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LDH, SDH AND MDH ENZYMATIC ACTIVITY OF CTENOPHARYNGODON IDELLA EXPOSED TO λ - CYHALOTHRIN (5 % EC)

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ABSTRACT

The synthetic pyrethroid λ- cyhalothrin widely used in agriculture, also absorb rapidly via different routes and slowly accumulate into various vital organs of freshwater fish like *Ctenoparyngodon idella*, ultimately cause adverse effects on health problems to human through food chain. The test fish was exposed to sublethal concentrations of λ-cyhalothrin for a period of 1, 4, 8 and 12days to evaluate the enzymatic levels of Lactate Dehydrogenase (LDH), Succinate Dehydrogenase (SDH) and Malate Dehydrogenase (MDH) in various organs such as gill, liver, kidney, muscle and brain. In the present study the activity level of LDH was elevated, whereas SDH and MDH were depleted in all the tissues of the experimental animal. Under exposure to λ- cyhalothrin, on day 12 the maximum percentage of elevation of LDH in brain (52.63%) and minimum percentage of elevation of LDH in muscle (17.94%) were observed. On day 12, the maximum percentage of depletion of SDH was in kidney (-34.75%), MDH in brain (-23.26%) and minimum depletion of SDH was in brain (-11.21%), MDH in kidney (-15.56%) were observed. All the exposures were compared with controls.

KEYWORDS: λ- cyhalothrin, *Ctenopharyngodon idella*, enzymatic activity, LDH, SDH and MDH.

INTRODUCTION

The use of agricultural chemicals like pesticides has been increased to preserve the standing crops from the attack of pests and to boost up crop production, in order to meet the ever-increasing food demand of the rising human population. Due to injudicious and indiscriminate use of pesticides the natural water resources are getting polluted all over the world (Ngidlo, 2013). Aquatic organisms can accumulate chemicals which are present in the aquatic media which leads to some changes in fish behavior (Ullah *et al.*, 2014; Rani and Kumaraguru, 2014). Due to extensive application of pesticide in agricultural field affected the enzymatic activity of non-target animals (Al-Ghanim *et al.*, 2020). Particularly fish are very sensitive to pesticides s because of their ability to uptake and retain the dissolved xenobiotic in water via active or passive processes (Bhuvaneshwari *et al.*, 2013). Different pesticide concentrations are susceptible to different fish species. The effects of pesticides were observed in almost all parts of the fish.

Certain antioxidant enzymes like LDH (Lactate dehydrogenase), SDH (Succinic dehydrogenase) and MDH (Malate dehydrogenase) are being extensively used as potential biomarkers for measurement of tissue and organ damage due to pesticidal toxicity. LDH and MDH isoenzymes are major stress related enzymes found in fishes (Neelanjana *et al.*, 2017). Hence this study is aimed to find out the biochemical changes in *Ctenopharyngodon idella* exposed to sublethal concentrations of λ-cyhalothrin.

MATERIAL AND METHODS

The freshwater fish *Ctenopharyngodon idella* were collected from ponds in Kuchipudi, Guntur District, brought and acclimatized to the laboratory conditions in large plastic tanks with unchlorinated ground water for two weeks at a room temperature of 28 ± 2^0 C prior to experimentation. LC_{50} for 96h was found out by using probit method (Finney, 1971). For biochemical studies fishes were exposed in sublethal concentration for a period of 1, 4, 8 and 12 days. The enzymatic activity of LDH was estimated by the method (Srikanthan and Krishnamurthy, 1955). Enzymes like SDH and MDH were estimated by the method with slight modifications (Nachlas, 1960).

