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Enzymatic alterations as biomarkers of Cypermethrin (25%EC) toxicity in a freshwater fish, *Cyprinus carpio* (Linn.)

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Abstract
An attempt has been made to assess the acute toxic effect of Cypermethrin (25%EC) on LDH, GDH and AChE in different tissues of *Cyprinus carpio*. Fishes were exposed to sub lethal concentrations (5, 10, 15 and 20 per cent of 96h LC_{50} value) of cypermethrin for three different exposure periods, 5, 10 and 15 days. Activity levels of LDH, GDH were found to increase in all the tissues of the fish. Maximum percentage of elevation in LDH activity was (74.29%) in liver and minimum elevation was (19.74%) in kidney at 15 days and 15% 96h LC_{50}. Initial increase was decreased in all the tissues studied at 20% 96h LC_{50} at all the exposure periods. Maximum percentage of elevation in GDH activity was (23.95%) in liver and minimum elevation was (19.04%) in kidney 15 days and 15% 96h LC_{50}. Increase was decreased in all the tissues studied at 20% 96h LC_{50} at all the exposure periods. Activity levels of AChE were found to decrease in all the tissues. Maximum percentage of decrement in AChE activity was (25.55%) in brain and minimum was (23.94%) in kidney at the longest exposure period and highest sublethal concentration. Dose and exposure period dependant alterations were observed which were statistically significant.

Keywords: Acute toxicity, Cypermethrin, Biomarkers, LDH, GDH, AChE, *Cyprinus carpio*.

1. Introduction
Environmental and chemical stress can interfere with normal physiological and biochemical functions such as growth, development, reproduction and circulatory system in fish. Numerous biochemical indices of stress have been proposed to assess the health of non-target organisms such as fish exposed to toxic chemicals in aquatic environments. Insecticides can cause serious impairment to physiological and health status of fish. Therefore, biochemical tests are routine laboratory tests useful in recognizing acute or chronic toxicity of insecticides [5]. and can be a practical tool to diagnose toxicity effects in target organs and to determine the physiological status in fish.

Lactate dehydrogenase (LDH) is one of the most sensitive enzymes to environmental pollutants. An alteration in its functioning would indicate the occurrence of pathological conditions in the organisms. LDH is the key enzyme located at the vital point between glycolysis and TCA cycle. Because of its strategic location and its relation to cori cycle, it is likely that any fluctuations in the cellular environment alters the activity of this enzyme. Lactate dehydrogenase enzyme is widely distributed in the tissues, more significantly in the metabolically active tissues and it catalyses the reversible oxidation-reduction reaction involving lactate, pyruvate, NAD+ and NADH. Lactate dehydrogenase occurs in animal tissues as five different isozymes and activity changes under pathological conditions [26].

GDH catalyses the amination of α-ketoglutarate by free ammonia [26]. GDH not only separates nitrogen from glutamate to ammonia, but also catalyses the oxidative deamination of glutamate to ammonia and α- ketoglutarate in the presence of NAD. Glutamate dehydrogenase is widely distributed in the tissues and its activity is very high. Glutamic acid will be acted upon by glutamate dehydrogenase to form ammonia and α-ketoglutarate. The other amino acids can transaminase with α-ketoglutarate to give glutamate which will further be acted upon by glutamate dehydrogenase. Thus the elimination of $-NH_2$ of amino acids can be considered to be routed through glutamic acid as the reaction involves transamination of amino acids and deamination of glutamic acid, it is referred as transdeamination, and the enzyme involved is as transaminase. The acetyl cholinesterase (AChE) activity is vital to normal behavior and

muscular function and represents a prime target on which some toxicants exert adverse effects. Inhibition of acetylcholinesterase (AChE), the enzyme involved in terminating the action of neurotransmitter acetylcholine (ACh), is perhaps the most often studied for organophosphorus pesticides [38, 8]. Many synthetic pyrethroid insecticides are also potent neurotoxins, functioning by inhibiting the action of acetylcholinesterase (AChE) in nerve cells. Neurotransmitters such as acetylcholine are profoundly important in the brain's development, and many pyrethroid pesticides have neurotoxic effects on developing organisms even at low levels of exposure [18]. The primary effect of pyrethroids on vertebrate and invertebrate organisms is the inhibition of AChE activity. Duration of exposure, type of pyrethroid, as well as species of fish has an effect on the extent of AChE expression. Acetylcholine (ACh) is the only classical neurotransmitter that after release into the synaptic cleft is inactivated by enzymatic hydrolysis rather than by reuptake. AChE activity is a biomarker extremely used in aquatic ecotoxicology studies [18], and is a fairly sensitive enzyme to low environmental concentrations of pyrethroid compounds.

Considering the role of lactate dehydrogenase (LDH), glutamate dehydrogenase (GDH) and acetylcholinesterase (AChE) as biomarkers in the field of eco-toxicology, the present study has been undertaken to understand the biochemical alterations induced by cypermethrin (25% EC) on exposure to sublethal concentrations to fish *Cyprinus carpio* in different tissues.

2. Materials and Methods

2.1 Maintenance of test organism

The freshwater fish *Cyprinus carpio* with length 6 - 8 cm, weight 6.5 to 7.5 g, irrespective of their sex, has been chosen as the test organisms for present investigation. Healthy and active fish were obtained from Ratna Singh Hatcheries, Kuchipudi, Guntur (A.P), India. Fish were washed with 0.1% $KMnO_4$ solution to avoid dermal infection. The fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at a room temperature of 28 ± 1°C. Water was renewed every day with12-12 h dark and light cycle. During the period of acclimatization, the fish were fed with groundnut oil cake and rice bran. Feeding was stopped one day prior to the acute toxicity test. All the precautions laid by committee on toxicity tests to aquatic organisms [3] were followed and such acclimatized fish only were used for the bioassay experiment. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish was discarded.

