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***Research Article** (Open Access)*

DELTAMETRIN SUBLETHAL CONCENTRATION IMPACT ON ORGAN SPECIFIC ENERGY CHARGE AND ATPASE ACTIVITY IN *LABEO ROHITA*

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ABSTRACT
Deltamethrin is a widely used insecticide, poses a serious threat to the environment as well as many aquatic organisms. In this context, the present investigation was aimed to evaluate the impact of sub-lethal concentration of deltamethrin on fresh water fish *Labeo rohita* upto 96h exposure using the adenylate nucleotide levels, energy charge and organ specific ATPase as novel biomarkers. Sub-lethal concentrations of deltamethrin influenced the ATP and ADP levels and were initially increased with a concomitant decrease of AMP concentrations in brain, gill, kidney, liver and muscle tissues. Similarly the energy charge levels were also fluctuated tremendously among the different tissues of the experimental model. The maximum decrease of Na^+, -K^+ ATPase was noticed at 48h in brain tissue, Whereas the maximum decrease levels of Mg^{2+}, Ca^{2+} and total ATPase were recorded at 72h in muscles, 48h in liver, 48h in brain respectively. Simultaneously the recovery levels were also noticed with Mg^{2+}, Ca^{2+} and total ATPase at 96h in muscles, kidney respectively. Therefore, the present study concluded that the deltamethrin alters the membrane permeability of ATPases, resulting in the breakdown of the active transport mechanism and would cause the alterations in physiology of whole organisms leading to death.

Keywords: *Deltamethrin, Sub-lethal Concentration, ATPase, Adenylate Nucleotides, Labeo rohita, Energy Charge*

INTRODUCTION
Tremendous increase in use of various pesticides to protect the crops that ultimately enhances the production in order to meet the global problem that ever growing demand for food of increasing population (Chandola *et al.* 2011). Among the different pesticides, pyrethroid group is widely used across the globe in general including India in particular (Sapna and Gupta, 2014). Unfortunately, non biodegradable pesticides are widely used for the agriculture fields and they could persist in environment for longer time as toxicants (Ngidlo, 2013). Indiscriminate use of these pesticides in agricultural fields triggers a series of environmental changes that may affect aquatic and land dwelling animals (Hussain *et al*. 2011, David *et al.* 2013) and also accumulated in agricultural based food materials (Chebbi and David 2009). However in third world nations, every year more than 25-77 million poison cases (Zhang *et al.* 2011) as well as 0.22 million casualties (Yashmashito *et al.*1997) have been reported due to pesticide infiltration.
Deltamethrin (DM) a pyrethroid synthetic pesticide has a huge acceptability for agricultural use due to their improved potency, photo stability and low biodegradability (Laskowski, 2002). It is severely toxic to both freshwater and marine forms (Vani et al. 2011) and thus interfering nerve transmission influx Bradberry *et al.* 2005). Hence it involves sodium (Na) channels at the axon

level which is a common effect on wide range of animals, by nerve blocking intermittently, and also extend the time interval of the impulse and ultimately lead to spasm, muscular paralysis and death (WHO 2005).

The potential biomarkers have been used to analyze type-II pyrethroid toxicity and other enzyme metabolisms in experimental fish (Vani et al. 2011). ATPases are involved in many physiological functions i.e., maintains of cellular water balance and osmo-regulation (Tucker and Matte 1998) pathological and toxicological processes. So variations in the activity of ATPases of synthetic peptides act as measurement of the toxic impact (Cotou et al. 2001). Many researchers have been addressed on the adverse effects of deltamethrin in fishes with reference to hematological and biochemical variables Srivastava *et al.* 1997, Kumar *et al.* 1999 and David *et al.* 2013). Therefore the information on deltamethrin impact on organ specific ion-regulatory enzymes in fresh water fish was very scanty. Hence an attempt was made to investigate the possible alterations in the levels of adenylate nucleotide with reference to the charge of Na^{+},-K^{+}, Mg $^{2+}$, Ca^{2+} ATPases and total ATPase in different tissues of fresh water fish *Labeo rohita* on exposure of sub-lethal concentrations of deltamethrin.

MATERIALS AND METHODS

Chemicals

Deltamethrin (DM) was selected to analyze its effect on energy charge and ATPase levels of freshwater fish *Labeo rohita,* because of uncontrolled usage in agricultural practices to manage the pests. It has enhanced photo stability leads to the lower the degradability and non-persistents. Sub-lethal concentration of deltamethrin for *Labeo rohita* was determined as the 1/10 of LC50/96hrs *i.e.*, 0.009ppb (Marali *et al.*2017).

Fish Maintenance and Treatments

The live fish weighed about 10±2 gm were purchased from local fisheries in and around Anantapuram, Andhra Pradesh, INDIA. To maintain the live fish, they stored in aquaria by frequent water changes and acclimatized to the laboratory conditions for a period of 10 days by providing natural photoperiod with ambient temperature. physico–chemical characteristics of the tap water were analyzed by standard protocols. The deltamethrin of commercial grade was obtained from local market, and fishes were subjected to expose at sub-lethal concentration of deltamethrin for 24, 48, 72 and 96 h with controls.

Sample Collection

Blood samples were collected from control and experimental groups by cardiac puncture. The collected blood was heparinized and placed in vials for freezing. Centrifuged the blood for 15 min. at 10,000 rpm and plasma was separated and used for further analysis. Later, the fish were cut and open to collect the internal organs like liver, muscle, brain, kidney and gills aseptically to assess the energy fluctuations and ATPase activity.

Analytical Methods

The energy changes and ATPase activity were investigated from the collected organs of exposed and control fish using the standard protocols at specified time intervals. Each experiment was carried out in triplicates and statistically analyzed.

Adenylate nucleotide levels and energy charge analysis

The concentration of adenylate nucleotides like ATP, ADP and AMP were tested separately in the liver, muscle, brain, kidney and gills of *Labeo rohita* at different exposure periods along with suitable controls. Prior to the estimation, to stop the movements of fish and thereby in order to keep the nucleotide concentrations unchanged, the fish were anaesthetized with methane

sulphonate (MS 222) and transferred to heptan (-90^{O}C) and thereafter the fish were put immediately in the liquid air and were frozen to death. Then the frozen tissues namely liver, muscle, brain, kidney and gills were separated and ground to a fine powder separately. Separate homogenates were prepared in ice cold 60% perchloric acid solution. The homogenate was centrifuged for 15min and the supernatants were neutralized to pH 7.4 and further supernatants used for determination of adenylate nucleotides such as ATP, ADP and AMP (Bergmayer 2012) and analyzed spectrophotometrically at a wavelength of 360nm and the ATP, ADP and AMP concentrations were expressed in moles/ gm.wet .wt.

