Acetylcholinesterase and acid phosphatase activity in Freshwater fish Ctenopharyngodon idella exposed to Profex

Haribabu Gogula, Lalitha V and Venkata Rathnamma V

Abstract

Insecticides are used extensively in agriculture, but their levels in superficial waters generally range far below lethal concentrations for aquatic organisms. However, sub lethal adverse effects might result from exposure of aquatic organisms to insecticides at environmentally relevant concentrations. Acetylcholinesterase activity is routinely used as a biomarker of the exposure to certain groups of pesticides, such as organophosphates. The fish Ctenopharyngodon idella was exposed to lethal (1/50th of LC50 of 96hrs = 24µgL⁻¹) and sub lethal (1/10th of LC20 of 96hrs = 12µgL⁻¹) concentrations of Profex (profenofos 50%EC) for 4 days. The gill, liver, kidney, brain and muscle tissues were analysed for the activities of Acetylcholinesterase and Acid Phosphatase and all the experiments were compared with controls.

Keywords: Profex (50%EC), Ctenopharyngodon idella, biochemical changes

1. Introduction

Pesticides are one of the most harmful chemicals which have posed serious threat to animal’s health worldwide (El-Shenawy et al., 2009; Magar and Bais 2013) and plays an important role in modern agriculture. However, pesticides may have a beneficial effect on agricultural productivity, their indiscriminate use causes many serious problems to the environment and human health, since these compounds are toxic to non-target species (Diez, 2010; Coutinho et al., 2005). Acetylcholinesterase enzyme is widely used for rapid detection to predict early warning of pesticide toxicity (Dutta and Arends, 2003). The role of AChE in cholinergic transmission is to regulate nervous transmission by reducing the concentration of acetylcholine (ACh) in the junction through AChE-catalyzed hydrolysis of ACh (Kopecka et al., 2004). AChE was identified as the enzyme responsible for termination of cholinergic transmission by cleavage of AChE to acetate and choline; AChE is found in cholinergic synapses in the brain as well as in autonomic ganglia, the neuromuscular junction, and the target tissues of the parasympathetic system (Silman and Sussman, 2005). The AChE activity is necessary to maintain normal behaviour and muscular function and represents a prime target called esteric site on which some toxicants can exert a detrimental effect by binding to it. Inhibition of the AChE activity results in a build-up process of acetylcholine causing prolonged excitatory postsynaptic potential. This results in repeated, uncontrolled firing of neurons leading to hyper stimulation of the nerve/muscle fibres, which leads paralysis, and eventual death. In the event of decreased ATPase system, phosphorylation may be preceded by activated phosphates to catalyse the liberation of inorganic phosphates from phosphate esters. Acetyl cholinesterase (AChE) activity is a frequently used in environmental monitoring, usually in areas contaminated with pesticides, heavy metals and effluents. It is an enzyme that catalyses three hydrolyses of acetylcholine to choline and acetate in synaptic cleft. The aim of this study was to verify the relationship between the lethal and sub lethal concentrations of Profex (profenofos 50%EC) and the activity levels of Acetylcholinesterase (AChE) and ACP in gill, liver, kidney, brain and muscle tissues of the fish as the possible early biomarkers of exposure to this organophosphate insecticide.

2. Materials and Methods

The fish Ctenopharyngodon idella with an average size of 6 to 7 cm total length (TL) and 6.5 to 7.5 g body weight were brought from a local fish farm Kuchipudi village, Guntur District of Andhra Pradesh, India, and acclimatized at 28 ± 2°C in the laboratory for 15 days. Such acclimatized fish were exposed to sub lethal (1/10th of LC50) and lethal (1/5th of LC50) concentrations of profenofos 50% EC commercial grade for 4 days.
The vital tissues like gill, liver, kidney, brain and muscle tissues of the fish exposed were taken for the estimation of activities of Acetyl chloride esterase (AChE) and ACP all the test fish were compared with controls. Profex (profenofos 50% EC) manufactured by Nagarjuna Agrichem Limited., Hyderabad, India was purchased from the local pesticide dealer in Guntur and used in the experiment to evaluate the effects in different tissues at lethal and sublethal concentrations on freshwater fish, *Ctenopharyngodon idella*.

### 2.1 Estimation of Acetyl Cholinesterase (AChE) Activity

Enzyme activity was determined by the method based on Ellman *et al.* (1961) [8].

### 2.2 Estimation of Acid Phosphatases (ACP)

The activity of acid phosphates was estimated by the method of Bodansky (1932) [9].

### 3. Results and Discussion

The AChE activity (µ moles/min/mg protein) was estimated in different tissues like gill, liver, kidney, brain and muscle of the fish *Ctenopharyngodon idella* exposed to sub lethal and lethal concentrations of profenofos 50% EC for a period of 4days along with controls and the percent change over the control are shown in table below.

