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Impact of Chlorpyrifos on biochemical constituents of the freshwater Cyprinid fish, *Cyprinus carpio* (L.)

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Abstract

Chlorpyrifos (O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)-Phosphorothioate second largest selling OP insecticide widely used to control pests in agriculture farms and orchards of fruit trees. In this study, *Cyprinus carpio* has been exposed to $1/10^{th}$ sub-lethal concentration (0.0318 mg/l) of 96 h LC50 of Chlorpyrifos for 7, 14, and 21 days. The current investigation sought to determine the detrimental effects of the insecticide chlorpyrifos on the levels of the biochemical components glycogen, glucose, total protein, and free amino acids, as well as the activities of the enzymes protease, aspartate aminotransferase (AST), and alanine aminotransferase (ALAT), lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), were studied in *Cyprinus carpio*. Exposure to a sub-lethal dose showed significant ($P<0.05$) alterations in the biochemical parameters observed in the tissues of *C. carpio*, and changes developed progressively with exposure time. Results of the present study indicated that exposure to sub-lethal concentrations of chlorpyrifos as low as 0.0318mg/l may induce a deleterious effect on cellular metabolism, leading to impaired carbohydrate and protein synthetic machinery.

Chlorpyrifos (CPF) O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)-Phosphorothioate second biggest selling synthetic organophosphate (OP), non-systemic, broad-spectrum insecticide and acaricide, which is used for controlling various pests belonging to the orders such as Diptera, Coleoptera, and Homoptera in soil or on foliage in over 100 crops[39]. Likewise, it is also used in the control of domestic pests and fruit trees against insects. However, due to its widespread use, it may cause adverse effects on the non-target organism, fish[1]. There are many pathways by which chlorpyrifos distribute throughout aquatic ecosystems. The major route of chlorpyrifos to aquatic ecosystems is through rainfall runoff and air drift[43].

[2]Professor

Different concentrations of chlorpyrifos were detected in the world's groundwater and surface waters[40]. Moreover, chlorpyrifos is moderately persistent in aquatic environments, especially in freshwater and estuaries[40], chlorpyrifos may be absorbed through the gill, skin, and digestive system of fish and distributed in different tissues via the blood. Chlorpyrifos is a neurotoxic inhibitor of acetylcholinesterase (AChE) in the central and peripheral nervous systems resulting in the accumulation of acetylcholine, CPF elicits several other responses such as hepatic dysfunction, genotoxicity, neurochemical, and neurobehavioral changes[17,24,29]. Earlier reports claimed that there were several hundred parts per billion of chlorpyrifos in the water, related to fish death and its half-life is 26.5 days in water[30], because of its low perseverance and prevalence of CPF in the aquatic environment making it the most potent agent to exert negative impacts on nontarget organisms, especially fish[1]. For these reasons, it is an excellent applicant for toxic studies on the health of aquatic organisms.

Due to the lipophilic property of this pesticide, it accumulates mainly in fatty tissues. Bioaccumulation and detoxification of chlorpyrifos in the liver may affect the physiological function of cells[23,41,44]. Dysfunction in the physiology of a cell exposed to toxic substances can change the levels of biochemical parameters which are very sensitive, conserved across species, and less variable than other parameters. Assessing biochemical parameters could be a useful tool to diagnose toxicity effects in target organs to determine the physiological status of fish exposed to pesticides[5,6]. *Cyprinus carpio* (L.) is a cool to temperate water fish species, due to its economic importance and status as a major element of many food chains around the world, is an ideal model indicator for toxicological investigations.

Oxidative stress brought on by pesticide poisoning causes the depletion of carbohydrate stores, and changes in carbohydrate metabolism are likely to be harmful to animal survival Due to the severe metabolic damage caused by the action of xenobiotic chemicals. Proteins are the most abundant macro-molecules of organisms. They are connected with all physical and chemical activity of life and also they are, essential to cell function, formation of cell structure, and maintaining the homeostasis of the cell. The assessment of protein content can be considered an identification tool to find the physiological process of the animal cell[19]. The proteome differs from cell to cell and is constantly changing as a result of biochemical interactions with the environment and genome. As a result, the expression of a particular group of proteins in the exposed organism, tissue, or cell type[21] is influenced by envir onmental factors thus biochemical components are very sensitive variables and are investigated as potential biomarkers which are conserved across a range of different species. Fishes are valuable bioindicators and integrators of toxicants. Since fishes are the most chlorpyrifos-sensitive aquatic organism, thus the present investigation deals to evaluate the effects of chlorpyrifos on selected metabolites and enzymes involved in protein and carbohydrate metabolism in the tissues of *C. carpio*.

