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Assessment of acute toxicity of chlorpyrifos and its sub-lethal effects on protein metabolism and electrophoretic protein patterns of *Cyprinus carpio* (L.) through SDS-PAGE

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Abstract
The present study was focused on the impact of chlorpyrifos (CPF) on the expression of electrophoretic protein patterns and metabolism of protein in the tissues of *Cyprinus carpio*, Fingerlings of *C.carpio* were subjected to various chlorpyrifos concentrations ranging from 0.1mg/L to 0.7mg/L for 96 h, and acute toxicity was determined as 0.318mg/L. Based on the LC_{50} value, the *Cyprinus carpio* were exposed to two sub-lethal concentrations viz., sub-lethal 1 ($1/5^{th}$ of LC_{50}, 0.0636mg/l) and sub-lethal 2 ($1/10^{th}$ of LC_{50},0.0318mg/l) in a semi-static system for 21 days. SDS-PAGE and estimation of protein metabolic rate were performed at the end of the exposure period to assess the changes within tissues of the muscle, liver, and gill, brain. The study has suggested that the degradation of protein caused due to proteolytic activity and oxidative stress of CPF would alter the set of genes resulting in impaired protein synthesis. The intensity of protein subunits in chlorpyrifos-exposed tissues decreased, and some protein subunits disappeared. Thus the variations in protein banding patterns and reduction in the metabolic rate are due to improper amino acid incorporation into the polypeptide chain or proteolysis which is attributed to functional and physiological alterations in cellular proteins. CPF can attack single-domain proteins as well as multiple domains proteins at both sub-lethal concentrations thus it is categorized as a hazardous pesticide in the world.

Keywords: Chlorpyrifos, *Cyprinus carpio*, Protein pattern, SDS-PAGE

Introduction

In India, Pesticides have become a vital constituent in agriculture progress since the tropical environment is incredibly advantageous to pest breeding (Kumar *et al* 2010) [17]. Indiscriminate use of this pesticide to enhance agricultural productivity and yield may pose a serious risk for non-target creatures, particularly aquatic life, and the environment. Pesticide pollution of the aquatic biota is a serious issue, and fish are more at risk of these pollutants, which might be ingested through gills, skin, and contaminated foodstuffs (Ling *et al* 2011) [19]. One of the foremost widely used pesticides in India is organophosphate chlorpyrifos (O, O-diethyl O- 3, 5, 6-trichlor-2-pyridyl phosphorthioate) highly toxic to fish than the organochlorine compounds (Tilak *et al*., 2001) [40].In India widely used for controlling various pests belongs to the orders Coleoptera, Diptera, and Homoptera in soil or on foliage in over 100 crops (Tomlin 2000) [42]. It also can be utilized in the control of other agricultural and domestic pests. However, its widespread use can lead to the development of adverse effects on the non-target organism, fish (Anita *et al* 2016) [2].

Chlorpyrifos is the irreversible inhibitor of acetylcholinesterase (AChE) in the central and peripheral nervous systems, hence a potential agent to exert neurotoxicity in aquatic organisms (Sturm *et al*., 2007) [39]. Chlorpyrifos is extremely toxic to fish, affecting their growth and reproduction (Levin *et al* 2004; Sledge *et al* 2011) [18,36] as well as their gills and liver (Xing *et al* 2012) [45]. Chlorpyrifos exerts several other effects *i.e.* affecting hepatic dysfunction, hematological changes, genotoxicity, and neurobehavioral and neurochemical changes. Decreases the activities of glutathione (GSH), catalase (CAT), and glutathione S-transferase (GST) in fish after exposure to CPF (Poet *et al* 2003; Mehta *et al* 2008;Alagoa *et al* 2009; Slotkin *et al* 2005; Goel *et al* 2005) [29,22,4,37,14], it also affects hatchlings and swimming behavior(Levin *et al* 2004) [18]. Earlier reports claimed that there were several hundred parts per billion of chlorpyrifos in the water, which led to an increase in the number of fish deaths (Abdel Halim *et al*., 2006) [1].

Since fishes are the most chlorpyrifos-sensitive aquatic organism, thus the present investigation deals to evaluate the toxic potential of chlorpyrifos and its sublethal impact on protein synthesis, and protein pattern expressions in different tissues of *Cyprinus carpio* using SDS-PAGE. Proteins are the principal effector molecules in all living systems, and any adaptive responses to environmental, physiological, or pathology will be reflected in changes in protein activity or content (Bradley et al. 2002) [7]. Sodium dodecyl sulfate or SDS-PAGE, an effective technique is widely employed in various disciplines to classify proteins based on electrophoretic mobility. Thus Proteomic content varies from cell to cell and changes constantly due to biochemical interactions with the genome and environment. As a result, environmental factors influence the expression of a distinct collection of proteins in the tissue or cell type of the exposed organism (Nesatyy and Suter 2007) [25]. SDS's powerful proteomic technique generates effective data on the interaction of macromolecules with environmental contaminants thus being accessed for toxicological investigations.

To our knowledge, no research has been done to analyze the protein patterns and subsequent degradation of protein levels in *C.carpio* tissues subjected to chlorpyrifos.