Change in energy charge

The adenylate energy charge (A. E. C) values were calculated by the following formula as given by Atkinson and Walton [25].

AEC = ATP + ½ ADP/ATP+ADP+AMP

ATPase

Separate analysis of metallic ion bound (Na^{+}, -K^{+}, Mg^{2+} and Ca^{2+}) ATPase and total ATPase activities were estimated in fish organs of test and controls.

Estimation of Na^{+}, -K^{+}, Mg^{2+} and Ca^{2+} ATPase and total ATPase activities (EC: 3.6.1.3)

Separate estimation of Na^{+}, -K^{+}, Mg^{2+} and Ca^{2+} ATPase activities were estimated in the tissues by the method described by Watson and Beamish (Watson and Beamish 1981) with slight modifications. 1% Tissue homogenate were prepared in 0.25 M ice-cold sucrose solution containing 5mM EDTA in 40 mM tris-HCl buffer with pH 7.5 and 0.01M imidazole. The homogenates were centrifuged at 2500rpm for 10 min and the supernatant used for crude enzyme assay of diversified ATPase activities.

Enzyme standardization were used for kinetic parameters by maintain three sets. In a total volume of 2ml, the first set consists of 100mM disodium adenosine triphosphate (prepared in 20mM tris-HCl buffer at pH 7.5), 100mM NaCl, 20mM KCl, 3mM $MgCL_{2,}$ and 0.3ml of enzyme crude extract; the second set composed of 100mM disodium adenosine triphosphate, 100mM NaCl, 20mM KCl, 3mM $MgCL_{2,}$ 1mM ouabain (potent inhibitor of Na^{+}, K^{+} ATPase) and 0.3ml of enzyme crude extract; the third set contains 100mM disodium adenosine triphosphate, 5mM $CaCl_{2,}$ and 0.3ml of enzyme extract. All the sets were incubated at 37^{0}C for exactly 15 min there after arrest the reaction by adding 2ml of cold 10% TCA. The inorganic phosphates liberated were estimated by the method of Fiske and subba row (Fiske and Subbarao 1981). The absorbance was read at 660nm along with blank. Disodium salt of ATP was used as co-factor to detect sodium salt stimulated activity.

The first set gives the total ATPase activities of Na^{+}, -K^{+} and Mg^{2+}. Second set gives only the Mg^{2+} ATPase, Hence the Na^{+}, -$K^{+,}$ ATPase activity was derived by subtracting the Mg^{2+} ATPase from the total of Na^{+}, -K^{+} and Mg^{2+} ATPase activities. The third set directly gives the Ca^{2+} ATPase activity. All the ATPase activities are expressed as µM Pi liberated / mg protein /h.

Statistical Analysis

Averages of six individuals for each experiment used for toxicity analysis. Hence triplicates were maintained for each set of experiment and the estimated values were calculated statistically by Mean, ±SD, standard error, percent changes and one-way ANOVA.

RESULTS AND DISCUSSION

Energy Rich Nucleotides and Deltamethrin Toxicity

The ATP and ADP concentrations initially increased with the concomitant decrease of AMP concentrations in brain, gill, kidney, liver and muscle of *Labeo rohita* during 24h and 48 h of

exposure of sub lethal concentration (0.009ppb) of deltamethrin. But reverse trend was noticed between ATP, ADP and AMP levels in all the tested tissues in 72h and 96h. Which indicates that the variations in all the nucleotide levels were remarkably showed an inverse relationship (Table 1, 2 and 3) (Figure. 1, 2 and 3). From the tables it was reported that the percent change of ATP levels were increased at 48h in muscle (29.008%) and gradually decreased at 96h in gills (2.890%). However the ADP and AMP levels were also significantly fluctuated and monitored that the 48h of exposure was increased in gills (45.358%), and decreased at 96h in brain (10.927%) and the 48h of exposure tremendously decreases in gills (-55.434%) and correspondingly the AMP levels were also recovered at 96h of exposure in brain (7.031%) (Table 2 & 3).

Adenylate nucleotides are known as primary energy source and served as allosteric modulators in many important metabolic reactions. The toxicity studies were also representing the levels of these nucleotides apparently decreased influenced by synthetic pesticide compounds. Therefore, the present investigation extensively reported that the estimated levels of adenylate nucleotides (ATP, ADP and AMP) and energy levels available in different tissues of the fish could be considered as an indicator of the pollution extent of aquatic environment. Similar to the present study, it has also been observed that cypermethrin play an important role in order to decrease the levels of nucleotides at different time intervals of its exposure in various tested tissues (Osman and Rabia 2014).

However the other investigators also noticed that the test and control tissues was elevated the levels of ATP, ADP and energy charge followed by decrease in AMP in different organs of *C. striatus* by exposure to sub-lethal concentration of Cypermethrin. In spite of this the maximum levels of ATP and energy charge of all the tested tissues of *C. striatus* during the pesticide exposure, which reflects the greater activity of mitochondrial electron transport system and also increasing the level of ATP turnover and energy (ATP) expenditure. Whereas the variations in energy rich nucleotide like ATP, ADP and their charge levels are ultimately correlated to the energy demands of the fish as suggested by Clark (Clark 1982 and Clark). Further the results obtained from the present investigation showed by the sub lethal exposure of the toxicants to the osmo regulatory tissues like kidney and gill are more affected then the non-osmo regulatory tissues viz., brain, liver and muscle.

Energy Charge Variations

The variations in the energy charge exhibited the same trend as that of the changes observed in the concentration of ATP. Whenever there is an increase in ATP concentration, as described earlier there is a corresponding increase in the energy charge. Thus the energy charge exhibits linear relationship with energy rich nucleotide ATP in all the tissues during different sub-lethal exposure periods of deltamethrin (Table 4 and Figure. 4). Like that of ATP concentration, the energy charge initially increased at 24h and 48h, later there is a stringent decrease in the energy charge at the remaining exposure levels at 72h and 96h.

The energy charge among the different tissues varied from each other in a similar way as noticed in ATP concentration. From the table 4 it was clearly noticed that the osmoregulatary tissues namely gill (10.451%) and kidney (10.109%) exhibited higher percentage change in energy charge than non osmo regulatory tissue of brain (6.908%), liver (7.448%) and muscle (6.483%). The level of energy charge depends upon the amount of ATP available in the tissues. Greater the ATP level that ultimately enhances the energy charge. On the whole, the inter tissue differences were found to be significant. Correspondingly our results were correlated with the findings of Atkinson (Atkinson 1968) who refers the ATP, ADP and AMP status of a cell as its energy

charge. Even a slight decrease in charge levels leads to many profound variations in the concentration of many metabolites.

