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Biochemical alterations in total proteins and related enzymes in tissues of *Cyprinus carpio* (L.) during sublethal exposure to karanjin based biopesticide Derisom

Shoeiba Tasneem* & Rafath Yasmeen,

Department of Zoology, University College of Science, Osmania University, Hyderabad, Telangana-500 007, India

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Excess usage of synthetic pesticides in agricultural fields perculates down the ecosystem to other livestock and fishes and thereby pose threat to humans. This has lead to prominence of bio pesticides which have always been thought to be harmless to the non-target organisms. However, literature on the safety of these biopesticides is limited. In this context, here, we studied the effect of Karanjin based botanical biopesticide Derisom on the biochemical parameters in various tissues of a fresh water fish species. We selected the edible common carp *Cyprinus carpio* (L.) for the study as it is an important protein food source for humans. The effect of the biopesticide - Derisom on certain metabolites and enzymes of protein metabolism were evaluated in gill, liver, kidney and the muscle of *C. carpio* during sublethal toxicity exposure of 21 days. About 0.28 ppm was taken as the sublethal value. The organs were taken from the exposed and control groups at the end of 1h, 7, 14 and 21 days, and were used for estimation of total proteins, free amino acids, glutamate dehydrogenase (GDH), alanine amino transferase (ALAT) and aspartate aminotransferase (AAT). All the four organs showed significant difference between control and exposed groups in all the estimated parameters on all the days of exposure. The present study considers the above biochemical parameters as an important biomarker in determining the level of toxicity caused by the biopesticide Derisom.

Keywords: Aquatic pollution, Common carp, Metabolism, Pesticide toxicity, *Pongamia pinnata*

Water contamination problems have been well documented worldwide and it has become a major problem at local, regional, national, and global levels[1]. Major sources of aquatic pollution are the continuous input of heavy metals and persistent organic pollutants (POPs) from agricultural runoffs which may easily get accumulated in organisms at various trophic levels and pose various health risks.[1] The surface waters contain a large number of chemical compounds, industrial and agricultural wastes. The insecticides form major pollutants of water bodies which contribute to the environmental problems. Rainfall, runoff and atmospheric deposition constitute the major routes of insecticides to aquatic ecosystems. Most insecticides finally reach the rivers, lakes and ponds[2] and have been found to be toxic to non-target organisms that inhabit the water bodies close to the agricultural fields. Presence of any toxicant in an aquatic ecosystem causes a reduction in the quality and level of dissolved oxygen (DO), pH, temperature, biological oxygen demand and chemical oxygen demand[3].

The contamination of water bodies by pesticides causes harmful effects on health, growth, survival and reproduction of aquatic animals. Different concentrations of pesticides present in the water bodies are mostly toxic to aquatic organisms especially fish[4]. Fishes are extremely sensitive to any contamination present in the water. Hence, pesticides may cause significant damage to certain biochemical processes when enter the organs and tissues of fish[5]. It has also been found that different kinds of pesticides can cause serious alterations in the physiological and health status of fishes[6]. Since fishes are important sources of proteins and lipids for humans, the health of fish is important for human beings.

The biochemical processes are considered as most sensitive and the earliest events of any pollutant damage. Earlier work has been done on effects of pesticides on biochemical processes in aquatic animals in India[7]. The metabolites and enzymes examined are one of the most important part of biological functions i.e. metabolism of proteins and carbohydrates. Similar kind of work on effect of pesticides on fishes has also been done by Begum[6]. Pesticide exposure causes severe alterations in the biochemistry of tissues of fishes[8]. Hence, biochemical parameters are best physiological indicators of the fish health. Therefore, they are important to be

*Correspondence:
E-Mail: shoeiba.tas@gmail.com

focused on while studying the toxic impact of pesticides and other toxicants on fish.