Materials and Methods

Experimental fish specimen and chemical

The fish *Cyprinus carpio* (L.) (Family: Cyprinidae, Order: Cypriniformes) is edible and commercially valuable. Live fish of size 5-6 cm and weight 3.5-4.5g weight were procured from State Fisheries Department, Bhadra Reservoir Project, Shimoga District, Karnataka State, India. To minimize cutaneous infections, fish specimens were bathed twice in 0.05 percent potassium permanganate (KMnO4) for 2 minutes. The specimens were then acclimatized for three weeks in a semi-static system under laboratory conditions.

During the acclimatization period, fish were supplemented with commercial fish pellets and rice bran twice a day. To reduce the ammonia content in the water, feces and other waste constituents were drained off daily. The physicochemical quality of test water such has, Temperature 25±1ºC, pH 7.2±0.2 at 25ºC, Dissolved Oxygen 6.7±0.8 mg/L, Carbon-dioxide 6.2±0.3 mg/L, Total Hardness 23.2±3.4 mg as CaCO3/L, Phosphate 0.37±0.002µg/L, Salinity 0.01 ppm, Specific Gravity 1.001 and the conductivity of the water is less than 10µS/cm were examined following the standard method (APHA 2005) [5].

For the present study, technical-grade of chlorpyrifos (50%EC) with the trade name 'Premain strong' (manufactured by ADAMA India Pvt. Ltd. Hyderabad) was purchased from the local market. It was found that chlorpyrifos (50%EC) grade chemical is heavily used in the agriculture fields.

Acute toxicity Test

To determine the 96-hour LC_{50} value of chlorpyrifos an acute toxicity bioassay was performed in a semi-static system in the laboratory by OECD guideline No. 203. (OECD 1992) [27], the stock solution was prepared by dissolving CPF in acetone and the maximum amount of acetone in the experimental medium was less than 0.1ml/L. To keep the chemical content consistent the test solution was changed every day. A total of ten acclimatised fish specimens were randomly selected and exposed to each of the seven concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7mg/L) of chlorpyrifos for 96 hours. These concentrations were selected after range-finding acute toxicity tests, and the experiment was run in triplicate to determine the 96-hour LC_{50} value for the species. To confirm the consistency of the experimental approach, a negative control (without pesticides) was included. During the experiment, dead fish were removed and mortality rates were calculated after 24, 48, 72, and 96 hours. A basic program from the Probit analysis was used to calculate the LC_{50} and 95 percent confidence limits of chlorpyrifos for *Cyprinus carpio* Finney (1971) [12].

In vivo sub-lethal exposure experiment

For *Cyprinus carpio*, the 96-hour LC_{50} value of chlorpyrifos was determined as 0.318 mg/L, based on the LC_{50} – 96 h value of the two test concentrations of chlorpyrifos *viz;* Sub-lethal Concentration 1 (SLC-1: $1/5^{th}$ of $LC_{50,}$ 0.0636mg/l) and sub-lethal 2 (SLC-2: $1/10^{th}$ of LC_{50},0.0318mg/l) were selected and used for the *in vivo* experiment another group was kept in control. To keep the pesticide content constant, the fish specimens were exposed to these two test concentrations in a semi-static system with test water changed every other day the exposure could last up to 21 days. Fish were sacrificed at the end of each exposure period, and organs such as the gills, brain, liver, and muscle were separated, and immediately processed for SDS-PAGE analysis.

For the estimation of protein level Sublethal concentration 1 (SLC-1 ofLC_{50}) was selected, as the nominal concentration for the analysis of protein level for the exposure periods 7^{th},14^{th}, and 21^{st} days and at the end of the exposure period liver, muscle, and gill and brain were removed and analyzed for the protein level in *Cyprinus carpio*.

Protein electrophoresis

The conventional method of Sodium Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Laemmli (1970) [20] method. was used to examine the change in protein fractions in the gill, brain, liver, muscle of control, and pesticide-exposed tissues.

Sample preparation

Muscle, liver, and Gill, brain homogenates were prepared in 10% TCA and centrifuged at 8000 rpm for 10 minutes in the cooling centrifuge. The pellet was centrifuged for 10 minutes at 8000 rpm after being washed twice with ice-cold acetone. The pellet was dissolved in sample buffer (0.5M Tris-HCL, pH 6.8-2ml, 40% glycerol-1.6 ml) and heated in a water bath at 95ºC for 10 minutes.

Preparation of Gel Slab

The sandwich was made by clamping two clean glass plates together and pouring 1 mm Teflon gel solution of 12.5% (1.5 M Tris-HCL, pH8.8-2 ml, 30% Acrymalide-3.2 ml, 10% SDS-0.5 ml double distilled water-1.8 ml, TEMED-0.015 ml, Ammonium persulphate-0.5 ml) was prepared and emptied in between the clamped glass plates. The gel solution was overlaid with distilled water to eliminate any air bubbles. The plates were left unperturbed for 30 minutes to allow the gel to polymerize. Overlaid water was removed and rinsed with stacking gel buffer after gel polymerization. The 5% stacking gel solution was prepared (0.5 M Tris HCL, pH6.8-2 ml, 30% acrylamide-0.8 ml, 10% SDS-0.5 ml, double-distilled water1.2ml, TEMED-0.015ml, 1.5% APS 0.5ml) and poured over the polymerized resolving gel. The comb was precisely fitted the gel arbor was left unperturbed for 15 min after the polymerization comb was loaded into the wells, and the gel was run at 60V.