RESULT AND DISCUSSION

The study of biochemical changes in fish has become an important tool for monitoring environmental exposure to contaminants in laboratory and field studies. The calculated values for *Ctenopharyngodon idella* exposed to sublethal concentrations ($1/10^{th}$ of lethal concentration, 0.26mg/l) of λ- cyhalothrin, the activities of LDH, SDH and MDH along with standard deviation and percent change over control is given. Present study LDH was elevated whereas SDH and MDH were depleted in all the tissues of test fish *Ctenopharyngodon idella* exposed to λ- cyhalothrin (5% EC), in sublethal concentrations for 1, 4, 8 and 12 days and compared with controls.

Lactate Dehydrogenase activity (LDH):

In the tissues of control fish, *Ctenopharyngodon idella* LDH was in the order: Liver > Gill > Muscle > Kidney > Brain. Present study the LDH activity was elevated in all the tissues of test fish exposed to λ-cyhalothrin in sublethal concentrations were compared with controls (Table 1, and Fig. 1).

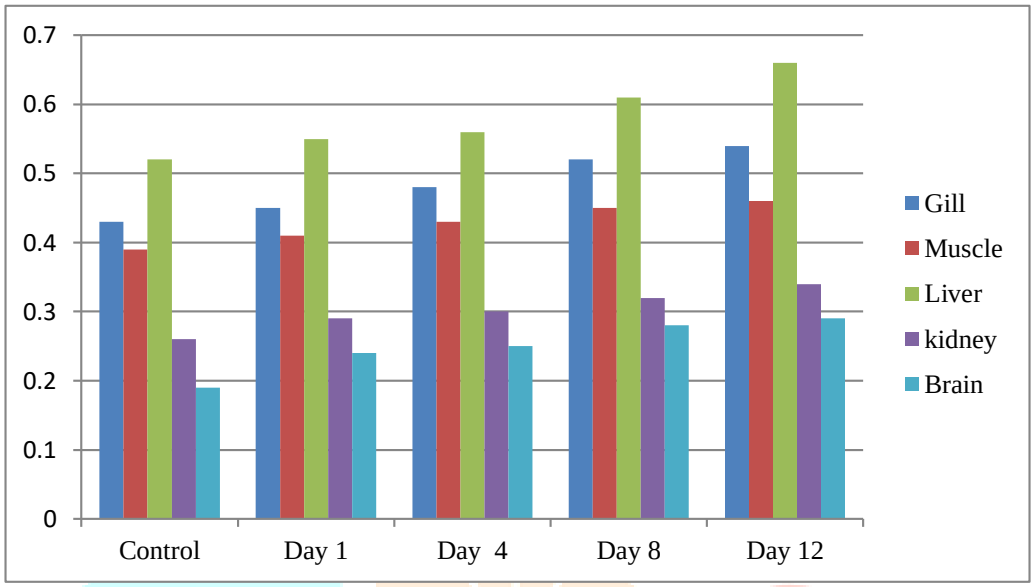
Normal activity of LDH patterns was altered in situations of chemical stress that leakage of LDH is a marker of membrane permeability and cell death and an increase in LDH activity might be due to stabilization of cytoplasmic membrane (Diamantino *et al.*, 2001). Disturbances in their catalytic process due to xenobiotic compounds can cause cellular homeostasis affecting different enzymatic systems, which leads to effects at higher levels of biological organization such as tissues, organs or individuals (Orrego *et al.*, 2011).

Table 1. Change in the Specific activity levels of LDH (µ moles of formazan/mg protein/h) and % change over the control in different tissues of *Ctenopharyngodon idella* on exposure to sub lethal concentrations of λ-cyhalothrin (5% EC)

