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Research Article

EFFECT OF AN INSECTICIDE CHLORANTRANILIPROLE ON BIOCHEMICAL CHARACTERISTICS OF SNAKEHEAD FISH, *CHANNA PUNCTATUS* (BLOCH, 1793)

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ABSTRACT

Objective: Pesticides are mainly released into lakes, ponds, and rivers due to the runoff from agricultural fields. Pesticides are generally toxic to many non-target organisms such as fish. Fish, generally accumulate contaminants from aquatic environments and have been largely used in studies of food safety. Hence, present investigation aims to determine the effects of chlorantraniliprole on biochemical characteristics (total protein, soluble and structural, free amino acids and lipid levels in muscle, kidney, and liver) of the snakehead fish *Channa punctatus*.

Methods: The water used for acclimatization and conducting experiments was clear unchlorinated groundwater. The soluble, structural and the total proteins in the organs were estimated using the Folin phenol reagent method.

Results: The result shows declined levels of biochemical parameters during all the exposure periods when compared with control.

Conclusion: The results of the current study show the toxic nature of the toxicant on the biochemical parameters of the fish, *C. punctuatus.* The changes in total soluble, soluble proteins, free amino acids, and lipid in the chlorantraniliprole treated fish.

Keywords: Accumulate, Food safety, *Channa punctatus*, Declined, Exposure.

INTRODUCTION

Pesticides were found to adversely affect a number of biological functions, thus causing harm to the non-target organisms and those compounds are known for this persistence in the environment and accumulation in the tissues for long periods for controlling the loss of productivity due to pest attack and as a consequence of the demand for producing more food, there has been an increasing use of pesticides in developed countries [1]. Proteins are the most versatile biomolecules in living organisms, proteins are the main enzymes in a cell and regulate metabolism by selectively accelerating chemical reactions. They function as bio-catalysts, they conveyance and supply supplementary molecule oxygen, provide mechanical provision and defense mechanism against foreign substances, intercellular signaling and they transfer nerve impulses [2]. They are the abundant macromolecules in a biological organization and are the byproducts of high molecular weight polypeptides. They not only assist as a fuel to produce energy but also play an energetic role in the structural and functional physiognomies of the living organism. Functionally, proteins show a prodigious diversity, establish a heterogeneous group, having diverse physiological roles and are involved in major physiological activities [3]. Therefore, the valuation of the protein content can be measured as an analytical tool to determine the physiological levels of animals [4,5]. The concentration of proteins in tissue is a balance between the rate of their synthesis and degradation or catabolism [6]; the overall protein turnover in an animal is the dynamic equilibrium between these two [7]. Hydrolysis of proteins is a quite common phenomenon wherein proteases split proteins stepwise into amino acids. Amino acids formed by protein degradation will also be utilized for energy production. Amino acids are vital intermediates in protein synthesis, and degradation products appear in the form of different nitrogenous substances [8]. Singh and Singh [9] reported that variations in lipids, phospholipids content in the *Heteropneustes fossilis* treated with endosulfan. The present investigation was intended to study the effect of chlorantraniliprole on soluble, and total proteins, free amino acids, and total lipid content of fish, *Channa punctatus*.

METHODS

Procurement and maintenance of fish

The freshwater fish *C. punctatus* size 12–13 cm and weight 18–20 g were brought from local freshwater bodies located at Kuchipudi, Guntur district of Andhra Pradesh, India. The fish were fed daily with commercial fish pellets and acclimatized to the laboratory conditions at 28 + 2°C for 15 days. During the acclimatization period daily fed with fish meal. If in any batch, mortality exceeds 5% during acclimatization, that entire batch of fish was discarded. The water used for acclimatization and conducting experiments was clear unchlorinated groundwater. The physical and chemical analyses of the water were carried out according to APHA [10]. The containers of the test media are of 15 L capacity, wherein each test five containers were used, and each container consisted of 10 fish. All the precautions laid by APHA [10] were followed. Hence, in the present investigation, 96 h LC50 and 1/10th of 96 h LC50 were selected as lethal and sublethal concentrations to study the behavioral responses and physiological alterations in the experimental animal.

Physicochemical analysis of water

Turbidity–8 Silicaunits, Electricalconductivityat 28°C –816 micro ohms/cm, alkalinity-1, phenolphthalein-nil, methylorange-472, total hardness (as CaCO3)-232,noncarbonatehardness(asCaCO3)-nil,calciumhardness(AsN) -nil, sulphate (as SO4) - trace, chloride (as Cl) - 40, fluoride (as Fl) - 1.8, iron (as Fe) - nil, dissolved oxygen - 8–10 ppm, Temperature - 28±2°C. All the precautions laid by the committee on toxicity tests to aquatic organisms (APHA, 2005) were followed.

