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Research Article

ENZYMATIC CHANGES IN TISSUES OF FISH, *CIRRHINUS MRIGALA* UNDER LETHAL AND SUBLETHAL EXPOSURE TO CYPERMETHRIN (10% EC)

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ABSTRACT

The effect of Cypermethrin (10% EC) formulations at sublethal and lethal concentrations on certain enzymes in tissue such as Aspartate Amino Transferase (AAT), Alanine Amino Transferase (ALAT), Acid Phosphatase (ACP)of the freshwater fish, *Cirrhinus mrigala* during and after the cessation of the exposure were observed. Ten fishes were exposed to the lethal and sublethal concentrations for a period of 4 days were sacrificed and tissues such as gill, kidney, liver, brain and muscle were analysed for biochemical parameters and remaining five fish were maintained in a clean well water for a period of seven days without test substance being spilled in the water and its tissues were collected after reversal period for biochemical analysis. Marked changes were observed in the biochemical parameters of the fish exposed and after the cessation of the exposure. Hence the present study has been under taken to study the effect of Cypermethrin 10% EC formulation at sublethal and lethal concentrations to fish.

Keywords: *Cirrhinus mrigala*, Cypermethrin toxicity, Aspartate aminotransferase , Alanine aminotransferase.

INTRODUCTION

The main causes for environmental health risk are industrialization, urbanization, over population, agriculture, forest fires, desert dust, and inadequate waste management, especially in developing countries. Pollution is one of the great existential challenges of the Anthropocene era. Pollution leads to the introduction of contaminants into the natural environment that causes adverse change on organisms. These pollutants ultimately find their way into groundwater, wetlands, rivers and lakes and finally to oceans in the form of sediment and chemical loads carried by rivers (Albanis *et al.,* 1998; EPA, 2003; Aydin and Koprucu, 2005). Exposure to chemicals and hazardous wastes cause debilitating and fatal illness, create harmful living conditions, and destroy ecosystems. Pollution endangers the stability of the earth's supporting system and threatens the continuing survival of human societies. All chemicals eventually find their way into the aquatic environment, where they prove to be toxic to many non-target organisms. The potential impacts of the pollutants are more on the aquatic organisms than on the terrestrial organisms.

Pesticides commercially available in the market generally are formulated products which contain a certain percentage of actual pesticide known as active ingredient. Pesticides kill the pest by inhibiting or blocking enzymes, neurotransmitters, hormones and secondary messengers in the various organ systems, kill adult pests or prevent growth and development in young ones. In effect, pesticides have the potential to kill not only the pests, but other animals, including humans. The major insecticides that are usually applied in agriculture and public healthy sections include organophosphates, organochlorines pyrethroids and carbamates. Most widely used pesticides are pyrethroids and organophosphates. Pyrethroids are one type of pyrethrin (Sunderlund *et al.*, 2002). Cypermethrin, a synthetic pyrethroid, is one of the most effective insecticides used in forestry, agriculture, buildings and farmyards (Casida *et al.*, 1983; Khan *et al*., 2006; Ullah *et al.*, 2015). Contamination of water by insecticides is mainly

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due to intensive agriculture combined with surface runoff and sub surface drainage usually within a few weeks after application (Banaee *et al.,* 2013). Fish due to its aquatic habitat is directly exposed to harmful insecticides which affects its profitable worth and rearing ability (Georgieva *et al.,* 2014; Firat *et al.,* 2011). Fish is highly at health risk to pyrethroids (De Moraes *et al.,* 2013).

Pesticides are accumulated in tissues like liver, brain, lungs, kidneys and muscle that result in dysfunction of organ and finally results in loss of fish (Srivastava and Kaushik, 2001). In fish, different insecticides can be absorbed through gills, skin or alimentary ducts (Schlenk, 2005; Banaee *et al.,* 2011; Banaee, 2012). The toxicity of fish increases with increase in value of toxin per litre (Guner, 2009). Fishes are particularly sensitive to environmental contamination of water. Hence, pollutants such as insecticides may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes (Banaee *et al.,* 2011). So, the effects of insecticides on fishes are of great concern.

Thus, the Cypermethrin (10% EC) can show its effect on biochemical parameters of the freshwater fish, *Cirrhinus mrigala* as well on metabolic enzymes such as Aspartate Amino Transferase (AAT), Alanine Amino Transferase (ALAT), and Acid Phosphates (ACP).*Cirrhinus mrigala* is popular as food fish. It is an important aqua cultured freshwater species throughout South Asia. It is widely farmed as a component of a polyculture system of three Indian major carps, along with *Labeo rohita and Catla catla* and hence chosen as a model systemin the study the effects of Cypermethrin (10% EC) at sublethal and lethal concentrations on certain enzymes.

MATERIALS AND METHODS

The test fish *Cirrhinus mrigala* with a size range of 5-6 ± cm and 3-4gm weight, irrespective of their sex have been chosen as the test organism in the present study. The freshwater fish were brought from fish hatcheries of Vissakoderu, Bhimavaram, West Godavari district, Andhra Pradesh, India which is 80 km away from the University. The fish were acclimated to laboratory conditions in large tubs with unchlorinated water for one week at a room temperature of 28±2ºC. During the period of acclimation, the fish were fed daily with Ground nut cake and rice bran as fish feed on an average at 3% of their body weight. The fish were not given feed a day prior to the experimentation. Fishes were exposed to static lethal and sublethal concentration (LC_{50} and $1/10^{th}$ of LC_{50} value for 24h and 96h) cypermethrin for 96 hours (4 days).