The pesticide used in the present work is a plant based biopesticide Derisom. The fish species used to study the toxicity of Derisom during sublethal exposure period is the common carp – *Cyprinus carpio* (L.), one of the most popular fish consumed worldwide and also common in aquaculture including integrated paddy fish farming. Being prolific breeder, it easily adapts to the laboratory conditions and that is why it has been selected for the present study.

In the present study, we studied the effect of Derisom, a Karanjin based biopesticide during sublethal exposure to freshwater edible fish *Cyprinus carpio* on various biochemical parameters, such as total proteins, free amino acids, and enzymes viz. glutamate dehydrogenase, alanine aminotransferase and aspartate amino transferase. The study showed that the Derisom – though a plant based pesticide showed alterations in the biochemical parameters of *Cyprinus carpio* at very small doses i.e., 1/10th of 96 hrs LC_{50} concentration.

Materials and Methods

Cyprinus carpio (Identified by Dr. C. Srinivasulu, Asst. Prof. Wildlife Biology & Taxonomy Lab, Dept. of Zoology, University College of Science, Osmania University, Hyderabad) samples ranging in length 14 ± 0.83 cm and weighing 28.53 ± 1.79 g were collected from Kaikaluru village of Andhra Pradesh State and transported to the laboratory in well aerated condition. The fishes were acclimatized in well aerated tanks for a period of one month. They were fed twice daily with commercially available fish feed pellets and the water was renewed daily. The 96 h LC_{50} value of Derisom was already estimated as 2.8 ppm according to method of Finney[9]. 1/10th of the 96 h LC_{50} value i.e., 0.28 ppm is taken as the sublethal value. The fishes were exposed to the sub-lethal concentration for a period of 21 days. After the completion of 1, 7, 14 and 21 days the fishes of both control and exposed groups were dissected and the organs gill, liver, kidney and muscle were collected and estimated for biochemical parameters –total proteins, free amino acids, glutamate dehydrogenase (GDH), alanine amino transferase (ALAT) and aspartate amino transferase (AAT).

The biopesticide used for the study was Derisom, product of Agri Life India Pvt Ltd., IDA Bollaram, Hyderabad. The product was directly procured from the manufacturer. The biopesticide is based on plant extract of *Pongamia pinnata.* Active ingredient is Karanjin.

Total protein was estimated by the method of Lowry *et al.*[10], free amino acids was estimated by the method of Moore & Stein[11], GDH was estimated by the method of Lee & Lardy[12] and the enzymes ALAT and AAT were estimated by the method of Reitman & Frankel[13] as described by Bergmeyer[14].

Total proteins were estimated by homogenizing 1% tissue in double distilled water, 1.0 mL 10% TCA added to 1.0 mL homogenate, centrifuged at 3000 rpm for 15min, the precipitate was used for protein estimation and the supernatant was used to estimate free amino acids. To the ppt 0.1N NaOH, 'C' solution 4 mL, 0.4 mL folins reagent were added and incubated at room temperature (32ºC) and colour was read at 540 nm on a spectrophotometer. For free amino acids estimation, to 1.0 mL of supernatant 2 mL ninhydrin reagent was added, boiled in hot water bath, cooled and 7 mL 50% ethanol was added and colour was read at 570 nm on a UV spectrophotometer. The enzyme GDH was estimated by taking 5% liver and 20% gill, kidney and muscle tissue (as the results were obtained by standardization of each organ and that organs in which 20% tissue was taken, formazon formed was not read when taken below 20%), homogenised in 0.25M ice cold sucrose solution. Centrifuged at 2000 rpm for 15 min and supernatant was used for enzyme estimation. The reaction mixture 2 mL included 0.5 mL supernatant, 100 µmoles phosphate buffer (pH-7.4), 4 µmoles INT, 0.1 µmoles NAD, and 40 µmoles sodium glutamate. Colour developed was read at 495 nm on a spectrophotometer.