2.2 Experimental toxicant and its exposure

Technical grade cypermethrin (25%EC) was obtained from United Phosphorus Ltd., Bombay. After the normal process of acclimatization, a group of ten fish each were transferred to plastic tubs (15L capacity) containing 10L of water. Fish were exposed to 4 sub-lethal concentrations i.e., 5, 10, 15 and 20 % of 96hLC_{50} (3.31µg/l) for 5, 10 and 15 days along with the control. Control and exposed fishes were sacrificed at end of each day. The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the estimation of nucleic acids (DNA & RNA).

2.3 Estimation of Lactate Dehydrogenase (LDH)

The Lactate Dehydrogenase activity (LDH) was estimated by the method of [43] with slight modifications. Two per cent homogenate of the tissue were prepared in 0.25M ice cold sucrose solution and centrifuged at 100rpm for 15 minutes. The supernatant served as the enzyme source. The reaction mixture of 2.0 ml contained 0.5ml of lithium lactate, 0.5ml of phosphate buffer, 0.2ml of INT [2-p-idophenol-3-(p-nitrophenyl)-5-(phenyl tetrazolium chloride)] and 0.2ml of NAD and 0.6ml of supernatant. The reaction mixture was incubated at 37ºC for 30 minutes and the reaction was stopped by adding 5ml of acetic acid. Zero time controls were maintained by adding 5ml of acetic acid prior to the addition of homogenate. The formazan formed was extracted overnight in 5ml of cold toluene. The intensity of colour developed was read at 495 nm against a reagent blank in a spectrophotometer. The activity was expressed as µ moles of formazan formed/mg protein/h.

2.4 Estimation of Glutamate Dehydrogenase (GDH)

GDH activity was assayed by the method of [22], with slight modification as described by [32]. 10% (W/V) homogenates of different tissues were prepared in ice cold 0.25M sucrose solution and centrifuged at 2500rpm for 15 minutes. The supernatant was used for the enzyme assay the reaction mixture in a final volume of 2ml contained 100µmoles of sodium glutamate, 100µ of moles phosphate buffer (p^H 7.4), 0.1µ of NAD, and 4µ moles of INT (Iodonitrotetrazolium) the reaction was initiated by adding 0.5 ml of homogenate and after incubation for 30 minutes at 37^{0}C, the reaction was stopped by the addition of 5ml of glacial acetic acid. The color was extracted overnight at 5^{0}C and read at 495nm in a spectrophotometer against a blank. The enzyme activity was expressed as µ moles of formazon formed/ mg protein/h.

2.5 Estimation of Acetyl Cholinesterase Activity (AChE)

AChE enzyme assays were performed spectrophotometrically by the method of [14]. The principle of the method is the measurement of the rate of the production of thiocholine as acetylcholine is hydrolysed. This is accomplished by the continuous reaction of the thiol with 5:5 dithiobis-nitrobenzoate ion to produce the yellow anion of 5-thio-2-nitro benzoic acid. The rate of production of colour is measured at 412 nm in a spectrophotometer. The reaction with the thiol is sufficiently rapid so as not to be rate limiting in the measurement of the enzyme and in the concentrations used does not inhibit the enzyme hydrolysis. The rate of enzyme hydrolysis can be recorded by using a recorder.

2.6 Enzyme Preparation

The fish were sacrificed and the tissues like muscle, brain, liver, gill and kidney were quickly excised into cold solution. The excess blood is washed with 0.15M KCl (cold) solution. The tissues were homogenized (10% w/v) in 0.1M pH 8 tris HCl buffer using potter-Elvehjam homogenizer fitted with Teflon pestle. The homogenates were centrifuged at 5000rpm for 10 minutes. The resultant supernatant was again centrifuged at 5000rpm for 10 minutes. The resultant supernatants were stored in ice and were used as enzyme source for the estimation of AChE activity. All the enzyme preparations were carried out at 0-40°C. Protein content for enzyme preparations were estimated by the method of [23] using Bovine serum albumin as standard.

2.7 Calculation

$$V = \blacktriangledown A/\min \times \frac{3}{\text{Protein}} \times \frac{1}{14.3} = \mu \text{ moles/min/mg protein}$$

▼ A/min is changes in optical density
3 is ml of solution in cuvette
14.3 is molar extinction coefficient of DTNB

2.8 AChE ASSAY

The reactions performed at 37^0C were initiated by adding small aliquots of varying concentrations of the substrate (acetyl-choline iodide) to yield a final volume of 3ml. The absorbances of 412nm were recorded continuously for 5 min. corresponding blanks lacking AChE were subtracted to yield the enzymatic activity rate. The typical runs for all experiments used were 2.7 ml buffer, 0.1 M phosphate buffer (pH 8), 50 µl (0.16mM) DTNB, 100 µl (1mg/ml) protein and 100 µl substrate.

