nicotinic acetyl choline receptor in the central nervous system, while Imidacloprid disrupts the correct signal transmission pathway, causing nerve cell excitation and, as a result, a nervous system malfunction that leads to the treated insect's death. Fipronil is predominantly an ingested toxicant with some contact effect, and it works by interfering with nerve impulse transmission.

Materials and Methods

Test pesticide

For the toxicity investigation on physiological changes in *C. carpio*, the insecticide Lesenta (Imidacloprid 40% $\pm$ Fipronil 40%) was bought from a local market. The

compound was dissolved in 1 mL of distilled water in a standard volumetric flask to make the stock solution. This study used 1/10th (sub-lethal concentrations) of lesenta based on the toxicity study. In distilled water, the various concentrations were made.

Test animal

Major carps, such as the common carp *Cyprinus carpio* (Linnaeus), are commercially important food fishes with high commercial value that may be found in abundance in fresh water tanks across the Adilabad district. As a result, these fish were chosen as the best experimental material for this study. About 200 fingerlings of *Cyprinus carpio* were obtained from a fish farm in Adilabad District. The average weight and length were 13.18±0.38 grams and 9.09±0.29 centimeters, respectively. The fish were brought to the lab in oxygenated plastic bags with as little stress as possible. The fish were kept in glass tanks that were well aerated. The fish were housed in eight tanks, each with a size of 100 gallons.

The fish were raised in tap water that had been dechlorinated and disinfected with a 0.1 percent potassium permanganate solution. Aeration was delivered by compressed air pumps via air stones, and daily water changes were performed manually in all of the tanks. Water was replaced daily during the acclimation phase, and fish were fed once a day with artificial food pellets purchased from the local market. The fish were acclimated for 15 days.

Determination of 96 hours sub-lethal concentration dose (LC_{50})

To select the suitable testing range of concentration, preliminary screening was carried out to estimate the concentration of the utilized insecticide that is most likely to cause 50% death (LC_{50}) for 96 hours exposure. This task was completed in accordance with the EPA process (1985). One hundred and ten healthy fish were distributed in eleven 100-liter aquaria (ten fish for each aquarium). The fish were not fed for 24 hours prior to the experiment or during it. The fish were divided into six groups of ten fish each (I, II, III, IV, V, VI, and VII). In the aquaria, different concentrations of Lesenta (0, 1, 5, 10, 15, 20, and 25 mg/L) were distributed and labeled as I, II, III, IV, V, VI, and VI groups, respectively. After 24, 48, 72, and 96 hours, fish mortality was observed and used for further analysis. According to Iqbal et al.(2005), LC_{50} values were computed. The LC_{50} concentration was determined using Finney's (1971) Probit Analysis LC_{50} determination method.

Experimental design

Following acclimation, healthy and similar-sized *Cyprinus carpio* (13.18±0.38gm and 9.09±0.29cm in length) were selected and divided into two groups of ten fish each. Group I fishes were served as control and Group II fishes exposed to sub-lethal concentration (1/10th of LC_{50} value) of Lesenta (1mg/L) for 28 days.

Fish were treated to their respective sub-lethal concentrations of Lesenta and kept at these levels for the duration of the experiment. The test medium was changed on a daily basis, allowing for the elimination of nitrogenous waste

emitted by the test fishes as well as unconsumed food. Fish were dissected after the control and experimental groups were completed to analyze biochemical changes in the liver, gills, and brain tissues.

Biochemical studies

The liver, gills, and brain, among other organs, were dissected and washed in physiological saline. The tissues listed above were employed for various biochemical analyses (proteins by Lowry et al, 1951; carbohydrates by Roe technique, 1955 and lipids by Folch et al, 1951).

Results and Discussions

The purpose of the acute toxicity study was to determine the safest concentration of test chemicals for future testing. To test acute toxicity, Lesenta was utilized as a toxicant. Test fishes were subjected to varied concentrations of lesenta (0, 1, 5, 10, 15, 20, and 25 mg/L) after a stock solution of 25 mg/L was created. At 1, 5, 10, 15, 20, and 25 mg/L there was 0%, 10%, 20%, 40%, 50%, and 60% mortality after 24 hours of exposure to lesenta respectively. At 1, 5, 10, 15, 20, and 25 mg/L, 10 percent, 20 percent, 40 percent, 50 percent, 60 percent, and 70 percent mortality was recorded at the end of a 48-hour exposure to lesenta. At 1, 5, 10, 15, 20, and 25 mg/L, respectively, 20 percent, 30 percent, 40 percent, 60 percent, 70 percent, and 80 percent mortality was recorded at 72 hours of lesenta exposure. At 1, 5, 10, 15, 20, and 25 mg/L, 30 percent, 40 percent, 50 percent, 70 percent, 80 percent, and 90 percent mortality was recorded at the conclusion of 96 hours of lesenta exposure. Figure-1 shows the results of the acute toxicity test at 96 hrs of exposure period.