Na^+, -K^+, Mg^{2+}, Ca^{2+} ATPase and total ATPase

The present investigation reveals that the activities of Na^+, -K^+, Mg^{2+}, Ca^{2+} and total ATPase (µMPi/mg/protein/h) from the tissues of brain, gills, kidney, liver and muscle of *Labeo rohita* exposed to sub-lethal concentration of deltamethrin at 24, 48, 72 and 96h, along with controls are represented in Tables 5, 6, 7 and 8 & Figures. 5, 6, 7 and 8. From the tables it was conformed that the comparison as well as the major differences were obtained in each parameter of the tested tissue of the fish at the said exposure period as well as the deltamethrin concentrations were converted as percentages. From the figures it was noticed that the maximum decrease of Na^+, -K^+, ATPase (-43.607%) at 48h in brain tissue, similarly the regeneration activity was recorded at 96h of exposure with liver tissue (-7.952%). Further Mg^{2+}, Ca^{2+} and total ATPase were tested for their activity, the maximum decrease levels of Mg^{2+}, Ca^{2+} and total ATPase were observed at 72h in muscles (-36.947%), 48h in liver (-24.542%), 48h in brain (-24.630%) respectively. Simultaneously the recovery levels were also noticed with Mg^{2+} ATPase at 96h in muscles (-0.841%), Ca^{2+} ATPase at 96h in kidney (-1.000%) as well as total ATPase at 96h in kidney (-2.608%).

The predominant function of Na^+/K^+-ATPase present in the baso-lateral membrane of gill epithelial cells was the electrolyte transport across the gills (Parvez *et al.* 2006), It is more prominent marker used for pollutant-induced osmoregulatory changes (Stagg *et al.* 1992 and Mathan *et al.* 2010). In the present study Na^+, -K^+ ATPase activities measured in order of gill > brain > kidney > muscle > liver. The decreased level of this enzyme indicates the demolition of cellular ionic regulation in the organs of the fish as reported by Marigoudar (Atkinson 1968).

It is presumed that Mg^{2+}ATPase have a unique role in energy synthesis present in all types of cells (Dogan *et al.* 2015), and is responsible for the trans epithelial regulation of Mg^{2+} ions, which is essential for cellular integrity, intracellular cements and the stabilization of bronchial permeability (Parvez *et al.* 2006). In general Mg^{2+}-ATPase are taken as an index of total ATPase activity because of its abundant distribution, dual localization in mitochondria and cytosol (Lehninger and Albert 1988). In the present investigation Mg^{2+} ATPase activities are quite different *i.e.*, liver > gill > muscle > brain > kidney. Shwetha (Fonseca et al. 2020) have suggested that the decrease in Mg^{2+}ATPase activity might be due to the damage of the mitochondria membranes, which may interfere with the conversion of oxidative energy to phosphate bond energy. The results of our study the present study was correlated with findings of Suvetha (Suvetha *et al.* 2010) observed inhibition of the gill ATPase activity in *C. carpio* exposed to cypermethrin.

Interestingly, we presumed to report that the Ca^{2+} ATPase activities are similar to the Na^+, -K^+ ATPase activities and followed the same order. A similar decrease order in Ca^{2+} ATPases has been reported by several other researchers Okolie and Audu (2004) and Unnisa and Devaraj (2007) observed that cyanide specifically inhibits the activity of Ca^{2+}ATPase in fish. Since this enzyme is directly involved in the oxidative phosphorylation the action of pesticide on this system correlates with toxicity of pesticide. Relatives to the controls, the Na^+, -K^+, $Mg^{2+}$and Ca^{2+} ATPase activities decreased significantly in the tissues of the fish exposed to the sub-lethal concentrations of deltamethrin. However, in all the organs of fish, the activities of the above said ATPases increased significantly from 48h exposure to 72h exposure. The increased activities were not observed in both test and control tissues. Being the functional stabilities of all these three ATPase activities, the total ATPase activity followed the same trend in all the organs of the

fish exposed to sub-lethal concentration of deltamethrin at respctive exposure periods (Table 8 and Figure 8). Similar to the present investigation, Dogan *et al.,* (2015) explained that individual and combined exposure to Ca^{2+} and Pb^{2+} altered the ATPase activities in tissues of *O. niloticus*. ATPases were considered as an index to determine the tolerance of oligodynamic components in environmental pollutions. The membrane localization is the key part to the physiological function of ATPases. Several other metal and non metallic compounds have been demonstrated as inhibitors of ATPases (Berrocal *et al.* 2021). Parvez *et al*. (2006) noticed that increasing of the exposure time of cyanide which causes a very less activity of ATPases in the tissues, especially in liver and muscle because of cyanide induced effect on the cell membrane. Jyoti and Rahul (2014) indicated that the time of exposure and duration depends on alterations of membrane bound ATPases. The inner and outer mitochondrial membranes consists of unsaturated lipids and the mitochondria are also highly susceptible to arsenic attack as well as by the free radicals produced by the other organelles Ramanathan *et al.* (2003) Hemalatha *et al.* (2013) reported that the significant reduction of Na+- K+ ATPase and Mg2+ ATPase levels in rat liver exposed to arsenic due to hepatotoxicity by inducing oxidative stress.

Table 1: Alterations in ATP concentrations (µ M/g of wet wt) in different tissues of the fish, *Labeo rohita*, exposed to sub lethal concentration of deltamethrin during different exposure periods.

S.No.	Name of the tissue		Exposure periods in hours				
			Control	24	48	72	96
1.	Brain	Mean	4.510	5.49	5.594	5.023	4.850
		SD	0.250	0.200	0.250	0.141	0.170
		% Change		(21.752)	(24.035)	11.374	7.539
		t Test		< 0.001	< 0.001	< 0.01	< 0.05
2.	Gill	Mean	2.630	3.022	3.093	2.984	2.706
		SD	0.180	0.121	0.130	0.26	0.192
		% Change		(14.905)	(17.605)	(10.038)	(2.890)
		t Test		< 0.001	< 0.001	< 0.05	N.S
3.	Kidney	Mean	3.282	3.820	4.014	3.860	3.411
		SD	0.124	0.161	0.281	0.264	0.215
		% Change		(16.462)	(22.255)	(17.682)	(3.890)
		t Test		< 0.001	< 0.001	< 0.001	N.S
4.	Liver	Mean	3.420	4.210	4.330	3.940	3.630
		SD	0.294	0.120	0.160	0.180	0.050
		% Change		(23.099)	(26.608)	(15.205)	(6.140)
		t Test		< 0.001	< 0.001	< 0.01	N.S
5.	Muscle	Mean	2.520	3.150	3.251	2.980	2.612
		SD	0.196	0.131	0.142	0.194	0.041
		% Change		(24.999)	(29.008)	(18.254)	(3.651)
		t Test		< 0.001	< 0.001	< 0.01	N.S

S.D : Standard deviation; N.S : Not significant
Each value is a six estimations. The % change over control is given in parenthesis.