Estimation of soluble and total proteins

The soluble, and the total proteins in the organs were estimated using the Folin phenol reagent method as described by Lowry *et al*. [11]. 1% homogenate (W/V) was prepared in ice-cold 0.25 M sucrose solution. For soluble and total proteins 1.0 ml of the homogenate were taken and centrifuged at 3000 rpm for 10 min. The supernatant was separated and to both the supernatant and residue 3 ml of 10% trichloroacetic acid

(TCA) was added and again centrifuged at 3000 rpm. The supernatants were discarded, and the residues were taken for experimentation. For total proteins, 1 ml of homogenate was taken; to it, 3 ml of 10% TCA was added and centrifuged at 3000 rpm. Supernatant was discarded, and the residue was taken for experimentation. All the three residues were dissolved in 5 ml of 0.1 N sodium hydroxide and to 1 ml of each of these solutions, 4 ml of reagent - D (mixture of 2% sodium carbonate and 0.5% copper sulfate in 50:1 ratio) was added. The samples were allowed to stay for 10 min, at the end of which 0.4 ml of Folin phenol reagent (diluted with distilled water in 1:1 ratio before use) was added. Finally, the optical density of the color developed was measured using spectrophotometer at a wavelength of 600 nm. A mixture of 4 ml of reagent–D and 0.4 ml of Folin phenol reagent was used as a blank. Bovine albumin was used for the preparation of protein standards. The protein content is expressed as mg/g wet wt of the tissue.

Estimation of free amino acids

Free amino acid levels in the tissues were estimated by the ninhydrin method as described by Moore and Stein [12]. Homogenates (4%) were prepared in cold phosphate extraction buffer (50 mmol, pH 7) and 2 ml of 15% TCA was added to 0.2 ml of the homogenate followed by centrifugation at 3000 rpm for 15 min. To the entire supernatant (2.2 ml), 2 ml of ninhydrin reagent was added, and the contents were boiled for exactly 5 min. The contents were cooled in ice-cold water, and the volume was made up to 10 ml with distilled water and n-propanol in 1:1 ratio). The optical density of the color developed was measured using spectrophotometer at a wavelength of 570 nm against a reagent blank. The free amino acid levels are expressed as µmol of tyrosine equivalents/g wet wt. of the tissue.

Estimation of lipids

Lipids were extracted as described by Folch [13], and estimated by the method of Barnes and Blackstock [14]. 50 mg of tissues were homogenized (5% w/v) in a waring blender in the chloroform-methanol mixture (2:1). The homogenates were filtered through Whatman No.1 filter paper, and the residue was homogenized as before and then filtered. The non-lipid matter from the pooled filtrate was removed by shaking vigorously with 0.88% KCL. 1 ml of filtrate was taken in a test tube and evaporated under nitrogen, and 1 ml of concentrated H_2SO_4 was added and boiled for 10 min. For estimation of total lipid, 0.2 ml of solution was taken, and 5 ml of vanillin reagent was added. The developed color was read in a spectrophotometer at 520 nm against reagent blank.

Protein electrophoresis

A change in protein fractions was done using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli [15] method.

Sample preparation

1% homogenates of brain, muscle, liver, kidney, and gill were prepared in 10% TCA and centrifuged at 8000 rpm for 10 min in a cooling centrifuge. The pellet was washed twice with ice cold acetone, again centrifuged at 8000 rpm for 10 min. The pellet was dissolved in sample buffer (0.5M Tris-HCL, pH 6.8–2 ml, and 40% glycerol-1.6 ml) and boiled in a water bath at 95°C for 10 min.

Preparation of gel slab

The glass plate's sandwich was assembled using two clean glass plates and 1 mm Teflon gel solution 12.5% (1.5M Tris-HCL, pH 8.8–2 ml, 30% acrylamide-3.2 ml, 10% SDS-0.5 ml double distilled water-1.8 ml, TEMED-0.015 ml, and ammonium persulphate-0.5 ml) was prepared and poured in between the clamped glass plates. To avoid entrapment of any air bubbles, the gel solution was overlaid with distilled water. The plates were left undisturbed for 30 min for the polymerization of the gel. After gel polymerization overlaid water was removed and rinsed with stacking gel buffer, now the 5% stacking gel solution (0.5M Tris HCL, pH 6.8–2 ml, 30% acrylamide –0.8 ml, 10% SDS-0.5 ml, double distilled water 1.2 ml, TEMED-0.015 ml, and 1.5% APS 0.5 ml) was prepared and poured over the polymerized resolving gel, and comb was inserted carefully. The gel slab was left undisturbed for 15 min after polymerization comb was loaded into the wells and gel was run at 60 V.