Experimental fish specimens and chemical:

The freshwater fish *Cyprinus carpio*

(Family: Cyprinidae, Order: Cypriniformes). Live fish of size 5-6 cm and weight 3.5-4.5g weight were procured from State Fisheries Department, Bhadra Reservoir Project, Shimoga District, Karnataka State, India. To minimize cutaneous infections, fish specimens were bathed twice in 0.05 percent potassium permanganate (KMnO4) for 2 minutes. The specimens were then acclimatized for three weeks in a semi-static system under laboratory conditions. Commercial fish pellets were added twice daily as a supplement to the fish during the acclimation period. Feces and other waste materials were drained off daily to lower the ammonia content in the water.

The physicochemical quality of test water such has, Temperature 25±1ºC, pH 7.2±0.2 at 25ºC, Dissolved Oxygen 6.7±0.8 mg/L, Carbon-dioxide 6.2±0.3 mg/L, Total Hardness 23.2±3.4 mg as CaCO3/L, Phosphate 0.37±0.002µg/L, Salinity 0.01 ppm, Specific Gravity 1.001 and the conductivity of the water is less than 10µS/cm. was measured following methods[3].

For the present study, commercial formulations of chlorpyrifos (50%EC) with the trade name 'Premain strong' (manufactured by ADAMA India Pvt. Ltd. Hyderabad) were purchased from the local market. This grade of chlorpyrifos was found to be predominantly used in agricultural settings.

In vivo exposure experiment :

Acute toxicity bioassay was performed in a semi-static system in laboratory[11], and the 96 h LC_{50} value of chlorpyrifos to *C. carpio* was determined as 0.318 mg/L, One-tenth ($1/10^{th}$)of LC_{50} was chosen as the nominal sublethal concentration (0.0318 mg/L) and was employed in the current study to examine the sublethal effects of chlorpyrifos in tissues (muscle, liver, gill, and brain) of *C. carpio* during exposure periods of 7, 14, and 21 days.

Protein metabolic profiles :
Estimation of organic constituents :

One percent of the tissues' homogenate was prepared in a 0.25 M ice-cold sucrose solution to determine the total proteins. Using the Folin phenol reagent and bovine albumen serum as standard [16]. One percent homogenate was precipitated with ten percent trichloro-acetic acid to estimate amino acids, and the protein-free supernatant was utilized to compute free amino acids (FAA) using the ninhydrin reagent[18]. Standard tyrosine was used.

Assay of enzymes :

For aspartate aminotransferase (AAT) and alanine aminotransferase (ALAT), a 5 percent homogenate of the tissues was prepared in 0.25 M ice-cold sucrose solution, and for protease, in ice-cold distilled water. These were centrifuged at 2500 rpm for 10 minutes in a refrigerated centrifuge at 4°C to remove cell debris, and clear cell-free extracts were used as enzyme sources. Protease activity was determined using a reaction mixture comprising 100 µm of phosphate buffer (pH 7.0) and 12 mg of denatured protein[18]. The activities of AAT (EC 2.6.1.1) and ALAT (EC 2.6.1.2) were measured[28] in the tissues. The AAT incubation mixture includes 100 µm of phosphate buffer (pH 7.4), 2 µm of ketoglutarate, and 50 µm of L-aspartic acid

(pH 7.4). The incubation stages for ALAT are the same as stated for AAT, except that the substrate employed was D-alanine (50 µm). Sodium pyruvate was used to create the standard graph and all spectrophotometric measurements were determined. Every homogenate that contains protein has had its specific enzyme activity evaluated.

Carbohydrate metabolism :