Tissue	Control	Exposure period (days)							
		1		4		8		12	
		Sub lethal	% Change	Sub Lethal	% Change	Sub Lethal	% Change	Sub Lethal	% Change
Gill	0.43 ±0.01	0.45 ±0.01	+4.65	0.48 ±0.01	+11.62	0.52 ±0.01	+20.9	0.54 ±0.01	+25.58
Muscle	0.39 ±0.01	0.41 ±0.01	+5.12	0.43 ±0.01	+10.25	0.45 ±0.03	+15.38	0.46 ±0.02	+17.94
Liver	0.52 ±0.01	0.55 ±0.02	+5.06	0.56 ±0.03	+7.69	0.61 ±0.01	+17.30	0.66 ±0.02	+26.92
Kidney	0.26 ±0.01	0.29 ±0.01	+11.53	0.30 ±0.02	+15.38	0.32 ±0.02	+23.07	0.34 ±0.03	+30.76
Brain	0.19 ±0.01	0.24 ±0.02	+26.31	0.25 ±0.02	+31.57	0.28 ±0.01	+47.3	0.29 ±0.01	+52.48

Results are the mean values of five observations and the Standard Deviation is indicated as ± and figures in % change over control and sub lethal respectively. Values are significant P< 0.05

Fig.1. Change in LDH activity (µ moles of formazan/mg protein/h) in different tissues of *Ctenopharyngodon idella* on exposure to sublethal concentration of λ- cyhalothrin (5% EC)



In the present study, under sublethal exposure the LDH activity was showed significant increase in all the tissues of fish *Ctenopharyngodon idella* exposed to λ-cyhalothrin. Further disruption of respiratory epithelium might caused tissue hypoxia resulting in a decreased oxidative metabolism which might responsible for increase in LDH activity in toxicant stress. Lactate dehydrogenase (LDH) converts the lactate to pyruvate and has plays a very important role in carbohydrate metabolism. LDH involved in carbohydrate metabolism, any change in protein and carbohydrate metabolism might cause change in LDH activity and depends on its five isoenzymes and the activity changes under pathological conditions (Banerjee and Bhattacharya, 2012).

Present study the significant alterations were observed in LDH activity, it indicates damage of organs producing particular enzyme either in kidney or liver injury because LDH is an important glycolytic enzyme and it present in almost all body tissues. Significant elevation of LDH in blood plasma was observed in rainbow trout on cypermethrin exposure (Velisek *et al.*, 2006). A similar elevation in LDH activity was observed in the tissues of fish *Labeo rohita* on exposure periods of sublethal concentrations of Deltamethrin,(Mohan *et al.*, 2017). LDH activity was increased after exposure to *Colisa fasciatus* in lethal concentration of cypermethrin (Shailendra *et al.*, 2010). Metabolic enzymes such as citrate synthetase and lactate dehydrogenase (LDH) are part of the respiratory enzymatic system, which can be affected by the detoxification enzyme systems under stress conditions in fish (Orrego *et al.*, 2011). LDH activity in fish *Channa punctatus* significantly increased in skeletal muscle (2.2) fold followed by liver (1.8) fold, gill (1.6) fold and brain (1.4) in response to treatment with alphamethrin for 14 days, due to an increase in anaerobic respiratory activity and production of more lactate for completion of metabolic process (Tripathi and Singh, 2013).

An increasing with ammonia concentration, there was a progressive increased LDH activity in gill, liver, kidney and brain of the exposed fingerlings *Cirrhinus mrigala*, might have due to stress induced increase in the rate of glycolysis and the pyruvate is not routed to Kreb's cycle, rather catalyses to lactate; thereby shifting the respiratory metabolism from aerobiosis to anaerobiosis (Das *et al.*, 2004). The activity of LDH was highly elevated following profenofos and carbosulfan exposure in the tissues of fish *Labeo rohita* indicating that the anaerobic respirations arrived and aerobic respiration inhibited so as to meet the increased metabolic stress and to overcome the toxic stress (Bantu *et al.*, 2017). Lactate dehydrogenase (LDH) is a glycolytic enzyme recognized as a potential biomarker for assessing chemical toxicity (Kubrak *et al.*, 2013). Elevated plasma LDH was reported in response to verapamil

exposure of juvenile rainbow trout (*O. mykiss)* and the release of LDH from injured tissues. The increase of LDH activity favouring anaerobic respiration to meet the energy demands lowers the aerobic respiration (Li *et al.*, 2011).