Staining method

The Coomassie Brilliant Blue Stain (CBBS) was prepared, and the gel was incubated for 6 hours overnight in a staining solution containing 40% methanol, 10% acetic acid, and 0.025% Coomassie Brilliant Blue R-250, filtered through Whatman #1 paper and shaken on a rotary shaker. The destaining solution is the same as the staining solution but without the Coomassie R-250 dye powder. The gel was incubated in the destaining solution for 30-60 minutes before being rinsed many times in double-distilled water and stored in water.

Determination of molecular weight of the protein subunits separated on SDS-Runner

The relative mobility of the individual protein subunits was estimated using the following formula to determine the molecular weight of the individual protein subunits.

$$\text{Relative mobility (Rm)} = \frac{\text{Distance traveled by the individual subunit}}{\text{Distance travelled by means of the marker dye}}$$

A standard curve was created by plotting the migration distances ('X'-axis) of known protein standards against their molecular weights ('Y'-axis) in Microsoft Excel. Based on the migration distance, the standard curve is used to compute the molecular weight of an unknown protein.

Protein Metabolic profile

One percent of the tissues' homogenate was prepared in a 0.25 M ice-cold sucrose solution to determine the total proteins. Using the Folin phenol reagent and bovine albumen serum as standards (Lowry *et al.*, 1951) [21]

Statistical Analysis

Percent mortality data of fish after 96 hours of exposure were analyzed using NCSS v. 22.0.3 software; confidential limits were calculated using probit analysis. Quantification of SDS-PAGE bands was done using Image J (version 1.46) software.

For the estimations of protein, one-way analysis of variance (ANOVA) followed by Tukey multiple range tests, and statistical significance was assessed at 5% ($P < 0.05$) levels using Graph Pad prism version (8.0) software.

Results

Acute toxicity test

The calculated acute toxicity values for Chlorpyrifos pesticide exposed for 96hr value were found 0.318mg/l and the lower and upper bound 95% lethal confidence limits indicated a range of values (0.250mg/L – 0.404mg/L) respectively were given in (Table.1). Mortality of fish *Cyprinus Carpio* in different concentrations along with log concentration of chlorpyrifos at 96hexposure period was given in the (Table. 2), Percentile mortality of fish *Cyprinus Carpio* in different concentrations of chlorpyrifos at 96h exposure period was given in (Table.3 and Fig.1).

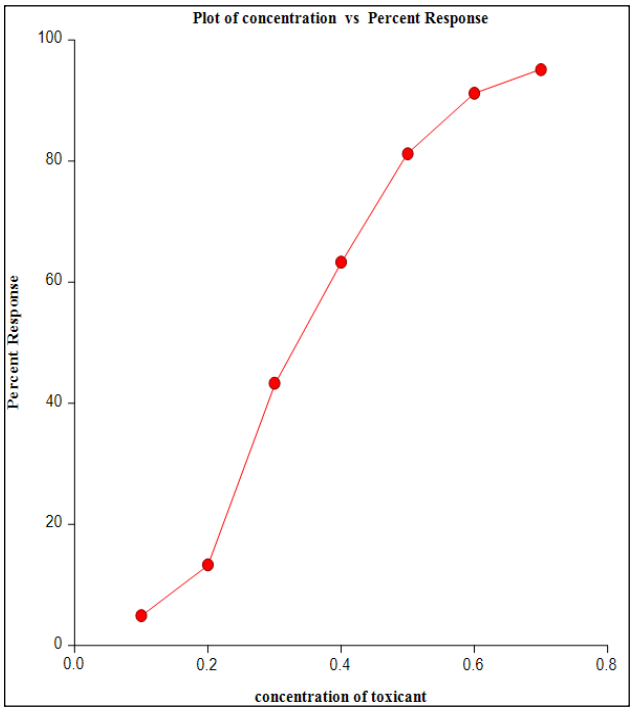

Fig 1: Percent response and concentration of chlorpyrifos

Table 1: LC_{50} values with 95% confidence limits for chlorpyrifos

Concentration	Exposed Fishes	% Mortality	Lower bound – Upper bound
0.318mg/L	10	50	0.250mg/L - 0.404mg/L

The LC_{50} of chlorpyrifos to *C. carpio* for 96 h of exposure was 0.318mg/L, and the lower and upper bound 95% lethal confidence limits indicated a wide range of values (0.250mg/L – 0.404mg/L) respectively

Table 2: Mortality of fish *Cyprinus carpio* in different concentrations of chlorpyrifos at 96 h exposure period