Table 2: Alterations in ADP concentrations (µ M/g of wet wt) in different tissues of the fish, *Labeo rohita*, exposed to sub lethal concentration of deltamethrin during different exposure periods.

S.no	Name of the tissue		Exposure periods in hours				
			Control	24	48	72	96
1.	Brain	Mean	3.011	3.560	3.824	3.697	3.340
		SD	0.173	0.210	0.202	0.230	0.300
		% Change		(18.233)	(27.000)	(22.783)	(10.927)
		t Test		< 0.001	< 0.001	< 0.001	< 0.05
2.	Gill	Mean	1.325	1.780	1.926	1.813	1.680
		SD	0.183	0.070	0.162	0.215	0.068
		% Change		(34.340)	(45.358)	(36.830)	(26.792)
		t Test		< 0.001	< 0.001	< 0.01	< 0.001
3.	Kidney	Mean	1.820	2.365	2.581	2.360	2.098
		SD	0.240	0.142	0.062	0.081	0.162
		% Change		(29.945)	(41.813)	(29.670)	(15.274)
		t Test		< 0.001	< 0.001	< 0.001	< 0.05
4.	Liver	Mean	2.791	3.640	3.888	3.400	3.249
		SD	0.042	0.320	0.364	0.240	0.145
		% Change		(30.419)	(39.305)	(21.820)	(16.410)
		t Test		< 0.001	< 0.001	< 0.001	< 0.001
5.	Muscle	Mean	1.680	2.171	2.320	2.104	1.986
		SD	0.180	0.042	0.124	0.106	0.040
		% Change		(29.226)	(38.095)	(25.238)	(18.214)
		t Test		< 0.001	< 0.001	< 0.001	< 0.01

S.D: Standard deviation; N.S: Not significant
Each value is a six estimations. The % change over control is given in parenthesis.

Table 3: Alterations in AMP concentrations (µ M/g of wet wt) in different tissues of the fish, *Labeo rohita*, exposed to sub lethal concentration of deltamethrin during different exposure periods.

S.no	Name of the tissue		Exposure periods in hours				
			Control	24	48	72	96
1.	Brain	Mean	1.920	1.784	1.630	1.712	2.055
		SD	0.132	0.05	0.111	0.032	0.034
		% Change		(-7.083)	(-15.104)	(-10.833)	(7.031)
		t Test		< 0.05	< 0.01	< 0.01	< 0.05
2.	Gill	Mean	0.920	0.522	0.410	0.563	0.736
		SD	0.190	0.031	0.062	0.040	0.063
		% Change		(-43.260)	(-55.434)	(-38.804)	(-20.000)
		t Test		< 0.001	< 0.001	< 0.01	< 0.05
3.	Kidney	Mean	1.413	1.022	0.890	1.088	1.240
		SD	0.180	0.110	0.063	0.042	0.066
		% Change		(-27.671)	(-37.013)	(-23.000)	(-12.243)

		t Test		< 0.001	< 0.001	< 0.01	< 0.05
4.	Liver	Mean	1.421	1.183	1.036	1.218	1.271
		SD	0.152	0.034	0.072	0.144	0.034
		% Change		(-16.749)	(-27.094)	(-14.286)	(-10.556)
		t Test		< 0.01	< 0.001	< 0.01	< 0.05
5.	Muscle	Mean	1.247	1.191	1.141	1.171	1.202
		SD	0.042	0.043	0.027	0.062	0.027
		% Change		(-4.491)	(-8.500)	(-6.095)	(-3.609)
		t Test		< 0.05	< 0.001	< 0.05	< 0.05

S.D: Standard deviation; N.S: Not significant
Each value is a six estimations. The % change over control is given in parenthesis.

Table 4: Alterations in energy charge in different tissues of the fish, *Labeo rohita*, exposed to sub lethal concentration of deltamethrin during different exposure periods.

S.no	Name of the tissue		Exposure periods in hours				
			Control	24	48	72	96
1.	Brain	Mean	0.637	0671	0.681	0.652	0.642
		SD	0.020	0030	0.021	0.010	0.045
		% Change		(6.907)	(6.908)	(2.355)	(0.785)
		t Test		< 0.05	< 0.01	N.S	N.S
2.	Gill	Mean	0.675	0.735	0.747	0.721	0.692
		SD	0.032	0035	0.029	0041	0057
		% Change		(8.709)	(10.451)	(6.677)	(2.468)
		t Test		< 0.01	< 0.01	< 0.05	N.S
3.	Kidney	Mean	0.643	0.694	0.708	0.690	0.661
		SD	0.010	0.022	0.031	0.035	0.015
		% Change		(7.931)	(10.109)	(7.309)	(2.799)
		t Test		< 0.001	< 0.001	< 0.01	< 0.05
4.	Liver	Mean	0.631	0.667	0.678	0.659	0.648
		SD	0.009	0.008	0.019	0.012	0.011
		% Change		(5.705)	(7.448)	(4.437)	(2.694)
		t Test		< 0.001	< 0.001	< 0.001	< 0.01
5.	Muscle	Mean	0.617	0.650	0.657	0.645	0.622
		SD	0.031	0.003	0.012	0.002	0.011
		% Change		(5.348)	(6.483)	(4.538)	(1.00)
		t Test		< 0.05	< 0.01	< 0.05	< 0.05

S.D: Standard deviation; N.S: Not significant
Each value is a six estimations. The % change over control is given in parenthesis.

Table 5: Alterations in Na^+, -K^+ATPase activity levels (µ M Pi/mg protein/h) in different tissues of the fish, *Labeo rohita*, exposed to sub lethal concentration of deltamethrin during different exposure periods.