Experiments were conducted to determine the toxicity of Cypermethrin in various concentrations with 10% EC formulation in static system. Experiments were conducted to select the mortality range from 10% to 90% for 24, 48, 72 and 96 h in static system. The data on the mortality percent of fish was taken into consideration to calculate LC_{50} values. The dead fish were removed immediately. The data were recorded from these tests at the end of each specific time period. The toxic tests were conducted to choose the mortality range from 10% to 90% for 4 days in static method. During the whole experiment, a suitable control was also maintained to nullify any other effects that likely to affect the fish. Then the fishes were sacrificed immediately and isolated fresh (wet) tissue of vital organs such as brain, gill, liver, kidney and muscle were taken for biochemical estimation of AAT, ALAT and ACP.

Estimation of Aspartate Amino Transferase (AAT) Activity

The reaction mixture of 1.5 ml contains 1ml phosphate buffer (Ph 7.4), 0.1ml of L-alanine, 0.1 ml of Ketoglutarate and 0.3 ml of supernatant as enzyme source. The contents were incubated at 37°C for 30 minutes. The reaction was stopped by the addition of 1 ml of 2, 4-dinitrophenyle hydrazine solution. After 20 minutes, 10ml of 0.4 N Sodium hydroxide was added and the colour developed was read at 545 nm in a spectrophotometer against a reagent blank. The enzyme activity was expressed as moles of pyruvate formed/mg protein/hour.

Estimation of Alanine Amino Transferase (ALAT) Activity

The reaction mixture of 1.5 ml contains 1ml phosphate buffer (pH 7.4), 0.1ml of L-aspartate (L-Aspartic acid), 0.1 ml of Ketoglutaric acid and 0.3 ml of supernatant as enzyme source. The reaction mixture was incubated at 37°C for 30 minutes. The reaction was stopped by adding 1 ml of 2, 4-dinitrophenyle hydrazine solution prepared in 0.1 N HCl and was allowed to stand for 20 minutes at room temperature. The rest of the details were the same as for alanine aminotransferase. The activity levels were expressed as moles of pyruvate formed/mg protein/hour.

Estimation of Acid Phosphates (ACP) Activity

The activity of acid phosphatase was estimated by the method of Bodansky *et al.*(1933). Two percent homogenates of the tissue were prepared in 0.25M ice cold sucrose solution and centrifuged at 1000 rpm for 15 minutes. The supernatant served as the enzyme source. The reaction mixture of 1.5 ml contains 1 ml of phosphate buffer (pH 5.3), 0.1 ml of α naphthyl phosphate, Fast Red TR 0.1 ml and tartrate 0.2 ml. The contents were incubated at 37^0C for 30 minutes. In acidic pH of buffer system, acid phosphatase hydrolyses α naphthyl phosphate to α naphthol and phosphate. The α naphthol is then coupled with diazotized Fast Red TR to form a diazo dye which has strong absorbance at 405 nm. The addition of L-tartrate inhibits the reaction. Zero-time controls were maintained by adding 5 ml of L-tartrate prior to the addition of homogenate. The intensity of colour developed was read at 405 nm against a reagent blank in a spectrophotometer. The activity was expressed as µg pi/g protein/hour.

RESULTS AND DISCUSSION

The calculated mean values of AAT activity along with standard deviation values and the percent change over control are represented in Table 1 during 24 hours Cypermethrin exposure. In control fish of *Cirrhinus mrigala*, the values of AAT activity in different tissues are in the order of Brain >Liver >Gill >Kidney >Muscle.

In the fish reared as control for 24 hours, the AAT activity was the highest in the Brain (254.882 µ moles of pyruvate formed /mg of protein /h) followed by Liver (214.146 µ moles of pyruvate formed /mg of protein /h) and gill (173.54 µ moles of pyruvate formed / mg of protein /h) moderate values were observed in Kidney (172.892 µ moles of pyruvate formed /mg of protein /h) and very low in muscle (159.1 µ moles of pyruvate formed /mg of protein /h) respectively. The total AAT content significantly increased ($p < 0.05$) during both sublethal and lethal exposures of cypermethrin (10% EC) when compared to the control fish group. The percent depletion of glycogen in the test fish *C. mrigala* under 10% EC of Cypermethrin exposure is in the following order (Figure 1and Tale 1)

Cypermethrin Sublethal for 24 h: Liver >Brain >Gill >Kidney >Muscle

Cypermethrin Lethal for 24 h:Liver >Brain >Kidney>Gill >Muscle

The calculated mean values of AAT activity along with the standard deviation values and the percent change over control are represented in Table 2. during 96 hours Cypermethrin exposure. In control fish *C.mrigala,* the values of AAT activity in different tissues are in the order of Brain >Liver >Gill >Kidney >Muscle.

In the fish reared as control for 24 hours, the AAT activity was the highest in the Brain (254.882 µ moles of pyruvate formed / mg of protein /h) followed by Liver (214.146 µ moles of pyruvate formed /mg of protein /h) and gill (173.54 µ moles of pyruvate formed /mg of protein /h) moderate values were observed in Kidney (172.892 µ moles of pyruvate formed /mg of protein /h) and very low in muscle (159.1 µ moles of pyruvate formed /mg of protein /h) respectively. The total AAT content significantly increased ($p < 0.05$) during sublethal and lethal concentrations of cypermethrin (10% EC) when compared to the control fish group. The percent depletion of AAT in the test fish *C. mrigala* 10% EC of Cypermethrin exposure is in the following order (Figure 2 and Table 2)

Cypermethrin Sublethal for 96 h: Liver >Gill>Kidney >Brain >Muscle

Cypermethrin Lethal for 96 h: Liver >Gill>Kidney >Brain >Muscle

In the present study, the levels of AAT activity were found to be increased in tissues of *C. mrigala* on administration of sublethal and lethal doses of cypermethrin for 96 hours when compared to control group. In the present investigation, the data on AAT activity levels elevated in both the nervous (brain) and non-nervous (liver, gill, kidney and muscle) organs of the fish C.*mrigala* exposed to sublethal and lethal toxicity of both the pesticides. The elevation in Aspartate Amino Transferase activity levels found to be more in lethal than sublethal exposure in 96 hours. The calculated mean values of ALAT activity along with standard deviation values and the percent change over control are represented in Table 3 during 24-hour Cypermethrin exposure. In control fish *C.mrigala*, the values of ALAT activity in different tissues are in the order of Muscle > Brain > Gill > Liver > Kidney.