The enzyme activity LDH in kidney of test fish *Gambusia affinis* was increased on exposure of choropyrifos (Sharma *et al.*, 2016). Increased LDH activity in the liver and muscle of fish *Cyprinus carpio* exposed to carbamazepine (CBZ), observed that disruption of respiratory epithelium might have caused tissue hypoxia resulting in a decrease in oxidative metabolism which might responsible for in LDH activity in toxicant stress (Annamalai *et al.*, 2012). Changes might have occurred due to tissue dysfunction and disturbances in the biosynthesis of these enzymes with alterations in the permeability of membrane. The activity of LDH was increased following sublehal exposures of fungicides azoxystrobin and hexaconazole in all the tissues of *Oreochromis mossambicus* and *Channa punctatus* through-out the experiment (Neelanjana *et al*., 2017).

Sublethal levels of cyanide on *Cirrhinus mrigala* showed an increase LDH activity in gill, brain and liver. The raise of LDH activity increases the permeability of cells as well as necrosis (Shwetha and Hosetti, 2013). Sublethal doses of copper, lead and pesticide cypermethrin, a comparative toxicity on *Oreochromis niloticus* observed elevated LDH activity levels (Firat *et al.*, 2011). Similar observations on LDH activity were also made (Mushigiri and David, 2004; Singh and Singh, 2004; Oropesa *et al.*, 2009; Aziza and Khadi, 2010; Al-Ghanim and Mahbood, 2012; Cristiana *et al.*, 2012). The LDH activity was elevated on exposure of Zebra fish *Danio rerio* to Butylbenzylphthalate (Sepperumal and Saminathan, 2014).

In the present study, it was observed that the LDH activity in the freshwater fish *Ctenopharyngodon idella* under exposure to sublethal concentration of λ-cyhalothrin was elevated indicating that the increased anaerobic respiration so as to meet the energy demands where aerobic oxidation is lowered. It suggests that aerobic catabolism of glycogen and glucose has shifted towards the formation of lactate, which might have adverse effects on the organism. LDH is associated with cellular metabolic action, particularly in conditions of chemical exposure and stress when high levels of energy required in a short period of time. LDH level in blood serum were increased in treated with cypermethrin intoxicating fish *Cirrhinus mrigala*, detoxifying property of *Cardiospermum halicacabum* was observed (Vasantharaja *et al.*, 2014). The increased LDH activity in brain and liver tissues of *Labeo rohita* treated with cypermethrin for 96hr, all above findings were supports the present study (Das and Mukherjee., 2003).

Succinate Dehydrogenase (SDH) activity:

In the tissues of control fish, *Ctenopharyngodon idella* the SDH was in the order: Brain > Muscle > Kidney > Gill > Liver. Present study, it can be visualized that there is a rapid depletion of SDH activity was observed in all the tissues of fish with sublethal doses of λ-cyhalothrin were compared with controls (Table 2. and Fig. 2).