S.no	Name of the tissue		Exposure periods in hours				
			Control	24	48	72	96
1.	Brain	Mean	6.210	4.400	3.502	3.750	4.113
		SD	1.062	1.021	1.041	1.062	1.012
		% Change		(-29.147)	(-43.607)	(-39.613)	(-33.768)
		t Test		< 0.01	< 0.001	< 0.01	< 0.01
2.	Gill	Mean	7.823	5.904	4.810	5.922	6.342
		SD	1.071	1.080	1.067	1.073	1.024
		% Change		(-24.530)	(-38.515)	(-24.300)	(-18.931)
		t Test		< 0.01	< 0.001	< 0.01	< 0.05
3.	Kidney	Mean	4.650	3.710	2.952	3.631	3.984
		SD	1.812	0.630	0.510	0.410	0.126
		% Change		(-20.215)	(-36.516)	(-21.914)	(-14.323)
		t Test		< 0.05	< 0.01	< 0.05	N.S
4.	Liver	Mean	3.760	3.250	3.124	3.292	3.461
		SD	0.290	0.300	0.280	0.310	0.160
		% Change		(-13.564)	(-16.915)	(-12.447)	(-7.952)
		t Test		< 0.01	< 0.01	< 0.05	< 0.05
5.	Muscle	Mean	4.520	4.030	3.751	3.984	4.136
		SD	0.420	0.300	0290	0.320	0.411
		% Change		(-10.840)	(-17.013)	(-11.858)	(-8.496)
		t Test		< 0.05	< 0.01	< 0.05	N.S

S.D: Standard deviation; N.S: Not significant
Each value is a six estimations. The % change over control is given in parenthesis.

Table 6: Alterations in Mg^{2+} ATPase activity levels (µ M Pi/mg protein/h) in different tissue of the fish, *Labeo rohita*, exposed to sub lethal concentration of deltamethrin during different exposure periods.

S.no	Name of the tissue		Exposure periods in hours				
			Control	24	48	72	96
1.	Brain	Mean	4.000	3.629	3.312	3.523	3.821
		SD	0.204	0.356	0.348	0.264	0.290
		% Change		(-9.275)	(-17.200)	(-11.925)	(-4.475)
		t Test		< 0.05	< 0.01	< 0.01	N.S
2.	Gill	Mean	4.900	3.952	3.741	4.023	4.406
		SD	0.322	0.294	0.423	0.310	0.368
		% Change		(-19.343)	(-23.653)	(-17.898)	(-10.082)
		t Test		< 0.001	< 0.001	< 0.001	< 0.05
3.	Kidney	Mean	3.751	3.124	2.982	3.241	3.572
		SD	0.210	0.380	0.421	0.244	0.360
		% Change		(-16.716)	(-20.501)	(-13.596)	(-4.772)

		t Test		< 0.01	< 0.01	< 0.01	N.S
4.	Liver	Mean	6.981	5.850	5.482	5.724	6.213
		SD	0.722	0.246	0.243	0.411	0.267
		% Change		(-16.201)	(-21.473)	(-18.006)	(-11.001)
		t Test		< 0.01	< 0.001	< 0.001	< 0.05
5.	Muscle	Mean	4.520	4.151	3.620	4.353	4.482
		SD	0.220	0.294	0.336	0.224	0.315
		% Change		(-8.164)	(-19.912)	(-36.947)	(-0.841)
		t Test		< 0.05	< 0.001	N.S	N.S

S.D: Standard deviation; N.S: Not significant
Each value is a six estimations. The % change over control is given in parenthesis.

Table 7: Alterations in Ca^{2+} ATPase activity levels (µ M Pi/mg protein/h) in different tissues of the fish, *Labeo rohita*, exposed to sub lethal concentration of deltamethrin during different exposure periods.

S.no	Name of the tissue		Exposure periods in hours				
			Control	24	48	72	96
1.	Brain	Mean	5.980	5.440	4.911	5.080	5.320
		SD	0.430	0.392	0.445	0.320	0.415
		% Change		(-9.030)	(-17.876)	(-15.050)	(-12.541)
		t Test		< 0.05	< 0.01	< 0.01	< 0.05
2.	Gill	Mean	8.750	8.102	7.184	7.703	8.031
		SD	0.620	0.360	0.320	0.710	0.310
		% Change		(-7.406)	(-17.897)	(-11.966)	(-8.217)
		t Test		< 0.05	< 0.01	< 0.01	< 0.001
3.	Kidney	Mean	5.021	4.814	4.566	4.770	4.982
		SD	0.151	0.121	0.171	0.125	0.191
		% Change		(-4.123)	(-9.062)	(-4.999)	(-1.000)
		t Test		< 0.05	< 0.001	< 0.01	N.S
4.	Liver	Mean	2.783	2.635	2.100	2.264	2.408
		SD	0.098	0.083	0.236	0.158	0.125
		% Change		(-5.318)	(-24.542)	(-18.649)	(-13.475)
		t Test		< 0.01	< 0.001	< 0.001	< 0.001
5.	Muscle	Mean	4.982	4.660	4.055	4.284	4.493
		SD	0.251	0.246	0.235	0.241	0.236
		% Change		(-6.463)	(-18.607)	(-14.010)	(-9.815)
		t Test		< 0.05	< 0.001	< 0.001	< 0.01

S.D: Standard deviation; N.S: Not significant
Each value is a six estimations. The % change over control is given in parenthesis.

Table 8: Alterations in Total ATPase activity levels (µ M Pi/mg protein/h) in different tissue of the fish, *Labeo rohita*, exposed to sub lethal concentration of deltamethrin during different exposure periods.

S.no	Name of the tissue		Exposure periods in hours				
			Control	24	48	72	96
1.	Brain	Mean	16.220	13.521	12.225	13.152	13.654
		SD	0.962	0.638	0.882	0.653	0.541
		% Change		(-16.634)	(-24.630)	(-18.150)	(-15.820)
		t Test		< 0.001	< 0.001	< 0.001	< 0.001
2.	Gill	Mean	20.930	17.520	16.830	17.450	18.630
		SD	1.029	0.980	1.026	1.031	1.035
		% Change		(-16.292)	(-19.589)	(-16.627)	(-10.989)
		t Test		< 0.001	< 0.001	< 0.001	< 0.01
3.	Kidney	Mean	13.420	11.642	10.451	12.030	13.070
		SD	1.261	1.114	1.151	1.030	0.862
		% Change		(-13.249)	(-22.124)	(-10.358)	(-2.608)
		t Test		< 0.05	< 0.01	< 0.01	N.S
4.	Liver	Mean	13.490	12.220	10.450	11.630	13.010
		SD	1.171	0.780	0.670	0.730	0.724
		% Change		(-9.414)	(-22.535)	(-13.788)	(-3.558)
		t Test		< 0.05	< 0.001	< 0.01	N.S
5.	Muscle	Mean	14.121	13.130	11.426	12.820	13.590
		SD	0.941	0.520	0.429	0.380	0.042
		% Change		(-7.018)	(-19.085)	(-9.213)	(-3.760)
		t Test		< 0.05	< 0.001	< 0.01	N.S

S.D: Standard deviation; N.S: Not significant
Each value is a six estimations. The % change over control is given in parenthesis.

