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Evaluation of Acute Toxicity of Karate and its Sub-lethal Effects on Protein and Acetylcholinestrase Activity in *Cyprinus carpio*

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Abstract

Karate is a locally used pesticide, has lambda cyhalothrin (λ-Cyhalothrin) as an active ingredient. To determine the acute toxicity, fry of *Cyprinus carpio* were exposed to 0.00, 0.08, 0.16, 0.2, 0.24, 0.32 and 0.4 µL L^{-1} of Karate for 96 h in a static bioassay. The 96 h LC_{50}value was determined by using Arithmetic method and found to be 0.160 µL L^{-1}. Fry of *C. carpio* were exposed to 10% (0.16 µL L^{-1}) and 20% (0.032 µL L^{-1}) lethal concentration of pesticide and observed the effects on total protein content and acetylcholinestrase (AChE) activity in brain, liver and muscle tissues. The total protein content and AChE activity in different tissues of fish decreased in concentration dependent manner and showed tissue specific pattern. The maximum reduction of AChE activity was observed in brain followed by muscle and liver tissues while liver showed higher decline in protein content as compared to muscle and brain tissues. The minimum reduction of protein content in response to Karate was observed in brain tissues. The study clearly indicated the toxicity of Karate to fish and suggest the prevention of indiscriminate use of this pesticide.

Keywords: Karate**;** λ-Cyhalothrin; Toxicity; Acetylcholinestrase; Total protein content

Introduction

The use of pesticides has increased several folds in Pakistan and is expected to increase in the forthcoming years. According to Hussain *et al*. (2002) more than one fourth (27%) of the pesticides being consumed are used on fruits and vegetables. Pesticides play an important role in modern agriculture on one hand by providing reliable, consistent and reasonably complete control against harmful pests with less cost and effort while on the other hand are considered as powerful aquatic pollutants. These chemicals can make their ways towards water reservoirs, rivers and streams, thus exerting detrimental effects on fish and other aquatic organisms (Atamnalp and Yanik, 2001; John and Prakash, 2003). Due to direct contact to pollutant, fish can act as a biological indictor of aquatic pollution and play an important role in assessing prospective risk related with contaminated aquatic environment (Lakra and Nagpure, 2009).

Nowadays, previously used pesticides have been replaced with synthetic pyrethroids like cypermethrin (CYP) and λ-cyhalothrins. Although these synthetic compounds are more beneficial pesticides, however recent reports indicates that they might be poisonous to fish and other water inhabiting organisms (Ahmad *et al*., 2012; Koprucu and Aydin, 2004; Saha and Kaviraj, 2003). The synthetic pyrethroids are widely used throughout the world for the control of insect pests in agriculture, gardens, homes and public health places (Amweg and Weston, 2005) and available in market with different brand names like Scimitar, Warrior, Karate, Icon, Demand and Matador. All these brands use λ-cyhalothrin as an active ingredient at different concentration.

The λ-cyhalothrin is a broad spectrum pyrethroid insecticide and is used for controlling variety of insect's pest on various crops. It is extensively applied in vegetable production and in cotton cultivation and the agricultural waste of these are most probably introducing λ-cyhalothrin to the land and aquatic reservoirs. During spraying on crops, some pesticide may also directly drift to water resources (Leistra *et al*., 2003). Therefore, residues of λ-cyhalothrin have been observed in runoff water, irrigation water and in their linked sediments and in surplus water resulting from residential and agriculture applications.

According to Paul and Simonin (2006), λ-cyhalothrin is poisonous to many fish and aquatic invertebrate. Data available on acute and sub-acute toxicity test clearly indicated the relevance of toxicity of pesticide with temperature (Singh *et al.,* 2010), species (Bradbury and Coats, 1989) and size of fish (WHO, 1992). The research has revealed that even at sub-lethal concentration, when fish exposed to λ-cyhalothrin, it induced biochemical and

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behavioral changes in fish, (Bao *et al*., 2007). Although due to good photostability and broad spectrum pesticidal activity, more than 520 tons of pyrethroids alone are used annually as an active ingredient in vector control programs throughout the world (Zaim and Jambulingam, 2004) but many investigators reported its fatal and neurotoxic effects to fish even at 10–1000 times lower concentration than analogous ranges in birds and mammals (Soderlund *et al.*, 2002; Jebakumar *et al.*, 1990).