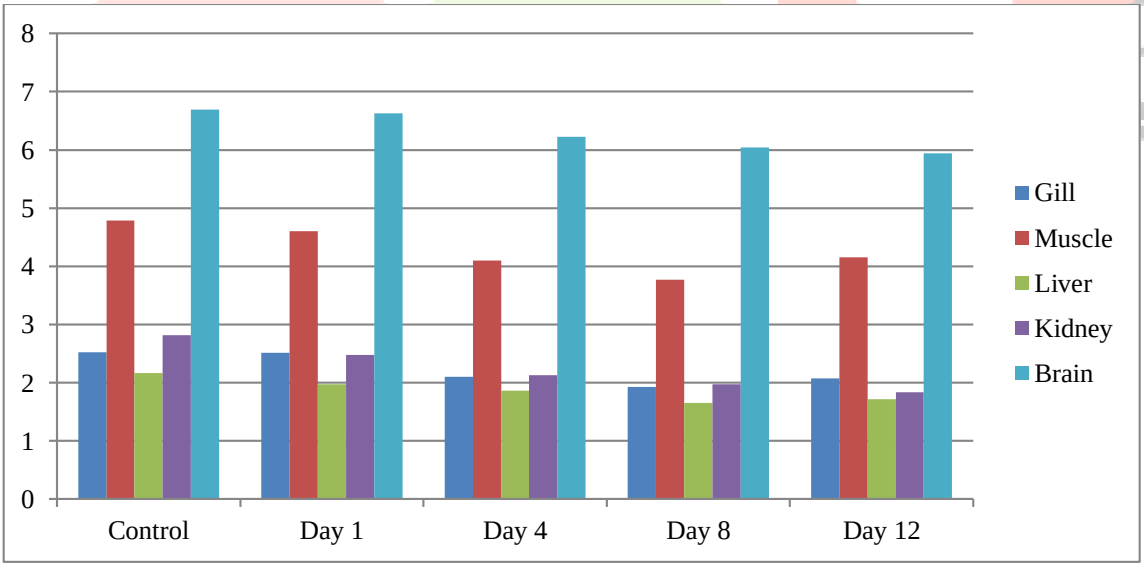
SDH is one of the important enzymes in Kreb's cycle and plays an important role in mitochondria, which are structures inside cells that the energy from food into a form that cells can use. Within mitochondria, the SDH enzyme links two important cellular pathways in energy conversion: the citric acid cycle and oxidative phosphorylation. It acts a vital role in citric acid cycle and catalyzes the reversible oxidation of succinate to fumarate. The mitochondrial enzyme in the oxidative catabolism of sugars and such is used effectively as a marker of mitochondrial activity. It was concentrated in the chloride cells within the gills and has used as an indicator of osmoregulatory activity (Sonia Mukherjee *et al.*, 2007). The higher activity of SDH in liver and muscle suggests higher distribution of mitochondria in the tissues, since succinate dehydrogenase is mitochondrial localized enzyme (Harper, 2012).

Table. 2. Change in the Specific activity levels of SDH (µ moles of formazan/mg protein/h) and % change over the control in different tissues of *Ctenopharyngodon idella* (Linnaeus) on exposure to Sub lethal concentrations of λ-cyhalothrin (5% EC)

Tissue	Control	Exposure period (days)							
		1		4		8		12	
		Sub lethal	% Change	Sub lethal	% Change	Sub lethal	% Change	Sub lethal	% Change
Gill	2.52 ±0.05	2.51 ±0.01	-0.39	2.1 ±0.06	-16.33	1.93 ±0.04	-23.11	2.07 ±0.02	-17.53
Muscle	4.79 ±0.08	4.6 ±0.05	-3.97	4.1 ±0.11	-14.41	3.77 ±0.21	-21.29	4.15 ±0.15	-13.36
Liver	2.17 ±0.02	1.97 ±0.01	-9.22	1.86 ±0.03	-14.29	1.65 ±0.03	-23.96	1.72 ±0.01	-20.73
Kidney	2.82 ±0.11	2.48 ±0.06	-12.06	2.13 ±0.04	-24.47	1.97 ±0.01	-30.14	1.84 ±0.01	-34.75
Brain	6.69 ±0.09	6.63 ±0.3	-0.9	6.23 ±0.1	-6.88	6.04 ±0.01	-9.72	5.94 ±0.04	-11.21

Results are the mean values of five observations and the Standard Deviation is indicated as ± and figures in % change over control and sub lethal respectively. Values are significant, $P<0.05$

Fig. 2. Change in SDH activity (µ moles of formazan/mg protein/h) in different tissues of *Ctenopharyngodon idella* on exposure to sublethal concentration of λ-cyhalothrin (5% EC)