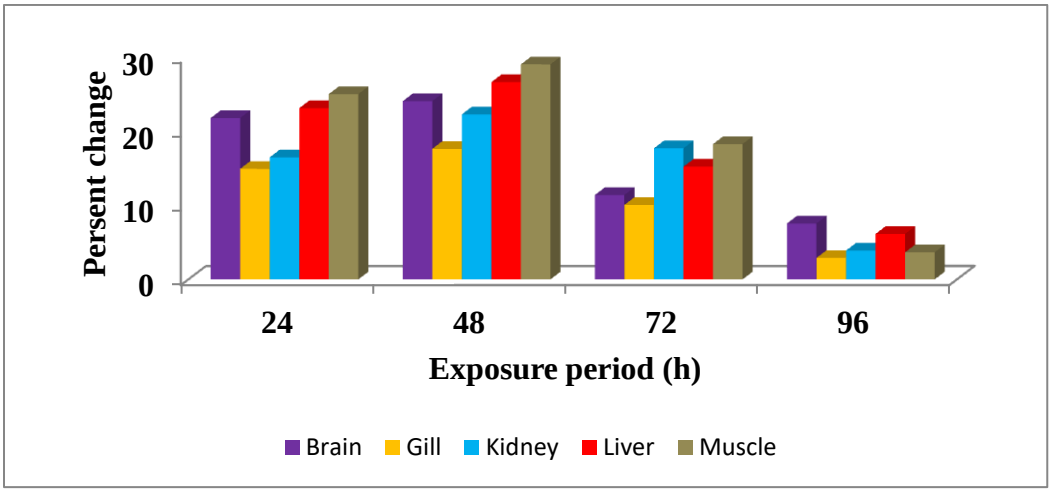

Figure 1: Percent change over control in ATP concentration in the tissue of fresh water fish. *Labeo rohita* at different exposure periods to sub lethal concentration of deltamethrin

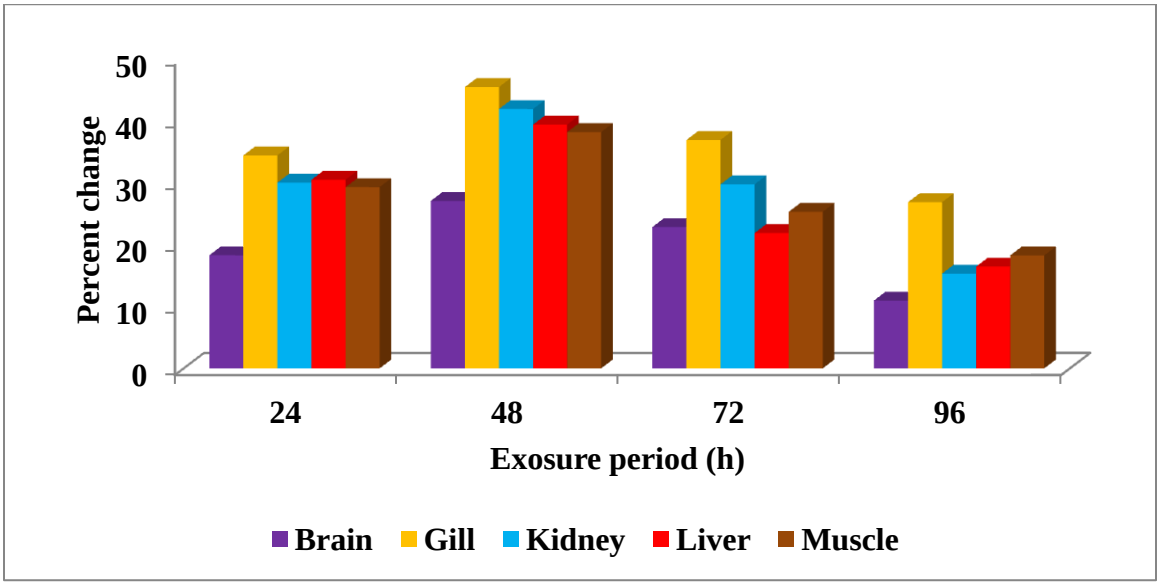

Figure 2: Percent change over control in ADP concentration in the tissue of fresh water fish *Labeo rohita* at different exposure periods to sub lethal concentration of deltamethrin

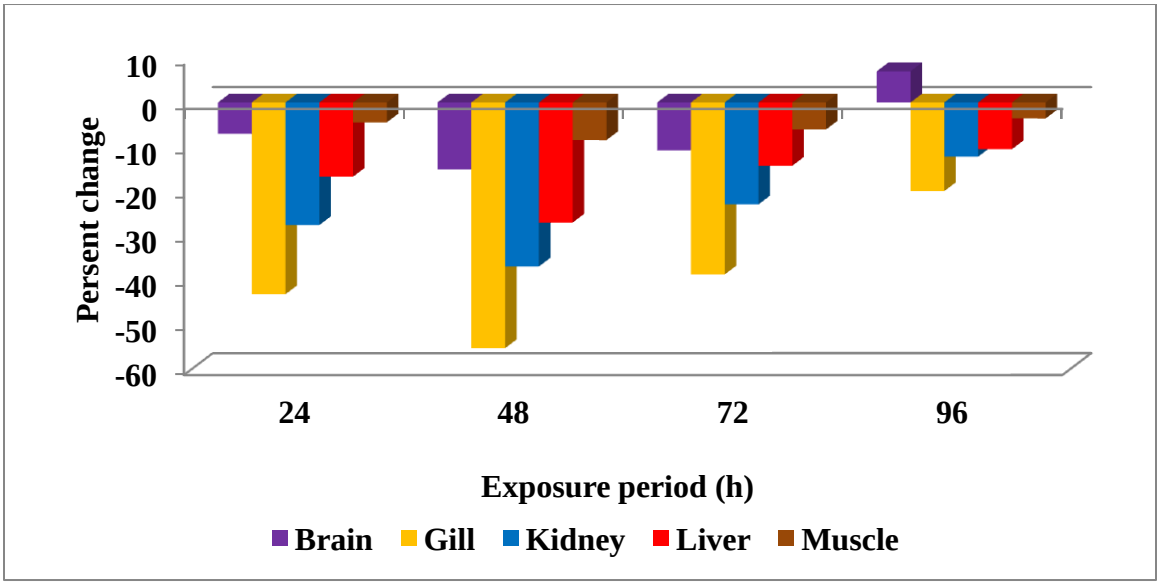

Figure 3: Percent change over control in AMP concentration in the tissue of fresh water fish. *Labeo rohita* at different exposure periods to sub lethal concentration of deltamethrin