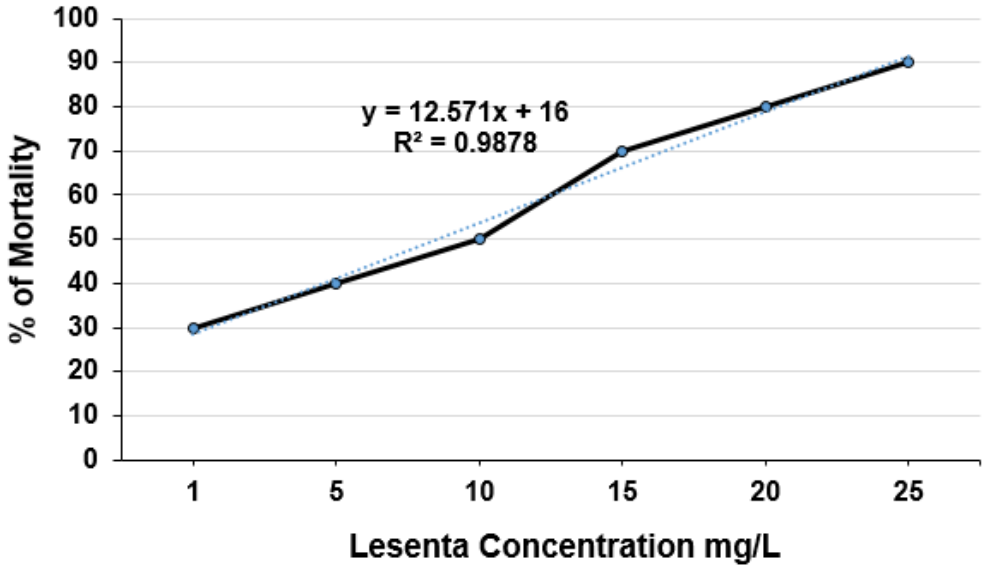

Figure 1. Linear graph of Percentage of mortality at diverse concentration of Lesenta for 96 hrs of exposure period

The LC50 values were derived using linear regression graphs. The LC50 values of Lesenta after 24, 48, 72, and 96 hours of exposure are 20, 15, 12.5, and 10 mg/L, respectively. Lesenta had the lowest LC50 after 96 hours of exposure. Following the LC50 concentration investigation, experimental groups were exposed to sub-lethal lesenta concentrations (i.e., 1 mg/L) for 24, 48, 72, and 96 hours. For testing the effect of sub lethal concentrations of lesenta on carp fish, *C. carpio*, ten times less concentration (1 mg/L) was taken from LC50 of 96 hrs (10 mg/L) of lesenta.

Total proteins in liver, brain and gills lesenta treated fishes

Changes in biochemical parameters such as carbohydrates, proteins, and lipids are crucial indicators of organ systems' sensitivity to pollution by affecting their function. The current study's findings revealed a considerable drop in protein content in the tissues examined. With increasing exposure time, the protein level in all of the treated tissue samples decreased.