The λ-Cyhalothrin appeared poisonous for many non-target aquatic organisms including fish (Paul and Simonin, 2006) and aquatic invertebrate (Mueller-Beilschmidt, 1990). It can cause neurotoxicity by making interaction with cholinergic neurotransmitter acetylcholine (ACh) (Sharbidre *et al.*, 2011; Chebbi and David, 2009; Chandra, 2008). Generally, in normal behavior and muscular function ACh after release in to synaptic cleft is hydrolyzed by an enzyme acetylcholinesterase (AChE) and synaptic transmission become terminated (Kopecka *et al*., 2004), whereas in the presence of pyrethroids, there is an accumulation of ACh due to the inhibition of enzyme AChE that result in a protracted excitatory postsynaptic potential. As a consequence, there is hyper stimulation of the muscle fibers due to over stimulation of neuron, which causes paralysis (Purves *et al*., 2008), and eventually death. Therefore, in aquatic ecotoxicological studies AChE activity is enormously used as a biomarker (Sharbidre *et al*., 2011; Kirby *et al.,* 2000).

Karate, a cheap and locally available pesticide is extensively used in Pakistan for boosting agriculture production and for elimination of pests from home, garden and laboratories. Its presence in aquatic environment and sediment may have serious impact on non-target aquatic organism including fish, common carp (*Cyprinus carpio*), a bottom dweller, and detritus feeding freshwater cyprinid. Therefore, the objectives of present study were to determine the 96 h LC_{50} value of locally available pesticide Karate for freshwater species, *C. carpio* and to investigate its sub-lethal effects on the AChE levels in liver, brain and muscle tissues of this species in order to test the hypothesis that Karate can cause neurotoxicity in fish and the effect may be mediated by its interaction with cholinergic neurotransmitter acetylcholine (ACh) and inhibition of enzyme AChE.

Materials and Methods

Healthy *C. carpio* fry, average body weight and length 2.19 g and 5.26 cm respectively were purchased from Rawal fish Hatchery Islamabad, Pakistan, and transported to the Fish laboratory, Department of Animal Sciences, Quaid-i-Azam University Islamabad in polythene bags filled with pure oxygen. The fish were transferred in the fiber circular tank containing well aerated dechlorinated water. Later, fish were acclimatized to the laboratory conditions for about 15 days before the start of the experiment. During the acclimatization period temperature was 23.5 ±0.08ºC, pH ranged from 7.5 to 8.11, oxygen concentrations was ~5.5 mg L^{-1} and ammonia was less than 0.25 ppm. During acclimatization, fish were fed twice daily up to satiation with semi moist diet containing 40% protein.

Pesticide Solution

Karate (Syngenta, PK) was purchased from local market and λ-cyhalothrin concentration was calculated on the basis of the active ingredient reported by the manufacturer (w/v, 25 g L^{-1}, 100%). The stock solution, 10 µg mL^{-1}(v/v, 100 µl 250 mL^{-1}) was prepared with 80 % acetone and used for the preparation of different concentrations 0, 0.08, 0.16, 0.18, 0.19, 0.2, 0.24, 0.32, 0.4 µL L^{-1} of pesticide for acute toxicity tests.