In the fish reared as control for 24 hours, the ALAT activity was the highest in the Muscle (156.352 µ moles of pyruvate formed /mg of protein /h) followed by Brain (153.838 µ moles of pyruvate formed /mg of protein /h) and gill (122.72 µ moles of pyruvate formed /mg of protein /h) moderate values were observed in Liver (67.794 µ moles of pyruvate formed /mg of protein /h) and very low in Kidney (53.208 µ moles of pyruvate formed /mg of protein /h) respectively. The total ALAT content significantly increased ($p < 0.05$) during both sublethal and lethal concentrations of cypermethrin (10% EC) when compared to the control fish group. The percent depletion of glycogen in the test fish *C. mrigala* under 10% EC of Cypermethrin exposure is in the following order (Figure 3 and Table 3)

Cypermethrin Sublethal for 24 h: Brain >Muscle Liver >Gill >Kidney

Cypermethrin Lethal for 24 h: Brain>Liver >Muscle > Kidney>Gill

The calculated mean values of ALAT activity along with standard deviation values and the percent change over control are represented in Table4 during 96 hour Cypermethrin exposure. In control fish *C.mrigala,* the values of ALAT activity in different tissues are in the order of Muscle >Brain >Gill >Liver >Kidney. In the fish reared as control for 96 hours, the ALAT activity was the highest in the Muscle (156.352 µ moles of pyruvate formed /mg of protein /h) followed by Brain (153.838 µ moles of pyruvate formed /mg of protein /h) and gill (122.72 µ moles of pyruvate formed /mg of protein /h) moderate values were observed in Liver (67.794 µ moles of pyruvate formed /mg of protein /h) and very low in Kidney (53.208 µ moles of pyruvate formed /mg of protein /h) respectively.

The total ALAT content significantly increased ($p < 0.05$) during both sublethal and lethal concentrations of cypermethrin (10% EC) when compared to the control fish group. The percent depletion of ALAT in the test fish *C. mrigala* under 10% EC of Cypermethrin exposure is in the following order (Figure 4and Table 4):

Cypermethrin Sublethal for 96 h: Muscle>Brain >Gill>Liver>Kidney.

Cypermethrin Lethal for 96 h: Brain>Liver>Kidney >Muscle>Gill.

In the present study, the levels of ALAT activity were found to be increased in tissues of *C. mrigala* on administration of sublethal and lethal doses of

cypermethrin for 96 hours when compared to control group. In the present investigation, the data on ALAT activity levels elevated in the nervous (Brain) and the non-nervous (liver, gill, kidney and muscle) organs of the fish *C. mrigala* exposed to the lethal and sublethal toxicity of the pesticide. The elevation in Alanine Amino Transferase activity levels found to be more in lethal than sublethal exposure in 96 hours.

The changes in the levels of AAT and ALAT were studied in different tissues of brain, liver, gill, kidney and muscle in the test fish *C. mrigala* under sublethal and lethal concentration of cypermethrin after 24 and 96 hours of exposure. The increase of AAT activity provides the oxaloacetate required for the gluconeogenesis path way to meet the additional supply of glucose for the production of energy under reduced phase of oxidative metabolism. Elevation in the levels of AAT and ALAT in different tissues of brain, liver, kidney, muscle and gill of the fish *C. mrigala* can be considered as a response to the stress induced by the cypermethrin to generate keto acids like alpha ketoglutarate and oxaloacetate for contributing to the gluconeogenesis and or energy production necessary to meet the excess energy demand under the toxic manifestations. The depletion of proteins under stress of cypermethrin toxicity observed in different tissues of *C. mrigala* indicates the proteolysis, prompting the suggestion that the proteins were utilized to meet the excess energy demands imposed by the toxic stress. The alterations in the levels of activity of aminotransferases induced by the pesticide cypermethrin clearly indicate that the stress brings about the metabolic orientation in the tissue by raising energy resources through transaminase systems. AAT and ALAT are placed in both mitochondrial and cytosol fractions of the cell. A close relation seems to exist between the mitochondrial integrity and transaminase levels and any modification in the organisation of mitochondria is bound to alter the enzyme systems associated with it. The activity of AAT, ALAT enzymes increased in Cypermethrin treated Nile tilapia, *O. niloticus* (OzgurFirat *et al.,* 2011). Sudhanshu Tiwari *et al.* (2012) have reported an increase in AAT and ALAT enzyme activities in gills, liver and kidney organs of fish, *C. carpio* and have proposed that elevated enzyme activity is with the intention to increase the role of proteins for energy production during stress or may be due to the synthesis of enzymes under pesticide stress. Al-Ghanim (2014) reported that the rapid increase in transaminase activity in *C.carpio* could have been due to tissue damage as a result of stress.

