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Research Article

Assessment of insecticide, lesenta toxicity on antioxidant enzymes in common carp (*Cyprinus carpio* L.)

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ABSTRACT

Indiscriminate discharge of pharmaceutical waste into the aquatic ecosystem may pose major health challenges to aquatic biota. The effect of acute exposure to ibuprofen was studied utilizing changes in haematological parameters under static bio-assay method in common carp (*Cyprinus carpio*). For this investigation, the fishes are split into four separate groups. Based on the sub-lethal toxicity research, fishes exposed to 1/10th of LC50 value of ibuprofen. The 96-h LC50 of Ibuprofen was 12.75 mg/L. in *C. carpio*. Therefore, for this experiment, fish were subjected to 0.25, 0.50 and 1 mg/L doses of ibuprofen. At the end of each exposure session (7th day, 14th day and 21st day), fish specimens were anesthetised with tricaine methane sulfonate to permit the collection of blood samples. The RBC count in group IV fishes of ibuprofen treated fishes, at 21 day of exposure, was lowered and it is statistically significant ($p<0.01$) to control fishes. WBC counts in fishes that had 7 and 14 days exposure to ibuprofen were not statistically altered, compared to control rats. Ibuprofen treated fish did not elicit any significant alterations ($p > 0.05$) with reference to monocytes, basophils and eosinophils. Based on the above results, it might be inferred that administration of ibuprofen, may elicit immunological perturbations, and their toxicity may rise depending on the dose.

1. Introduction

The common carp (*Cyprinus carpio* L.) is one of the most important fish cultivated worldwide, either for food or for pleasure fishing. Carp is frequently recommended for the baseline assessment of emerging contaminants in aquatic ecosystems (Sudisha et al, 2019) and is commonly utilized in experimental models due to its availability and its adaptability to laboratory conditions (Ningthoujam et al, 2013).

Pesticides of various varieties are used in modern agricultural systems to manage pests, weeds, and plant diseases. Pesticides are usually recognized as very effective and approved for protecting plants from insect assault, and they have significantly contributed to increased agricultural productivity (Sudisha et al, 2010). Lesenta (Imidacloprid 40% + Fipronil 40%) is an effective treatment for chronic white grub infestations. Imidacloprid, an antagonist to the nicotinic acetyl choline receptor in the central nervous system, disrupts the correct signal transmission pathway, causing nerve cell stimulation and, as a result, a nervous system malfunction, ultimately leading to the death of the treated insect.

Insecticide use causes an increase in reactive oxygen species (ROS), which causes lipid peroxidation, cell membrane damage, protein oxidation, enzyme inactivation, and DNA and RNA damage. Plants use a variety of ways to cope with pesticide-induced harm (Badraoui et al, 2012). Under stressful situations, they collect a variety of metabolites, including amino acids and osmoprotectants (compatible solutes). Compatible solutes such as sucrose, proline, trehalose, polyols, and quaternary ammonium compounds protect plants from stress through a variety of mechanisms such as ROS detoxification, membrane integrity maintenance, osmotic adjustment, and protein/enzyme stability (Ningthoujam et al, 2013). Plants have also evolved a complex antioxidant system to prevent and repair the damage caused by ROS. One of the most important plant defense mechanisms against environmental challenges is the enzymatic antioxidative system. Toxic organic compounds, in general, can disrupt the function of antioxidant enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD),

catalase (CAT), glutathione reductase (GR), and glutathione S-transferase (GST), which reflect not only the level of toxicity but also the stress tolerance capacity of plants (Kaya et al, 2016).

The current study investigated the effect of the insecticide lesenta at various dosages on antioxidant enzymes (CAT and SOD).

2. Material and Methods

2.1 Chemicals and reagents

Test substance Lesenta insecticide purchased from local market of Adilabad district, Telangana, India. Its stock solution was prepared by dissolving the pesticide in 1 mL of distilled water in a standard volumetric flask.

2.2 Fish

Carp specimens weighing 30.0±5.2 g and measuring 8-11 cm in length were obtained from a local fish market in Adilabad, Telangana, India, and acclimated to laboratory conditions for 15 days prior to the start of the experiment. Fish were kept in separate tanks (1 m0.4 m0.5 m, 160 effective litres) in two batches (control and exposed group) of ten individuals each. Throughout the trial, fish were fed a commercial dry diet ad libitum on a daily basis until the end of the investigation. The fish were starved for 24 hours before being sacrificed.