Concentration (mg/L)	Probit Percent	Actual Percent	No. of Fish	Mortality Result	Expected Result	Difference	Chi-Square
0.1	4.90	1.41	10	0.49	0.14	0.35	0.87
0.2	13.30	18.95	10	1.33	1.90	-0.57	0.21
0.3	43.30	45.57	10	4.33	4.56	-0.23	0.02
0.4	63.30	66.78	10	6.33	6.68	-0.35	0.05
0.5	81.20	80.42	10	8.12	8.04	0.08	0.00
0.6	91.20	88.54	10	9.12	8.85	0.27	0.07
0.7	95.10	93.25	10	9.51	9.32	0.19	0.05

Total Chi-Square (χ^2): 1.28 D. F.: 5 Prob Level: 0.94

Table 3: Concentration of Chlorpyrifos and Percentile responses of *Cyprinus carpio*

Percentile	Probit	Log (Concentration)	Std. Error Log (Conc.)	Concentration	Std. Error Conc.
1	2.6737	-1.0305	0.1344	0.0932	0.0289
5	3.3551	-0.8743	0.1033	0.1336	0.0318
10	3.7184	-0.7911	0.0874	0.1618	0.0326
20	4.1584	-0.6903	0.0694	0.2041	0.0326
25	4.3255	-0.6520	0.0631	0.2229	0.0324
30	4.4756	-0.6176	0.0579	0.2412	0.0322
40	4.7467	-0.5554	0.0498	0.2783	0.0319
50	5.0000	-0.4974	0.0446	0.3181	0.0327
60	5.2533	-0.4393	0.0424	0.3637	0.0355
70	5.5244	-0.3772	0.0440	0.4196	0.0425
75	5.6745	-0.3428	0.0465	0.4542	0.0486
80	5.8416	-0.3045	0.0504	0.4960	0.0576
90	6.2816	-0.2037	0.0646	0.6257	0.0931
95	6.6449	-0.1204	0.0789	0.7579	0.1377
99	7.3263	0.0358	0.1085	1.0859	0.2713

Protein Pattern Analysis

The electrophoretogram (Fig.2) represents the muscle protein subunits of Chlorpyrifos (SLC-1) and Chlorpyrifos (SLC-2) which showed a decrease in the intensity of banding patterns subunits when compared to the control. The Rm values (Table 4) of control muscle protein subunits 0.16, 0.29, 0.39, 0.59, 0.64, and 0.75 corresponded to ~87, ~62, ~47, ~27, ~24, and ~18kDa molecular weights respectively. In chlorpyrifos-exposed tissue samples, the Chlorpyrifos (SLC-1) muscle protein subunits had a lower intensity in the banding pattern than the Chlorpyrifos (SLC-2) tissue sample. In the Chlorpyrifos (SLC-1) exposed sample, the Rm values of protein subunits 0.29,0.59, 0.64, and 0.75 with a molecular weight closer to ~62, 27, 24, and 18kDa respectively completely disappeared. A protein subunit with an Rm value of 0.29, 0.75 with a molecular weight closer to ~62,18kDa was absent in both concentrations of exposed tissue samples. A protein subunit with Rm value 0.36 at ~51kDa was observed in (SLC-1) which was not observed in (SLC-2) and control tissue samples.

The electrophoretogram (Fig. 3) represents the liver protein subunits of Chlorpyrifos (SLC-1) and Chlorpyrifos (SLC-2) exposed samples, which showed a decrease in the intensity of liver protein subunits when compared to the control. The Rm values (Table.5) of control liver protein subunits 0.16, 0.26, 0.31, 0.42, 0.48, 0.66, and 0.75 corresponded to ~89, ~68, ~58, ~43, ~37, ~23 and ~18kDa molecular weights respectively. In chlorpyrifos-exposed tissue samples, the Chlorpyrifos (SLC-1) liver protein subunits had a greater reduction in the intensity of the banding pattern than the Chlorpyrifos (SLC-2) tissue sample. In the Chlorpyrifos (SLC-1) exposed sample, the Rm values of protein subunits are 0.26,0.31,0.66, and 0.75 with a molecular weight closer to ~68,58and 23,18kDa, respectively completely disappeared. A protein subunit with an Rm value of 0.66 and 0.75 with a molecular weight closer to 89,18kDa was absent in both concentrations of exposed tissue samples. The protein subunit with an Rm value of 0.42 and 0.66 with a molecular weight closer to ~43, 23kDa shows a low-intensity banding pattern in the concentration (SLC-2) exposed tissue sample.

The electrophoretogram (Fig.4) represents the gill protein subunits of Chlorpyrifos (SLC-1) and Chlorpyrifos (SLC-2) exposed samples, which showed a greater decrease in the intensity of gill protein subunits when compared to the control. The Rm values (Table 6) of control gill protein subunits 0.17, 0.23, 0.30, 0.36, 0.53, 0.64, 0.71, and 0.80 corresponded to ~86, ~73, ~61, ~51, ~33, ~24, ~20, and ~15kDa molecular weights respectively. In chlorpyrifos-exposed tissue samples, the Chlorpyrifos (SLC-1) gill protein subunits had a lower intensity in the banding pattern than the Chlorpyrifos (SLC-2) tissue sample. In the Chlorpyrifos (SLC-1) exposed sample, the Rm value of protein subunit 0.17, and 0.64,0.71,0.80 with a molecular weight closer to ~86,24,20,15kDa was absent and protein band with Rm value 0.23 at ~73 shows light intensity in (SLC-1). In the Chlorpyrifos (SLC-2) exposed sample, the Rm values of protein subunits 0.23,0.64, 0.71, and 0.80 with a molecular weight closer to ~73, ~24, ~20, and ~15kDa, respectively completely disappeared.