Similar elevation in aminotransferases also have been reported by peer researchersin cypermethrin exposed to fish *C. carpio* (Al-Ghanim, 2014;Velmurugan *et al.,* 2007;Neelima *et al.,*2015;Jee *et al.,* 2005) who found that an increase in activity of AAT and ALAT in Korean rockfish (*Sebastes schlegeli*) exposed to cypermethrin which is due to the increased utilization of amino acids for energy synthesis as a consequence of this, fish suffer from toxic stress and energy crisis. The changes in the levels of AAT and ALAT were studied in different tissue of liver, brain, muscle, gill, kidney in the test fish *C.mrigala* under sublethal and lethal concentrations of cypermethrin after 24 and 96 hours of exposure. The increase of AAT activity provides the oxaloacetate required for the gluconeogenesis pathway to meet the additional supply of glucose for the production of energy under reduced state of oxidative metabolism. Elevation in the levels of AAT and ALAT in different tissues of liver, brain, muscle, gill, kidney in the test fish *C.mrigala* can be considered as a response to the stress induced by cypermethrin to generate ketoacids like ketoglutarate and oxaloacetate for contributing to gluconeogenesis and or energy production necessary to meet the excess energy demand under the toxic manifestations. In the present study, it was observed that the reduction of proteins under the stressful condition due to cypermethrin toxicity observed in different tissues of *C. mrigala* indicates proteolysis. This condition is because of utilisation of proteins to meet the excess energy demands imposed by the toxic stress. Thus, the changes in the activity levels of aminotransferases induced by both the pesticides in the experiment clearly indicated that the stress brings about the metabolic reorientation in the tissues by raising energy resources through transaminase systems. The statistical analysis shows significant increase ($p < 0.05$) in the AAT and ALAT activity levels in all the tissues except intestinal tissue of the fish *C. mrigala* under cypermethrin activity.

Table 1. Changes in the specific activity levels of Aspartate Aminotransferase (AAT) (μ moles of pyruvate formed /mg of protein /h) and % change over the control in different tissues of *Cirrhinus mrigala* on exposure to sublethal and lethal concentration of cypermethrin (10% EC) for 24 hours.

/	Control (M ± SD)	Sublethal (M ± SD)	% Change	Lethal (M ± SD)	% Change
Liver	214.416±3.5028	311.436±17.1546	45.2484	341.874±29.5538	59.4443
Brain	254.882±4.0236	305.686±14.7706	19.9324	331.368±58.1440	30.0084
Muscle	159.1±2.0877	133.682±4.5077	15.9761	117.170±26.3179	26.3507
Gill	173.54±2.4578	235.19±3.4335	35.525	259.554±31.9427	49.5644
Kidney	172.892±7.0805	233.416±3.6807	35.0068	262.092±13.7294	61.5929

(M±SD) = Mean of 5 values ± standard deviation. Values are significant at $p<0.05$

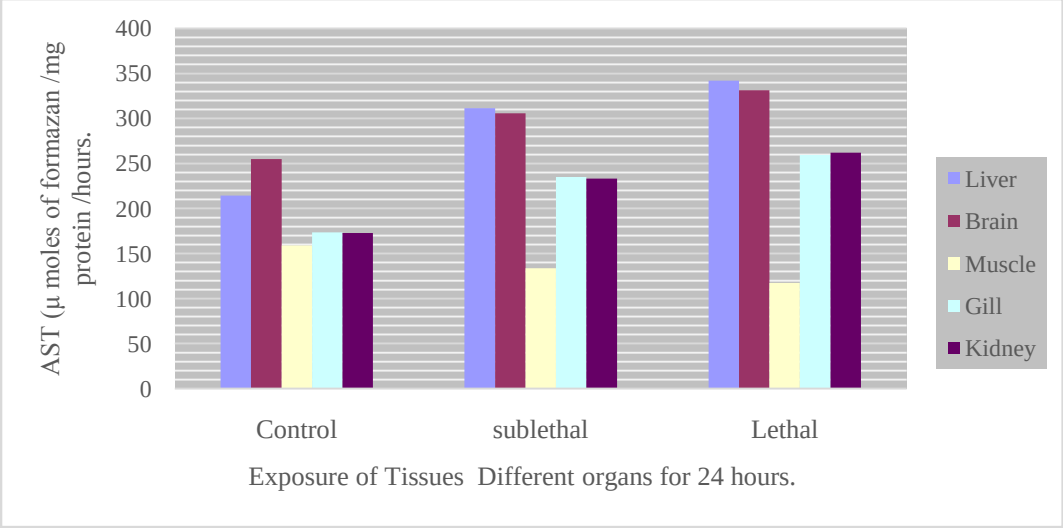

Figure 1.Changes in the specific activity levels of Aspartate aminotransferase (AAT) (µ moles of pyruvate formed /mg of protein /h) and % change over control in different tissues of *Cirrhinusmrigala* on exposure to sublethal and lethal concentrations of cypermethrin (10% EC) for 24 hours.

Table 2. Changes in the specific activity levels of Aspartate aminotransferase (AAT) (µmoles of pyruvate formed /mg of protein /h) and % change over the control in different tissues of *Cirrhinus mrigala* on exposure to sublethal and lethal concentration of cypermethrin (10% EC) for 96 hours.

Tissue	Control (M±SD)	Sublethal (M±SD)	% Change	Lethal (M±SD)	% Change
Liver	214.41±3.5028	311.546±2.7010	45.7662	359.278±3.1411	67.5612
Brain	254.92±4.0274	272.51±3.1635	6.9009	307.322±10.6799	20.5563
Muscle	159.1±2.0877	125.114±15.3626	21.3616	103.918±15.6783	34.6838
Gill	173.54±2.4578	308.78±10.7599	77.9313	330.162±13.48211	90.2512
Kidney	172.892±7.0805	308.222±11.2329	78.2743	329.134±18.5081	90.416

(M±SD) = Mean of 5 values ± standard deviation. Values are significant at $p<0.05$.