Results and Discussion

3.1 Lactate Dehydrogenase Activity (LDH)

Calculated values for LDH along with standard deviations are given in table 1. Percent change of LDH in experimental fish over control is graphically represented in figure 1. The control values of LDH in different tissues of *Cyprinus carpio* was in the order of Muscle > Gill > Liver > Brain > Kidney. Under sublethal exposure of cypermethrin the activity levels of LDH were found to increase in all the tissues of the fish

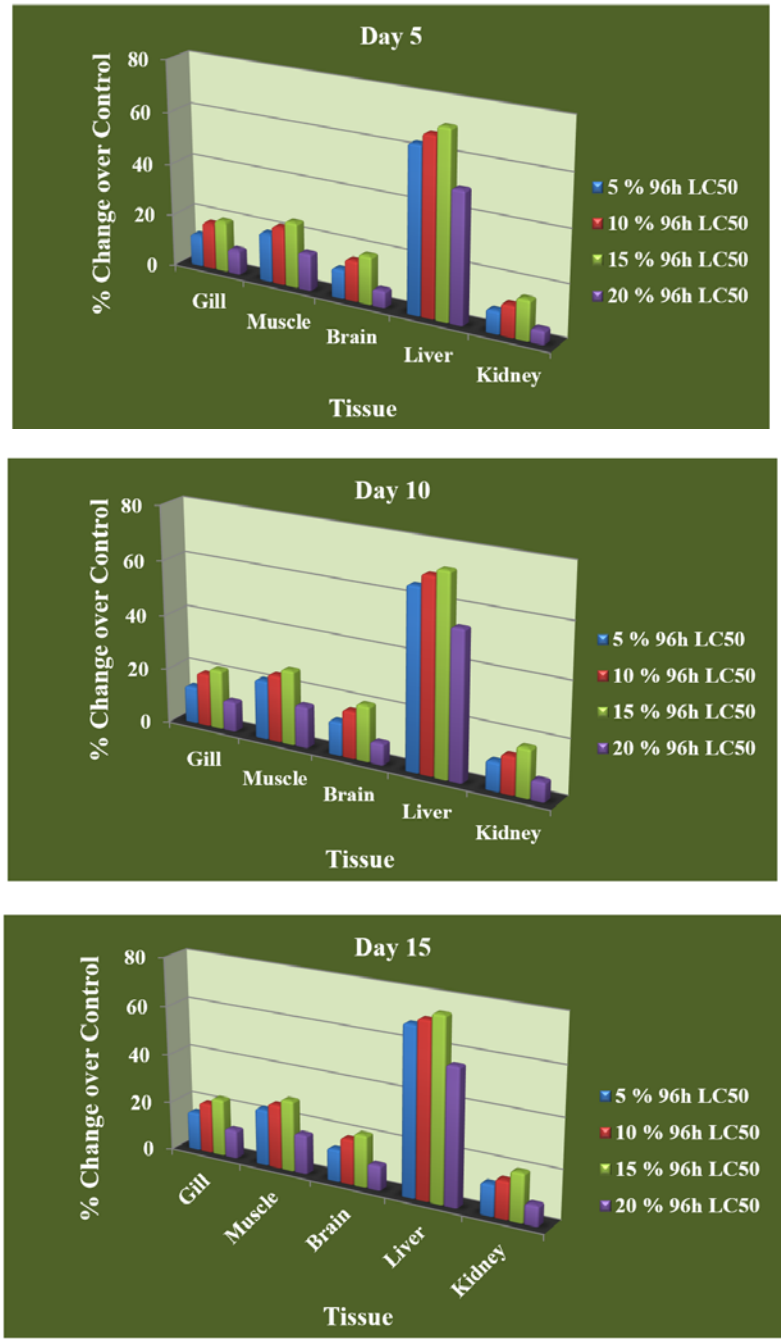

Fig 1: Per cent change over control in LDH activity (µ/m formazan/mg protein/h) in different tissues of *Cyprinus carpio* exposed to sublethal concentrations of cypermethrin (25%EC)

Table 1: LDH activity levels (µ/m formazan/mg protein/h) in different tissues of *Cyprinus carpio* exposed to sublethal concentrations of cypermethrin (25% EC)

Exposure period in Days	Tissue	Control	Cypermethrin Concentration (% 96h LC_{50})			
			5% 96h LC_{50}	10% 96h LC_{50}	15% 96h LC_{50}	20% 96h LC_{50}
5	Gill	0.1097 ± 0.021	0.1229 ± 0.030 (12.03)	0.1291 ± 0.025 (17.68)	0.1312 ± 0.011 (19.59)	0.1200 ± 0.052 (09.38)
	Muscle	0.1410 ± 0.036	0.1672 ± 0.029 (18.58)	0.1722 ± 0.040 (22.12)	0.1764 ± 0.014 (25.10)	0.1610 ± 0.019 (14.18)
	Brain	0.0201 ± 0.019	0.0223 ± 0.022 (10.94)	0.0232 ± 0.015 (15.42)	0.0238 ± 0.021 (18.40)	0.0214 ± 0.034 (06.46)
	Liver	0.0812 ± 0.024	0.1327 ± 0.033 (63.42)	0.1362 ± 0.021 (67.73)	0.1391 ± 0.011 (71.30)	0.1218 ± 0.017 (50.00)
	Kidney	0.0151 ± 0.045	0.0164 ± 0.037 (08.60)	0.0169 ± 0.029 (11.92)	0.0174 ± 0.020 (15.23)	0.0159 ± 0.032 (05.29)
10	Gill	0.1101 ± 0.020	0.1248 ± 0.012 (13.35)	0.1312 ± 0.010 (19.16)	0.1340 ± 0.022 (21.70)	0.1221 ± 0.018 (10.89)
	Muscle	0.1414 ± 0.033	0.1712 ± 0.042 (21.41)	0.1752 ± 0.026 (24.25)	0.1793 ± 0.017 (27.16)	0.1623 ± 0.019 (15.10)
	Brain	0.0204 ±0.024	0.0229 ± 0.039 (12.25)	0.0239 ± 0.026 (17.15)	0.0245 ± 0.039 (20.29)	0.0220 ± 0.039 (07.84)
	Liver	0.0815 ± 0.032	0.1350 ± 0.030 (65.64)	0.1386 ± 0.032 (70.06)	0.1407 ± 0.025 (72.63)	0.1255 ± 0.041 (53.98)
	Kidney	0.0154 ± 0.050	0.0170 ± 0.030 (10.38)	0.0175 ± 0.042 (13.63)	0.0181 ± 0.026 (17.53)	0.0165 ± 0.019 (07.14)
15	Gill	0.1106 ± 0.034	0.1279 ± 0.015 (15.64)	0.1332 ± 0.022 (20.43)	0.1365 ± 0.027 (23.41)	0.1235 ± 0.019 (11.66)
	Muscle	0.1417 ± 0.031	0.1744 ± 0.051 (23.07)	0.1786 ± 0.019 (26.04)	0.1829 ± 0.035 (29.07)	0.1645 ± 0.051 (16.09)
	Brain	0.0207 ± 0.062	0.0234 ± 0.026 (13.04)	0.0245 ± 0.039 (18.35)	0.0251 ± 0.039 (21.25)	0.0228 ± 0.030 (10.14)
	Liver	0.0817 ± 0.026	0.1378 ± 0.032 (68.66)	0.1400 ± 0.042 (71.35)	0.1424 ± 0.026 (74.29)	0.1272 ± 0.019 (55.69)
	Kidney	0.0157 ± 0.054	0.0177 ± 0.039 (12.73)	0.0181 ± 0.026 (15.28)	0.0188 ± 0.019 (19.74)	0.0170 ± 0.026 (08.28)