The electrophoretogram (Fig.5) represents the brain protein subunits of Chlorpyrifos (SLC-1) and Chlorpyrifos (SLC-2) exposed samples, which showed a decrease in the intensity of brain protein subunits when compared to the control. The Rm values (Table 7) of brain control protein subunits 0.19, 0.56, 0.63, 0.72, and 0.81 corresponded to ~81, ~30, ~24, ~19, and ~15kDa molecular weights respectively. In chlorpyrifos-exposed tissue samples, the Chlorpyrifos (SLC-1) brain protein subunits had a slightly lower intensity in the banding pattern than the Chlorpyrifos (SLC-2) tissue sample. In the Chlorpyrifos (SLC-1) exposed sample, the Rm values of protein subunits 0.63,0.72,0.81 with a molecular weight closer to ~24,19,15kDa completely disappeared whereas a protein subunit with Rm value 0.42 at ~43kDa was observed only at (SLC-1) of brain tissue. The protein band with an Rm value of 0.19 at ~81kDa shows a low-intensity banding pattern in the (SLC-1) exposed sample.

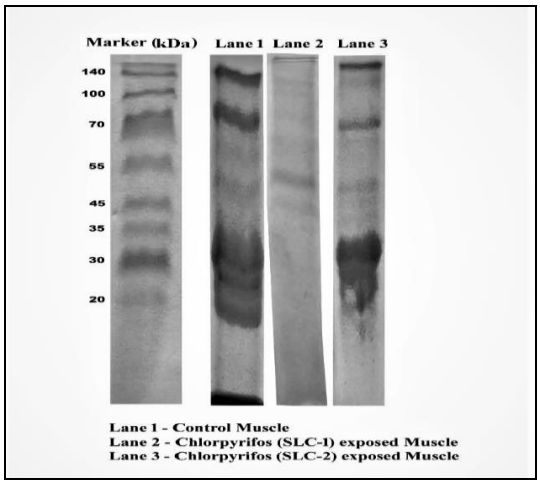

Fig 2: Changes in protein subunits in muscle tissues of *C. carpio* exposed to chlorpyrifos

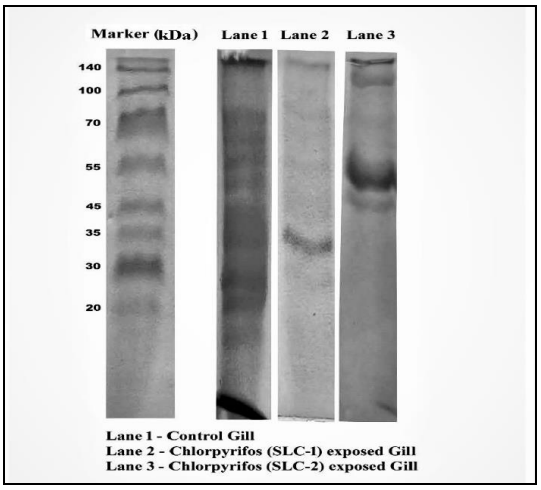

Fig 4: Changes in protein subunits in gill tissues of *Cyprinus carpio* exposed to chlorpyrifos.

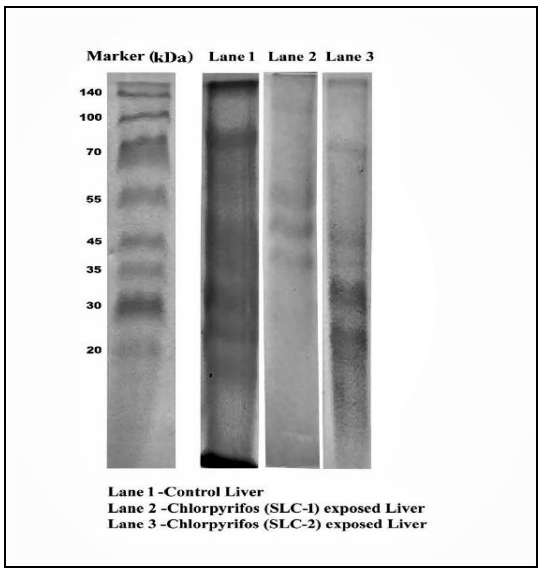

Fig 3: Changes in protein subunits in liver tissues of *Cyprinus carpio* exposed to chlorpyrifos