Staining method

The coomassie brilliant blue staining was based on the method of Barnes and Blackstock [14]. Incubate the gel in staining solution of 40% methanol, 10% acetic acid 0.025%, and Coomassie Brilliant Blue R-250, which has been filtered through Whatman#1 paper. Incubate the gel for 6 h to overnight in the staining solution with shaking on a rotary shaker. Destaining solution is the same as staining solution, but not containing the Coomassie R-250 dye powder add destaining solution to the gel and incubate for 30–60 min. The gel was washed several times in double distilled water, and the electrophoretogram gel was preserved in water.

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

To determine the molecular weight of the individual subunits of the protein, the relative mobility of the individual subunits was calculated using the following formula:

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

A standard curve is prepared by plotting migration distances ("X"-axis) of known protein standards against their molecular weights ("Y"-axis) on semilog graph paper. From the migration distance of an unknown protein, the molecular weight of the protein is being calculated from the standard curve.

RESULTS AND DISCUSSION

The data are presented on the levels of soluble, total proteins, free amino acids, and lipids in the organs of the fish *C. punctatus* on exposure to lethal 24 h and 1, 10, 20, and 30 days of sublethal concentrations of chlorantraniliprole. All results are presented in the tables from 1 to 4 and Figs. 1-4, a substantial decrease comparative to controls is seen in the soluble, and total proteins, amino acids, and lipids of all the vital organs of fish, *C. punctatus* at all the exposure periods in the lethal and sublethal concentrations of chlorantraniliprole. These protein levels also recorded a significant decrease in the organs of fish on day 1 and 10 on exposure to sublethal concentration but on further exposure gradual reduction in the increase was observed at 20 and 30 days (Tables 1-2 and Figs. 1 and 2). During the exposure periods, the levels of soluble, total proteins, amino acids, and lipids significantly decreased in the kidney, muscle, and liver compared to control fish. The lowest decrease was observed in kidney (12.49%) at 24 h and maximum (121.78%) in liver at 96 h on exposure to the lethal concentrations. The similar observation was not the circumstance at sublethal concentration, among the tissues of fish, the reduction in protein content was greater in liver than kidney, and muscle exposed to the lethal and sublethal concentrations of chlorantraniliprole. The data presented in Table 4, corresponding to the reduction in protein content a rapid increase in free amino acid levels in all the organs of fish at all the exposure periods in the lethal concentration of chlorantraniliprole was observed. Furthermore, in under sublethal concentration, however, free amino acid levels was decreased, it is mainly more in the tissues of the fish exposed to lethal than the sublethal concentration.

The relative mobility's of the protein fractions of the freshwater fish *C. punctuatus* exposed to chlorantraniliprole in sublethal concentrations for 30 days are given in Table 5 and Fig. 5. The electrophoretogram (Fig. 5) represents the decrease in the intensity of liver protein subunits compared to control. Under the chlorantraniliprole exposure, liver protein subunits showed more decreased intensity in banding pattern compared to the control sample. The Rm value of protein subunit 0.66 nearer to 87 daltons (Kda) was absent in 10 days exposure and Rm value of protein subunit 0.92 in between molecular weight of 21 daltons and 43 daltons was absent in both 20 and 30 days treated fish tissue samples when compared to control.