Values are the mean of 5 observations Standard Deviation is indicated as (±) Values are significant at $p < 0.05$ Percent changes over control are given in Parenthesis

Lactate dehydrogenase (LDH) is found in the cellular cytoplasm and is active during glycolysis, converting pyruvate from glucose to lactic acid. The activity of LDH in muscle of control fish was observed to be 0.1410±0.036µm formazan /mg protein/h and in sublethal concentrations of cypermethrin, the increase was observed to be 0.1672±0.029, 0.1722±0.040, and 0.1764±0.014µm formazan/mg protein/h equal to 12.03, 17.68, and 19.59 % respectively over control for 5, 10 and 15% 96h LC_{50} after 5 days of exposure period. Later increase was decreased at 20%. This increase was much intensified after maximum period of 15 days as the sublethal concentrations increase from 5% to 15% 96h LC_{50}. Maximum increase of 0.1829±0.0035 µm formazan/mg protein/h is equal to 29.07% at 15% 96h LC_{50} and maximum exposure period of 15 days.

Similarly gill, liver, brain and kidney LDH activity was found to increase to 0.1365±0.027, 0.1424±0.026, 0.0251±0.039 and 0.0188±0.019 from the control values after 15% 96h LC_{50} and 15 days respectively. In cypermethrin 25% EC sublethal exposure, maximum percentage of elevation in LDH activity was (74.29%) in liver and minimum elevation was (19.74%) in kidney at the longest exposure period (15 days) and 15% 96h LC_{50}. Increase was decreased in all the tissues studied at 20% 96h LC_{50} at all the exposure periods.

LDH is an important glycolytic enzyme which is present in the cells of almost all body tissues and changes in the enzyme activity may provide direct and indirect evidence of the cellular damage and can indicate the toxic mechanism. LDH is a terminal enzyme of anaerobic glycolysis, therefore, being of crucial importance to the muscular physiology, particularly in conditions of chemical stress, when high levels of energy may be required in a short period of time. The significant changes in enzymes activity of LDH indicate damage to any or all organs producing this enzyme such as liver or kidney injuries.

Increases of LDH activities are related to liver damage and change in hepatic function [25]. Increase of LDH attributed to the leakage of these enzymes to the blood stream [48]. The hepatic injury might be attributed to oxidative damage by free radicals [30]. Increased activity of LDH may specify a shift towards anaerobiosis resulting in boosted production of lactic acid [25]. Lactate dehydrogenase is an enzyme involved in carbohydrate metabolism and has been used as indicative criteria of exposure to chemical stress [11]. In the present study it is observed that the activity of LDH was highly elevated following cypermethrin exposure indicating increased anaerobic respiration to meet the energy demands where aerobic oxidation is lowered.

Cyprinus carpio exposed to acute dose of cypermethrin (7.5µg/l) for 1, 3, 5, 7 and 9 days and sub-lethal concentrations (1.5µg/l) for 1, 7, 14, 21 and 28 days induced both time

dependent and tissue-specific elevation in LDH activity in gill, liver and muscle [2]. Inhibited activity of LDH was observed in gill, kidney, liver and muscle plasma of African catfish *Clarias gariepinus* exposed to sublethal concentrations of cypermethrin (0.05, 0.10, 0.20 and 0.25ml/l) for 10 days [16]. While studying the effect of cypermethrin (0.05µg/l) for 4 and 21 days, [15] reported elevated activity of LDH in Nile tilapia, *Oreochromis niloticus*. Acute exposure to cypermethrin resulted in a significant higher concentration of LDH rainbow trout. In common carp cypermethrin resulted in a significant decrement in LDH [45].

Cyprinus carpio exposed Alimetrin 10EM in the concentration of 29.1µg/l corresponding to 29.1µg/l of cypermethrin for 96h showed a significant ($P<0.01$) decrease in its LDH activity [12]. In rainbow trout cypermethrin caused a significant increase in concentration of LDH [46]. In *Labeo rohita*, [9] observed that sublethal exposure to cypermethrin produced a significant increase in the activity LDH. A significant increase was found in LDH in rainbow trout (*Oncorhynchus mykiss*) following cypermethrin exposure at different sublethal doses [4].

