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Innovation The Research Concept

To Study the Effect of Endosulfan and Its Withdrawal on the Activity and Specific Activity of cMDH in the Liver and the Skeletal Muscle of a FW Teleost, *Heteropneustesfossilis*

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Abstract

The sublethal concentration of endosulfan reduced the activity of cMDH significantly, about 54% reduction was obtained after 7 days of exposure in case of liver and 64% reduction was obtained in skeletal muscle. The withdrawal of END from the medium after one week of exposure showed recovery in the activity and specific acitivity of cMDH close to the control value.

Keywords: cMDH, END, LC_{50},*H. fossilis*.

Introduction

To control various agricultural pests, a large number of pesticides have been in common use which is harmful to non-target species. They get accumulated in fishes and inturn affect human health via ecological cycling and biological magnifications (Hernandez et. al., 1992; Neogrohati et. al., 1992). Among several types of pesticides, the organochlorines are the most commonly used agricultural pesticides which become bioconcentrated along the food chain due to their great stability, high lipophilicity and low aqueous solubility. Thus, they enter the human body through various food stuffs and are accumulated in human adipose tissues (Alawi et.at., 1991). Energy metabolism plays a key role as the animal is forced to expend more energy to overcome toxic stress. One of the widely used organochlorine is endosulfan (6,7,8,9,10,10 hexachloro- 1,5,5a,6,9a-hexahydro - 6,9 - methano - 2,4,3 - benzodioxathiepine - 3 - oxide). It has been reported that the endosulfan inhibits the activity of ATPase in a few tissues of *Channagachua* (Dalela et.al., 1978; Sharma, 1988). A reduction in the activities of cMDH, mMDH and LDH in the liver of FW catfish, *Clariasbatrachus*, exposed to endosulfan has been reported (Tripathi and Shukla, 1990). Keeping the importance of cMDH in the processes related to energy production, the study was made on cMDH in the FW catfish, *H. fossilis*. The liver and the skeletal muscle were choosen for the estimation of activity of enzyme as these tissues represent aerobic and anaerobic nature of the cells, respectively. The study was carried out on the effect of endosulfan and its withdrawal on the activity and specific acitivity of cMDH in the liver and the skeletal muscle.

Aim of the Study

The enzyme cMDH plays a vital role in the processes related to energy production; therefore, a study was carried out on the effect of endosulfan and its withdrawal on the activity and specific activity of cMDH in the liver and the skeletal muscle. The liver and the skeletal muscle were chosen for the estimation of enzyme activity as these tissues represent aerobic and anaerobic nature of the cells, respectively.

Materials and Methods

Live and healthy specimens of freshwater cat fish, *Heteropneustesfossilis*, weighing 35.50 ± 2.5 gm having length of 16.4 ± 3.6 cm were collected locally and acclimatized in tap water for 15 days under natural photoperiod (11.58 – 12.38 light hours) and ambient temperature of 24.4 ± 1.8^{o}C in 50 litre glass aquaria. The fish were fed on minced goat liver on alternate day. Pilot experiments were performed for various experimental designs.

Stock solutions were prepared by dissolving the commercial grade endosulfan (85%) in acetone and the required volume was added from the

stock solution to maintain a desired concentration of the endosulfan in each aquarium. The sublethal and the median lethal concentrations (LC_{50}) for 7 days of endosulfan were determined by employing the trimed-Spearman Karber method (Hamilton et.al., 1977).

The fish were exposed to a sublethal concentration ($1X10^{-5}$ppm) of the commercial grade (85%) endosulfan. The impact of endosulfan and its withdrawal were studied. All substrates including NAD+, NADH, Oxaloacetate was freshly prepared at every 3 hours whenever required. The cytoplasmic fraction of the liver and skeletal muscle was taken for the assay of cMDH (cytoplasmic malate dehydrogenase). The procedure adopted for the assay of cMDH was those of Ochoa (1955).

One unit of enzyme activity was taken as that amount of the enzyme catalyzing the oxidation of one µmole of NADH per minute. The activity was expressed as units per gm wet weight of the tissue and the specific activity as units per mg protein. The student's t - test and ANOVA were applied to determine the level of significance.

Results and Discussion

The LC_{50} of the commercial grade endosulfan (END) for 21 days was 3.5×10^{-4} ppm and its sublethal concentration used in the experiments was 1×10^{-5} ppm. The low LC50 value of endosulfan indicates its high toxicity to the fish *H. fossilis*.