Acute Toxicity Test

Acute toxicity test was performed as described by Yaji *et al*. (2011). Healthy and uniform sized fish, regardless of sex were randomly selected, weighed and evenly distributed into 12 glass aquaria (60 x 30 x 30 cm), each containing 20 L of dechlorinated water. Initially, three test concentrations were selected based on literature for the determination of lethal concentration 96 h LC_{50} and experiment was conducted in replicate. All aquaria were equipped with air stones and a heater to maintain oxygen levels and a constant temperature 23.5°C. After 48 h of acclimatization, fish were exposed to different concentrations of pesticide which were 0, 0.08, 0.16, 0.18 µL L^{-1} whereas control fishes were kept in dechlorinated water only. Water quality parameters such as pH, temperature and dissolved oxygen were monitored every 24 h. The experiment was lasted for 96 h. Mortality data was recorded after every 24 h. On the basis of preliminary experiment, further five concentrations 0.19, 0.2, 0.24, 0.32, 0.4 µL^{-1}, were selected and repeated the experimental procedure for the determination of LC_{50} for 96 h.

Experimental Design

Sub-lethal concentrations i.e., one fifth (20%, 0.032 µL L^{-1}) and one tenth (10%, 0.016 µL L^{-1}) of LC_{50} for 96 h of Karate were selected for further studies. Healthy and uniform sized fish regardless of sex were randomly chosen and evenly distributed in to 9 glass aquaria. Experiment was conducted in replicate and fish were stocked at stocking density 1.5 g L^{-1}. First three aquaria served as control group whereas others were divided in to two treatment groupsI and II receiving 10 and 20% of LC50 of Karate, respectively. All aquaria were fitted with air stones and heaters for constant temperature and dissolve oxygen. After 72 h of acclimatization, fish in experimental groups were exposed to sub-lethal concentrations, whereas the control group received acetone used in the preparation of the maximum Karate concentration. After every 72 h, water from each

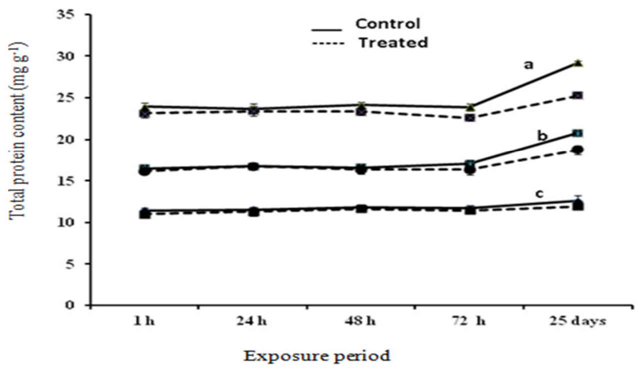

Fig. 1: Changes in total protein content in (a) liver (b) muscle and (c) brain tissues of juvenile *Cyprinus carpio* exposed to 10% LC_{50} (96 h) of Karate at different time period

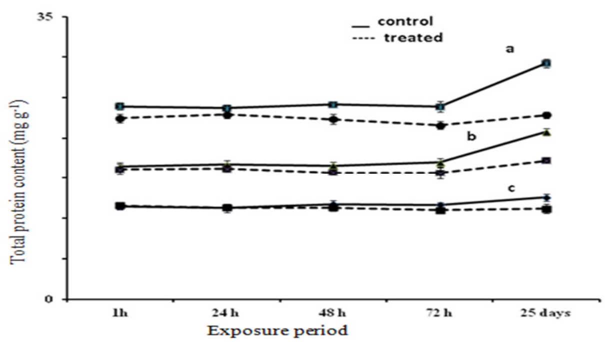

Fig. 2: Changes in total protein content (mg g^{-1}) in (a) liver (b) muscle and (c) brain tissues of juvenile *Cyprinus carpio* exposed to 20% LC_{50}(96 h) of Karate at different time period

aquarium was exchanged with fresh decholrinated water and different concentrations of pesticide were maintained afresh. The experimented was conducted for 25 days.

After 1, 24, 48, 72 h and 25 days, three fish from each aquaria were removed and anesthetized with MS222 (60 mg L^{-1}). The fish were scarified, liver, brain and muscle tissues were removed and immediately deep frozen in liquid nitrogen and saved in Ziploc bag at -20ºC for total protein content and AChE enzyme analysis.