**Table 1:** Changes in the AChE activity (µ moles/min/mg protein) in different tissues of the fish, *Ctenopharyngodon idella* exposed to sub lethal and lethal concentrations of profenofos 50% EC.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Control</th>
<th>Profenofos 50% EC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sub lethal (4days)</td>
<td>Lethal (4days)</td>
</tr>
<tr>
<td>Gill</td>
<td>0.126±0.0012</td>
<td>0.056±0.0013</td>
</tr>
<tr>
<td>Liver</td>
<td>0.280±0.0022</td>
<td>0.136±0.0007</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.121±0.0051</td>
<td>0.072±0.0013</td>
</tr>
<tr>
<td>Brain</td>
<td>0.391±0.0043</td>
<td>0.138±0.0005</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.317±0.0042</td>
<td>0.127±0.0002</td>
</tr>
</tbody>
</table>

Values are the mean of five observations: Standard Deviation is indicated as (±). Value are significant at $p< 0.05$

In profenofos 50% EC sub lethal exposure for 4days maximum percentage of depletion was (-64.71%) in brain followed by muscle (- 59.93), gill (-55.71), liver (-51.43) and minimum percentage was (-40.66%) in kidney. In lethal exposure maximum percentage of depletion was (-71.35%) in brain and followed by muscle (-63.74), gill (-62.96) liver (-56.43), and minimum percentage was (-48.11%) in kidney, all these were compared with controls.

Fish immune system, important for defence against a variety of harmful pathogens is very sensitive to homeostatic adjustments via endocrine regulation and is influenced by biochemical profile of the nervous system. Insecticides can alter the immune functions of the body and result in immune-depression, uncontrolled cell proliferation, and alterations of the host defence mechanism against pathogens (Banaee M, 2012) [10]. Pesticides have been shown to cause alterations in the activities of many enzymes concerning to cellular metabolisms (Vani *et al.*, 2011) [11]. Organophosphate pesticides act by inhibiting the acetyl cholinesterase (AChE) at its esteratic site, resulting in an accumulation of the neurotransmitter acetylcholine in nerve tissue and at the effector organ (Ernest Hodgson, 2010; Mirjana B Colovic *et al.*, 2013) [12, 13].

According to Jaqueline Ineu Golombieski *et al.*, 2017) [14], accumulation of pesticides of organophosphate result in the continued stimulation of cholinergic synapses. Responses to organophosphate insecticides by aquatic organisms are broad ranged depending on the compound, exposure time, water quality and the species (Fisher, 1991; Richmonds and Dutta, 1992) [15, 16]. Low concentrations of the compounds can inhibit AChE, which leads to an accumulation of acetylcholine at central cholinergic synapses and neuromuscular junctions (Sancho *et al.*, 1997; Varó *et al.*, 2003) [17, 18]. The inhibition of the Acetyl cholinesterase by pesticides can affect...
locomotion and equilibrium of exposed organisms (Saglio & Trijasse, 1998; Bretaud et al., 2000) [19, 20].

According to the study of Jaqueline Ineu Golombieski et al., 2008) [21] after diafuran exposure, the AChE activity decreased (p< 0.05) for all concentrations in both tissues in relation to the control. Maximum inhibition of the AChE activity for all species was reached when exposed to 1 mg L-1 diafuran.

Maximum percentage AChE activity for 1 mg L-1 of diafuran in brain and muscle tissue compared to control was 28.92 and 28.89% for common carp, 30.17 and 55.45% for grass carp, and 55.22 and 64.54% for bighead carp AChE inhibition was higher in the brain of common and grass carp exposed to 1 and 2 mg L-1 of diafuran than in bighead carp. Changes in brain and muscle AChE activity observed in common, grass and bighead carp exposed to diafuran probably reflected in movement disturbances, with fish lethargic and immobile in the boxes, help to explain behaviour alterations induced by insecticides.

Erratic swimming, convulsions and lethargy were also observed in fathead minnows (Pimephales promelas) exposed to carbofuran (0.2 g L-1) (Health et al., 1997) [22] and European eel (Anguilla anguilla) (Sancho et al., 1997; Fernández-Vega et al., 2002) [17, 23] after exposure to fenitrothion and thiobencarb, respectively, and silver catfish (Rhamdia quelen) fingerlings exposed to 10 mg L-1 clomazone for 96 h (Miron et al., 2005) [24]. Cholinesterase inhibition in brain and muscle produce adverse effects in movement because the AChE participates in neuronal and neuromuscular transmissions (Fernández-Vega et al., 2002) [23].

When inhibition occurs in AChE activity the neurotransmitter, acetylcholine is not hydrolysed in the nerve synapse and neuromuscular junction, causing an abnormal amount of Ach in these areas, which leads to an over activation of the brain and muscular tissues (Roex et al., 2003; Candida Toni et al., 2011) [25, 26]. The mechanism of a toxic effect of diazion, a thiophosphoric acid ester is the same as of other organophosphorus substances. There is an inhibition of a whole series of enzymes and mainly of Acetylcholinesterase (Mahboob et al., 2011); Akhtar et al., 2012) [27, 28]. Organophosphate compounds phosphorylate AChE and inhibit its activity causing accumulation of acetylcholine (Ach) at the nerve synapse, which leads to disruption of the central nervous system and eventually death of the animal. AChE activity decreased at both the concentrations in liver, kidney and gills of Ctenopharyngodon idella as compared to the control.