The enzymes Alanine amino transferase and Aspartate amino transferase were estimated by preparing 5% tissue homogenate in 0.25M ice cold sucrose solution, centrifuged at 2000 rpm for 10 min and supernatant used for enzyme estimation. The incubation mixture 1.0 mL for ALAT consisted of 100 µmol of dl-alanine (pH 7.4), 2 µmol of α-ketoglutaric acid (pH 7.4), 100 µmol of phosphate buffer (pH 7.4) and 0.2 mL of supernatant. The mixture was incubated for 30min. For AAT reaction mixture 1.0 mL consisted of 100 µmol of L-aspartic acid (pH 7.4), 2 µmol of α-ketoglutaric acid (pH 7.4), 100 µmol of phosphate buffer (pH 7.4) and 0.2mL of supernatant. The reaction mixture was incubated for 1 h. To all the tubes 1.0 mL of 2, 4-DNPH solution was added and incubated at room temperature. Finally, 10 mL of 0.4N NaOH solution was added for colour to develop and read at 540 nm on a spectrophotometer.

Results

The fish *Cyprinus carpio* during sublethal exposure (0.28 ppm) of 21 days to the biopesticide Derisom showed the following significant results in gill, liver, kidney and muscle when compared to the control group. All the results are expressed as Mean ± Standard Deviation. *P <0.05.

The total protein content was found to be highest in muscle followed by gill, kidney and liver, respectively. The concentration of total proteins in gill showed significant decrease after 24 h, the concentration then increased after 7 days and 14 days and then again decreased after completion of 21 days when compared to the control. The overall amount of protein in exposed gill was decreased when compared to control. Total proteins were found to be highest in gill of control group and least in 24 h exposure group. The total proteins in liver increased after 24 h and 7 days then showed a significant decrease after completion of 14 days and further decrease after 21 days when compared to the control. Total proteins in liver was highest in 7 days exposure group and least in control group. The total proteins in kidney decreased after 24 hrs later increased after 7 days and 14 days and then again decreased after 21 days when compared to the control. Total proteins in kidney was highest in 14 days group and least in 24 h group. The total proteins in muscle decreased throughout the exposure period up to 21 days. Total protein in muscle was highest in control fish and least in 7 day group. (Fig. 1A)

The total free amino acid content was highest in muscle followed by gill, liver and kidney, respectively. The free amino acids concentration in gill decreased significantly after 1, 7 and 14 days and increased to higher than control after 21 days. The free amino acid concentration in gill was highest on 21 day and least on 14 day of exposure when compared to the control. The free amino acids concentration in liver and muscle showed similarity, the value decreased after 24 h and 7 days and then increased after 14 and 21 days when compared with the control. The liver and kidney showed highest value on 21 day and least on 7 day of exposure. The free amino acid concentration in kidney increased after 24 h then decreased after 7 days and the increased significantly after 14 days and was higher than control after 21 days exposure. Kidney showed highest concentration of amino acids on 21st day of exposure and least on 7th day when compared to the control. The amino acid concentration in muscle decreased after 24 h and 7 days exposure and then gradually increased on 14th and 21st day of exposure when compared to the control. The muscle tissue showed highest value of free amino acids in control fish and least value on 7 days exposure. (Fig. 1B)

The G.D.H content was found to be highest in liver, followed by kidney, muscle and least in gills. The G.D.H concentration in gill, liver and kidney