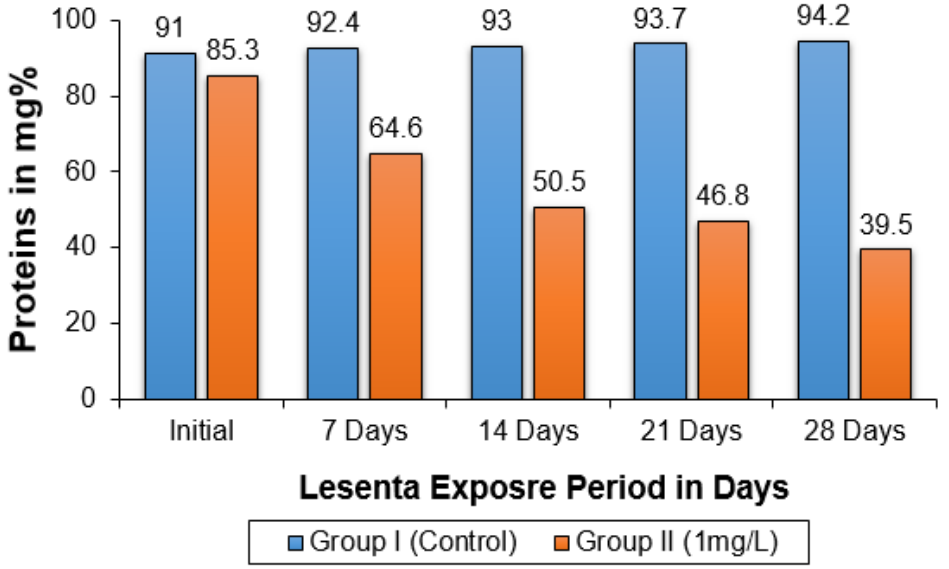

Figure 2. Effect of Lesenta on the protein (mg%) content of the liver of *Cyprinus carpio* in different exposure periods

The protein content of control liver tissue was 94.2±4.23 mg/L, and it gradually decreased to 39.5±0.67 and 25.9±1.0 mg/L for Lesenta treated fishes at the 28th day in 1/10th of LC50 value of Lesenta (1mg/L) (Figure-2).

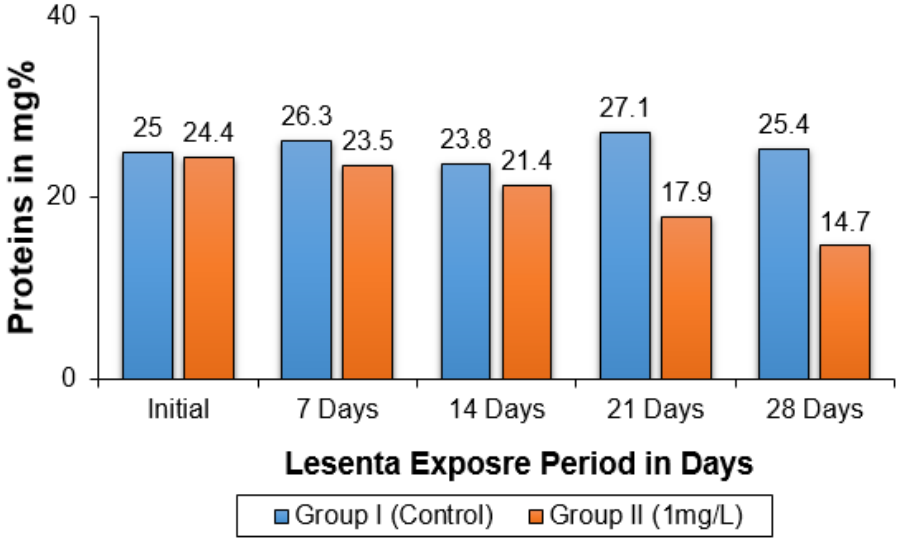

Figure 3. Effect of Lesenta on the protein (mg%) content of the brain of *Cyprinus carpio* in different exposure periods

After 0, 7, 14, 21, and 28 days, the mean total protein content in control brain tissue was 25.0±2.14, 26.3±2.11, 23.8±1.21, 27.1±1.24, and 25.4±2.34 mg/L, respectively. The protein content was reduced to 24.4±1.62, 23.5±1.90, 21.4±1.22, 17.9±2.00 and 14.7±1.04 mg/L after 0, 7, 14, 21, and 28 days exposure respectively (Figure-3).