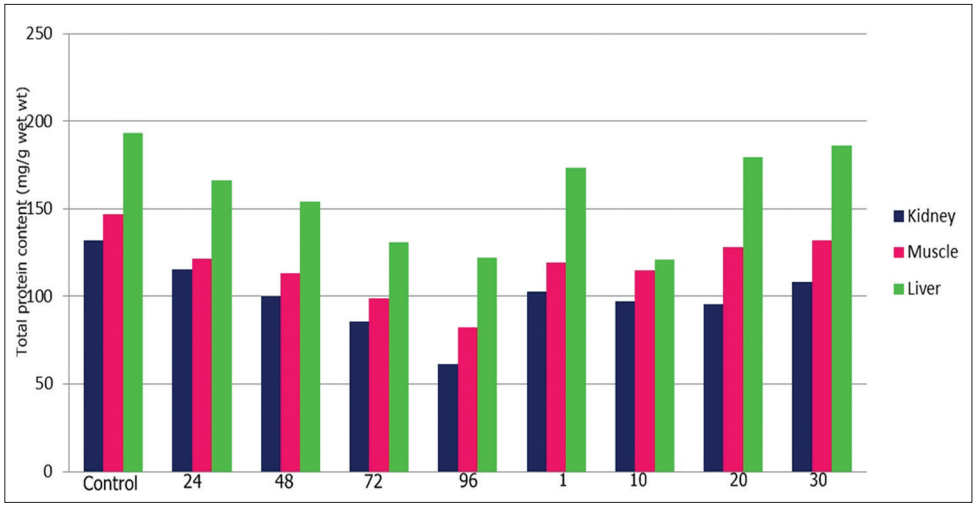

Fig. 1: Total protein content (mg/g wet wt) in the organs of fish, *Channa punctatus* on exposure to the lethal and sublethal concentrations of chlorantraniliprole for 10, 20, and 30 days

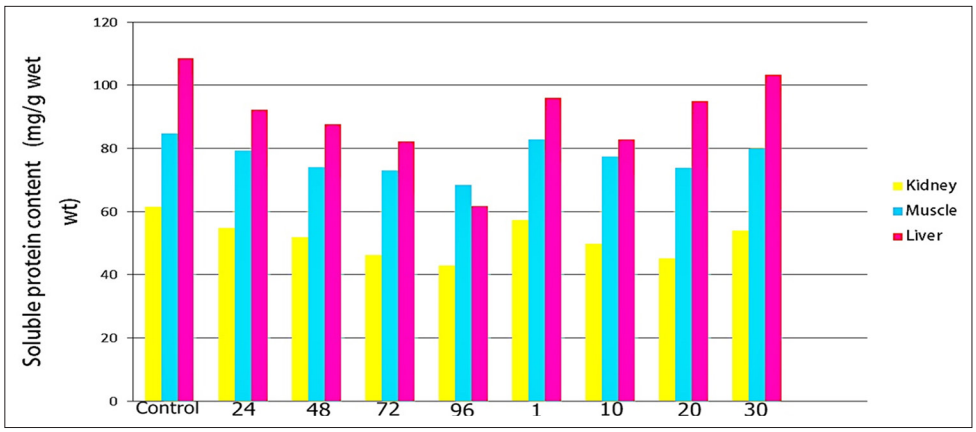

Fig. 2: Soluble protein content (mg/g wet wt.) in the organs of fish, *Channa punctatus* on exposure to the lethal and sublethal concentrations of chlorantraniliprole for 10, 20, and 30 days

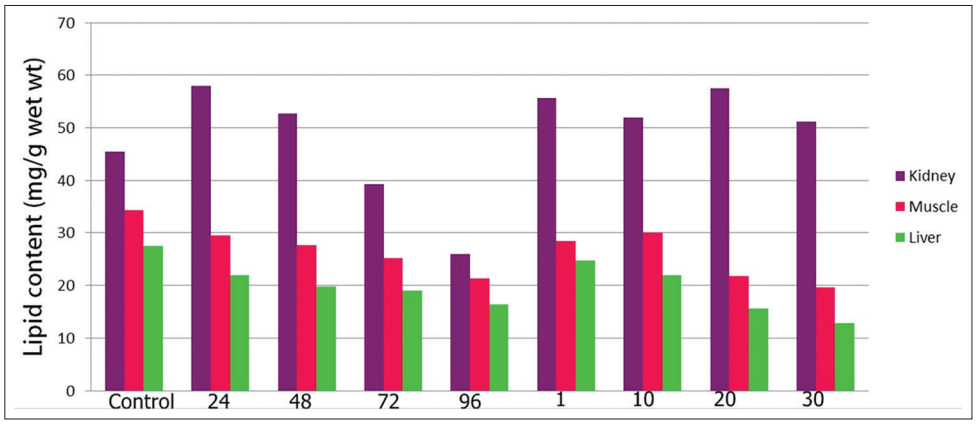

Fig. 3: Lipid content (mg/g wet wt.) in the organs of fish, *Channa punctatus* on exposure to the lethal and sublethal concentrations of chlorantraniliprole for 10, 20, and 30 days

In the present study, the toxic effects of chlorantraniliprole on the total protein content, free amino acid levels, and lipids content in the tissues of the fish, *C. punctatus* showed time-dependent alterations. Total proteins, and soluble protein, free amino acid, and lipid levels were exhausted in all the vital organs exposed to the lethal concentration of chlorantraniliprole representative the breakdown of these proteins due to the severe pesticidal stress. Usually, the breakdown of proteins dominates over synthesis under enhanced proteolytic activity [16]. It is evident in the current study that the hypoproteinemia is related with the sudden raise free amino acid levels in the tissues of the fish exposed to the lethal concentrations. The maintenance of proteins in a highly organized state requires an active and incessant supply of energy. Similar observations were recorded with other pesticides in numerous fishes, as reported in *Cirrhinus mrigala* [17] exposed to