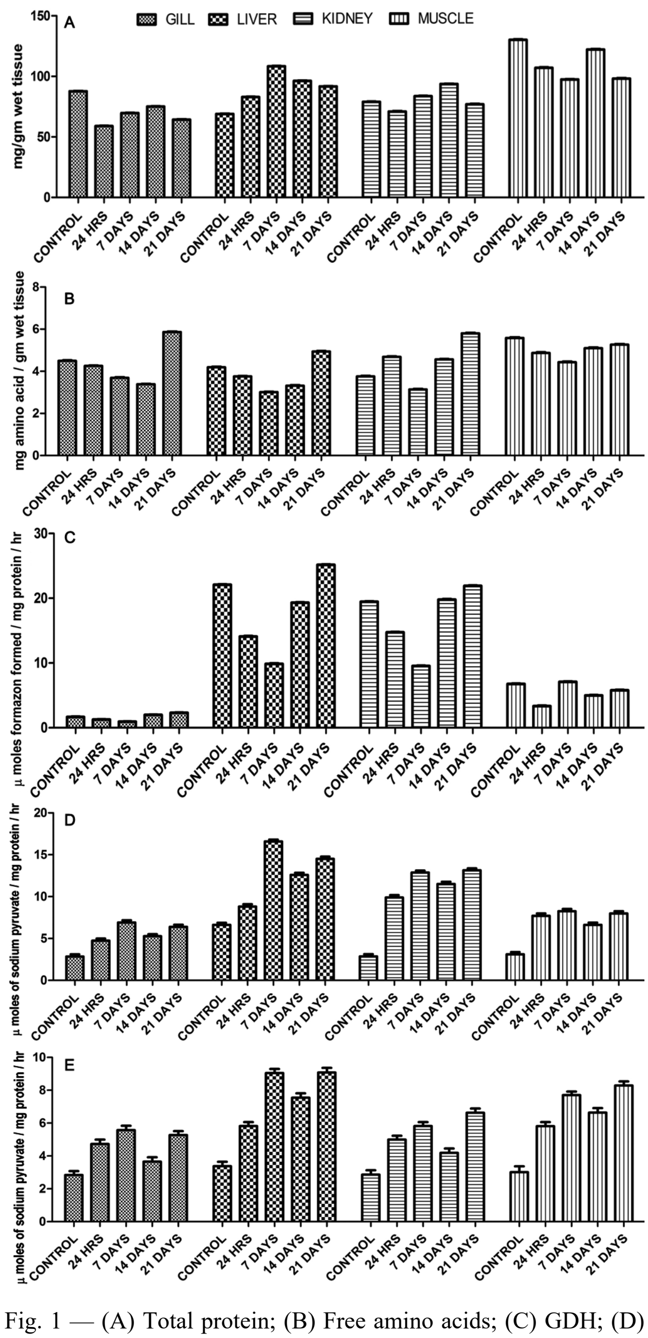

Fig. 1 — (A) Total protein; (B) Free amino acids; (C) GDH; (D) ALAT; and (E) AAT concentration in gill, liver, kidney and muscle of common carp *Cyprinus carpio* during exposure to sublethal concentration of Derisom

showed similarity, the value decreased after 24 hrs and also after 7 days later increased after 14 days and then further increased after 21 days when compared to the control. The content of G.D.H in gill, liver and kidney was highest in 21 days exposure and least in fish of 7 days exposure. The G.D.H value in muscle tissue decreased after 24 hrs, increased on 7th days of exposure, again decreased after 14th day and finally increased on 21st day when compared to the control. The G.D.H concentration in muscle was highest on 7th day and least on 24 hrs exposure. (Fig. 1C)

Both A.L.AT and A.A.T content was found to be highest in liver tissue, followed by muscle, kidney and least in gills. The A.L.A.T concentration showed similarity in gill, liver, kidney and muscle. The value for both A.L.A.T and A.A.T in all the four tissues increased after 24 hrs and 7 days, then decreased after 14 days and finally showed an increase after 21 days exposure when compared to the control. The A.L.A.T content in gill and muscle was found to be highest on 7 days exposure and least in control fish. The A.L.A.T concentration in liver and kidney was highest on 21st day and least in control group. The A.A.T concentration in liver, kidney and muscle was highest on 21st day and least in control group. The A.A.T content in gill was highest on 7th day and least in control group. (Fig. 1 D & E).