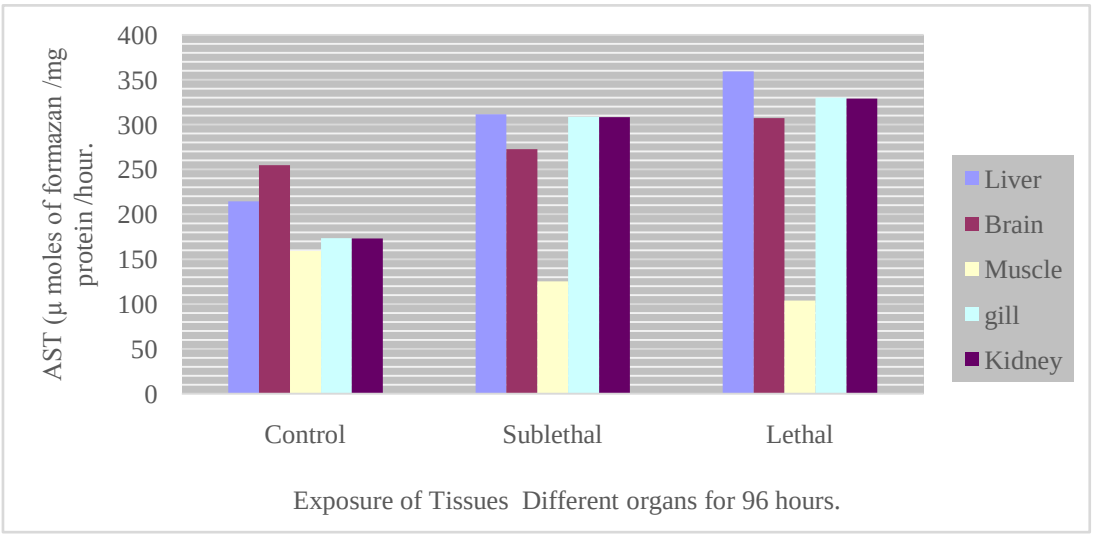

Figure 2. Changes in the specific activity levels of Aspartate aminotransferase (AAT) (µ moles of pyruvate formed /mg of protein /h) and % change over control in different tissues of *Cirrhinus mrigala* on exposure to sublethal and lethal concentrations of cypermethrin (10% EC) for 96 hours.

Table 3. Changes in the specific activity levels of Alanine aminotransferase (ALAT) (µ moles of pyruvate formed /mg of protein /h) and % change over the control in different tissues of *Cirrhinus mrigala* on exposure to sublethal and lethal concentration of cypermethrin (10% EC) for 24 hours.

Tissue	Control (M±SD)	Sublethal (M±SD)	% Change	Lethal (M±SD)	% Change
Liver	67.794±2.6237	113.358±21.6919	67.2095	127.336±4.2651	87.8227
Brain	153.838±10.165	244.926±57.8769	59.2103	283.828±53.4360	84.498
Muscle	156.352±6.2853	132.102±8.7644	15.5099	111.966±19.6182	28.3885
Gill	122.72±1.6453	99.016±4.1468	19.3155	84.718±27.5908	30.9664
Kidney	53.208±15.6173	82.342±14.9226	54.7549	103.162±35.2873	93.8844

(M±SD) = Mean of 5 values ± standard deviation. Values are significant at p<0.05.

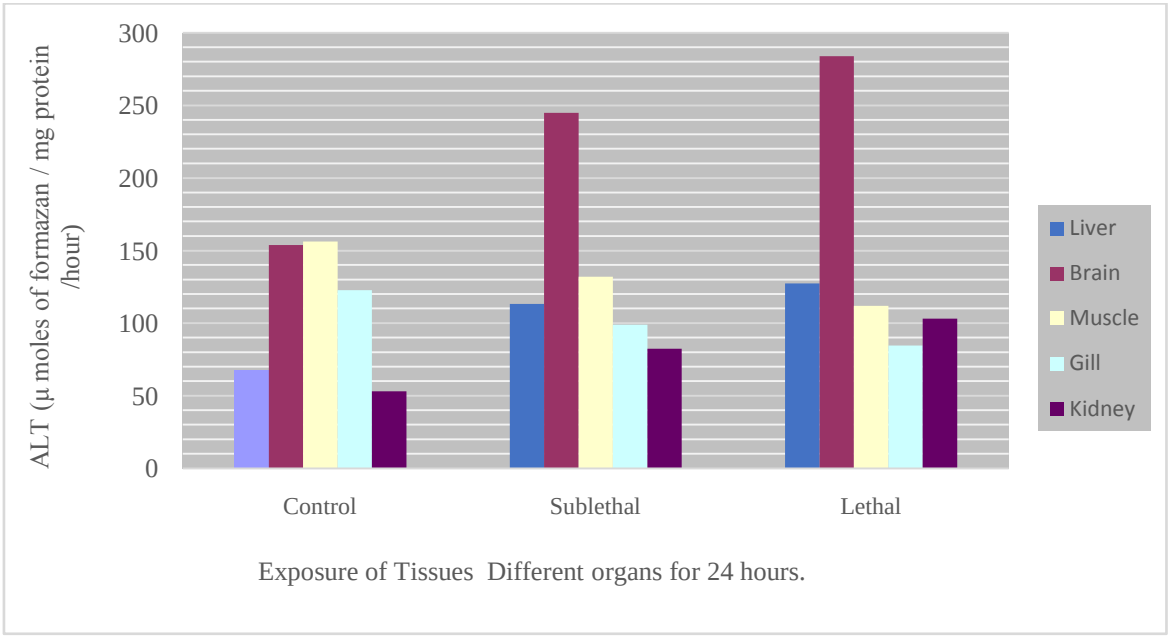

Figure 3. Changes in the specific activity levels of Alanine aminotransferase (ALAT) (µ moles of pyruvate formed /mg of protein /h) and % change over control in different tissues of *Cirrhinus mrigala* on exposure to sublethal and lethal concentrations of cypermethrin (10% EC) for 24 hours.