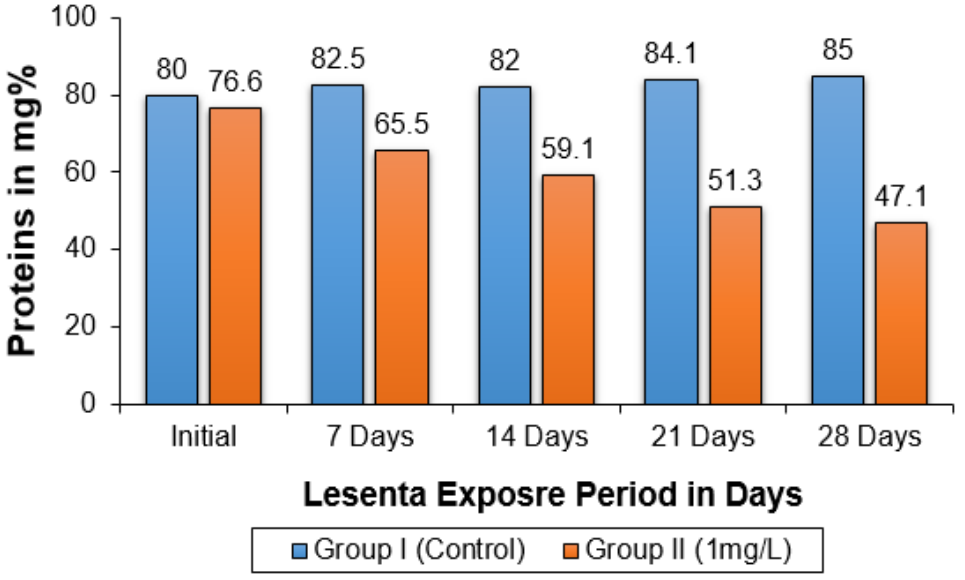

Figure 4. Effect of Lesenta on the protein (mg%) content of the gills of *Cyprinus carpio* in different exposure periods

On 7, 14, 21, and 28 days of exposure to sub-lethal concentrations (1mg/L Lesenta), the total protein content in the gills of Lesenta-treated fishes was

reduced from 82.5±2.62, 82.0±1.90, 84.1±1.99, and 85.0±3.21, respectively, to 65.5±1.42, 59.1±1.19, 51.3±1.09, and 47.1±1.90 mg/L. The results were determined to be statistically significant (P<0.01) (Figure-4).

Total carbohydrates in liver, brain and gills Lesenta treated fishes

Carbohydrates are a necessary component of living cells and provide energy to animals. The present findings revealed a considerable reduction in carbohydrate content in all of the tissues examined.

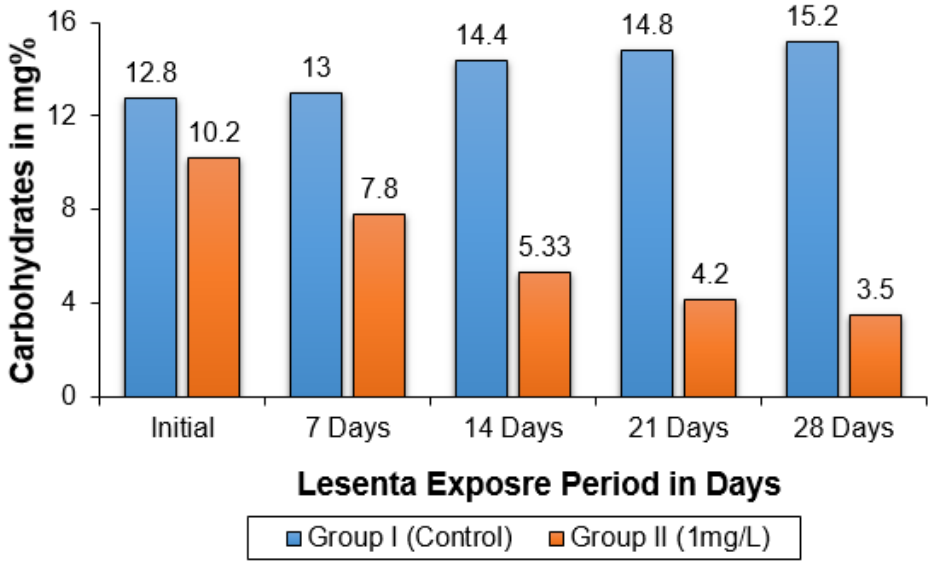

Figure 5. Effect of Lesenta on the carbohydrate (mg%) content of the liver of *Cyprinus carpio* in different exposure periods

After 7, 14, 21, and 28 days of exposure the carbohydrate content of control liver tissue was 13.0±1.01, 14.4±1.12, 14.8±0.08, and 15.2±0.23, and it steadily decreased to 7.80±1.30, 5.33±0.93, 4.20±0.52 and 3.50±0.87 in sub-lethal concentration treated animals. In exposed fish, the drop was substantial (P<0.05) (Figure-5).