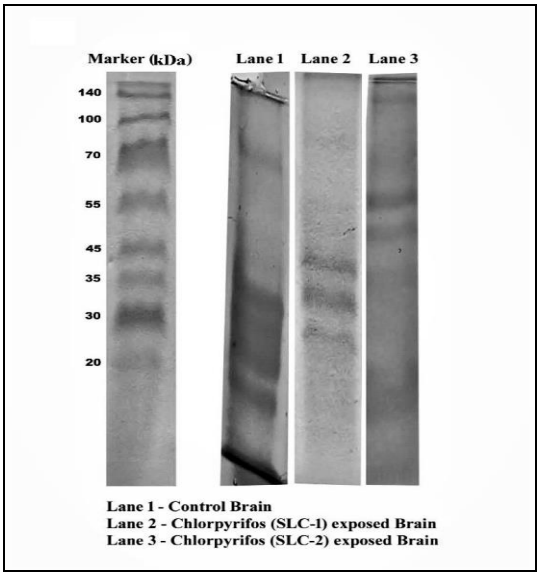

Fig 5: Changes in protein subunits in brain tissues of *C. carpio* exposed to chlorpyrifos

Table 4: Rm values for Control Muscle, Chlorpyrifos (SLC – 1) exposed Muscle, and Chlorpyrifos (SLC – 2) exposed Muscle

Marker	Lane 1 - Control Muscle	Lane 2 – Chlorpyrifos (SLC – 1) exposed Muscle	Lane 3 – Chlorpyrifos (SLC – 2) exposed Muscle
0.03	-	-	-
0.10	-	-	-
-	0.16	0.16	0.16
0.22	-	-	-
-	0.29	-	-
0.31	-	-	-
-	-	0.36	-
-	0.39	0.39	0.39
0.42	-	-	-
0.50	-	-	-
-	0.59	-	0.59
0.60	-	-	-
-	0.64	-	0.64
0.70	-	-	-
-	0.75	-	-

Table 5: Rm values for Control Liver, Chlorpyrifos (SLC – 1) exposed Liver, and Chlorpyrifos (SLC –2) exposed Liver

Marker	Lane 1-Control Liver	Lane 2 – Chlorpyrifos (SLC – 1) exposed Liver	Lane 3 – Chlorpyrifos (SLC – 2) exposed Liver
0.03	-	-	-
0.10	-	-	-
-	0.16	0.16	0.16
0.22	-	-	-
-	0.26	-	0.26
0.31	0.31	-	-

0.42	0.42	0.42	-
-	0.48	0.48	0.48
0.50	-	-	-
0.60	-	-	-
-	0.66	-	-
0.70	-	-	-
-	0.75	-	-

Table 6: Rm values for Control Gill, Chlorpyrifos (SLC – 1) exposed Gill, and Chlorpyrifos (SLC – 2) exposed Gill

Marker	Lane 1 - Control Gill	Lane 2 – Chlorpyrifos (SLC – 1) exposed Gill	Lane 3 – Chlorpyrifos (SLC – 2) exposed Gill
0.03	-	-	-
0.10	-	-	-
-	0.17	-	0.17
0.22	-	-	-
-	0.23	0.23	-
-	0.30	-	-
0.31	-	-	-
-	0.36	0.36	0.36
0.42	-	-	-
0.50	-	-	-
-	0.53	0.53	0.53
0.60	-	-	-
-	0.64	-	-
0.70	-	-	-
-	0.71	-	-
-	0.80	-	-

Table 7: Rm values for Control Brain, Chlorpyrifos (SLC – 1) exposed Brain, and Chlorpyrifos (SLC – 2) exposed Brain

Marker	Lane 1 - Control Brain	Lane 2 – Chlorpyrifos (SLC – 1) exposed Brain	Lane 3 – Chlorpyrifos (SLC – 2) exposed Brain
0.03	-	-	-
0.10	-	-	-
-	0.19	0.19	0.19
0.22	-	-	-
0.31	-	-	-
			-
0.42	-	0.42	-
0.50	-	-	-
-	0.56	0.56	0.56
0.60	-	-	-
-	0.63	0.63	0.63
0.70	-	-	-
-	0.72	-	0.72
-	0.81	-	-

Protein metabolic profile

Each datum represents the Mean ± SD, of six individuals (n=6). Values are significant at P<0.05. The total protein content in control fish *Cyprinus carpio* at (SLC-1) exposure was in the order Gill>Liver>Muscle>Brain. In the Present study depletion in the total protein level was observed in the order Liver>Gill> Muscle>Brain of test fish exposed to chlorpyrifos in sublethal concentrations were compared with controls (Fig. 6).