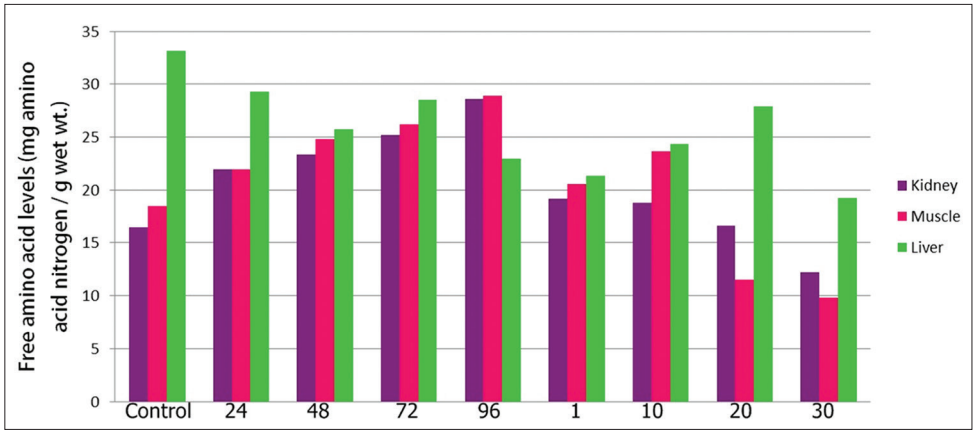

Fig. 4: Free amino acid levels (mg amino acid nitrogen/g wet wt.) in the organs of fish, *Channa punctatus* on exposure to the lethal and sublethal concentrations of chlorantraniliprole for 10, 20, and 30 days

Table 1: Total protein content (mg/g wet wt.) in the organs of fish, *C. punctatus* on exposure to the lethal and sublethal concentrations of chlorantraniliprole

Organs	Control	Exposure periods							
		Lethal (h)				Sublethal (days)			
		24	48	72	96	1	10	20	30
Kidney	131.78	115.32^{b}	99.76^{a}	85.72^{d}	61.44^{a}	102.51^{c}	97.26^{a}	95.79^{d}	108.42^{d}
SD±	0.22	0.29	0.35	0.44	0.22	0.51	0.59	0.44	0.32
% Change	---	12.49	17.04	34.95	53.37	22.21	26.19	27.31	17.72
Muscle	146.87	121.39^{d}	113.45^{d}	98.77^{b}	82.36^{d}	119.43^{a}	114.89^{d}	128.22^{b}	131.92^{c}
SD±	0.51	0.32	0.39	0.44	0.33	0.22	0.52	0.59	0.29
% Change	---	17.34	22.75	32.75	43.92	18.68	21.77	12.69	10.17
Liver	193.31	166.29^{c}	153.92^{a}	130.75^{d}	121.78^{c}	173.52^{d}	120.65^{c}	179.32^{a}	186.29^{b}
SD±	0.01	0.22	0.32	0.35	0.51	0.52	0.44	0.22	0.29
% Change	---	13.97	25.39	32.36	56.11	10.23	0.3758	0.07.23	0.3.63

Means are SD± (n=5) for a parameter in a row, different letters indicate significant differences between the values of control and pesticide chlorantraniliprole exposed groups are based on 24, 48, 72, and 96 h and 1, 10, 20, and 30 days exposure. (a) p≤0.02 denotes significant when compared with control values, (b) p≤0.05 denotes significant when compared with control values, (c) p≤0.005 denotes significant when compared with control values, (d) p≤0.01 denotes significant when compared with control values. SD: Standard deviation, *C. punctatus: Channa punctatus*

Table 2: Soluble protein content (mg/g wet wt.) in the organs of fish, *C. punctatus* on exposure to the lethal and sublethal concentrations of chlorantraniliprole