Protein Estimation

For this purpose 90 mg tissue was homogenized in 100 mM KH_2PO_4 buffer containing 1 mM EDTA, using an ultrasonic processor. The homogenate was centrifuged at 12000 × g for 20 min at low temperature (4˚C). The supernatant was decanted in other tube and stored at -20˚C until the analysis of protein. Lowry *et al*. (1951) method was adopted for the estimation of total protein content in different tissues of Juvenile common carp, *C. carpio* while crystalline bovine serum albumin (BSA) was used as a standard.

AChE Assay

Liver, brain and muscle tissues of control and λ- cyhalothrin treated group of fish were selected for the estimation of AChE activity. Briefly, 20 mg of sample was homogenized in phosphate buffer (0.1 M, pH 7.5) and then homogenate was centrifuged at low temperature (4°C) at a speed of 5000×*g* for 10 min. The supernatant was collected in separate tube and centrifuged again at a speed of 5000×*g* for 10 min. The supernatant was collected and used for the estimation of AChE activity. AChE activities were measured using a commercially available AmpliteTM Colorimetric AChE Assay Kit, obtained from Advancing assay and test technologies, AAT Bioquest, Inc. California. All samples were run in duplicate.

Statistical Analysis

The results were presented as means ± SE. The data obtained were analyzed using one way analysis of variance followed by Tukey's multiple comparison test (HSD) in Statistic for windows Soft- ware version 8.1. Values, $P < 0.05$ were considered statistically significant.

Results

Acute Toxicity Test of Karate

The fish remained normal and healthy and no mortality was recorded in the control aquaria. However in treated groups at concentrations of 0.08, 0.16, 0.18, 0.19, 0.2, 0.24, 0.32 and 0.4 µL L^{-1} of Karate*,* the percent mortalities were 20, 50, 60, 60, 70, 70%, 100 and 100% respectively. After 96 h of exposure, the LC_{50} value for Karate on the basis of mortality of fish was calculated by using arithmetic method of Kaber and found to be 0.160 µL L^{-1} for juvenile *Cyprinus carpio* (Table 1).

AChE Activity

Results showed that Karate caused a considerable decreased in the level of AChE in brain, muscle and liver tissues of *C. carpio* exposed to sub lethal concentrations. Response was time and concentration dependent (Table 2, 3). Exposure of fish to 10% (0.016 µL L^{-1}) of acute toxicity value (LC_{50}) of Karate showed no significant change in the level of AChE in brain, liver and muscle tissue after 1 h exposure (Table 3) while inhibition of enzyme activity was pronounced even after one 1 h exposure of Karate at the concentration of 0.032 µL L^{-1} (Table 2). AChE activity showed tissue specific pattern, maximum reduction was observed in brain followed by muscle and liver tissues. The AChE activity showed increasing trend after 48 h but significantly low level of activity was observed after prolong exposure i.e., 25 days.

Table 1: Determination of LC_{50} value of Karate for 96 h based on arithmetic method

Concentration (µL L^{-1})	Concentration difference	Number of fish exposed	Number of dead fish	Mean death	Mean death× concentration difference
0	0	10	0	0	0
0.08	0.08	10	2	1	0.08
0.16	0.08	10	5	3.5	0.28
0.18	0.02	10	6	5.5	0.11
0.19	0.01	10	6	6	0.06
0.2	0.01	10	7	6.5	0.065
0.24	0.04	10	7	7	0.28
0.32	0.08	10	10	8.5	0.68
0.4	0.08	10	10	10	0.8
					∑=2.36

Summation indicates sum of Mean death × concentration difference
LC_{50} for Karate = LC_{100} - ∑ (Mean death × concentration difference)/number of fish per group LC_{50} for Karate = 0.4 - (2.36/10) = 0.4 - 0.24 =0.160 µL L^{-1}