Table 4. Changes in the specific activity levels of Alanine aminotransferase (ALAT) (µ moles of pyruvate formed / mg of protein /h) and % change over the control in different tissues of *Cirrhinus mrigala* on exposure to sublethal and lethal concentration of cypermethrin (10% EC) for 96 hours.

Tissue	Control (M±D)	Sublethal (M±SD)	% Change	Lethal (M±SD)	% Change
Liver	67.794±2.6237	114.408±15.2138	68.7583	124.798±18.9601	84.0841
Brain	153.838±10.1615	169.666±6.1128	10.2887	193.748±4.2491	25.9429
Muscle	156.352±6.2853	125.288±2.7018	19.868	98.664±26.9228	36.8962
Gill	122.72±1.6453	111.246±7.5661	9.3497	89.48±24.9955	27.08
Kidney	53.208±15.6173	88.97±37.6122	67.2117	100.63±5.3907	89.1257

(M±SD) = Mean of 5 values ± standard deviation. Values are significant at p<0.05

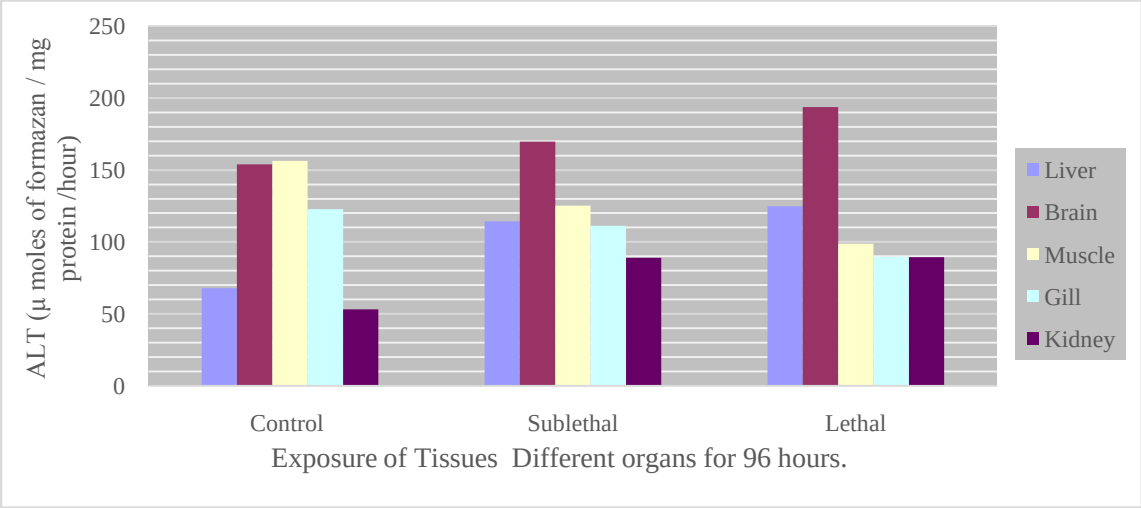

Figure 4.Changes in the specific activity levels of Alanine aminotransferase (ALAT) (µ moles of pyruvate formed / mg of protein /hr) and % change over control in different tissues of *Cirrhinus mrigala* on exposure to sublethal and lethal concentrations of cypermethrin (10% EC) for 96 hours.

Table 5. Changes in the specific activity levels of Acid Phosphatase (ACP) (mg/pi/gram protein/h) and % change over the control in different tissues of *Cirrhinus mrigala* on exposure to sublethal and lethal concentration of cypermethrin (10% EC) for 24 hours.

Tissue	Control (M±SD)	Sublethal (M±SD)	% Change	Lethal (M±SD)	% Change
Liver	8.692±0.8265	11.748±0.9898	14.1981	9.996±2.4097	26.9939
Brain	10.824±1.1839	16.148±1.8583	49.187	19.916±3.4012	83.9985
Muscle	8.4156±1.7582	11.05±0.5157	30.6764	13.262±0.8611	66.8354
Gill	8.622±1.4291	8.77±1.2048	17.4355	7.262±2.8736	31.6325
Kidney	24.162±3.4468	15.092±2.2587	33.3996	13.89±1.1391	42.513

(M±SD) = Mean of 5 values ± standard deviation. Values are significant at $p<0.05$.