The amount of glucose and glycogen was estimated using the calorimetric micro method[7]. After standardization, the activities of lactate dehydrogenase (EC 1.1.1.27) were measured by the following approach[32]. Tissue homogenates were created in a 0.25 M sucrose solution and centrifuged for 15 min at 3000 rpm in a cooled high-velocity rotator; the supernatant was aided as the enzyme source. LDH (2 ml) reaction mixture included 100 µmol of phosphate buffer (pH 7.4), 2 µmol of 2-(P-indophenol) 3-(P-nitrophenyl)-5-phenyl tetrazolium chloride (INT), 50 µmol of sodium lactate, 0.1 µmol of NAD, and 0.5 ml of the enzyme. For the assessment of Succinate dehydrogenase (SDH) (EC 1.3.99.1), and Malate dehydrogenase (MDH) (EC 1.1.1.37)[20] were taken on.100 mol of phosphate buffer (pH 7.4), 2 mol of INT, 100 mol of sodium succinate, and 0.5 ml of enzyme made up the SDH incubation mixture. The same preparation was made for MDH in a final volume of 2 ml, except for the substrate, which was 50 mol of sodium malate. After an hour of incubation at 37ºC, the reaction between the three dehydrogenases was stopped with 5 ml of glacial acetic acid. Before adding the enzyme extract to the reaction mixture for each tissue, glacial acetic acid was added to maintain zero time controls (ZTC). The color was eliminated by refrigerating it overnight after adding 5 ml of toluene, and it was then measured at 495 nm. Every homogenate that contains protein has had its specific enzyme activity evaluated.

Statistical analysis :

The data obtained from the experimental investigation were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey multiple range tests, and statistical significance was assessed at 5% ($P < 0.05$) levels using GraphPad prism version (8.0) software.

Carbohydrate metabolic profiles :
Glucose

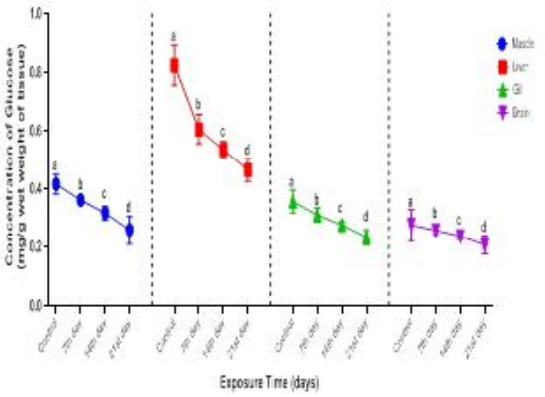

Fig. 1. Changes in the glucose content in different tissues of *Cyprinus carpio* exposed to sublethal concentrations ($1/10^{th}$) of chlorpyrifos for 7, 14, and 21 days.

The calculated values for Glucose level in the Muscle, liver, gill, and brain of the treated fish *Cyprinus carpio* at a sub-lethal concentration of Chlorpyrifos after 7^{th}, 14^{th}, and 21^{st} days exposure and percent change

over control along with standard deviations of six individuals (n=6). Were graphically exemplified in Fig. 1. Values are presented as mg/g of wet tissue weight. Since P<0.05 in all the tissues, there is a significant variation between the various exposure days was found across all tissues. The glucose content of the control fishes was in the order Liver>Muscle> Gill>Brain and the percentage decrease of glucose in the treated fishes was in the order Liver>Muscle>Gill>Brain for all the exposure time.

Glycogen

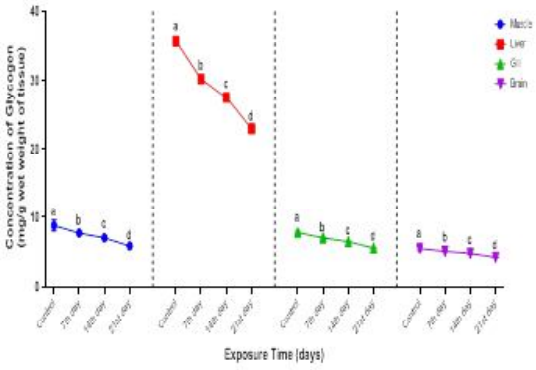

Fig. 2. Changes in the glycogen content in different tissues of *Cyprinus carpio* exposed to sublethal concentration (1/10th) of chlorpyrifos for 7, 14, and 21 days.

The calculated values for Glycogen level in the Muscle, liver, gill, and brain of the treated fish *Cyprinus carpio* at a sub-lethal concentration of Chlorpyrifos after 7th, 14th, and 21st days exposure and percent change over control along with standard deviations of six individuals (n=6). were graphically exemplified in Fig. 2. Values are presented as mg/g of wet tissue weight. Since P<0.05 in all the tissues, there is a significant variation between the various exposure days was found across all tissues. The glycogen content of the control fishes was in the order Liver>Muscle> Gill>Brain and the percentage decrease of glycogen in the treated fishes was in the order Liver>Muscle>Gill>Brain for all the exposure time.