Table 2: AChE activity (µmol min^{-1} mg^{-1} protein) in the brain, muscle and liver tissues of the fish, *Cyprinus carpio* following exposure to 20% 96 h LC_{50} of karate

Tissues	Sub-lethal exposure period					
	0 h	1 h	24 h	48 h	72 h	25 days
Brain	340.18±0.82[a]	268.43±4.30[b]	153.56±0.32[e]	159.05±0.95[e]	230.67±1.4[c]	208.86±094[d]
Muscle	295.74±1.64[a]	269.92±3.51[b]	155.087±2.88[e]	161.45±0.459[e]	197.10±0.84[d]	219.05±0.98[c]
Liver	238.98±4.1[a]	215.87±0.32[b]	159.96±0.17[d.]	161.45±0.459[d]	186.34±2.50[c]	188.79±0.79[c]

Data are presented as mean ± S.E. (n=15). Means with different superscripts are significantly different (P<0.05)

Table 3: AChE activity (µmol min^{-1} mg^{-1} protein) in the brain, muscle and liver tissues of the fish, *Cyprinus carpio* following exposure to 10% of 96 h LC_{50} of karate

Tissues	Sub-lethal exposure periods					
	0 h	1h	24 h	48 h	72 h	25 days
Brain	340.18±0.82[a]	299.43±1.10[a]	162.85±1.81[e]	217.97±4.52[d]	241.94±2.20[c]	269.32±1.99[b]
Muscle	295.74±1.64[a]	261.83±0.82[a]	163.38±0.33[e]	173.22±1.40[d]	235.62±2.20[c]	249.22±1.02[b]
Liver	238.98±4.1[a]	225.58±5.88[a]	164.56±0.44[c]	199.03±0.82[b]	218.20±1.28[a]	203.32±1.032[b]

Data are presented as mean ± S.E (n=15). Means with different superscripts are significantly different (P < 0.05)

Table 4: Percentage inhibition of protein in brain, muscle and liver tissues of juvenile *Cyprinus carpio* exposed to 10% LC_{50} (96 h) of Karate at different time period

Tissues	Exposure period				
	1 h	24 h	48 h	72 h	25 days
Brain	8.173±1.13[a]	2.43±1.36[d]	2.24±0.17[e]	2.85±0.32[c]	7.38±0.86[b]
Muscle	2.18±0.52[d]	1.50±0.6[e]	2.28±1.08[c]	5.75±0.52[b]	11.88±1.19[a]
Liver	3.51±0.80[c]	1.43±0.65[e]	3.35±0.60[d]	5.80±1.29[b]	15.62±0.60[a]

Data are presented as mean ± S.E (n=15). Means with different superscripts are significantly different (P < 0.05).

Table 5: Percentage inhibition of protein in brain, muscle and liver tissues of juvenile *Cyprinus carpio* exposed to 20% LC_{50} (96 h) of Karate at different time period

Tissue	Exposure period				
	1 h	24 h	48 h	72 h	25 days
Brain	14.96±2.13[c]	12.77±1.85[d]	19.95±2.73[b]	5.82±0.86[e]	26.01±2.06[a]
Muscle	11.53±1.53[c]	6.67±1.12[e]	10.56±1.73[d]	13.40±1.67[b]	20.77±1.21[a]
Liver	6.73±0.75[e]	8.52±0.50[d]	9.88±0.72[c]	10.94±0.75[b]	27.84±1.46[a]

Data are presented as mean ± S.E (n=15). Means with different superscripts are significantly different (P < 0.05)

Protein

Exposure of fish to sub-lethal concentration of Karate resulted in significant time and concentration dependent decrease in protein contents in all tissues of *C. carpio* studied (Table 4 and 5). The decreased in total protein content was considerably more pronounced at 20% than at 10% 96 h LC_{50} of Karate. The decreased in protein content was significantly higher in liver followed by muscle and then brain tissues (Fig 1 and 2).