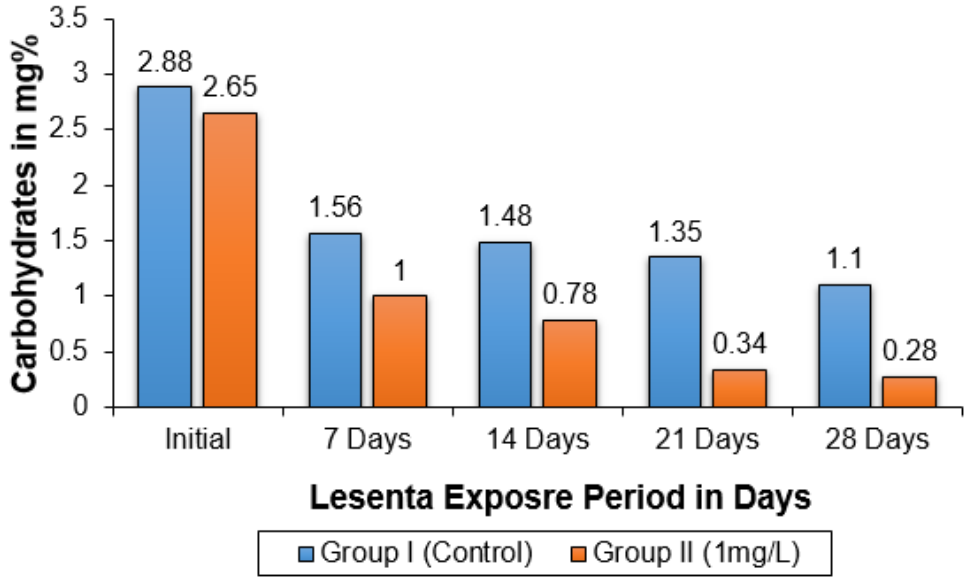

Figure 6. Effect of Lesenta on the carbohydrate (mg%) content of the brain of *Cyprinus carpio* in different exposure periods

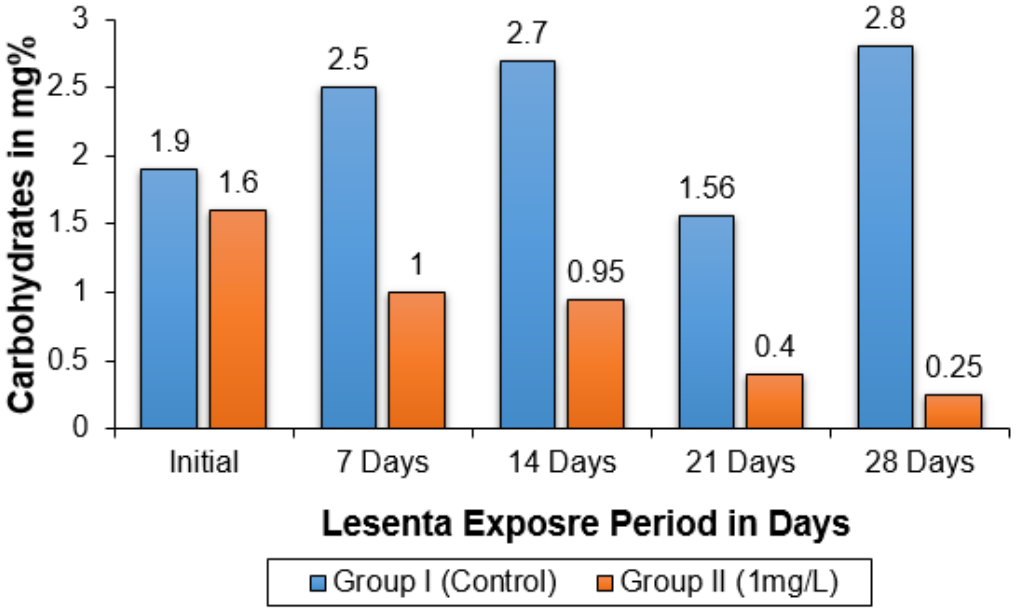

Figure 7. Effect of Lesenta on the carbohydrate (mg%) content of the gills of *Cyprinus carpio* in different exposure periods

When compared to group I control fishes, carbohydrate levels in brain tissue of group II treated fishes were considerably lower after 7, 14, 21, and 28 days. When compared to control fishes, carbs were reduced from 1.10±0.01 to 0.28±0.04 mg/L after 28 days of Lesenta exposure in group II (1 mg/L lesenta treated fishes) brain tissue (Figure-6)..