The general decrease in SDH activity during pesticide stress was associated with the inhibition of mitochondrial respiratory mechanism of dearrangement on ultra-structure, architectural integrity and permeability of mitochondria (Shwetha and Hosetti, 2013). This prevents the transfer of electron to molecular oxygen, resulting in the inhibition of SDH activity and shifting to aerobic metabolism to anaerobiosis (Kamalaveni *et al.*, 2001). The inhibition of NAD dependent, LDH activity and SDH activity indicated a decreased pass of intermediates into the citric acid cycle. This might be responsible for suppression of oxidative phase of tissue metabolism under pesticidal impact showing a shift from aerobic metabolism to anaerobic metabolism under pesticidal stress (Mohamed *et al.*, 2010). The decreased SDH activity indicate inhibition of O_2 at mitochondrial level (Srivastava *et al.*, 2016). Similar decrement in the SDH activity was also observed by various workers in different species of fish exposed to different

pesticides. Decrease in activities of LDH and SDH on fish *Colisa fasciatus* and *Labeo rohita* after exposure to cypermethrin (Shailendra *et al.*, 2010; Jacob Doss *et al.*, 2007).

The inhibition of LDH and SDH activities were observed in fish *Colisa fasciatus* due to toxicity of ethanolic extract of *Nerium indicum* mill latex (Tiwari and Singh, 2009). There is a rapid depletion in SDH activity in all tissues of fish *Labeo rohita* treated with sublethal and lethal concentrations of profenofos and carbosulfan due to pesticidal stress was associated with the inhibition of mitochondrial respiratory mechanism of dearrangement on ultra-structure, architectural integrity and permeability of mitochondria (Bantu *et al.*, 2017). The SDH activity was elevated on exposure of Zebra fish *Danio rerio* to Butylbenzylphthalate (Sepperumal and Saminathan, 2014). Biochemical alteration in fresh water teleost fish *Colisa fasciatus* on exposure to synthetic pyrethroid cypermethrin for different water temperatures and different concentrations were studied Kubrak *et al.*, 2013). On exposure to 24, 48, 72 and 96 h to 0.009, 0.008 and 0.007 and 0.006 mg/L for 16^0C and 24, 48, 72 and 96 h to 0.06, 0.04, 0.03 and 0.02 mg/L at 28^0C were measured. SDH activity was decreased to 62 and 54% at 16^0C on exposure to 96 h to 40 and 60% of LC_{50} of cypermethrin.

The decreased SDH activity in fish *Tilapia mossambica* and *Clarias gariepinus* exposed to different types of chemicals, due to depletion in oxidative metabolism at the level of mitochondria leading to depression of TCA cycle (Sudharsan *et al.*, 2000; Al-Ghanim and Mahboob, 2012). Decrement in the SDH activity was observed by in fish exposed to Malathion and fenitrothion pesticides (Mohamed *et al.*, 2010). On exposure of 96 h LC_{50} cypermethrin in brain, liver and kidney tissues of *Labeo rohita*, SDH activity was depleted [33]. A rapid depletion in SDH activity was observed in all the tissues of fish *Labeo rohita* treated with lethal and sublethal doses of fenvalerate and endosulfan (Suneetha, 2012). A similar decrement in SDH activity was observed in the tissues of fish *Labeo rohita* on exposure periods of sublethal concentrations of Deltamethrin,(Mohan *et al.*, 2017).