Succinate dehydrogenase (SDH)

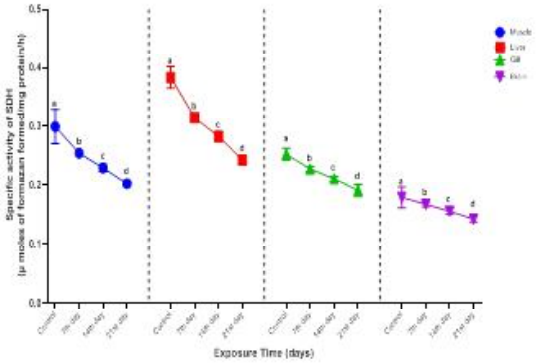

Figure 3. Changes in the Succinate dehydrogenase (SDH) content in different tissues of *Cyprinus carpio* exposed to sublethal concentration (1/10th) of chlorpyrifos for 7, 14, and 21 days.

The calculated values for Succinate dehydrogenase (SDH) level in the Muscle, liver, gill, and brain of the treated fish *Cyprinus carpio* at a sub-lethal concentration of Chlorpyrifos after 7th, 14th, and 21st days exposure and percent change over control along with standard deviations of six individuals (n=6). were given graphically exemplified in Fig. 3. Values are presented as µ moles of formazan formed/mg protein/h. Since P<0.05 in all the tissues, there is a significant variation between the various exposure days was found across

all tissues. The SDH activity of the control fish was in the order Liver>Muscle>Gill>Brain and the percentage decrease of SDH in the treated fishes was in the order Liver>Muscle>Gill>Brain for all the exposure time.

Malate dehydrogenase (MDH)

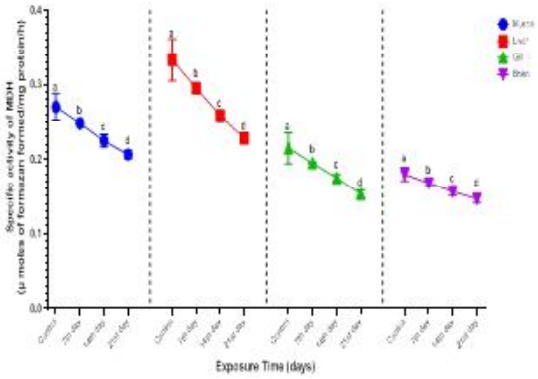

Figure 4. Changes in the Malate dehydrogenase (MDH) content in different tissues of *Cyprinus carpio* exposed to sublethal concentration (1/10th) of chlorpyrifos for 7, 14, and 21 days.

The calculated values for Malate dehydrogenase (MDH) level in muscle, liver, gill, and brain of the treated fish *Cyprinus carpio* at a sub-lethal concentration of Chlorpyrifos after 7th, 14th, and 21st days exposure and percent change over control along with standard deviations of six individuals (n=6). Were graphically exemplified in Fig. 4. Values are presented as µ moles of formazan formed/mg protein/h. Since P<0.05 in all the tissues there is a significant variation between the various exposure days was found across all tissues. The MDH activity of the control fish was in the order Liver>Muscle>Gill>Brain and the percentage decrease of MDH in the treated fishes was in the order Liver> Gill> Muscle>Brain for all the exposure time.

Lactate Dehydrogenase (LDH)

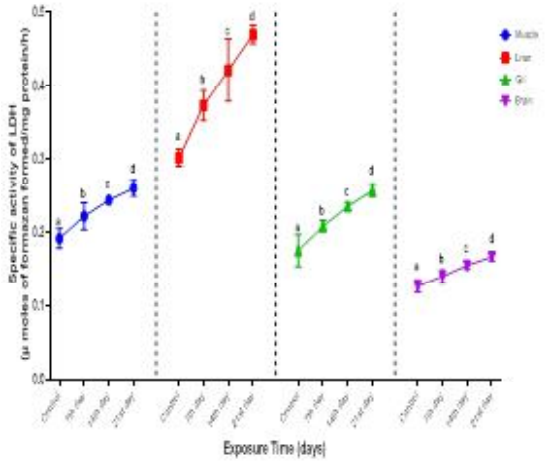

Figure 5. Changes in the Lactate Dehydrogenase (LDH) content in different tissues of *Cyprinus carpio* exposed to sublethal concentration (1/10th) of chlorpyrifos for 7, 14, and 21 days.