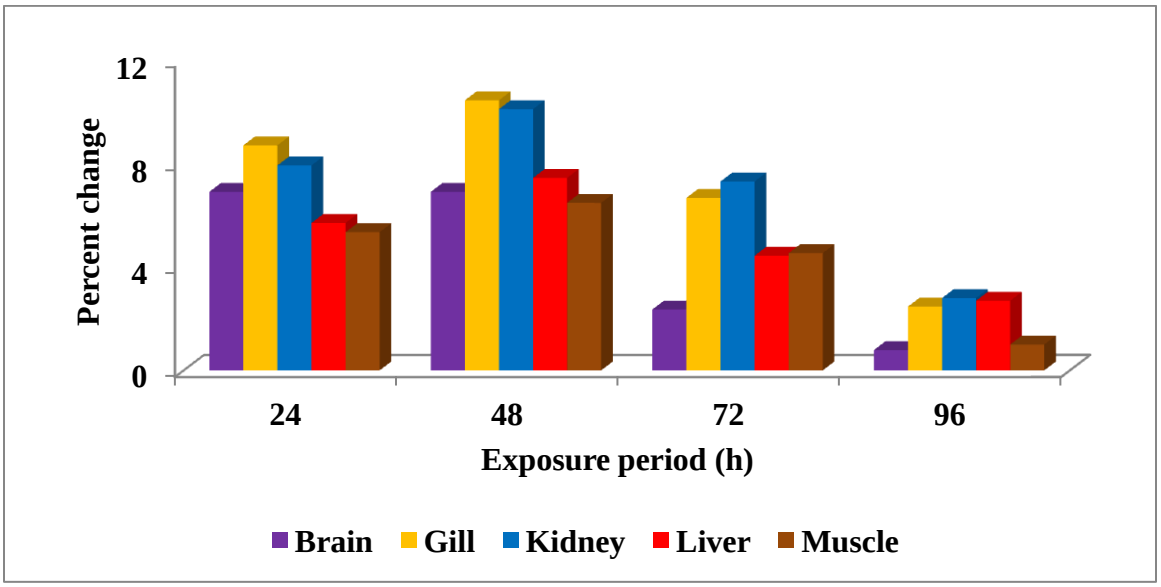

Figure 4: Percent change over control in energy charge in the tissues of fresh water fish *Labeo rohita* at different exposure periods to sub lethal concentration of deltamethrin.

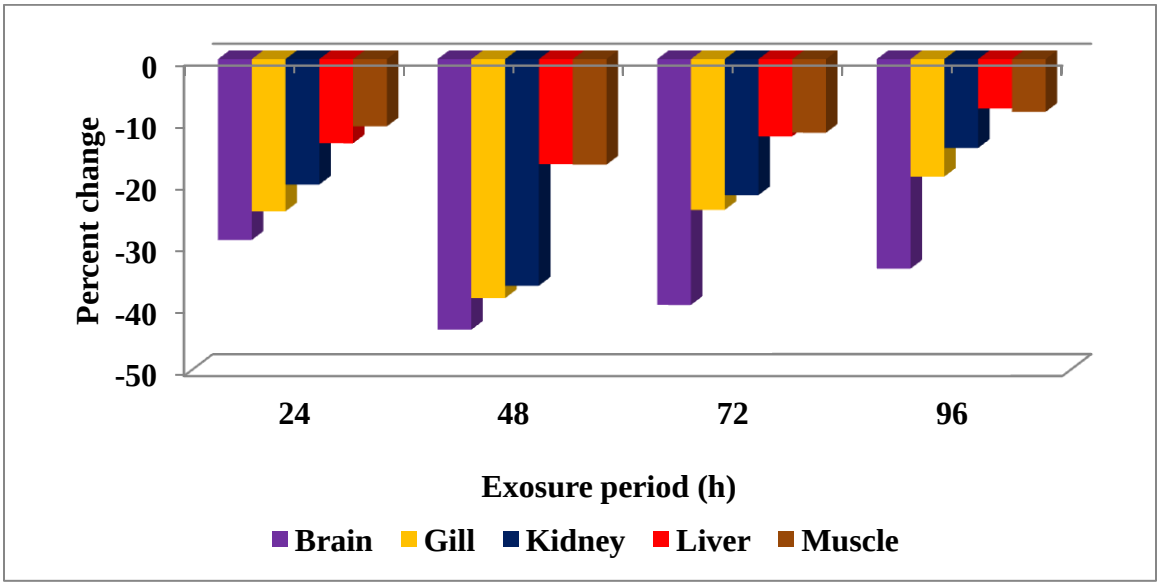

Figure 5: Percent change over control in Na^+, K^+ ATP ase in the tissues of fresh water fish *Labeo rohita* at different exposure periods to sub lethal concentration of deltamethrin.