3.2 Glutamate Dehydrogenase Activity (GDH)

Calculated values for GDH along with standard deviations are given in table 2. Percent change of GDH in experimental fish over control is graphically represented in figure 2. The control values of GDH in different tissues of *Cyprinus carpio* was in the order of Liver > Muscle > Gill > Brain > Kidney. Under sublethal exposure of cypermethrin the activity levels of GDH were found to increase in all the tissues of the fish

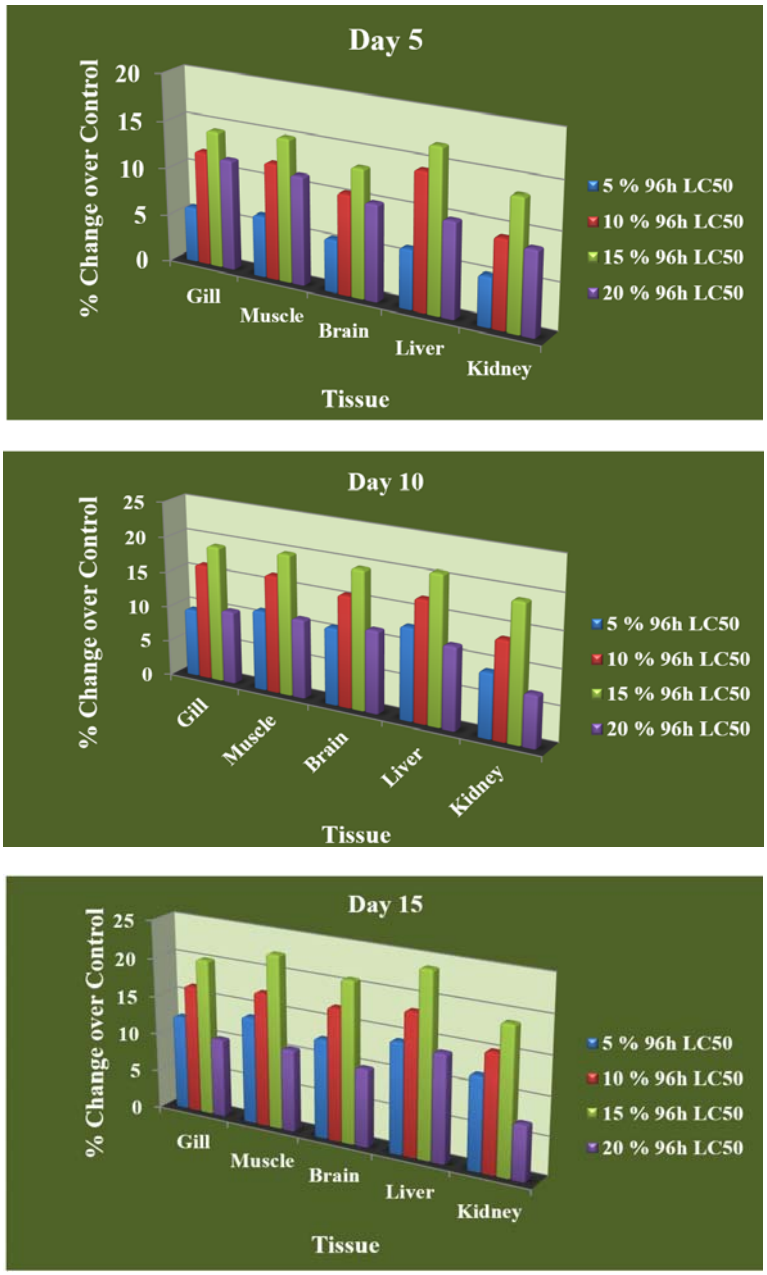

Fig 2: Percent change over control in GDH activity (µ/m formazan/mg protein / h) in different tissues of *Cyprinus carpio* exposed to sublethal concentrations of cypermethrin (25%EC)

Table 2: GDH activity levels (µ/m formazan/mg protein/h) in different tissues of *Cyprinus carpio* exposed to sublethal concentrations of cypermethrin (25% EC)

Exposure period in Days	Tissue	Control	Cypermethrin Concentration (% 96h LC_{50})			
			5% 96h LC_{50}	10% 96h LC_{50}	15% 96h LC_{50}	20% 96h LC_{50}
5	Gill	0.1242 ± 0.011	0.1314 ± 0.020 (5.797)	0.1391 ± 0.029 (11.99)	0.1421 ± 0.041 (14.41)	0.1387 ± 0.025 (11.67)
	Muscle	0.1597 ± 0.035	0.1700 ± 0.026 (6.449)	0.1792 ± 0.042 (12.21)	0.1838 ± 0.045 (15.09)	0.1780 ± 0.029 (11.45)
	Brain	0.1148 ± 0.063	0.1212 ± 0.042 (5.574)	0.1275 ± 0.039 (10.54)	0.1302 ± 0.026 (13.41)	0.1264 ± 0.039 (10.10)
	Liver	0.3768 ± 0.021	0.4002 ± 0.019 (6.210)	0.4308 ± 0.028 (14.33)	0.4410 ± 0.011 (17.03)	0.4144 ± 0.047 (09.97)
	Kidney	0.1003 ± 0.044	0.1055 ± 0.038 (5.184)	0.1096 ± 0.030 (09.27)	0.1140 ± 0.028 (13.65)	0.1091 ± 0.037 (08.77)
10	Gill	0.1245 ± 0.020	0.1364 ± 0.012 (9.558)	0.1448 ± 0.010 (16.30)	0.1485 ± 0.022 (19.27)	0.1374 ± 0.018 (10.36)
	Muscle	0.1601 ± 0.033	0.1782 ± 0.042 (11.30)	0.1868 ± 0.026 (16.67)	0.1920 ± 0.017 (19.92)	0.1780 ± 0.019 (11.18)
	Brain	0.1150 ± 0.024	0.1275 ± 0.039 (10.86)	0.1331 ± 0.026 (15.73)	0.1376 ± 0.039 (19.65)	0.1284 ± 0.039 (11.65)
	Liver	0.3774 ± 0.032	0.4264 ± 0.030 (12.98)	0.4421 ± 0.032 (17.14)	0.4565 ± 0.025 (20.95)	0.4210 ± 0.041 (11.55)
	Kidney	0.1007 ± 0.050	0.1098 ± 0.030 (9.030)	0.1145 ± 0.042 (13.70)	0.1200 ± 0.026 (19.16)	0.1080 ± 0.019 (07.24)
15	Gill	0.1249 ± 0.034	0.1402 ± 0.010 (12.24)	0.1456 ± 0.022 (16.57)	0.1503 ± 0.027 (20.33)	0.1376 ± 0.019 (10.16)
	Muscle	0.1605 ± 0.031	0.1828 ± 0.051 (13.89)	0.1884 ± 0.019 (17.38)	0.1968 ± 0.035 (22.61)	0.1776 ± 0.051 (10.65)
	Brain	0.1153 ± 0.062	0.1301± 0.026 (12.83)	0.1351 ± 0.039 (17.17)	0.1395 ± 0.039 (20.98)	0.1269 ± 0.030 (10.06)
	Liver	0.3778 ± 0.026	0.4321 ± 0.032 (14.37)	0.4475 ± 0.042 (18.44)	0.4683 ± 0.026 (23.95)	0.4305 ± 0.019 (13.94)
	Kidney	0.1003 ± 0.054	0.1124 ± 0.039 (12.06)	0.1156 ± 0.026 (15.25)	0.1194 ± 0.019 (19.04)	0.1075 ± 0.026 (07.17)