The sublethal concentration of END reduced the activity of cMDH significantly, about 54% reduction was obtained after 7 days of exposure in case of liver and 64% reduction was obtained in skeletal muscle. Subsequently, no significant change was reported till 21 days (Table 1). In case of liver, the specific activity of cMDH was increased, about 22% after 7 days of exposure which was mainly due to a large decline (66%) in the total cytoplasmic protein content (Table 2). In case of skeletal muscle, the specific activity of cMDH maximally reduced by 30% after 4 days of exposure and the total cytoplasmic protein was reduced by 45% after 7 days of exposure (Table 2).

In case of liver, the withdrawal of endosulfan after one week of exposure showed recovery in the activity of cMDH, it was increased by 77% after 21 days (Tables 3,4). Likewise the specific activity and the total cytoplasmic protein content also reached close to the control value after 21 days withdrawal of endosulfan. In case of skeletal muscle, the withdrawal of END significantly increased the activity of cMDH and the value reached close to the control value after 21 days withdrawal of END. The specific activity showed no significant change after 21 days of END withdrawal (Tables 3,4). The total cytoplasmic protein content was also increased by 85% after 21 days withdrawal of END which was close to the control value. These observations suggest that the activity of cMDH of the liver and skeletal muscle is inhibited by the exposure of endosulfan and the withdrawal of END from the medium restore the activity of the enzyme.

Table - 01

The effect of endosulfan (END) on the activity (units per gm wet wt.) of cytoplasmic malate dehydrogenase (cMDH) from the liver and skeletal muscle of the FW catfish, *H. fossilis*

END Exposure (day)	Liver	Skeletal Muscle
	Activity (U/gm)	Activity (U/gm)
Control	272.348 ± 6.568	87.124 ± 4.677
1D	252.476 ± 8.766	85.276 ± 5.105
2D	242.675 ± 9.285	71.050 ± 3.201
3D	212.768 ± 7.422	53.586 ± 4.342
4D	182.152 ± 9.700	36.246 ± 3.398
7D	124.650 ± 6.664	31.367 ± 3.580
14D	128.772 ± 6.253	33.145 ± 3.366
21D	126.535 ± 5.361	29.078 ± 3.349

Each datum represents mean ± SEM of five individuals (n=5)

Table - 02

The effect of endosulfan (END) on the specific activity (units per mg protein) of cytoplasmic malate dehydrogenase (cMDH) from the liver and skeletal muscle of the FW catfish, *H. fossilis*

END Exposure (day)	Liver	Skeletal Muscle
	Specific Activity (U/mg protein)	Specific Activity (U/mg protein)
Control	4.990± 0.258	4.394 ± 0.316
1D	4.794± 0.191	4.193± 0.291
2D	4.592± 0.215	3.924± 0.176
3D	4.661± 0.239	3.406± 0.103
4D	5.442± 0.284	2.841± 0.106
7D	6.100± 0.209	2.946± 0.195
14D	6.929± 0.193	2.927± 0.252
21D	6.248± 0.247	2.995± 0.137

Each datum represents mean ± SEM of five individuals (n=5)

Table - 03

The effect of endosulfan withdrawal (END-W) on the activity (units per gm wet wt.) of cytoplasmic malate dehydrogenase (cMDH) from the liver and skeletal muscle of the FW catfish, *H. fossilis*

Each datum represents mean ± SEM of five individuals (n=5)		
Group	Liver	Skeletal Muscle
	Activity (U/gm)	Activity (U/gm)
Control	268.686± 4.875	86.368± 2.720
END	126.474± 3.512	32.532± 3.683
END-W (7D)	145.079± 4.253	41.765± 2.752
END-W (14D)	167.577± 3.044	48.162± 2.807
END-W (21D)	224.594± 3.252	80.752± 3.139

Group of fish from which END was withdrawn (END-W) for 7,14 & 21 days after one week of exposure.

Table - 04

The effect of endosulfan withdrawal (END-W) on the specific activity (units per mg protein) of cytoplasmic malate dehydrogenase (cMDH) from the liver and skeletal muscle of the FW catfish, *H. fossilis*

Each datum Represents mean ± SEM of five individuals (n=5)		
Group	**Liver**	**Skeletal Muscle**
	Specific Activity (U/mg protein)	**Specific Activity (U/mg Protein)**
Control	5.151± 0.292	4.169± 0.182
END	5.974 ± 0.211	2.961± 0.176
END-W (7D)	6.756± 0.199	3.213± 0.213
END-W (14D)	6.418± 0.231	3.290± 0.202
END-W (21D)	6.052± 0.251	3.971± 0.327

Group of fish from which END was withdrawn (END-W) for 7, 14 & 21 days after one week of exposure.

Conclusion

A sublethal concentration of endosulfan inhibited significantly the activity of cMDH of the liver and the skeletal muscle after 7 days of exposure. The withdrawal of endosulfan from the medium after one week of exposure resumed the activity of cMDH of the liver and the skeletal muscle.

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