The amount of carbohydrates in the gill tissue of Lesenta-treated fishes was similarly significantly reduced (P <0.05). After 7, 14, 21, and 28 days, the mean carbohydrate levels in control fish were 2.50±0.42, 2.77±0.60, 1.56±0.09, and 2.80±0.11 mg/L, respectively, while in group II fish (1 mg/L Lesenta treated), they were 1.00±0.02, 0.95±0.09, 0.40±0.02 and 0.25±0.03 mg/L (Figure-7).

Total lipids in liver, brain and gills of Lesenta treated fishes

Lipids are essential components of biological architecture. Lipids are also necessary for maintaining appropriate cell permeability and cell membrane structural integrity. The levels of total lipids in the liver, brain, and gills of fish *C carpio* were measured after 7, 14, 21, and 28 days of exposure to sub-lethal doses (1/10th of LC50 i.e. 1mg/L and 1/5th of LC50 i.e. 2mg/L) of Lesenta, as well as control levels, in the present study (Figure-8).

At 7, 14, 21, and 28 days after exposure, total lipid content in the liver of normal fish was estimated to be 26.2±1.10, 26.7±1.01, 27.1±1.29, and 26.1±1.08 mg/L. On the 7th, 14th, 21st, and 28th days after exposure to the insecticide Lesenta, there was a very considerable drop. When compared to the control group, the experimental group II (1mg/L concentration of Lesenta) had a substantial drop in lipid content of the liver from 26.1±1.08 mg/L to 9.50±0.87mg/L on the 28th day of treatment.

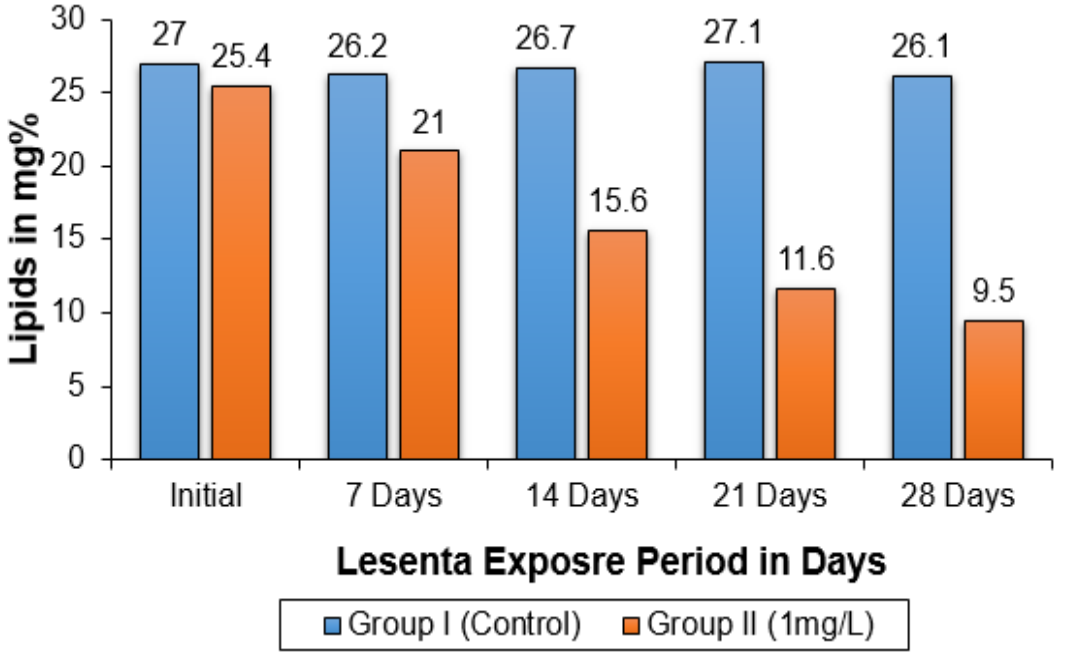

Figure 8. Effect of Lesenta on the total lipid (mg%) content of the liver of *Cyprinus carpio* in different exposure periods

On the 7th, 14th, 21st, and 28th days of exposure, the total lipid in the brain tissue of normal fish was estimated to be 86.1±2.10, 85.1±2.58, 84.8±2.09, and 85.9±2.80 mg/L, respectively, and the values gradually decreased to 72.0±3.10, 67.6±2.02, 61.0±2.67 and 56.1±2.02 mg/L in group II fishes (1 mg/L Lesenta treated). These differences were statistically significant at $p<0.01$ (Figure-9).