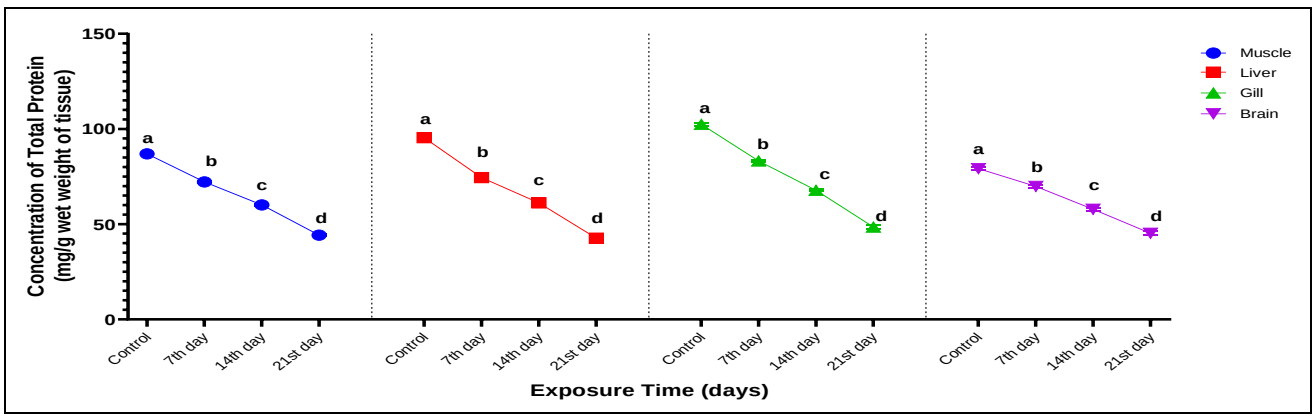

Fig 6: Changes in the Total Protein content (mg/g wet weight of tissue) (mg/g wet weight of tissue) in different tissues of *Cyprinus carpio* exposed to (SLC-1) of chlorpyrifos for 7, 14, and 21 days.

Discussion

Chlorpyrifos does not persist in the environment because of their low perseverance and the prevalence of CPF bioaccumulation occurs to some extent in fish, owing to their slow metabolic rate, in the aquatic environment. However, with the extensive exploitation and use of chlorpyrifos in agriculture, its frequent detection has increased its residues in aquatic biota, soil, sediment, and water thus making it the most potent agent to exert negative impacts on nontarget organisms especially fish (Ali *et al*. 2009) [3].

Data obtained from Acute toxicity provides water quality guidelines for regulatory purposes (*Sundaram et al.*,1994) [38] the present study reveals that chlorpyrifos is toxic to fish. In addition, pesticide intoxication is dose and time-dependent. The current study's 96-hour LC_{50} value of 0.318 mg/L for *Cyprinus carpio* exposed to chlorpyrifos is slightly higher than the 96-hour LC_{50} values of 0.219mg/L of chlorpyrifos estimated in *Puntius chola* (Verma and Saxena 2013) [44] and 0.280mg/L for *Ictalurus punctatus* (Johnson and Finley 1980) [16]; 0.176mg/L for *Poecila reticulate* (Sharbidre 2011) [34] and 0.136mg/L for *Cyprinodon variegates* (Clark *et al*., 1985)[8] and for *Oreochromis mossambicus* 96 h LC_{50} value is 0.0022mg/L (Padmanabha 2015) [28]; 0.0041mg/L for *Fundulus similis* (Schimmel 1983) [33] our obtained 96 h LC_{50} value (0.318mg/L) is lower than 96 h LC_{50} value 0.35mg/L of *Gibelion catla*, 0.47mg/L for *Labeo rohita* and 0.65mg/L for *Cirrhinus mrigala* (Tilak *et al.,* 2004) [41] and 1.023mg/Lfor *Oreochromis niloticus* (Díaz and Girón,2014) [10] 0.92mg/L for *Clarias gariepinus* (Okechukwu *et al.,* 2103) [26]; 16.5mg/L for *Clarias batrachus* (Reddy *et al*.,2012) [31]; 1.57mg/L for Nile tilapia Gul (2005) [14].

Many factors, including species, different conditions of pesticides, such as its specification,manifestation, stereochemistry (Ullah 2015) [43], and also water parameters can affect the concentrations at which a compound is lethal. Our results are in good consonance with the previous reports validating the high toxicity of chlorpyrifos in various fish species (Tilak *et al.,* 2004; Díaz and Girón, 2014; Okechukwu *et al.,* 2103: Reddy *et al*.,2012; Gul, 2005) [41, 10, 26,14].

Proteins are the significant natural constituents of the cell Protein synthesis is an important mechanism in maintaining physiological homeostasis. In the present investigation liver, muscle, and gill, brain tissues of *Cyprinus carpio* exposed to sublethal concentration of chlorpyrifos were subjected to protein metabolic profile and SDS polyacrylamide gel electrophoresis showed significant variations in the intensity of protein banding patterns and protein turnover due to toxic stress resulting in deprivation of cellular proteins.

The present study reveals that chlorpyrifos (SLC-1) influenced all molecular weight protein components in all tissues the variations in protein subunits indicate *Cyprinus carpio* is under stress. CPF induced changes in tissues over high-intensity bands at 89,86,73,68,62kDa, and less dense protein bands at 20-15kDa.It was observed that high molecular weight proteins greater than 50kDa usually have peptide folding results in multiple domain dimensions independently (Erickson, 2009) [11] whereas in low molecular weight 10 to 30kDa proteins peptide folding results in a single domain. Each domain leads to a compact three-dimensional structure. Thus CPF can degrade protein subsequently at both sub-lethal concentrations, it attacks low molecular weight proteins, single domain as well as high molecular weight proteins, multiple domains at both high and less concentrations. Thus it is categorized as a hazardous insecticide in the world (ITRC, 1989) [15].To overcome the toxicant stress, new protein subunits were observed in muscle and brain tissue, this is evident that CPF significantly inhibits gene expression or it may activate some set of genes that synthesize mRNAs, to survive in a stress situation, mRNAs are translated into stress-induced proteins.