The calculated values for Lactate Dehydrogenase (LDH) level in the Muscle, liver, gill, and brain of the treated fish *Cyprinus carpio* at a sub-lethal concentration of Chlorpyrifos after 7th, 14th, and 21st days exposure and percent change over control along with standard deviations of six individuals (n=6). Were graphically exemplified in Fig. 5. Values are presented as µ moles of formazan formed/mg protein/h. Since P<0.05 in all the tissues there is a significant variation between the various exposure days was found across all tissues. The LDH activity of the control fish was in the order Liver>Muscle>Gill>Brain and the percentage increase of LDH in the treated fishes was in the order Liver> Gill>

Muscle> Brain for all the exposure time.

Protein metabolic profiles

Total Protein

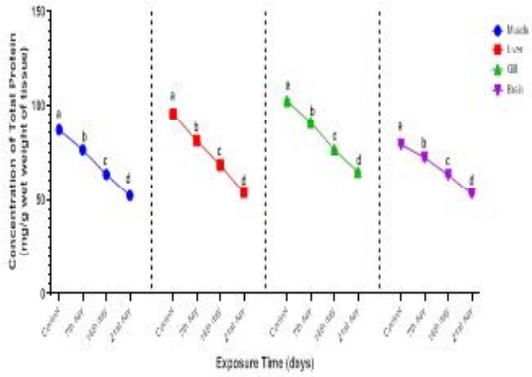

Figure 6. Changes in the Total protein content in different tissues of *Cyprinus carpio* exposed to sublethal concentration ($1/10^{th}$) of chlorpyrifos for 7, 14, and 21 days.

The calculated values for Total protein level in muscle, liver, gill, and brain of the treated fish *Cyprinus carpio* at a sub-lethal concentration of Chlorpyrifos after 7^{th}, 14^{th}, and 21^{st} days exposure and percent change over control along with standard deviations of six individuals (n=6). were graphically exemplified in Fig. 6. Values are presented as mg/g wet weight of tissue Since $P<0.05$ in all the tissues, there is a significant variation between the various exposure days found across all tissues. The protein content of the control fish was in the order Gill>Liver>Muscle>Brain and the percentage decrease of protein in the treated fishes was in the order Liver >Gill>Muscle> Brain for all the exposure time.

Free Amino Acid

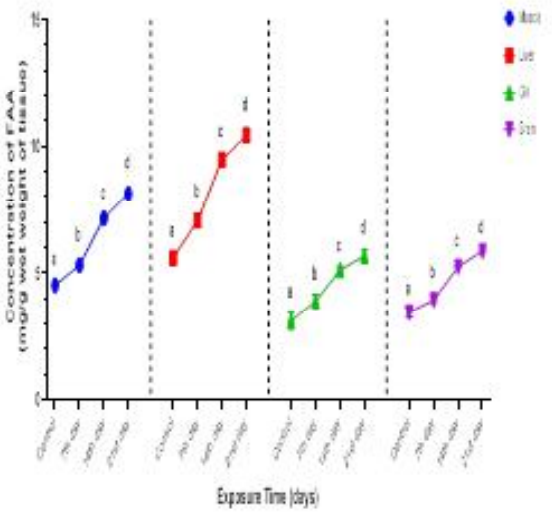

Figure 7. Changes in the Free Amino acids (FAA) content in different tissues of *Cyprinus carpio* exposed to sublethal concentration ($1/10^{th}$) of chlorpyrifos for 7, 14, and 21 days.

The calculated values for Free Amino Acids (FAA) level in muscle, liver, gill and brain of the treated fish *Cyprinus carpio* at a sub-lethal concentration of Chlorpyrifos after 7^{th}, 14^{th}, 21^{st} days exposure and percent change over control along with standard deviations of six individuals (n=6) were graphically exemplified in Fig. 7. Values are expressed as mg/g wet weight of tissue. Since $P<0.05$ in all the tissues, there is a significant variation between the different days of exposure within each tissue. Free Amino Acids (FAA) content of the control fish was in the order Liver> Muscle>Brain>Gill and the percentage increase of FAA in the treated fishes was in the order Liver>Gill> Muscle>Brain for all the exposure time.