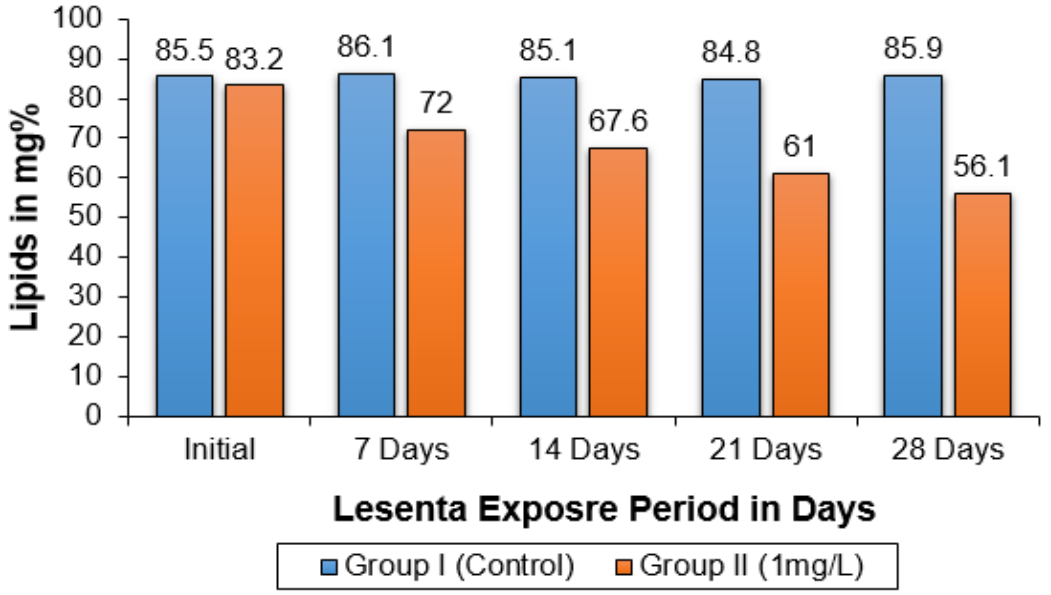

Figure 9. Effect of Lesenta on the total lipid (mg%) content of the brain of *Cyprinus carpio* in different exposure periods

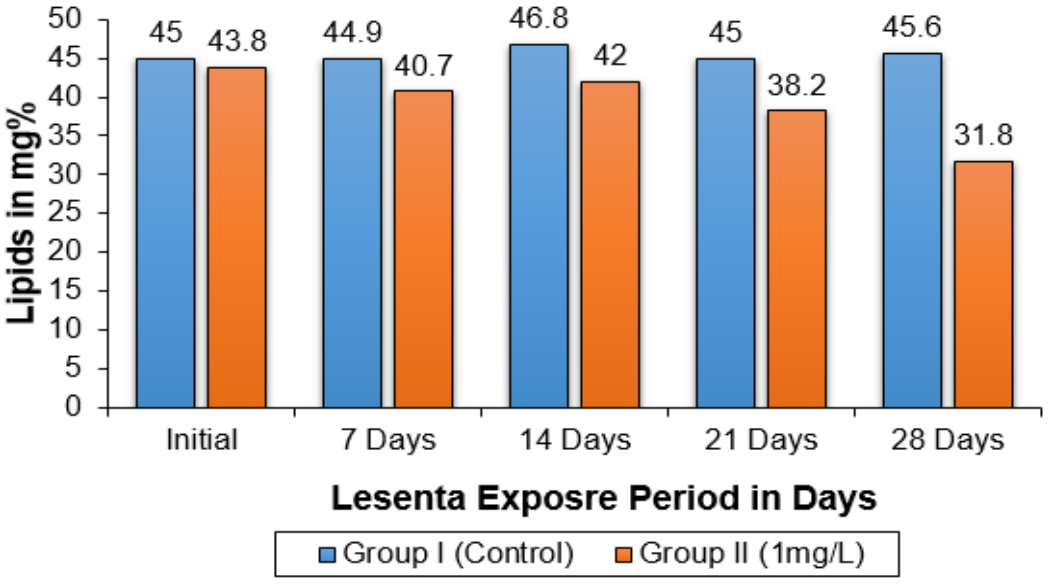

Figure 10. Effect of Lesenta on the total lipid (mg%) content of the gills of *Cyprinus carpio* in different exposure periods

