Evaluation of Acute Toxicity of Karate and its Sub-lethal Effects on Protein and Acetylcholinesterase 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 LC₉₀ 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 acetylcholinesterase (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. © 2014 Friends Science Publishers

Keywords: Karate; λ-Cyhalothrin; Toxicity; Acetylcholinesterase; 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; Kopruçu 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
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 LC50 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 ±0.08°C, 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 LC50 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 L\(^{-1}\), were selected and repeated the
experimental procedure for the determination of LC50 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 LC50 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
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Aquarium was exchanged with fresh dechlorinated water and different concentrations of pesticide were maintained afresh. The experiment was conducted for 25 days.

After 1, 24, 48, 72 h and 25 days, three fish from each aquarium 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 -20°C for total protein content and AChE enzyme analysis.

**Protein Estimation**

For this purpose 90 mg tissue was homogenized in 100 mM KH\(_2\)PO\(_4\) 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 \(\lambda\)-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 Amplite™ 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.
Protein exposure of fish to sub-lethal concentration of Karate content was considerably more pronounced at 20% than at 10% resulting in significant time and concentration dependent decrease in total protein contents in all tissues of juvenile *Cyprinus carpio* studied (Table 4 and 5). The decreased in protein content was significantly higher in liver followed by muscle and then brain tissues (Fig 1 and 2).

### Table 1: Determination of LC50 value of Karate for 96 h based on arithmetic method

<table>
<thead>
<tr>
<th>Concentration (µL L-1)</th>
<th>Concentration difference</th>
<th>Number of fish exposed</th>
<th>Number of dead fish</th>
<th>Mean death</th>
<th>Mean death× concentration difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.08</td>
<td>0.08</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>0.08</td>
</tr>
<tr>
<td>0.16</td>
<td>0.08</td>
<td>10</td>
<td>5</td>
<td>3.5</td>
<td>0.28</td>
</tr>
<tr>
<td>0.18</td>
<td>0.02</td>
<td>10</td>
<td>6</td>
<td>6.6</td>
<td>0.06</td>
</tr>
<tr>
<td>0.19</td>
<td>0.01</td>
<td>10</td>
<td>7</td>
<td>6.5</td>
<td>0.065</td>
</tr>
<tr>
<td>0.2</td>
<td>0.01</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>0.28</td>
</tr>
<tr>
<td>0.24</td>
<td>0.04</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>0.28</td>
</tr>
<tr>
<td>0.32</td>
<td>0.08</td>
<td>10</td>
<td>10</td>
<td>8.5</td>
<td>0.68</td>
</tr>
<tr>
<td>0.4</td>
<td>0.08</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Summation indicates sum of Mean death × concentration difference

\[\text{LC50} = \frac{\sum \text{(Mean death × concentration difference)}}{\text{number of fish per group}}\]

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 LC50 of karate

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Sub-lethal exposure period</th>
<th>0 h</th>
<th>1 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>25 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td></td>
<td>340.18±0.82a</td>
<td>268.43±4.30b</td>
<td>153.56±0.32a</td>
<td>159.05±0.95a</td>
<td>230.67±1.4c</td>
<td>208.86±0.94d</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td>295.74±1.64a</td>
<td>269.92±5.1b</td>
<td>155.087±2.88a</td>
<td>161.45±0.45a</td>
<td>197.10±0.84a</td>
<td>219.05±0.98a</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>238.98±4.1d</td>
<td>215.87±0.32a</td>
<td>159.96±0.17a</td>
<td>161.45±0.45a</td>
<td>186.34±2.50a</td>
<td>188.79±0.79a</td>
</tr>
</tbody>
</table>

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% 96 h LC50 of karate

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Sub-lethal exposure periods</th>
<th>0 h</th>
<th>1 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>25 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td></td>
<td>340.18±0.82a</td>
<td>299.43±1.10a</td>
<td>162.85±1.81a</td>
<td>217.97±4.52a</td>
<td>241.94±2.20a</td>
<td>269.32±1.99a</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td>295.74±1.64a</td>
<td>261.83±0.82a</td>
<td>163.84±0.40a</td>
<td>192.12±1.40a</td>
<td>235.62±2.20a</td>
<td>249.22±1.02a</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>238.98±4.1d</td>
<td>225.58±5.88a</td>
<td>164.56±0.44a</td>
<td>199.03±0.82a</td>
<td>218.20±1.28a</td>
<td>203.32±1.032a</td>
</tr>
</tbody>
</table>

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% LC50 (96 h) of Karate at different time period

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Exposure period</th>
<th>1 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>25 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td></td>
<td>8.17±1.13a</td>
<td>2.43±1.36a</td>
<td>2.24±0.17a</td>
<td>2.85±0.32a</td>
<td>7.38±0.86a</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td>2.18±0.52a</td>
<td>1.50±0.6a</td>
<td>2.28±1.08a</td>
<td>5.75±0.52a</td>
<td>11.88±1.19a</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>3.51±0.80c</td>
<td>1.43±0.65a</td>
<td>3.35±0.60a</td>
<td>5.88±0.12a</td>
<td>15.62±0.60a</td>
</tr>
</tbody>
</table>

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% LC50 (96 h) of Karate at different time period

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Exposure period</th>
<th>1 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>25 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td></td>
<td>14.96±2.13a</td>
<td>12.77±1.85a</td>
<td>19.95±2.73a</td>
<td>5.82±0.86a</td>
<td>26.01±2.06a</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td>11.53±1.53a</td>
<td>6.67±1.12a</td>
<td>10.56±1.73a</td>
<td>13.40±1.67a</td>
<td>20.77±1.21a</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>6.73±1.75c</td>
<td>8.52±5.05c</td>
<td>9.88±0.72c</td>
<td>10.94±0.75c</td>
<td>27.84±1.46c</td>
</tr>
</tbody>
</table>

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 LC50 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 et al., 2003) and depending on percentage of \( \lambda \)-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 \( \mu \)L L\(^{-1}\) or 4 \( \mu \)g L\(^{-1}\)on the basis of commercial formulation (25 g \( \lambda \)-cyhalothrin L\(^{-1}\) of Karate solution). This value was somehow greater than reported by Hill (1985a, b, c) for \( C. \ carpio \), 0.50 \( \mu \)L L\(^{-1}\); sheep shead minnow (\( Cyprinodon variegates \) variegates) 0.81 \( \mu \)g L\(^{-1}\)and rainbow trout (\( Salmo gairdneri \), 0.93 \( \mu \)g L\(^{-1}\). This discrepancy in \( LC_{50} \) values of pesticides containing \( \lambda \)-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 \( \lambda \)-cyhalothrin showed variable results in different fish species like \( LC_{50} \) values were 2–2.8 \( \mu \)g L\(^{-1}\) for brown trout (Charles and Hance, 1968) and 7.92 \( \mu \)g 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 23.5\(^{\circ}\)C 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 20\(^{\circ}\)C (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 1 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, \( \lambda \)-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), \( Oreochromis niloticus \) (Trídio et al., 2010) and \( Poecilia reticulata \) (Sharbidre 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 \( \lambda \)-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 LC₅₀ (24 h) of cypermethrin, while Ahmad et al. (2012) reported decrease in total protein and free amino acids contents in zebra fish, *Danio rerio* (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 dependent 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 decrease 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.

**References**


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Toxicity of Karate to *Cyprinus carpio* / *Int. J. Agric. Biol.*, Vol. 16, No. 4, 2014


(Received 12 June 2013; Accepted 31 December 2013)