The activity of GDH in liver of control fish was observed to be 0.03768±0.021 µm formazan / mg protein / h and in sublethal concentrations of cypermethrin, the increase was observed to be 0.4002±0.019, 0.4308±0.028, and 0.4410±0.011µm formazan / mg protein / h equal to 6.21, 14.33, and 17.03% respectively over control for 5, 10 and 15% 96h LC_{50} after 5 days of exposure period. Later increase was decreased at 20%. This increase was much intensified after maximum period of 15 days as the sublethal concentrations increase from 5% to 15% 96h LC_{50}. Maximum increase of 0.4683±0.026 µm formazan / mg protein / h is equal to 23.95% at 15% 96h LC_{50} and maximum exposure period of 15 days.

Similarly muscle, gill, brain and kidney GDH activity was found to increase to 0.1968±0.035, 0.1503±0.027, 0.1395±0.039 and 0.1194±0.019 from the control values after 15% 96h LC_{50} and 15 days respectively. In cypermethrin 25%EC sublethal exposure, maximum percentage of elevation in GDH activity was (23.95%) in liver and minimum elevation was (19.04%) in kidney at the longest exposure period (15 days) and 15% 96h LC_{50}. Increase was decreased in all the tissues studied at 20% 96h LC_{50} at all the exposure periods.

Progressive increase in GDH in all the organs of the fish exposed to cypermethrin suggests the active transdeamination of amino acids for the incorporation of ketoacids into the TCA cycle to release necessary energy required for the synthesis of new proteins [42, 40]. Enhancement in GDH activity in the tissues provided ketoglutarate and reduced nucleotides, which may fulfill the energy requirements during toxicity manifestations [7].

GDH is also known to play crucial role in ammonia metabolism and is known to be affected by a variety of effectors [10]. After several metabolic functions with great physiological significance and known to be closely associated with the detoxification mechanisms of tissues. GDH in extrahepatic tissue could be utilized for its ultimate detoxification to urea in the liver. In the present study the significant elevation in activity of GDH in the organs of fish exposed to sublethal concentration of cypermethrin indicates greater association of oligomers of these enzymes in response to toxic stress. This shows that oxidative deamination is contributing higher ammonia production. The high levels of ammonia produced is not eliminated but is salvaged through GDH activity which is utilized for amino acid synthesis through transaminases [33, 35]. The increase in GDH activity could also be attributed to the mitochondrial permeability or to the lysosomal damage or to the induced synthesis of the enzyme [17].

Lethal (5µg/l for 1,2,3,4 days) and sublethal concentration (1µg/l for 1, 7, 14, 21 days) of cypermethrin caused an increase in the activity of GDH in all the tissues with increase in exposure time [34]. 10 days of sublethal exposure to cypermethrin caused marked increase in the activity GDH in different organs of *Clarias batrachus* [6]. While studying the perturbations in carbohydrate metabolism of *Tilapia*

mossambica under cypermethrin stress [36] reported cypermethrin caused an increase in GDH.

3.3 Acetylcholinesterase Activity (AChE)

The AChE activity was estimated in different tissues like gill, liver, kidney, brain and muscle of the fish *Cyprinus carpio* exposed to sublethal concentrations of cypermethrin for 5, 10 and 15 days and calculated values along with standard deviations are given in table 3. Percent change of AChE in experimental fish over control is graphically represented in figure 3. The control values of AChE in different tissues of *Cyprinus carpio* was in the order of Muscle > Gill > Liver > Kidney > Brain. Under sublethal exposure of cypermethrin the activity levels of AChE were found to decrease in all the tissues of the experimental fish, *Cyprinus carpio*.