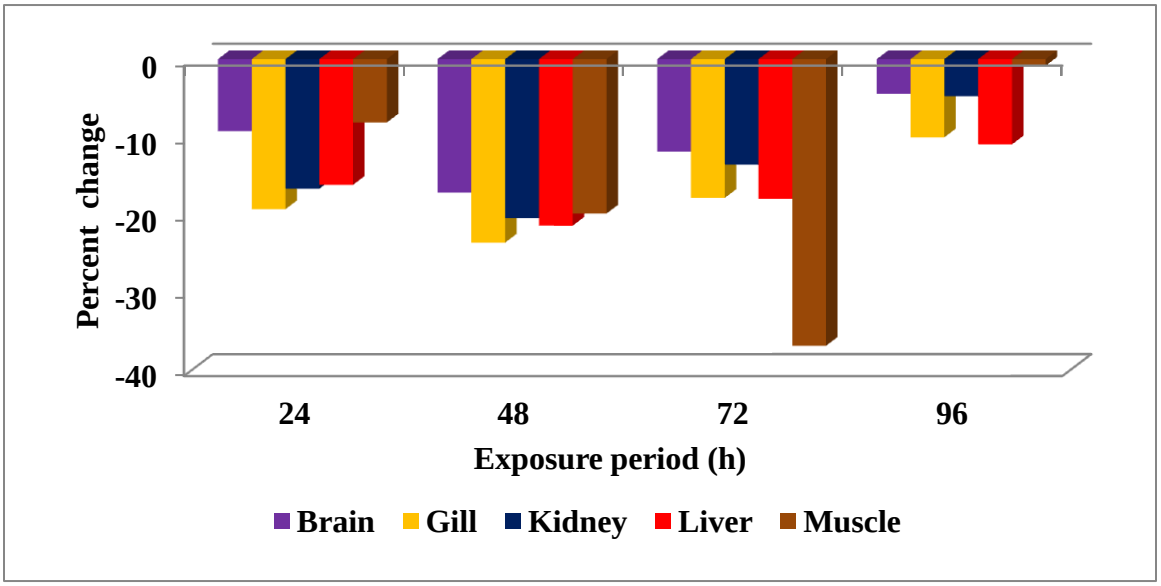

Figure 6: Percent change over control in Mg^{2+} ATPae in the tissues of fresh water fish *Labeo rohita* at different exposure periods to sub lethal concentration of deltamethrin.

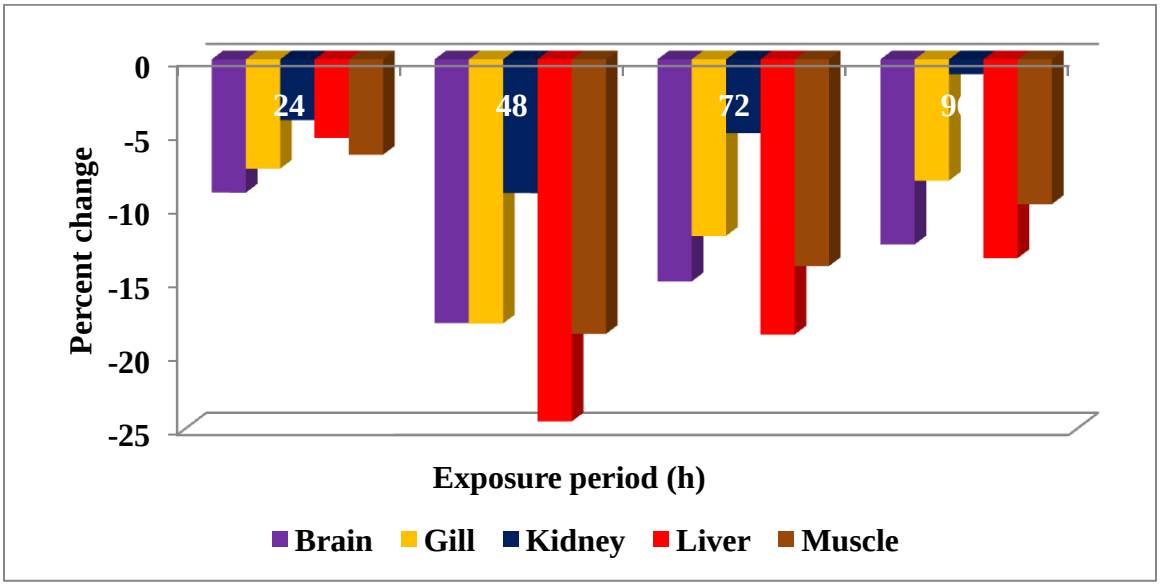

Figure 7: Percent change over control in Ca^{2+} ATPae in the tissues of fresh water fish *Labeo rohita* at different exposure periods to sub lethal concentration of deltamethrin.

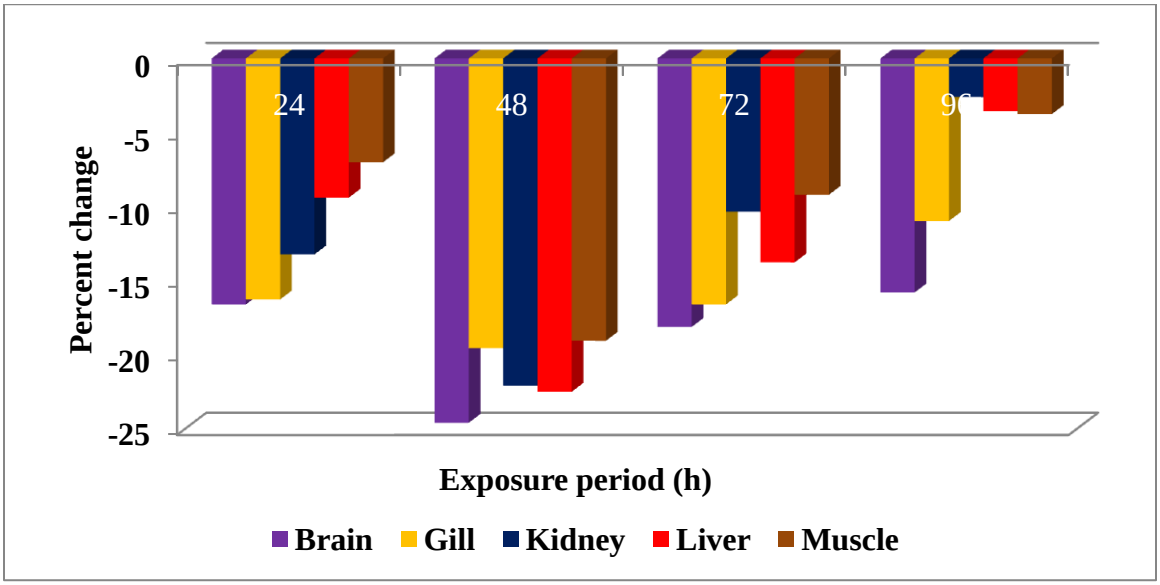

Figure 8: Percent change over control in total ATPae in the tissues of fresh water fish *Labeo rohita* at different exposure periods to sub lethal concentration of deltamethrin.

CONCLUSIONS

Sub-lethal exposure of deltametrin significantly alters the nucleotide concentrations, energy charge and various ATPase activities in exposed tissue. Thus the toxic potential of deltamethrin was clearly illustrated by increased or decreased activity levels of Na^{+}, $-K^{+}$, $Mg^{2+}$and Ca^{2+} ATPase in different tissues of exposed tissues. The decrease in percentage of energy charge is maximally noticed in brain at 96h exposure which leads to many profound variations in the concentration of several metabolites. Further it indicates that the severe disruption in the cellular ionic regulation and may have greater influence on the membrane permeability. Hence these biomarker fluctuations were also act as model study to measure the toxic impact of synthetic pesticides.

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