At the 7th, 14th, 21st, and 28th days after exposure, the total lipid of the gills was 44.9±2.04, 46.8±1.99, 45.0±2.01, and 45.6±2.81 in the untreated fish. On the 7th, 14th, 21st, and 28th days of exposure, total lipid in the gills of 1mg/L Lesenta treated fishes was 40.7±1.82, 42.0±2.01, 38.2±1.56, and 31.8±1.50 mg/L, respectively, on all days of exposure, a decrease in lipid content was seen in the fish treated with sub-lethal concentration of Lesenta, which was significant at $p<0.01$ (Figure-10).

The initial step in the development of toxic effects is the interaction between pollutants and biomolecules. Understanding the changes caused by pollution exposure may aid in the prediction of harmful consequences that may occur at higher levels of biochemical organization (Rosety-Rodriguez et al., 2005). Biologically active substances such as carbohydrates, proteins, and lipids are affected by toxicants (Ghosh and Chatterjee, 1985).

Proteins are the most basic and plentiful biological constituents found in fish. Proteins are the most vital energy source to have on hand during a stressful period (Yadav et al., 2003). Animals exposed to toxicants, even at sublethal levels, undergo significant metabolic stress throughout the toxicant's detoxification (Yadav et al., 2003). Proteins are engaged in major physiological events, hence measuring protein levels can be used as a diagnostic tool for determining an organism's physiological stages. Toxicological depletion of protein content has been found in the muscle, gut, and brain of the fish *Catla catla*. When an animal is under toxic stress, it diversifies its energy sources to meet the approaching energy demands, and its protein stores are reduced as a result (Neff, 1985). The degradation of protein into free amino acids could be the cause of the decrease in total protein content.

Carbohydrates are an organism's primary source of energy (Saravanan et al., 2000). These are abundant in the liver and muscle tissues, but only in trace levels in the kidney and brain. When fishes are exposed to toxic stress, their carbohydrate, protein, and lipid metabolism is disrupted (Shaffi, 1978). The decrease in carbohydrate content in muscle and brain could be related to glucose use to meet the increased energy demand imposed by mercury intoxication's acute anaerobic stress. Anabas testudineus and Anabas scandens showed similar responses when exposed to copper, lead nitrate, and mercuric chloride, respectively (Mary Chandravathy et al., 1991).

Lipids are essential components of biological architecture. Lipids are also necessary for maintaining appropriate cell permeability and cell membrane structural integrity. The synthetic pyrethroid cypermethrin was likewise observed to reduce the lipid content in *Labeo rohita* liver and muscle tissue (Das and Mukherjee, 2003). The levels of total lipids in the muscle, liver, and brain of Gambusia affinis treated to the pesticide phosphamidon were significantly reduced, according to Govindan et al. (1994). Cyprinus carpio, a freshwater fish, was exposed to sublethal concentrations of cypermethrin and showed significant alterations in protein fractions of the liver, brain, and muscle tissue (Asztalos etal., 1990).

The stress caused by changes in the biochemical make-up of fish exposed to toxicants causes rapid mobilization of these energy-producing biomolecules to provide excess energy required by stressed animals (Saravanan et al., 2003) to combat the heavy physical exercise in the form of erratic and rapid movements and high respiratory rates under toxicant stress (Saravanan et al., 2003). The carbohydrate, protein, and fat contents were low in all of the experimental groups in virtually all of the tissues in the current investigation, in the experiment involving sequential synergism. It definitely implies a very low metabolic rate, which could be owing to the fish's initial stress.

Conclusion

When compared to the control, the result demonstrates decreased levels of biochemical parameters during all exposure durations. Lesenta pesticide has an impact on the cellular and molecular level, resulting in physiological and metabolic alterations. The current study's findings clearly demonstrate the hazardous nature of the toxicant on the biochemical parameters of the *C. carpio* fish. All of the metabolites studied are found to be sensitive changes in the normal indictors, which reflect changes in the normal activities of various functional systems, and the changes in total proteins, carbohydrates, and lipid in the lesenta treated fish will unusually affect the nutritive value of these fishes.

Acknowledgments

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