Organs	Control	Exposure period in days							
		Lethal (h)				Sublethal (days)			
		24	48	72	96	1	10	20	30
Kidney	61.5^{b}	54.76^{d}	51.93^{a}	46.25^{a}	42.88^{a}	57.33^{b}	49.72^{b}	45.25^{c}	53.88^{b}
SD±	0.05	0.01	0.12	0.05	0.12	0.16	0.30	0.29	0.01
% Change	---	−11.08	−15.68	−24.90	−30.37	−6.91	−19.27	−26.53	12.51^{a}
Muscle	84.65	79.20^{a}	74.11^{d}	72.99^{d}	68.29^{d}	82.90^{d}	77.29^{d}	73.84^{a}	79.99
SD±	0.29	0.61	0.29	0.45	0.31	0.41	0.20	0.45	0.21
% Change	---	−6.43	−12.45	−13.77	−19.32	−2.09	−8.69.	−12.77	−5.50
Liver	108.5^{a}	92.29^{d}	87.66^{c}	82.21^{b}	61.77^{c}	95.99^{c}	82.85^{a}	95.04^{b}	103.22^{d}
SD±	0.05	0.05	0.01	0.41	0.29	0.45	0.29	0.41	0.29
% Change	--	−14.97	−19.23	−23.75	−43.09	−11.56	−23.66	−12.43	−4.90

Means are SD± (n=5) for a parameter in a row, different letters indicate significant differences between the values of control and pesticide chlorantraniliprole exposed groups are based on 24, 48, 72, 96 h and 1, 10, 20 and 30 days exposure. (a) p≤0.02 denotes significant when compared with control values, (b) p≤0.05 denotes significant when compared with control values, (c) p≤0.005 denotes significant when compared with control values, (d) p≤0.01 denotes significant when compared with control values. *Channa punctatus: C. punctatus*, SD: Standard deviation

Rogor. Depletion of proteins in tissues may constitute a physiological mechanism and may play a key role of compensatory mechanism below pesticidal stress, to distribute intermediates to the Kreb's cycle or to enhance osmolarity, by retaining free amino acid content in hemolymph, to compensate osmoregulatory problems encountered due to the seepage of ions and other indispensable molecules, during the pesticide stress [18,19]. The depletion of protein endorses increased proteolysis and possible utilization of the products of their degradation for metabolic purposes [20]. The reduction of protein level induces to diversification of energy to meet the imminent energy demands during the toxic stress [21].

The depletion of protein level induces to diversification of energy to meet the impending energy demand during toxic stress [22]. Under

Table 3: Lipid content (mg/g wet wt.) in the organs of fish, *C. punctatus* on exposure to the lethal and sublethal concentrations of chlorantraniliprole

Organs	Control	Exposure periods							
		Lethal (h)				Sublethal (days)			
		24	48	72	96	1	10	20	30
Kidney	45.44	57.99[a]	52.66[d]	39.33[b]	25.96[d]	55.65[a]	51.99[c]	57.54[d]	51.20[a]
SD±	0.92	0.51	0.52	0.41	0.29	0.27	0.45	0.05	0.29
% Change	---	−27.61	−15.88	−13.44	−42.86	−22.46	−14.41	−26.64	−12.76
Muscle	34.32	29.57[d]	27.65[a]	25.22[c]	21.33[b]	28.43[d]	29.98[a]	21.76[a]	19.66[d]
SD±	1.06	0.44	0.39	0.31	0.25	0.46	0.42	0.49	0.51
% Change	---	−13.84	−19.43	−26.51	−37.84	−17.16	−12.64	−36.59	−42.71
Liver	27.54	21.96[a]	19.72[b]	18.99[a]	16.44[d]	24.75[b]	21.97[c]	15.65[c]	12.78[c]
SD±	1.45	0.51	0.47	0.41	0.32	0.29	0.11	0.51	0.29
% Change	---	−10.43	−28.39	−31.04	−40.31	−10.14	−20.22	−43.17	−53.59

Means are SD± (n=5) for a parameter in a row, different letters indicate significant differences between the values of control and pesticide chlorantraniliprole exposed groups are based on 24, 48, 72, and 96 h and 1, 10, 20, and 30 days exposure. (a) p≤0.02 denotes significant when compared with control values, (b)p≤0.05 denotes significant when compared with control values, (c) p≤0.005 denotes significant when compared with control values, d) p≤0.01 denotes significant when compared with control values. *C. punctatus: Channa punctatus*, SD: Standard deviation

Table 4: Free amino acid levels (mg amino acid nitrogen/g wet wt.) in the organ soffish, *C. punctatus* on exposure to the lethal and sublethal concentrations of chlorantraniliprole