Malate Dehydrogenase (MDH) activity:

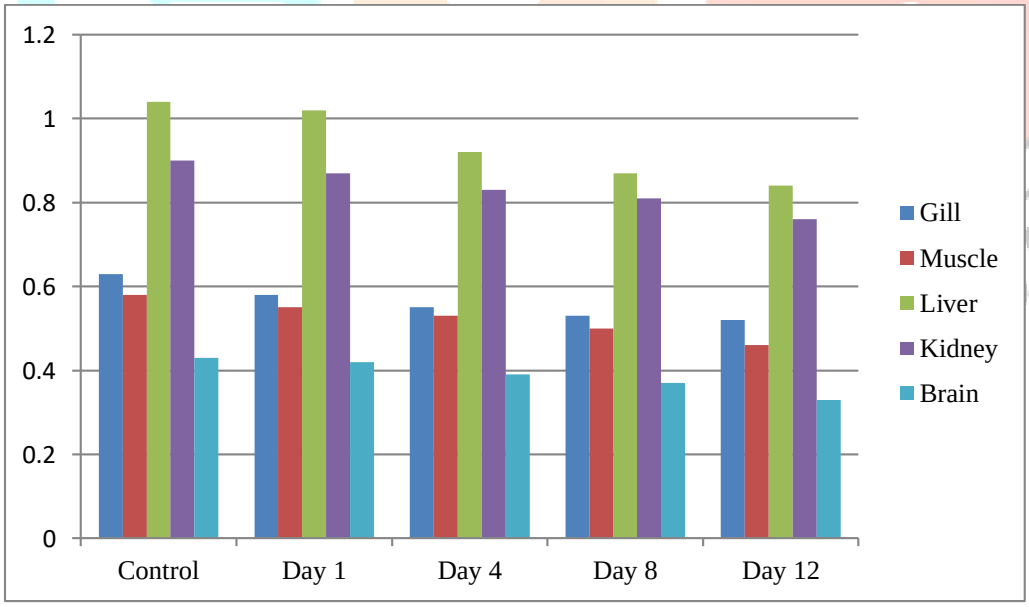
In the tissues of control fish, *Ctenopharyngodon idella* MDH activity was in the order of: Liver > Kidney > Gill > Muscle > Brain. Present study, the MDH activity was depleted in all the tissues of test fish exposed to λ-cyhalothrin in sublethal concentrations were compared with controls (Table 3, and Fig. 3).

Table 3. Change in the Specific activity levels of MDH (µ moles of formazan/mg protein/h) and % change over the control in different tissues of *Ctenopharyngodon idella* on exposure to sublethal concentrations of λ-cyhalothrin (5% EC)

Tissue	Control	Exposure period (days)							
		1		4		8		12	
		Sub lethal	% Change	Sub lethal	% Change	Sub lethal	% Change	Sub Lethal	% Change
Gill	0.63 ±0.01	0.58 ±0.02	-7.93	0.55 ±0.02	-12.7	0.53 ±0.02	-15.87	0.52 ±0.03	-17.46
Muscle	0.58 ±0.01	0.55 ±0.02	-5.17	0.53 ±0.01	-8.62	0.50 ±0.01	-13.79	0.46 ±0.01	-20.69
Liver	1.04 ±0.04	1.02 ±0.06	-1.92	0.92 ±0.01	-11.54	0.87 ±0.01	-16.35	0.84 ±0.01	-19.23
Kidney	0.90 ±0.02	0.87 ±0.01	-3.33	0.83 ±0.01	-7.78	0.81 ±0.01	-10	0.76 ±0.01	-15.56
Brain	0.43 ±0.01	0.42 ±0.01	-2.33	0.39 ±0.01	-9.3	0.37 ±0.01	-13.95	0.33 ±0.01	-23.26

Results are the mean values of five observations and the Standard Deviation is indicated as ± and figures in % change over control and sub lethal respectively. Values are significant $p<0.05$

Fig. 3. Change in MDH activity (µ moles of formazan/mg protein/h) in different tissues of *Ctenopharyngodon idella* on exposure to sublethal concentration of λ-cyhalothrin (5% EC)



Malate dehydrogenease is an NAD dependent enzyme which converts malate to oxaloacetate and reversible oxidation of fumarate to malate. It exists in two isozymic forms: (a) mitochondrial (b) cytosolic. This enzyme not only converts malate to oxaloacetate but also play a significant role in CO_2 fixation and in gluconeogenesis (Lehninger, 2008). Any alterations in mitochondrial structure it inhibits the activity of MDH. Most of the TCA cycle enzymes are of mitochondrial origin and any structural change in these enzymes induced by the pesticide might influence their activity levels. Pesticides are known to effect the structure of mitochondria which in turn alter the enzyme activities associated with it (Venkata Rathnamma *et al.*, 2008).