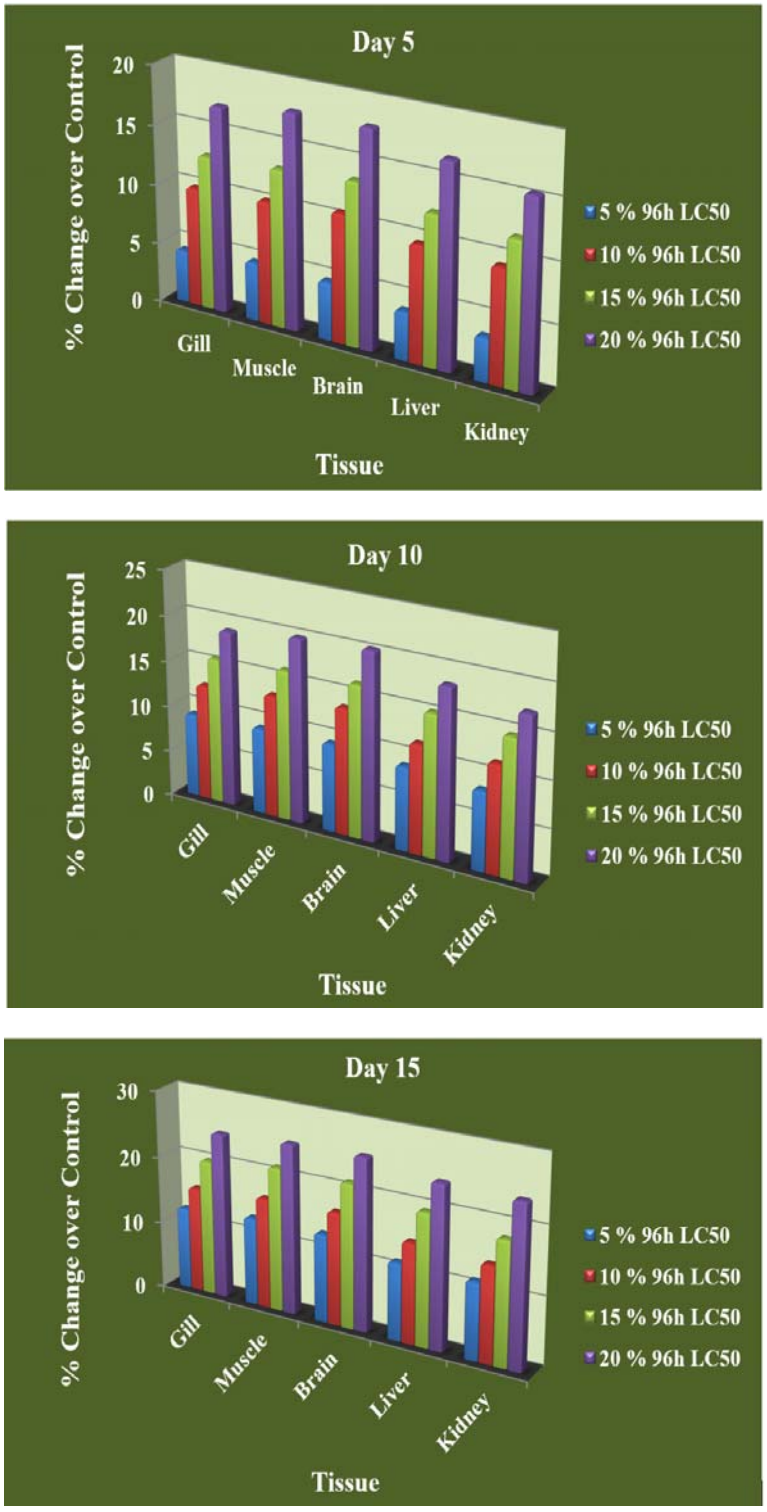

Fig 3: Percent change over control in AChE activity levels (µm acetylcholine hydrolyzed / mg / protein /h) in the tissues of *Cyprinus carpio* on exposure to sublethal concentrations of cypermethrin (25%EC)

Table 3: AChE activity levels (µm acetylcholine hydrolyzed / mg / protein /h) in the tissues of *Cyprinus carpio* on exposure to sublethal concentrations of cypermethrin (25%EC)

Exposure period in Days	Tissue	Control	Cypermethrin Concentration (% 96h LC_{50})			
			5% 96h LC_{50}	10% 96h LC_{50}	15% 96h LC_{50}	20% 96h LC_{50}
5	Gill	4.83 ± 0.021	4.62 ± 0.030 (4.34)	4.35 ± 0.025 (9.93)	4.21 ± 0.011 (12.83)	4.00 ± 0.052 (17.18)
	Muscle	5.57 ± 0.036	5.30 ± 0.029 (4.84)	5.00 ± 0.040 (10.23)	4.84 ± 0.014 (13.10)	4.57 ± 0.031 (17.95)
	Brain	3.55 ± 0.019	3.38 ± 0.022 (3.38)	3.17 ± 0.015 (9.01)	3.07 ± 0.021 (11.54)	2.91 ± 0.034 (14.36)
	Liver	4.76 ± 0.024	4.57 ± 0.033 (3.99)	4.30 ± 0.021 (9.66)	4.17 ± 0.011 (12.39)	3.96 ± 0.017 (16.80)
	Kidney	4.45 ± 0.045	4.29 ± 0.037 (3.59)	4.03 ± 0.029 (9.43)	3.92 ± 0.020 (11.91)	3.76 ± 0.032 (15.50)
10	Gill	4.86 ± 0.020	4.42 ± 0.012 (9.05)	4.25 ± 0.010 (12.55)	4.09 ± 0.022 (15.84)	3.93 ± 0.018 (19.13)
	Muscle	5.60 ± 0.033	5.08 ± 0.042 (9.28)	4.86 ± 0.026 (13.21)	4.69 ± 0.017 (16.25)	4.48 ± 0.019 (20.00)
	Brain	3.58 ±0.024	3.24 ± 0.039 (8.65)	3.09 ± 0.026 (11.17)	2.99 ± 0.039 (13.96)	2.85 ± 0.039 (17.03)
	Liver	4.78 ± 0.032	4.35 ± 0.030 (8.99)	4.22 ± 0.032 (11.71)	4.05 ± 0.025 (15.27)	3.90 ± 0.041 (18.41)
	Kidney	4.48 ± 0.050	4.10 ± 0.030 (8.48)	3.96 ± 0.042 (11.60)	3.82 ± 0.026 (14.73)	3.70 ± 0.019 (17.41)
15	Gill	4.87 ± 0.034	4.28 ± 0.015 (12.11)	4.11 ± 0.022 (15.60)	3.89 ± 0.027 (20.12)	3.67 ± 0.019 (24.64)
	Muscle	5.62 ± 0.031	4.89 ± 0.051 (12.98)	4.70 ± 0.019 (16.37)	4.42 ± 0.035 (21.35)	4.20 ± 0.051 (25.26)
	Brain	3.60 ± 0.062	3.13 ± 0.026 (10.55)	3.00 ± 0.039 (13.88)	2.83 ± 0.039 (17.77)	2.68 ± 0.030 (21.94)
	Liver	4.81 ± 0.026	4.26 ± 0.032 (11.43)	4.10 ± 0.042 (14.76)	3.87 ± 0.026 (19.54)	3.65 ± 0.019 (21.11)
	Kidney	4.51 ± 0.054	4.00 ± 0.039 (11.30)	3.87 ± 0.026 (14.19)	3.69 ± 0.019 (18.18)	3.43 ± 0.026 (23.94)