Enzyme Assay

Protease activity

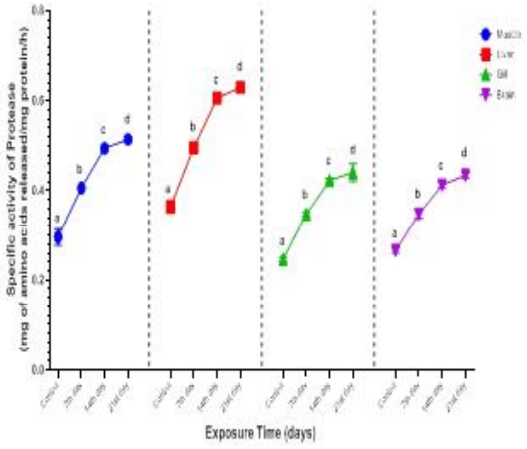

Figure 8. Changes in the Protease activity in different tissues of *Cyprinus carpio* exposed to sublethal concentration ($1/10^{th}$) of chlorpyrifos for 7, 14, and 21 days.

The calculated values for Protease activity level in muscle, liver, gill, and brain of the treated fish *Cyprinus carpio* at a sub-lethal concentration of Chlorpyrifos after 7^{th}, 14^{th}, and 21^{st} days exposure and percent change over control along with standard deviations of six individuals (n=6). Were graphically exemplified in Fig. 8. Values are presented as mg of amino acids released/mg protein/h. Since $P<0.05$ in all the tissues there is a significant variation between the various exposure days was found across all tissues. Protease activity of the control fish was in the order Liver>Muscle>Brain>Gill and the percentage increase of protease in the treated fishes was in the order Gill> Muscle> Liver> Brain for all the exposure time.

Aspartate Aminotransferase (AAT)

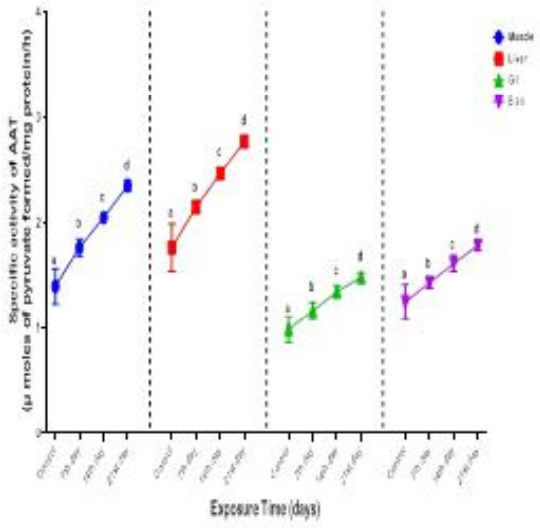

Fig. 9. Changes in the specific activity of aspartate aminotransferase in different tissues of *Cyprinus carpio* exposed to sublethal concentration ($1/10^{th}$) of chlorpyrifos for 7, 14, and 21 days

The calculated values for Aspartate Aminotransferase (AAT) level in muscle, liver, gill, and brain of the treated fish *Cyprinus carpio* at a sub-lethal concentration of Chlorpyrifos after 7^{th}, 14^{th}, and 21^{st} days exposure and percent change over control along with standard deviations of six individuals (n=6). were graphically exemplified in Fig. 9. Values are presented as µ moles of pyruvate formed/ mg protein/h. Since $P<0.05$ in all the tissues there is a significant variation between the various exposure days was found across all tissues. AAT activity of the control fish was in the order Liver>Muscle>Brain>Gill and the percentage increase of AAT in the treated fishes was in the order Muscle>Liver>Gill> Brain for all the exposure time.

Alanine Aminotransferase (ALAT)

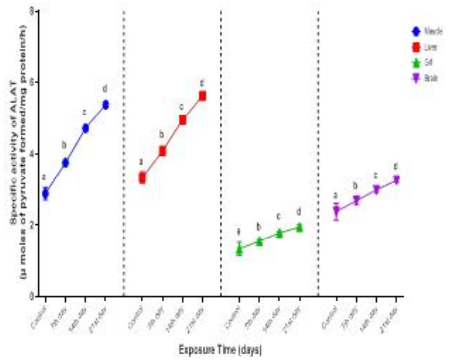

Fig. 10. Changes in the specific activity of alanine aminotransferase in different tissues of *Cyprinus carpio* exposed to sublethal concentration ($1/10^{th}$) of chlorpyrifos for 7, 14, and 21 days.

The calculated values for Alanine Aminotransferase (ALAT) level in muscle, liver, gill, and brain of the treated fish *Cyprinus carpio* at a sub-lethal concentration of Chlorpyrifos after 7^{th}, 14^{th}, and 21^{st} days exposure and percent change over control along with standard deviations of six individuals (n=6). were graphically exemplified in Fig. 10. Values are presented as µ moles of pyruvate formed/ mg protein/h. Since $P<0.05$ in all the tissues there is a significant variation between the various exposure days was found across all tissues. ALAT activity of the control fish was in the order Liver>Muscle>Brain>Gill and the percentage increase of ALAT in the treated fishes was in the order Muscle> Liver> Gill> Brain for all the exposure time.