Discussion

The changes in the biochemical constituents in gill, liver, kidney and muscle of *Cyprinus carpio* exposed to sublethal concentration of Karanjin were thoroughly investigated in the present study. The changes in the metabolites and enzymes of carbohydrate and protein metabolism exhibited significant alterations in all the tissues when compared to the control group. As the gills are in constant contact with the external environment, they are the first targets of pollutants present in waters[15]. The liver is the first organ to check on any kind of environmental toxicants that enter the hepatic portal vein from the digestive system. And liver function can be drastically altered by acute or chronic exposure to toxicants[16]. Liver is the site for the synthesis of various proteins and regulatory centre of metabolism. Kidney is an important organ of excretion, detoxification of some toxic substances to some extent and osmoregulation, but many times the kidney is indirectly affected by various pollutants including pesticides[17,18]. Muscle is rich in protein, it forms mechanical tissue, muscle undergoes different metabolic processes and is also affected by toxic substances including pesticides. The impact of any kind of pollutants of aquatic ecosystems can be assessed by measuring the biochemical parameters in fish because fishes are the biota which respond specifically to any kind and degree of contamination.

Tissue protein content is used as a very good indicator of xenobiotic-induced stress in aquatic organisms[19]. Sathyanarayana[20] suggested that the physiological condition of an animal is indicated by the metabolic status of proteins. Jrueger *et al.*[21] reported that the fish can get energy through breakdown of proteins. In the present study gill and muscle tissues showed decrease protein content during the sub-lethal exposure period, whereas kidney showed slight increase and liver showed significant increase. These variations in response of fish to pesticide (Karanjin) suggests difference in metabolic rates of individual tissue, these results are in correlation with work of Venkataramana *et al.*[22]. The decrease in the protein content in tissues in the present study may be due to the impairment of protein synthesis or increase in the rate of their degradation to amino acids due to stress induced by the pesticide exposure, similar results were also obtained by the work done by Al – Ghanim *et al.*[23]. The results of the present study showed increase in protein content in liver and kidney which are also in agreement with the findings of Tripathi *et al.*[24] which showed increase in protein content in liver, heart, kidney and gill of fish *Clarias batrachus* (Linn.) after exposure of Cypermethrin. The free amino acid level showed an increasing trend in all the four organs throughout the exposure period. The protein content showed a decreasing trend in the liver, brain and kidney tissues of *Channa punctatus* on exposure to lihocin was concluded by the studies done by Abdul *et al.*[25].Sana Ullah *et al.*[26] reported lterations in the protein content and antioxidant enzymes in tissues gill, liver, brain and brain of *Labeo rohita* under Endosulfan stress. Palaniappan & Vijayasundaram[27] observed in their study that the decrease in protein content may be either due to liver cirrhosis or nephrosis or due to alteration in enzymatic activity involved in protein biosynthesis. Omprakasam *et al.*[28] in their study of toxicity of DMSO to the Indian major carp, *Catla* reported that protein remained in higher concentration in the muscle tissue because of the need of growth factors and energy required for swimming. The increased levels of free amino acid was a result of protein breakdown was concluded by the studies of Singh *et al.*[29]. The elevated free amino acid levels were used for producing energy, which supplied them as keto acids into TCA cycle to contribute energy required during toxic stress.

Glutamate dehydrogenase (GDH) plays a key role in oxidative metabolism to form glutamate to ammonia[30]. The increase in GDH activity in various tissues indicates that there may be an increased mitochondrial permeability or the lysosomal damage or the induced synthesis of enzymes[31]. Increased free amino acid levels in tissues of various organs and their subsequent transamination results in production of higher amount of glutamate. Hence, increasing the availability of intracellular substrate - glutamate for consequent oxidative deamination reaction through GDH. Therefore in the present study the concentration of GDH in all the tissues showed a trend of decrease and increase when compared to the control. This might be as a result of the fact that the biopesticide caused tissue or cell damage that caused the cells lysosomes to rupture and as a consequence the amount of GDH increased. There has been seen a decrease in GDH concentration which may be due to the fact that the time spent by the fishes in sub-lethal concentration might have made the fish tolerant to the biopesticide and after long days of exposure periods the fishes could not tolerate the concentration and showed the alterations in the concentration of GDH in all the four tissues.