2.3 Determination of 96 hours sub-lethal concentration dose (LC50)

To define the appropriate testing range of concentration, preliminary screening was performed to estimate the concentration of the utilized insecticide that is most likely to cause 50% death (LC50) after 96 hours exposure. This task was completed in accordance with the EPA process (1985). One hundred and ten healthy fish (weighing 5.021.2 g and measuring 6.420.43 cm) were placed in eleven 100-liter aquaria (ten fish for each aquarium). The aquaria were fed dechlorinated tap water and were kept at constant aeration, temperature, and pH. The fish were not fed for 24 hours prior to and during the experiment. Lesenta concentrations of 0.5, 1.0, 2.0, 4, 8, and 1.6 g/L were disseminated in the aquaria. After 24, 48, 72, and 96 hours, fish mortality was found. Iqbal et alLC50 .'s values were used to calculate LC50 values (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 linearization of the concentration response curve by logarithmic transformation of concentrations (log+2).

2.4 Experimental Design

Fish (n=24) were gathered and randomly placed into four glass aquariums after being fasted for 24 hours. Each tank had six fish and 25L of test fluid, and three tanks were utilized in each treatment group. For this study, a sub-lethal concentration was used. The fish are classified into four groups. Group I fish served as controls, whereas Group II fish were treated to lesenta 0.12 g/L for 21 days. Group III fish are subjected to 0.25 g/L lesenta for 21 days, while Group IV fish are treated to 0.5 g/L lesenta for 21 days. Except for group I, the remaining three groups of fish were exposed to their respective sub-lethal concentrations (0.12, 0.25, and 0.5 g/L) of lesenta for 21 days. Group I was kept as the control group. The meal was the same for all groups, and the other conditions were the same. Fish were slain at the end of each exposure period (7th, 14th, and 21st day), and parts such as gills, liver, and brain were dissected and extracted. The tissues (10 mg) were homogenized in 80% methanol, centrifuged for 15 minutes at 3500 rpm, and the clear supernatant was utilized to analyze several antioxidant parameters (SOD and CAT).

2.5 Measurement of Antioxidant Enzyme Activity

2.5.1 Catalase

At room temperature, catalase (CAT) activity was evaluated spectrophotometrically by monitoring the drop in absorbance at 240 nm caused by H2O2 breakdown. Catalase activity was determined using Aebi's technique [11]. Under assay circumstances, one unit (U) of catalase activity was defined as the amount of enzyme that generated an absorbance change of 0.001 per minute. In a total volume of 3.0 mL, the reaction mixture contained 100 mM sodium phosphate buffer (pH 7.0), 30 mM H2O2, and 100 uL test compound.

2.5.2 Superoxide dismutase

The suppression of photoreduction of nitroblue tetrazolium (NBT) by SOD enzyme was used to measure superoxide dismutase (SOD) activity [14]. In a final volume of 3.0 mL, the reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 12 mM L-methionine, 50 mM NBT, 10 mM riboflavin, and 100 mL crude extract. A control reaction was carried out in which no crude extract was used. The SOD reaction was carried out by exposing the reaction mixture to white light at room temperature for 15 minutes. A spectrophotometer was used to measure absorbance at 560 nm after 15 minutes of incubation. One unit (U) of SOD activity was defined as the amount of enzyme that inhibited photochemical degradation of NBT by 50%.

2.6 Statistical analysis

All experiments were done in triplicate, and the results were given as the average standard deviation of the readings. The standard deviation was calculated using Excel (Microsoft Co, Redmond, WA, USA).

3. Results and Discussion

3.1 Determination of LC50.

The median lethal doses (LC50) of lesenta for C. carpio at 24, 48, 72, and 96 hours are described. The probit numerical values and associated 95% confidence intervals are also computed. Lesenta's 96-hour LC50 was 6.12 g/L. According to the 96-hour LC50 of lesenta in C. carpio, fish were exposed to 0.5 g/L of lesenta. Taking into account that the effect of an insecticide on fish becomes consistent after 96 hours of exposure, the LC50 of lesenta was chosen as the lethal quantity to evaluate the antioxidant enzymes level of the fish, Cyprinus carpio. Furthermore, studies on acute toxicity have significant limitations, such as the occurrence of test animal adaptation to the imposed toxicity. Sub-lethal studies are required because distinct changes involving a sequence of events in the responses of test animals may occur at sub-lethal concentrations. As a result, 1/10th of the 96h LC50 (0.5 g/L) was chosen as the sublethal concentration of lesenta for future research.