Carbohydrate metabolic profile :

Carbohydrates are the principal organic nutrients to be degraded and depleted in response to stress conditions in fish bodies. Carbohydrate provides the energy required for the animal to perform different body processes. Metabolism of carbohydrates is very essential for animal survival[35]. These sources are likely to be most depleted to meet the energy demand and for regulating cellular function and maintaining permeability, structural stability enzymes are required to monitor the cell's integrity[9]. carbohydrates facilitate structural stability and fill in as supplements, and put away energy as glycogen in fish tissue and the organs like the liver when there is a hypoxic condition and dearth of food[22]. In almost all the tissues of fish muscle, liver, gill, and brain tested at a sublethal concentration ($1/10^{th}$) chlorpyrifos show a reduction in the glucose, and glycogen levels it could be the result of direct use for energy production, a need brought on by pesticide stress which suppresses the expression of enzymes like glucose-6-phosphatase, responsible for facilitating glycogen synthesis as a result of its reduction leads to depletion in glycogen and glucose content. The findings of the present study are correlated with the works of [2,33].

Environmental contaminants like pesticides can affect the function of some enzymes. According to the result obtained a decrease in Succinate dehydrogenase (SDH), Malate dehydrogenase (MDH), and an increment in Lactate Dehydrogenase (LDH) was observed in all the tissues against the control, Succinate dehydrogenase (SDH) an oxidative enzyme showed a declined activity in all the tissues exposed which indicate the oxidative metabolism is completely shattered in fish *C. carpio* exposed to chlorpyrifos if the enzyme activity of SDH is altered then it will affect Kreb's cycle thus the whole metabolism is altered. Since SDH is an oxidative enzyme it catalyzes the reversible oxidation of succinate to fumarate thus playing a crucial role in the Krebs cycle such is used as a biomarker of mitochondrial activity[12], Pesticidal intoxication resulting in loss of permeability and integrity of the mitochondria[4]

results in inhibition of the mitochondrial respiratory mechanism as the cause of pesticide stress favors anaerobic respiration. The present investigation is in agreement with the findings of [34]. Further Malate dehydrogenase activity is decreased in the present study along with succinate dehydrogenase activity, which indicates a reduction in oxidative metabolism attributed to impaired aerobic pyruvate consumption and a consequent reduction in fumarate-malate conversions as it is a NAD-dependent enzyme attributed to decreased tissue oxygen uptake and intracellular accumulation of chlorpyrifos toxic stress impairs the Krebs cycle's ability to function, because of enzyme-inhibitor complex, which is difficult to dissolve thus reduces the activity of particular enzymes. According to to[14] due to the accumulation of chlorpyrifos in the intracellular network, there is decreased availability of cofactors inside subcellular structures could be the cause of these two dehydrogenase enzyme inhibition. The reduction in muscle MDH activity may result from either change in the active site's conformation or the development of an enzyme inhibitor component that affects carbohydrates metabolism. on the other hand, the enzyme lactate dehydrogenase (LDH), which converts lactate to pyruvate, is crucial for the metabolism of carbohydrates, as it is a glycolytic enzyme found to be an important marker for assessing the toxicity of various chemicals. increment in LDH level is due to the hypoxic condition resulting from the damage of the respiratory epithelium which in turn may have reduced oxidative metabolism due to toxicant stress. In the present study, under sublethal exposure, LDH activity is increased implying increased anaerobic respiration to fulfill the energy needs when aerobic oxidation was declined and also reduced oxidative metabolism due to hypoxia in tissue attributed to stabilizing cytochrome c oxidase complex as these enzymes are mitochondrial localized[38]. Modifications to the LDH Activity have shown to be very effective functions as a useful marker and a tool for diagnosis in toxicological research for fish tissue damage.

In the present study decreased glucose, glycogen, and SDH, MDH while the increase in LDH level was maximum in the liver while brain tissue showed lower effects on carbohydrate metabolic contents as it is dependent on blood glucose for all its metabolic activities. The results of the present investi-gations are in agreement with the findings of [4,8].