Discussion

The present results clearly indicated the toxicity of Karate that contains λ-cyhalothrin as an active ingredient. λ-cyhalothrin is the broad-spectrum pyrethroid that is extensively used in the formulation of various brand name pesticides like Scimitar, Warrior, Karate, Icon, Demand and Matador (CDPR, 2006). These products are commonly used for controlling variety of insect's pests on various crops (Leistra e*t al*., 2003) and depending on percentage of λ-cyhalothrin give variable results. Although cyhalothrin show wide spread application and beneficial effects in agriculture sector but appeared toxic to aquatic organism including fish (Velmurugan *et al*., 2006). In present study the acute toxicity 96 h LC_{50} value of Karate for juvenile *C. carpio* was found to be 0.16 µL L^{-1} or 4 µg L^{-1}on the basis of commercial formulation (25 g λ- cyhalothrin L^{-1} of Karate solution). This value was somehow greater than reported by Hill (1985a, b, c) for *C. carpio,* 0.50 µg L^{-1}; sheep shead minnow (*Cyprinodonvariegatus variegates*) 0.81 µg $L^{-1}$and *rainbow trout (Salmo gairdneri*), 0.93 µg L^{-1}. This discrepancy in LC_{50} values of pesticides containing λ-cyhalothrin as active ingredients, even for same fish species may be related to formulation of pesticide and stereochemistry of the molecule (FMC Agricultural chemical group, 1989). In our study we used locally available pesticide, whereas Hill (1985 a,c) used 5% EC lambda-cyhalothrin.

Toxicity of pyrethroid is greatly related to the stereochemistry of the molecules and every isomer in pesticide formulation differ in its specific toxicity. According to Bradbury and Coats (1989) single isomer base formulations of pesticides are relatively more toxic compared to formulation used combination of various isomers. In addition to formulation, toxicity of the pesticide also depends on the carrier of the active ingredients, contaminants and inert ingredients (FMC Agricultural Chemicals Group, 1989). Immense literature is available, where acute toxicity (96 h) test of technical grades λ-cyhalothrin showed variable results in different fish species like LC_{50} values were 2–2.8 µg L^{-1} for brown trout (Charles and Hance, 1968) and 7.92 µg L^{-1} for *Channa punctatus* (Kumar *et al*., 2007). Beside formulation and stereochemistry of ingredients, there are number of other factors like temperature, health, age and size of the species, also affect the toxicity of chemicals to aquatic organisms (Abdul-Farah *et al.,* 2004). Though we have conducted the acute toxicity test at 23.5ºC and have not find relationship between temperature and LC_{50} value of this pesticide but reports are available that suggested the inverse relationship between the toxicity of pyrethroid and temperature (Kumaragura and Beamish, 1981). Pyrethroids were more toxic in winter season than in summer season and about ten-fold variation observed in the 96 h LC_{50} values at 10, 15 and 20ºC (Singh *et al.,* 2010). The same inverse relationship was also observed between body weight and pesticide toxicity, therefore 200 g trout showed higher tolerance to pesticides than fish of about I g (WHO, 1992).

The present results indicated that treatment of common carp, *C. carpio* to sublethal concentrations of Karate resulted in a significant decrease in total protein contents and AChE activity in liver, brain and muscle tissues. The decrease appeared in time and concentration dependant manner (Table 2, 3). Acetylcholine (ACh) is an important cholinergic neurotransmitter which acts on postsynaptic membrane excitatory receptors and initiate action potential in the neuron. When ACh released, the enzyme AChE split it into acetate and choline and therefore cause inactivation (Kopecka *et al.,* 2004). This mechanism prevents continued action and is important for normal transmission of nerve impulse but in case of neurotoxicity, there is an inhibition of AChE activity and accumulation of ACh at nerve ending that cause over excitation and interruption of normal nervous activity.