Organs	Control	Exposure periods							
		Lethal (h)				Sublethal (days)			
		24	48	72	96	1	10	20	30
Kidney	16.45	21.93[b]	23.38[c]	25.22[a]	28.61[d]	19.20[b]	18.76[a]	16.65[c]	12.24[b]
SD±	0.05	0.01	0.29	0.10	0.11	0.12	0.11	0.08	0.10
% Change	---	33.31	42.12	53.31	73.92	16.71	14.04	12.15[a]	25.59[a]
Muscle	18.45	21.96[d]	24.78[a]	26.21[d]	28.92[d]	20.55[a]	23.62[d]	11.49	9.85
SD±	0.01	0.05	0.11	0.12	0.14	0.12	0.08	0.01	0.05
% Change	---	19.02	34.30	42.05	56.74	11.38	28.02	37.72[b]	46.61[d]
Liver	33.18	29.27[a]	25.75[c]	28.53[a]	22.98[a]	21.34[c]	24.32[c]	27.93	19.28
SD±	0.05	0.10	0.12	0.29	0.12	0.22	0.11	0.01	0.05
% Change	---	11.78	22.39	14.01	30.74	35.68	26.70	15.82	41.89

Means are SD± (n=5) for a parameter in a row, different letters indicate significant differences between the values of control and pesticide chlorantraniliprole exposed groups are based on 24, 48, 72, 96 h and 1, 10, 20 and 30 days exposure. (a) p≤0.02 denotes significant when compared with control values, (b) p≤0.05 denotes significant when compared with control values, (c) p≤0.005 denotes significant when compared with control values, (d) p≤0.01 denotes significant when compared with control values. *C. punctatus: Channa punctatus*, SD: Standard deviation

Table 5: Relative mobility values for fish *C. punctatus* exposure to the lethal and sublethal concentrations of chlorantraniliprole for 10, 20, and 30 days

Marker	Lane1 control	Lane 2 chlorantraniliprole exposed liver for 10 days	Lane 3 chlorantraniliprole exposed liver for 20 days	Lane 4 chlorantraniliprole exposed liver for 30 days
-	0.16±0.01	0.11±0.21	0.10±0.45	0.09±0.05
-	0.18±0.09	0.13±0.5	0.12±0.21	0.15±0.49
0.39	-	0.15±0.03	-	0.17±0.01
-	0.22±0.51	-	0.18±0.29	0.19±0.29
-	0.35±0.40	0.22±0.22	0.24±0.14	0.20±0.22
0.42	-	0.24±0.21	-	-
-	0.42±0.01	0.39±0.41	-	0.25±0.05
0.58	-	-	0.28±0.05	0.22±0.08
-	0.49±0.29	0.41±0.01	0.53±0.29	0.44±0.14
0.64	-	-	-	-
-	0.55±0.14	0.45±0.03	0.69±0.45	0.59±0.01
0.77	-	0.58±0.05	0.78±0.22	0.66±0.55
-	0.66±0.55	0.74±0.59	-	-
0.82	0.72±0.03	0.81±0.14	0.89±0.03	0.79±0.22
-	0.89±0.21	0.83±0.01	0.92±0.25	0.85±0.29
−0.89	-	0.91±0.03	-	-
−0.97	0.91±0.08	0.96±0.29	0.94±0.05	0.91±0.45
-	-	0.94±0.21	0.96±0.49	0.94±0.22

Values are theme office observations; standard deviation indicated as (±), values are significant at p<0.05. *C. punctatus: Channa punctatus*

proteolysis, enhanced breakdown dominates over synthesis while in the case of anabolic process; increased synthesis dominates the protein breakdown [16]. This is further corroborated through the increased levels of free amino acids in all the tissues. These amino acids might

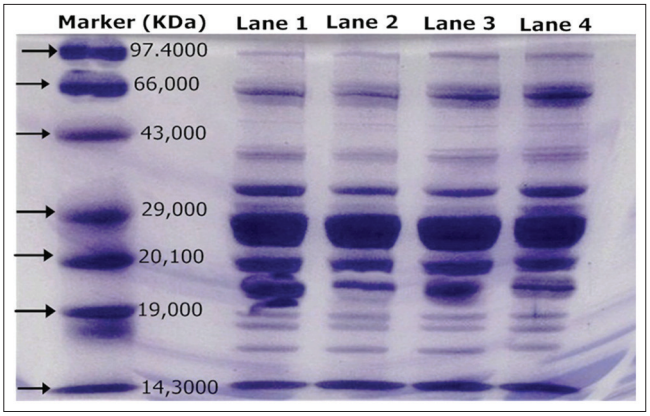

Fig. 5: Changes in protein subunits in liver tissue of fish, Channa punctatus exposure to the lethal and sublethal concentrations of chlorantraniliprole for 10, 20, and 30 days

be fed into the TCA cycle as keto acids by way of transamination since transaminases are known to be elevated during pesticide intoxication [22]. The increased levels of free amino acids might also be due to increased synthetic potentiality. This possibility might exist in the tissues of toxicant exposed fish.