Protein metabolic profiles and enzyme assay

The indiscriminate use of pesticides in the agriculture field has been causing environmental problems. Biochemical studies help to analyze the effect of various pesticides in different tissues of fish. Pesticides often act as metabolic inhibitors, stressing metabolically active molecules like proteins, carbohydrates, and enzyme metabolites. The metabolic rate which depends on the site of activity will determine the biochemical constraints that can upsurge or decline. Proteins act as building blocks of cells and are vital for all biological processes. Protein synthesis is an important mechanism in maintaining physiological homeostasis in humans and other animals. In the present study tissues such as the liver, gill, muscle, and brain shows decrement in the proteins of freshwater fish *Cyprinus carpio* treated with sublethal convergences of chlorpyrifos recommends the presence of high proteolytic action, and the inhibition of protein biosynthesis and breakdown is caused by

oxidative stress, cell necrosis, and as a result, inhibition of protein synthesis. In the present study, the liver is more affected where protein level is decreased to a greater extent due to pesticidal intoxication causing cellular impairment due to hormonal imbalance and induced stress causes hepatocytic necrosis thus increased degradation of proteins in the liver was observed and this trend of decrease in the protein level agrees with the earlier findings of [27,31]. In the present study, protein level declined while an increment in amino acids level is observed this is due to increased proteolysis activity under chlorpyrifos toxicity, and increased FAA an emergency source of energy during persistent stress. Proteolytic enzymes break down the protein molecules into free amino acid subunits, thus expansion in free amino acid (FAA) levels indicates possible tissue damage brought on by improper incorporation of amino acids during polypeptide chain formation under chlorpyrifos stress[26]. Released FAA was utilized to generate energy which aided them as keto acids into the TCA cycle via aminotransferase reaction supporting to provide the energy needed under toxic stress[13].

In the present study increase in FAA may be attributed to enhancing the activity of protease enzyme in all the tissues of *Cyprinus carpio* to overcome tissue necrosis due to oxidative stress. The elevated level of Protease which correlates with the present results, under toxic stress proves that chlorpyrifos generates higher protease activity resulting in an increased breakdown that Surpasses the synthesis. Although in the occurrence of anabolic metabolism increased synthesis overwhelms the protein dissolution[12]. Proteolysis occurrence results in increasing protease action which is reflected in the decrease of the protein levels and increase in free amino acids contents in various tissues of *Cyprinus carpio* presented to chlorpyrifos agrees with the works of [13,25].

A decline in proteins and elevated levels of free amino acids are seemingly connected and are indicative of metabolic consumption giving possible energy to fulfill the energy demand during stress conditions.

Increased Aspartate Aminotransferase (AAT) and Alanine Aminotransferase (ALAT) activity in all the fish tissues Muscle, liver, gill, and brain tissues is noticed it may be due to enhanced transamination activity or even enhanced amino acid synthesis from sources like glucose or fatty acids or may be due to toxic stress. Elevated levels of AAT and ALAT were observed in different tissues as they are biomarkers of tissue degeneration under stress resulting in alteration in aminotransferase activity. In the present study, fish stress may have increased internal oxidative stress, which affects cellular membrane permeability thus leakage of this enzyme from liver cytosol through the membrane could be the reason for an increase in the activities of transaminases in stressed fish. Since stress is known to promote aminotransferase activity, the elevation in transaminases indicates that there was a substantial loss of metabolites during the chlorpyrifos intoxication. Transaminase levels and mitochondrial integrity appear to have a close relationship, and any change in the association of mitochondria will undoubtedly modify the chemical frameworks related to it. Similar trend of increase in AAT and ALAT levels was observed [15]in various fish tissues. The reduced protein levels and increased FAA level and activity of protease, AAT, and ALAT enzymes, in *C. Carpio* tissues, suggest a link

and probable use of their products for metabolic processes that cause tissue injury. In support of the present investigation, few of the investigators reported the same trend[10,37].

The current study shows that a Sub-lethal concentration of chlorpyrifos altered the protein and carbohydrates metabolism in *Cyprinus carpio,* decrease in the protein levels reflects altered protein synthesis due to toxicant buildup in the organs of fish as a result of alterations in the functioning of organs and changes in carbohydrates metabolism due to toxic stress of chlorpyrifos may inhibit aerobic and anaerobic metabolism. Thus chlorpyrifos exerts significant alterations in biochemical constraints even at sublethal convergence and inevitably varies the nutritional value of fish it is necessary to assess the hazardous effect of pesticides on fish and thereby establish subsequent management strategies for safeguarding aquatic organisms and their associated fauna.

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