Table-1. Effect of lesenta on the activity of catalase in gills, liver and brain of fresh water fish *Cyprinus carpio*

Tissue	Day of Exposure	Experimental Groups (U/mg protein)			
		Group I (Control)	Group II (0.12 µg/L)	Group III (0.25 µg/L)	Group IV (0.5 µg/L)
Liver	7	42.2+2.54	36.5+0.75	31.0+1.42	26.9+1.23*
	14	45.5+5.43	33.2+0.33	30.9+1.45	25.7+1.15*
	21	44.6+1.42	30.6+0.23	27.9+0.99	23.5+0.25*
Gills	7	71.3+2.11	68.7+1.80	62.6+1.56	59.0+0.13*
	14	70.4+3.34	65.7+1.35	60.7+1.36	53.2+0.23*
	21	68.5+1.62	61.8+0.99	57.8+0.53	48.9+0.16 *
Brain	7	69.4+2.43	61.6+0.54	58.5+0.74	51.4+0.64 *
	14	68.6+1.22	62.5+0.32	56.1+0.34	47.9+0.55 *
	21	65.3+1.35	58.3+0.33	49.4+0.35	41.0+1.66*

The results are expressed as Mean±SEM of five observations.
Levels of significance values are *p<0.05, considered to be statistically significant compared with control group.

Table-2. Effect of Lesenta on the activity of superoxide dismutase in gills, liver and brain of fresh water fish *Cyprinus carpio*

Tissue	Day of Exposure	Experimental Groups (U/mg protein)			
		Group I (Control)	Group II (0.12 µg/L)	Group III (0.25 µg/L)	Group IV (0.5 µg/L)
Liver	7	80.0+1.24	77.4+0.75	73.0+1.77	71.0+1.73*
	14	81.6+2.23	71.2+0.36	70.2+1.05	69.7+1.35*
	21	81.7+2.22	68.4+0.53	66.3+0.64	66.8+0.58*
Gills	7	72.6+1.44	69.3+1.60	61.6+1.57	59.2+0.73*
	14	69.4+2.33	64.5+1.85	61.7+1.35	53.7+0.53*
	21	73.5+2.52	60.5+0.99	57.8+0.53	48.9+0.16 *
Brain	7	85.4+2.55	72.6+1.53	68.5+0.64	61.4+1.34 *
	14	83.6+1.87	70.5+0.11	66.4+1.34	62.4+1.53 *
	21	85.2+1.55	68.3+0.23	69.5+1.55	57.6+2.65*

The results are expressed as Mean±SEM of five observations.
Levels of significance values are *p<0.05, considered to be statistically significant compared with control group.

3.2 Effect of lesenta on catalase

The activity of the antioxidant enzyme catalase in the gills, liver, and brain of control and experimental animals is shown in Table 1. Catalase activity was highest in the brain and gills of control fishes, followed by the liver. Catalase activity was significantly lowered in the gills (p0.001), liver (p0.01), and brain (p0.001). At 0.5 g/L concentration, the activity was reduced by 18% in the gills and 15% in the brain, but only 13% in the liver after 21 days of exposure. At 0.12 g/L, no significant change in activity was observed in all three organs.

3.3 Effect of lesenta on superoxide dismutase

Table-2 shows the activity of the antioxidant enzyme superoxide dismutase in gills, liver, and brain subjected to

varying doses of lesenta. Superoxide dismutase activity was highest in the brain of control fishes, followed by the liver and gills. Lesenta treatment after 21 days significantly reduced superoxide dismutase activity in the gills, liver, and brain (p0.01). The reduction was nearly identical in all organs. At 0.5 g/L, 11 percent of enzyme activity is lowered in the gills and liver, and 9 percent is reduced in the brain.

According to Isani et al., the observed decrease in the activity of antioxidant enzymes in the gills, liver, and brain of Cyprinus carpio after lesenta exposure may be attributed to the flux of superoxide radicals (2003). Under chemical stress, the activities of antioxidant enzymes may be boosted or suppressed depending on the degree and duration of the stress, as well as the susceptibility of the exposed species. The response of the antioxidant system to oxidative stress in diverse tissues differs between species and organs due to changes in antioxidant capability of these tissues (Isla et al, 2000).

Fish exposed to lesenta demonstrate a decrease in antioxidant enzyme activity. In the current investigation, pesticide exposure reduced catalase activity in the organs of experimental fish. Catalase activity may be reduced due to the flux of superoxide radicals, which have been shown to impair catalase activity (Pandey et al., 2001). The same decrease in catalase activity was detected in tissues of Channa punctatus exposed to deltamethrin (Sayeed et al., 2003). Several researchers have observed similar findings (Orun et al., 2008).

Ibuprofen-treated fish did not show any significant changes in monocytes, basophils, or eosinophils (p 0.05). Neutrophils are important in the body's defense against harmful invading pathogens, and tissue damage caused by toxicant stress can cause an increase in their number in the blood. Ibuprofen caused neutropenia and lymphocytosis in this study, which was dose and duration dependent. The duration-dependent declines and increases in lymphocyte and neutrophil percentage subpopulations could be linked to drug-induced stress and stress defense. As a result, lymphocytosis is a fish's response to stress caused by the medicine. Neutrophils are phagocytotic, hence a decrease indicates that the fish blood's phagocytotic function has been damaged. In experiments involving fish exposed to fenthion, the neutrophil count was likewise suppressed (Nwani et al. 2016). Diminished neutrophil counts, according to Lohner et al. (2001), may indicate reduced or disturbed phagocytotic capacity and disease resistance. Nwani et al. (2016) discovered a substantial rise in WBCs in *Clarias gariepinus* exposed to pharmaceutical chloramphenicol, while Reddy (2013) found the same in *Catla catla*, a freshwater fish exposed to cadmium.