It appears that protein degradation is in active phase over synthesis in the kidney, muscle, and liver of fish at sublethal concentration of toxicant as evidenced from the decrease in soluble and total proteins with the significant increase in protease activity and amino acid levels. Similar reports were observed in Mus boodoja on exposure to BHC [23]. However, reduced decrease in soluble and total proteins along with gradual rise in protease activity and free amino acid levels in the kidney, muscle, and liver of fish at day 10 and 15 indicates the onset of acceleratory phase of protein synthesis over breakdown. The reduced decrease in total proteins could be helpful to the animal to fortify its organs for developing resistance to the imposed sublethal toxic stress; further, the reduced magnitude of decrease in soluble protein fraction could indicate the synthesis of enzymes necessary for detoxification. Protein synthesis being an energetically expensive process, the increase in oxidative metabolism of the fish during sublethal toxicant stress also strengthens the increase in its protein synthetic potentials. Degradation of proteins by proteolytic enzymes results in increased amino acid pool. Further, prevalence of pathological conditions in the organ systems of an animal may decrease protein synthetic acid pool. The above two factors could be responsible for the increase in free amino acid levels in the organs of fish exposed to the lethal concentration of toxicant. High concentrations of amino acids in tissues can lead to hyper amino academia which, in turn, can cause a number of side effects on the physiological conditions of the cell. The increase in the free amino acids in the organs of fish exposed to sublethal concentrations can be partly due to the increased proteolytic activity and partly due to certain transaminases reported to be indicators of protein degradation in salmonids and liver intoxication in rainbow trout [24].

The slow increase in soluble protein in the fish exposed to the sublethal stress could also support the elevation in these enzyme activities. The increase could be due to the stepwise induction of these enzymes greater and eater association of their oligomers [25]. The increase in these enzyme activities could be helpful to the fish for structural reorganization of proteins and incorporation of keto acids into the TCA cycle to favor gluconeogenesis or energy production.

Lipids support as energy reserves to meet the metabolic requirement for more energy to mitigate toxic stress. Srinivas *et al*. [26] reported that decreased lipid content in *Tilapia mossambica* exposed to atrazine. In general, the decreases in lipid contents in kidney, muscle, and liver tissues were found to be increased with the time of exposure. Decline in the lipid levels may be due to the inhibition of cholesterol biosynthesis in the liver or due to reduced absorption of dietary cholesterol as reported by Mishra *et al*. [27], Arockia and Mitton [28] have showed decreasing trend of lipid content in the brain, gill, kidney, liver, and muscle tissues on exposure to Lannate in the fish *Oreochromis mossambicus*. Various authors studied similar reduction of lipids content in different tissues. Ram and Sathyanesan [29] observed a reduced lipid content in the liver tissue of fish *C. punctuatus* exposed to emisan. A decrease in the lipid content of the liver, muscle, and kidney tissues exposed to chlorantraniliprole recommends that lipid might have been directed for energy production for other metabolic function in which these products play a key role during toxicant stress condition. Ramadan [5] reported that plasma protein band pattern in fish Nile tilapia using SDS-PAGE, the number of protein bands declined in fish on exposure to 0.20 ppm, 0.002 ppm, 0.004 ppm, 0.008 ppm, and 0.02 ppm concentrations of butataf. The number of plasma protein bands decreased due to toxicity of butataf, when compared to control. SDS-PAGE was performed for the liver tissue of *C. punctatus* exposed to chlorantraniliprole, similar results also reported by Nagaraju *et al*. [30], in freshwater fish, *Labeorohita* on exposure to profenofos and carbosulfan. The protein subunits of liver showed decrease in intensity, and some protein subunits were disappeared. Inhibition of proteins may be due to tissue necrosis which leads to losses of intracellular enzymes or other proteins.

CONCLUSION

A influence of chlorantraniliprole and its effect at cellular, molecular level and ultimately cause physiological and biochemical changes. The results of the current study obviously show the toxic nature of the toxicant on the biochemical parameters of the fish, *C. punctuatus*. The changes in total soluble proteins, free amino acids, and lipid in the chlorantraniliprole treated fish will unusually affect the nutritive value of these fishes and all the metabolites studied are found to be sensitive changes in the normal indictors, which reflect changes in the normal activities of various functional systems.

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