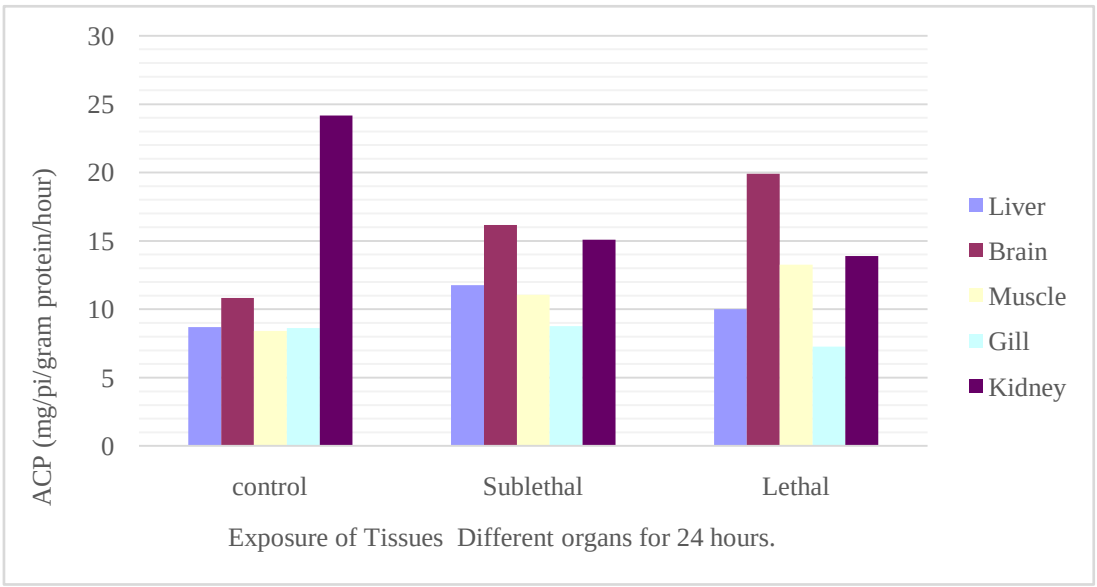

Figure 5. Changes in the specific activity levels of Acid Phosphatases (ACP) (mg/pi/g of protein/h) and % change over control in different tissues of *Cirrhinus mrigala* on exposure to sublethal and lethal concentrations of cypermethrin (10% EC) for 24 hours.

Table 6. Changes in the specific activity levels of Acid Phosphatase (ACP) (mg/pi/g of protein/hour) and % change over the control in different tissues of *Cirrhinus mrigala* on exposure to sublethal and lethal concentration of cypermethrin (10% EC) for 96 hours.

Tissue	Control (M±SD)	Sublethal (M±SD)	% Change	Lethal (M±SD)	% Change
Liver	8.69±0.82	11.09±1.29	19%	12.14±1.23	12%
Brain	10.82±1.18	14.89±1.04	37%	13.82±1.24	27%
Muscle	8.45±1.75	10.15±0.98	20%	11.11±1.19	31%
Gill	8.62±1.42	7.62±1.02	29%	8.52±1.05	20%
Kidney	24.162±3.44	11.486±0.84	53%	13.03±1.26	47%

(M±SD) = Mean of 5 values ± standard deviation. Values are significant at $p<0.05$

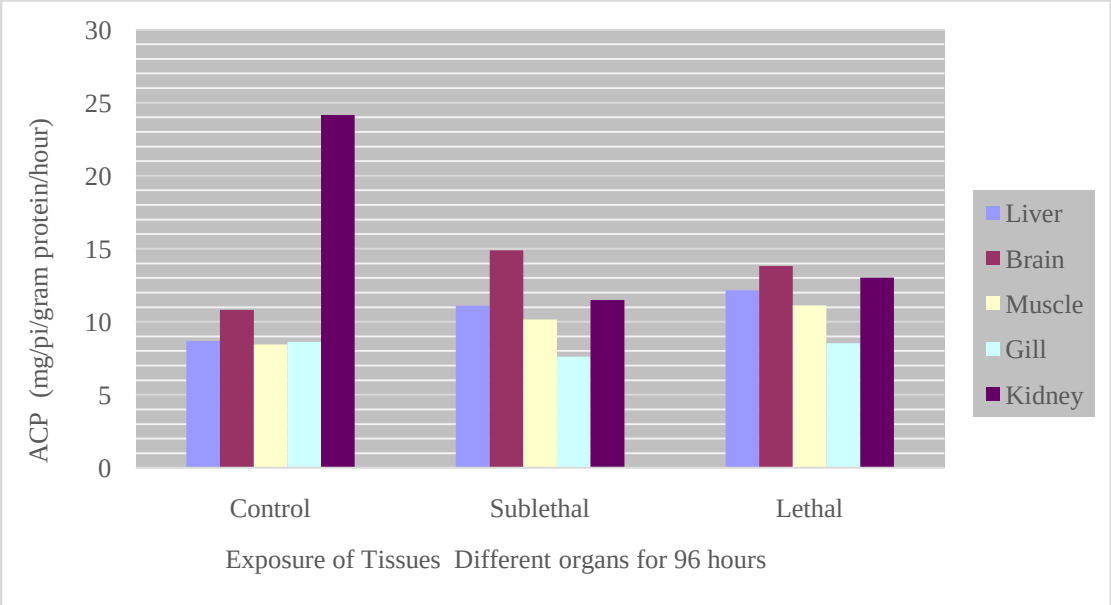

Figure 6. Changes in the specific activity levels of Acid Phosphatases (ACP) (mg/pi/g of protein/hour) and % change over control in different tissues of *Cirrhinus mrigala* on exposure to sublethal and lethal concentrations of cypermethrin (10% EC) for 96 hours.

The calculated mean values of ACP activity along with standard deviation values and the percent change over control are represented in Table 5 during 24 hours Cypermethrin exposure. In control fish of *Cirrhinusmrigala*, the values of ACP activity in different tissues are in the order of Kidney >Brain >Liver >Gill >Muscle. In the fish reared as control for 24 hours, the ACP activity was the highest in the Kidney (24.162 mg pi/g of protein /h) followed by Brain (10.824 mg pi/g of protein /h) and Liver (8.692 µ mg pi/g of protein /h) moderate values were observed in Gill (8.622 mg pi/g of protein /h) and very low in muscle (8.415 mg pi/g of protein /h) respectively. The total ACP content significantly increased ($p <0.05$) during both sublethal and lethal exposures of cypermethrin (10% EC) when compared to the control fish group. The percent depletion of glycogen in the test fish *C. mrigala* under 10% EC of Cypermethrin exposure is in the following order (Figure 5 and Table 5).