Depletion in the protein level and subsequent fading or decrease in intensity of protein subunits expressions is found to be more in liver and gill tissues followed by muscle and brain of *Cyprinus carpio* treated with sublethal convergence(SLC-1) of chlorpyrifos recommends the presence of high proteolytic action, and oxidative stress which attributes to produce stress-induced proteins resulting in degradation of proteins causing reduced protein synthesis and also substantial changes in the expression of proteins results in greater decrement of intensity in protein bands or complete disappearance or fading of protein bands are found to be more in (SLC-1) having a high concentration of chlorpyrifos than the (SLC-2) of chlorpyrifos. CPF significantly inhibits gene expression or it may activate some set of genes that synthesize mRNAs, to survive in a stressful situation, mRNAs are translated into stress-induced proteins. According to Muhammad (2018) [23], SDS-PAGE analysis is an important biomarker for toxicological studies in fish.

In the present study protein subunits and banding patterns showed variations it may be due to protein synthesis turnover and degradation of proteins after pesticidal exposure leading to cytoplasmic alterations in protein patterns resulting in an inhibitory effect on protein anabolism. The rate of protein synthesis or the rate of protein breakdown or protein depletion in treated animal was the physiological strategy played by the animal to adopt itself to

the changed metabolic system. This leads to degradation process like proteolysis and utilization of degraded products for increased metabolism. Protein amount may also be affected as a result of insufficient amino acid incorporation into the polypeptide chain or proteolysis the understanding of the protein components of the cell becomes necessary in the light of the radical changes that take place in protein profiles during pesticide intoxication in addition to the quantity of protein. Both the protein degradation and synthesis are sensitive over a wide range of conditions and show changes to a variety of physical and chemical modulators

In the present study, high and low molecular weight proteins are denatured as a result of CPF toxicity decrease in intensity of protein bands with higher molecular weights was accompanied by an increase in intensities of protein bands with lower molecular weights was observed this variations in the intensity of protein banding patterns and protein biosynthesis due to toxicant stress resulting to alters the functioning of cellular and structural proteins hence typical metabolic function is impaired.

Throughout the exposure time, the protein subunits displayed a consistent decreasing trend in the intensity of all the fractions, indicating a chlorpyrifos inhibitory effect on *Cyprinus carpio*. The current findings are in agreement with

the observations on the same pesticide CPF, Slaninova (2009)[35] stated that as the concentration of pesticide chlorpyrifos in goldfish *Carassius auratus (var*.,) *auratus* was increased resulting in the alterations in protein banding patterns of liver tissue and the findings of (Bantu Nagaraju and Zenebe Hagos 2016) [24] reveal that reduction in the intensity of various protein subunits and protein turnover in distinct tissues of fish *Labeo rohita* treated with profenofos and carbosulfan. Bheem Rao *et al*., (2018) [6] examined the tissue protein banding patterns changed in freshwater fish *H. fossilis* (Bloch) the strength of the tissue protein banding pattern has decreased, and certain protein subunits have faded entirely. Some studies infer variations in protein subunit band patterns may be due to changes in protein turnover (synthesis/degradation). Pesticides may inhibit (or) activate the expression of some genes, causing them to produce specific mRNAs that are then translated into specific proteins known as stress-induced proteins Ramadan (2007) [30] and Sandal et.al (2011) [32], decreased protein content in the tissues of *Cyprinus carpio* may be the result of stress-induced proteolysis or impairment of protein synthesis when exposed to cypermethrin (David *et al.*, 2004) [9]. All these reviews uphold our current examination, depletion in total protein and decreased expression of protein patterns in tissues exposed to chlorpyrifos implies a degradation of proteins due to the toxic stress of pesticides, and also it could be due to hormonal imbalance, impaired tissue repair which affects the protein levels in tissues, or maybe hepatocytic necrosis of cells which subsequently dysfunction the protein biosynthesis.

Conclusions

According to the aforementioned findings and discussion, Data obtained from acute toxicity bioassay provides guidelines for water quality management. Studies using SDS-PAGE and protein modulations revealed impaired protein synthesis and alterations in protein banding patterns because insecticidal stress may alter the peptide sequences in *Cyprinus carpio*. Alterations in the protein degradation due to pesticide intoxication may provide an early warning signal for determining pesticide toxicity and its impact on aquatic species and also help predict the possible mechanisms of toxicity in humans because of its diagnostic significance. It would be very advantageous in assessing the associated environmental risk of these pesticides and thus establishing subsequent management strategies for safeguarding aquatic organisms and their associated fauna.

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Ethical approval

Not applicable. As the fish used for the study used has a high economic food value in India.

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