The variation in activity levels of acetyl cholinesterase in different tissues of fish suggests the neural activities of those particular organs. In cypermethrin 25% EC sublethal exposure, maximum percentage of decrement in AChE activity was (25.55%) in brain and minimum was (23.94%) in kidney at the longest exposure period (15 days) and highest sublethal concentration (20% 96h LC_{50}).

AChE, is found in cholinergic synapses in the brain as well as in autonomic ganglia, the neuromuscular junction and the target tissues of the parasympathetic system [37, 41]. Acetylcholinesterase enzyme involves in the maintenance of the structural and functional integrity of cellular membranes. The role of AChE in cholinergic transmission is to regulate nervous transmission by reducing the concentration of acetylcholine (ACh) in the junction through AChE catalyzed hydrolysis of Ach [19]. The AChE activity is vital to normal behaviour and muscular function and represents a prime target on which some toxicants can exert a detrimental effect. Inhibition of AChE results in a build-up of acetylcholine, causing a continuous and excessive stimulation of the nerve/muscle fibers, which leads to tetany, paralysis, and eventual death.

The inhibition of AChE results in buildup of acetylcholine within the nerve synapses leading to a variety of neurotoxic effects and decreased cholinergic transmission [28]. Results obtained by different workers [31, 44, 39, 27, 20, 21, 1, 24] independent of tissues and species used are quite similar in the AChE inhibitory effects. The greater inhibition of AChE in the fish was in brain. This may be due to the pesticide activity on the brain. Since the compound is neurotoxic the activity levels of AChE was inhibited in brain.

Cypermethrin (20µg/l) exposure to *Clarias gariepinus* for 5 days significantly ($p<0.05$) lowered the activity of cholinesterase to 1/3rd that of control [31]. Laboratory evaluation of cypermethrin toxicity made by [44] revealed sublethal doses of cypermethrin (0.129µg/l, 0.258µg/l for 24h and 0.082µg/l, 0.164µg/l for 96h exposure period caused significant ($P<0.05$) reduction in AChE activity. Time and dose dependent decreased activity of AChE in nervous tissue of *Colisa fasciatus* was observed by [39]. Cypermethrin showed positive correlation between concentration and inhibition/alteration of AChE activity in *Channa punctatus* and the inhibition of AChE activity in brain were significantly higher than muscle followed by gill [20].

Cypermethrin at 1/7th (0.57mg/l) and 1/12th (0.33mg/l) of the lethal concentration (4mg/l) for a period of 1, 7, or 14 days and allowed recovery for another 7 days caused inhibition in acetyl cholinesterase (AChE) activity [27]. Maximum inhibition was found in brain followed by muscle, gill, and liver on day 14 at both the sublethal concentrations. Recovery showed a rise in AChE activity but significantly decreased compared to controls. Brain acetylcholinesterase activity was decreased significantly over a period of 45 days in *Labeo rohita* exposed to 0.139ppm of cypermethrin [1].

In the present study, the results showed a time and concentration dependent inhibition of AChE activity by cypermethrin in the tissues of the fish. Inhibition of AChE results in nerve impulses as nerves become permeable to sodium, allowing sodium to flow into the nerve. Pyrethroids delay the closing of the gate that allows sodium flow [47] and thus, multiple nerve impulses rather than the usual single one occur. In turn, these impulses release the neurotransmitter ACh, which stimulates other nerves [13]; ultimately resulting in buildup of ACh within the nerve synapses leading to a variety of neurotoxic effects and decreased cholinergic transmission [28]. It is also known pyrethroid compounds which inhibit AChE activity were known to disrupt the normal behavioral patterns in the effected animals [29].

Inhibition of AChE activity in functionally vital organs like gill, muscle and liver lead to impaired critical neurophysiological activity and block sodium channels of nerve filaments, thereby lengthening the depolarization phase. Further, cypermethrin affects the GABA receptors in the nerve filaments and other related processes. In addition, the reduction in AChE activity and ACh levels may be attributed to *in vivo* biotransformation of sequestered cypermethrin in the storage organs.

4. Conclusion

Cypermethrin is highly toxic to fish, and impose catastrophic effect on fish at and sublethal concentrations. Altered biochemical responses sush as changes in LDH, GDH and AChE activities can be used as tools in bioassessment to monitor ecotoxicological risks associated with cypermethrin to the test species, *Cyprinus carpio* in particular and freshwater fish species in general. Definitely this study will provide valuable scientific data for formulating biomonitoring programmes in this region or elsewhere.

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