Cypermethrin Sublethal for 24 h: Brain >Kidney >Liver >Muscle >Gill

Cypermethrin Lethal for 24 h: Brain>Kidney>Muscle >Liver>Gill

The calculated mean values of ACP activity along with standard deviation values and the percent change over control are represented in Table6 during 96 hours Cypermethrin exposure. In control fish *Cirrhinusmrigala*, the values of ACP activity in different tissues are in the order of Muscle >Brain >Gill >Liver >Kidney. In the fish reared as control for 96 hours, the ACP activity was the highest in the Muscle (156.352 mg pi/g of protein /h) followed by Brain (153.838 mg pi/g of protein /h) and gill (122.72 mg pi/g of protein /h) moderate values were observed in Liver (67.794 mg pi/g of protein /h) and very low in Kidney (53.208 mg pi/g of protein /h) respectively. The total ACP content significantly increased ($p <0.05$)

during both sublethal and lethal exposures of cypermethrin (10% EC) when compared to the control fish group. The percent depletion of glycogen in the test fish *C. mrigala* under 10% EC of Cypermethrin exposure is in the following order (Figure 6 and Table 6).

Cypermethrin Sublethal for 96 h: Kidney >Brain >Liver >Muscle>Gill.

Cypermethrin Lethal for 96 h: Brain>Muscle >Kidney >Liver >Gill

In the present investigation, the levels of ACP activity were found to be increased in all fish tissues of *C. mrigala*(liver, brain, muscle, gill and kidney) exposed to cypermethrin at sublethal and lethal concentrations for 96 hours, when compared to the control fish group. In the present study, the ACP activity level increases in all the tissues of fish *C. mrigala* exposed to toxicity of cypermethrin during sublethal and lethal exposure. The elevation in ACP level was found to be high during 96 h sublethal and lethal exposure periods. The ACP is a hydrolytic enzyme released by lysosomes for the hydrolysis of and the raise in its activity is probably related to the cellular damage. The increased ACP activity seems to resulted from enhanced enzyme turn over under pesticide stress. Borges *et al.* (2007) recorded increased values of ACP during cypermethrin exposure to fish *Rhamdiaquelen* and *Labeo rohita* (Das and Mukherjee 2003) respectively. Ogueji and Auta (2007) analysed increased values of ACP in different organs like gill, liver and kidney during lamda-cyhalothrin exposure to fish *Clariasgariepinus*. Long-term exposure to deltamethrin also caused the increased values of ACP of Nile tilapia. Tilak (2009) have been reported the increase in acid phosphatase activity exposed to the toxicant Dichlorvos, in the fish *Labeorohita* can be interpreted as a shift of the tissue on energy break down path way from normal ATPase system to phosphatase system. Pesticides reduce glycogen levels and increase phosphorylase activities (Mishra and Srivastava, 1984). Al-Attar(2010) recorded increased values of ACP was affected by different doses of Malathion. The researchers concluded that necrosis of liver and subsequent leakage of this enzyme into blood stream might be responsible for increase of this enzyme in blood. Magar and Shaikh (2012) reported significant decrease in acid phosphatase activity of liver and muscle in malathion treated group compared with control.

In the Present study, the mean value of ACP activity is increased during the 96h exposure. This increased activity was due to the cellular damage caused by hepatotoxins or as a response to overcome toxicity of Cypermethrin. The increased levels of phosphatase indicate an increase in the rate of phosphorylation and transport of molecules across the membrane.The significant difference in phosphatases activities between the control and experimental groups of fish species might be considered due to the damage of hepatic tissue with dysfunctions of organs. the elevation in ACP activity. The enzyme ACP activity elevation in brain, gills and kidney tissue was described in stress-exposed freshwater murrel, *Channa punctatus* exposed to mercuric chloride (Sastry and Rao, 1984). The effect of exposure to the sublethal concentrations of quinalphos in muscle, brain, liver, kidney of Indian major carp, *Labeorohita* showed the elevation of acid phosphatase activity was studied by MukherjeeandDas (2003). Banaee*et al.* (2014) reported a significant increase in the ACP activity in freshwater fish, *Alburnus mossulensis* exposed to sublethal concentrations of Fenpropathrin and ACP plays a significant role in phosphate hydrolysis and in membrane transport and it also acts as a good bio-indicator of stress in biological systems. Increase in ACP activity was reported in the crayfish after exposure to endosulfans and cell necrosis in gills and kidneys of tilapia exposed to the monocrotophos toxicity. Marked increase in ACP activity was observed in common carp, *Cyprinus carpio* exposed to Ammonia and nitrite and suggested that single or combined presence of pesticides cause adverse effects in blood indices and antioxidant defences in common carp. Molayemraftar*et al.*(2022) andAshok Kumar &Prakash(2020) investigated the significant elevation of ACP of a freshwater fish, *Mystusvittatus*exposed to sublethal concentrations of heavy metal, Arsenic and studied as a biomarker of heavy metals.

CONCLUSION

In the present study, it is concluded that exposure to sublethal and lethal concentrations of Cypermethrin, the activity of AAT, ALAT and ACP levels are elevated in different organs of freshwater fish, *Cirrhinus mrigala* and these alterations might be attributed to increased autolysis in the tissues due to cytotoxicity. In the present study, the results of the present experiment are in correlation with the previous work done on various fish species exposed to different toxicants. It can be concluded that Cypermethrin was highly toxic to *C. mrigala* exposure to sublethal concentrations of Cypermethrin resulted in significant biochemical alterations and behavioural changes which may be potentially disruptive for the survivability of *C.mrigala*.

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