Transaminase enzymes play a crucial role in carbohydrate–protein metabolism in fish and determination of transaminases especially alanine aminotransferase (ALAT) and aspartate aminotransferase (AAT) and can be used for aquatic biomonitoring according to the research done by Vutukuru *et al.*[32]. Enzyme ALAT is cytosolic whereas the enzyme AAT has both cytosolic and mitochondrial forms. The enzymes ALAT and AAT indicate organ damage or dysfunction in aquatic organisms during conditions of stress[33]. Under normal conditions there is baseline activity of both of these enzymes. But when an organism is under stressful conditions the levels of these enzymes significantly increase in order to meet the increase in ATP demands. The alterations in enzyme activity during stress is one of the most important biochemical parameter and levels of these enzymes is of diagnostic importance[34,35]. The enzymes ALAT and AAT are liver specific enzymes which are useful to measure the sensitivity of hepatotoxicity[36]. According to alterations in the levels of ALAT and AAT indicate tissue damage not only in liver but also in other tissues like kidney, muscle and gills. In the present study, compared to the control group, ALAT and AAT activities were found to be highly elevated in all the tissues of fish during sub-lethal exposure to Karanjin. In fish, one of the primary energy currencies is amino acids as suggested by Oluah[37] and Varadarajan[38]. Alanine aminotransferases and Aspartate aminotransferases are important in conversion of amino acids to keto acids, like pyruvate and oxaloacetate which may be later used as intermediates in Kreb's cycle or directed into the gluconeogenic pathway. Significant elevations in AAT activity was recorded in *Cyprinus carpio* exposed to copper sulphate according to the studies conducted by Karan *et al.*[39] .Elevated activity of ALAT was noticed in *Cyprinus carpio* and *Oreochromis niloticus* exposed to cadmium by Almeida *et al*[40]. Yildirim *et al.*[41] observed an increase in AAT and ALAT enzyme activities in gills, liver and kidney of a fish species exposed to a toxicant. The elevation in aspartate aminotransferase in *Heteroclarias* exposed to tobacco leaf dust was observed by Adamu[37]. The alterations in the ALAT levels play a crucial role in glucose – alanine cycle and also the elevated levels of enzymes ALAT & AAT stimulate the process of gluconeogenesis & the metabolism of L-amino acids[42].

The observations of the present study when Common carp was exposed to the sub-lethal concentration of Derisom showed marked alterations in tissue protein, amino acids and enzymes related to protein metabolism in different organs, these results are also in correlation with the work done by other researchers in their studies when different species of fish were exposed to various categories of pesticides[43-46]. It is always assumed that only synthetic pesticide formulations show biochemical alterations in tissue proteins and enzymes in fish which are the non-target organisms. Our work done in the present study revealed that like synthetic pesticides, botanical pesticide formulations also elicit comparable biochemical alterations in tissues of edible fish species which form an important group of non-target organisms.

Conclusion

The study showed that the karanjin based biopesticide Derisom showed significant alterations in the biochemical parameters of *Cyprinus carpio* at small doses i.e., 1/10th of 96 h LC_{50} concentration The total protein content and enzymes related to protein metabolism i.e., glutamate dehydrogenase, alanine amino transferase and aspartate amino transferase in organs gill, liver, kidney and muscle were disrupted on exposure to Derisom to some extent. There were some similarities in the extent of biochemical changes caused due to the biopesticide exposure among the organs. The variations in biochemical parameters serve as an important marker in monitoring the status and health of fish being affected by the pesticides. As the results indicate, use of biopesticides in agricultural fields needs monitoring.

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Conflict of Interest

Authors declare no conflict of interest.

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