4. Conclusion

Pharmaceuticals discharged from sewage treatment plants are one of the most common sources of medications in the aquatic environment. The consequences on human, aquatic, and animal health must be thoroughly explored through bio-safety and toxin research. To lessen the problem, conscious efforts are required, as well as proper legislation to assess and control it. The 96-h LC50 of Ibuprofen in *C. carpio* was 12.75 mg/L in this study, indicating that the fish is very susceptible to the medication even at low acute doses. Though this is modest in comparison to literature values, numerous variables could have contributed to the difference, including the drug's heterogeneous metabolism by different fish species. The toxicity of ibuprofen was tested in *Cyprinus carpio* at the behavioral and haematological levels, and fish health was negatively affected at each of these levels. Based on the existing findings, it is possible to conclude that ibuprofen administration may cause immunological disturbances, with toxicity increasing with dose. More research is needed to determine their toxicity in the biological system. Additional research on the toxicokinetics and toxicodynamics of ibuprofen and ibuprofen in freshwater fishes is needed, however, to have a complete knowledge of the drug's mechanism.

Conflicting Interests

The authors have declared that no conflicting interests exist.

References

[1] Isani G., Monari M., Andreani G., Fabbri M and Carpenè E (2003). Effect of copper exposure on the antioxidant enzymes in bivalve mollusk Scapharca inaequivalvis. Veterinary Research Communications, 27(1): 691-693

[2] Isla MI., Moreno MIN., Sampietro AR and Vattuone MA (2001). Antioxidant activity of Argentine propolis extracts. Journal of Ethnopharmacology, Lausanne, 76(2): 165-170.

[3] Pandey G and Jain GC (2013). A review on toxic effects of aluminium exposure on male reproductive system and probable mechanisms of toxicity International Journal of Toxicology and Applied Pharmacology, 3(3): 48-57.

[4] Sayeed I., Parvez S., Pandey S., Bin-Hafeez B., Haque R and Raisuddin S (2003). Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, Channa punctatus Bloch. Ecotoxicology and Environmental Safety, 56: 295-301

[5] Orun I., Talas ZS., Ozdemir I., Alkan A and Erdogan K (2008). Antioxidative role of selenium on some tissues of (Cd2+, Cr3+) induced rainbow trout. Ecotoxicology and Environment Safety, 71: 71-75

[6] Sudisha, J. *et al*. Relative efficacy of strobilurin formulations in the control of downy mildew of sunflower. *J. Pest Sci.* 83, 461–470.

[7] Ningthoujam, M., Habib, K., Bano, F., Zutshi, S. & Fatma, T. Exogenous osmolytes suppresses the toxic effects of malathion on Anabaena variabilis. *Ecotoxicol. Environ. Saf.* (2013).

[8] Kaya, A. & Doganlar, Z. B. Exogenous jasmonic acid induces stress tolerance in tobacco (Nicotiana tabacum) exposed to imazapic. *Ecotoxicol. Environ. Saf*. (2016)

[9] Badraoui, R., Nasr, H. B., Louati, R., Ellouze, F., and Rebai, T. (2012). Nephrotoxic effect of tetradifon in rats: a biochemical and histomorphometric study. *Exp. Toxicol. Pathol*. 64, 645–650.

[10] United States Environmental Protection Agency (US EPA) Fish Sampling and Analysis: Guidance for Assessing Chemical Contamination Data for Use in Fish Advisories (EPA 823-B-00-008). Washington: US EPA; 2000.

[11] Cavalheiro de Menezes C, Leitemperger J, Santi A, Lópes T, Veiverberg CA, Peixoto S, Bohrer Adaime M, Zanella R, Vargas Barbosa NB, Loro VL. The effects of diphenyl diselenide on oxidative stress biomarkers in Cyprinus carpio exposed to herbicide quinclorac (Facet®). Ecotoxicol Environ Saf 2012;81:91-7.

[12] Aebi HE. Catalase. In: Methods of enzymatic analysis. Bergmeyer, H.U. (Ed.). Verlag Chemie Weinhem. 1983;273-286.

[13] Kumar A, Dutt S, Bagler G, Ahuja PS, Kumar S. Engineering a thermo-stable superoxide dismutase functional at subzero to >50°C, which also tolerates autoclaving. Sci Rep. 2012;2:387