Decreased MDH activity levels due to the inhibition exerted by oxaloacetate, because of decrease in the activity of TCA cycle dehydrogenase is consistent with the disintegration in mitochondria of CO_2 formation from acetate (Ravisekar *et al.*, 2009). The decrease in the malate dehydrogenase activity is in line with the decreased succinate dehydrogenase activity indicating suppressed oxidative metabolism which in turn lowers fumarate-malate conversions. The decreased MDH activity might suggest the lower level of functioning of TCA cycle due to

inadequate supply of substrate or decreased oxygen uptake to the tissue level during λ-cyhalothrin toxicity stress. A similar decrement in MDH activity was observed in the tissues of fish *Labeo rohita* on exposure periods of sublethal concentrations of Deltamethrin, which suggests that the lower level of functioning of Krebs cycle due to inadequate supply of substrate or decreased oxygen uptake at the tissue level during toxicant toxicity stress (Mohan *et al.,* 2017).

The present study indicates λ-cyhalothrin caused alterations in the MDH activity of fish *Ctenopharyngodon idella*. Pesticide significantly inhibits aerobic, as well as anaerobic metabolism in exposed animals (Shailendra *et al.*, 2010). The effect of fenvalerate on *Labeo rohita* was showed rapid decline in MDH activity in all the tissues of fish (Suneetha, 2012). Depletion in MDH levels in different tissues of rat exposed to pesticide cypermethrin stress. It suggests that there is a shift in the respiratory metabolism (Ravisekar, 2009). The activity of MDH levels decreased in the tissues of freshwater mussel *Lamellidens marginalis* exposed to sublethal concentration of copper sulphate, decreased MDH activity due to inhibition of oxidative metabolism in mussel by copper stress (Satyaparameshwar *et al.,* 2006). Similar trend of reduction was observed in the activity of MDH in *Oreochromis mossambicus* and *Channa punctatus* exposed to the sublethal concentrations of fungicides azoxystrobin and hexaconazole, (Neelanjana *et al.,* 2017).

In the present investigation, malate dehydrogenase was gradually decreased in all the tissues of λ-cyhalothrin exposed fish. It is the mitochondrial matrix and cytosol catalyzes in the inter conversion of malate to pyruvate as well as oxalo acetate pyruvate. Reduction in MDH activity was observed in matrinxa, *Bryconcephalus* on exposure to Folidol 600 (Lucia *et al.,* 2004). It is also evident from the results that the tissues most affected are gill and muscle. The decrease trend in malate dehydrogenase activity is similar to the decreased activity as encountered for SDH and LDH. From all these studies, it is evident that λ-cyhalothrin has an effect on the oxidative metabolism even at sublethal concentrations. There is a reduction in MDH level in all tissues of fish *Labeo rohita* treated with sublethal and lethal concentrations of profenofos and carbosulfan due to inhibition exerted by oxaloacetate might be decrease in the activity of TCA cycle dehydrogenase is consistent with the disintegration of mitochondria of CO_2 formation from acetate (Bantu *et al.,* 2017).

CONCLUSION

The present finding revealed that the synthetic pyrethroid λ-cyhalothrin toxicity caused impairment metabolism in *Ctenopharyngodon idella*. An increase in the activity of LDH and decreased levels of SDH and MDH indicates that these enzyme activities can be potent diagnostic tool for λ-cyhalothrin induced to *Ctenopharyngodon idella.*

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