It was observed that both sub-lethal concentrations of Karate showed significant inhibition of AChE activities in different tissues of juvenile common carp (Table 2, 3) but after 24 h, the acute symptoms of pesticide start disappearing and AChE level showed increasing trend in brain, liver and muscle tissues of fish exposed to 10% of LC_{50} but this increasing trend appeared after 48 h when sub lethal concentration of Karate was increased (20% LC_{50}). The increasing trend of AChE activity explain the non-accumulation of Karate in *C. carpio* and disappearance of acute toxicity but prolong exposure up to 25 days resulted in a low level of activity that may be due to some neural impairment that persist long and have long term effects. Our results are in accord with previous results where other pesticides malathion, Diazinon, λ-cyhalothrin and cypermethrin showed positive correlation between concentration and inhibition / alteration of AChE activity in catfish *Heteropneutes fossilis* (Chandra, 2008), *Seriola dumerilli* (Jebali *et al*., 2006), *Oreochromis niloticus* (Tridico *et al*., 2010) and *Poecilia reticulata* (**S**harbidre *et al*., 2011) and freshwater fish, *Channa punctatus* (Kumar *et al*., 2009).

Many scientists reported the tissue specific decrease in AChE activity in response to pesticide. In *Channa punctatus* the inhibition of AChE activity in brain was significantly higher than muscle followed by gill in response to λ-cyhalothrin and cypermethrin (Kumar *et al*., 2009). Similar tissue specific decrease in AChE activity was also observed in *C. carpio* in response to quinalphos (Chebbi and David, 2009). Our results followed the same tissue-specific pattern, maximum inhibition in brain followed by muscle and liver. It might be due to the fact that AChE exist in different molecular form that differs in their interaction with pyrethroid (Szegletes, 1995).

Proteins play a key role in the structure and function of the cell and occupy a major position in cellular metabolism (Murray *et al*., 2007). According to Nelson *et al*. (2005) the physiological activity of animal was indicated by the metabolic status of proteins. It is well documented that pesticides alter the total protein content in different tissues of fish (Ahmad *et al*., 2012). Singh *et al. (*2010) reported the significant ($P < 0.05$) dose dependent decrease in total protein levels in liver and muscle tissues of freshwater teleost *Colisa fasciatus* exposed for 40 and 60% of LC_{50} (24 h) of cypermethrin, while Ahmad *et al*. (2012) reported decrease in total protein and free amino acids contents in zebra fish, *Daniorerio* (Hamilton) in response to λ-cyhalothrin. Similar reduction in total protein contents in the muscle and liver tissues of the same species exposed to sub-lethal doses of malathion and carbaryl pesticide was also reported by Tripathi and Singh (2003). We also observed the concentration dependant decline in total protein contents in brain, liver and muscle tissues of *C. carpio*, exposed to sub-lethal concentration of Karate (Table 4, 5). The protein inhibition was higher in liver followed by muscle and brain tissues (Fig. 1 and 2). This decreased in protein contents may be due to low feeding activity of fish and catabolism of protein to fulfill the energy demand and other metabolic process that augmented during stress. The fish can get its energy through the catabolism of protein during stress was also suggested by Mommsen and Walsh (1992). David *et al.* (2004) and Parthasarathy and Joseph (2011) also demonstrated the similar decreased in protein contents in *C. carpio* and *Oreochromis mossambicus* exposed to cypermethrin and λ-cyhalothrin respectively. Several others investigators also reported the depletion of tissue protein in fish exposed to toxicants while Ray and Banerjee (1998) suggested that stress in response to pesticide exposure influence the conversion of tissue protein in to soluble fraction moving in the blood for utilization.

In conclusion, Karate is toxic to fish and even at sub lethal concentration altered the AChE activity and total protein content in brain, liver and muscle tissues of fish *C. carpio,* Therefore, there is great need to prevent the indiscriminate use of pesticide because they are contributing in decreasing the population fish in the natural water bodies.

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