A significant inhibition in the activity of AChE was observed and it increased with the increase in the exposure to chlorpyrifos in the organs of the fish studied (R. Jindal and M. Kaur, 2014) [29]. Chronic exposure to low levels of pesticides might have more significant effects on fish populations than acute poisoning (Capkin and Altinok, 2013) [30]. Biochemical changes lead to metabolic disturbances, inhibition of enzymatic activities and retardation of growth of aquatic organisms (Dogan, 2006) [31]. However, disturbances in their catalytic properties as results of pesticide interactions can cause impairment in cellular homeostasis affecting other biochemical parameters and enzymatic activities, which can lead to adverse effects at higher levels of biological organizations such as tissues, organs or individuals (Orrego et al., 2011) [12]. Incidentally, a great deal of attention has been paid to evaluate the hazardous effects of various pesticides on physiology of many non-target organisms.

Acid phosphatase activity (ACP)
The calculated values of acid phosphatase (ACP) activity along with controls and percent change over the control are given in table and graphically represented in figure. The acid phosphatase activity in the control fish is in the order of:

**Controls: Kidney > Gill > Liver > Muscle > Brain**

Under exposure to sub lethal and lethal concentration of profenofos 50%EC for 4days. The percent change in acid phosphatase activity is in the order of:

**Sublethal 4days:** Gill > Brain > Muscle > Liver > Kidney

**Lethal 4days: Brain > Gill > Liver > Muscle > Kidney**

In sub lethal exposure maximum percentage of elevation in ACP activity was (74.86%) in gill followed by brain (59.06), muscle (54.43), liver (44.07) and minimum elevation was (32.25%) in kidney. But in lethal exposure maximum percentage of elevation was (86.57%) in brain followed by gill (83.52), liver (69.62), muscle (68.98) and minimum percentage of elevation was (56.27%) in kidney. The acid phosphatase (ACP) is a lysosomal enzyme and the raise in this activity probably released to the cellular damage. It is difficult, however, to relate the decrease in ACP activity with tissues damage. Increase in acid phosphatase activities can be interpreted as a shift of the tissues emphasis on energy breakdown pathway from normal specific pH and are usually termed phosphomonoesterases. Pesticide poisoning increases ACP activity in the fish (Abdel et al., 2004); Carla et al., 2005) [33, 34]. Pesticides are reported to reduce glycogen levels and increase phosphorylase activities; the boost of phosphatases activity reveals the increase the transportation of metabolites through cellular membrane (Abdel et al., 2010) [35].

### Table 2: Changes in the specific activity levels of Acid Phosphatase (ACP) (mg pi/gram protein/hr) in different tissues of the fish, *Ctenopharyngodon idella* exposed to sub lethal and lethal concentrations of profenofos 50% EC.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Control</th>
<th>Sublethal</th>
<th>% Change</th>
<th>Lethal</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill</td>
<td>1.87±0.0316</td>
<td>3.27±0.044</td>
<td>74.86</td>
<td>5.38±0.051</td>
<td>83.52</td>
</tr>
<tr>
<td>Liver</td>
<td>2.76±0.058</td>
<td>3.89±0.048</td>
<td>44.07</td>
<td>4.58±0.054</td>
<td>69.62</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.79±0.048</td>
<td>3.69±0.050</td>
<td>32.25</td>
<td>4.36±0.032</td>
<td>56.27</td>
</tr>
<tr>
<td>Brain</td>
<td>1.49±0.057</td>
<td>2.37±0.048</td>
<td>59.06</td>
<td>2.78±0.051</td>
<td>86.57</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.58±0.041</td>
<td>2.44±0.047</td>
<td>54.43</td>
<td>2.67±0.051</td>
<td>68.98</td>
</tr>
</tbody>
</table>

Values are the mean of five observations: Standard Deviation is indicated as (±). Values are significant at p<0.05.
Alterations in alkaline phosphatase and acid phosphatase (ACP) activities in tissues and serum have been reported in fish (B. Jyothi and G. Narayan, 2000) [36]. Borah et al., 1996 [37] noticed significant decline in the activity of acid phosphatase of Heteropephus fossilis on exposure to rogor. The decrease in acid phosphatase activity might be due to histopathological changes such as necrosis. It may be due to decrease in the rate of trans-phosphorylation. Dalela et al., 1980) [38] are also on the opinion that uncoupling of oxidative phosphorylation has been the main reason for inhibition of acid phosphatase. The acid phosphatase activity in the liver and muscle tissues after exposure to the Malathion. They were showed significant decrease after exposing Channa punctatus to malathion for four days thus the activity of alkaline phosphatase was found to decrease in the experimental fish when compared with that of the control fish, Magar RS and Afsar Shaikh, 2013) [39].

4. Conclusion

In the present study, it is concluded that exposure to sub lethal and lethal concentrations of profenofos for a period of 4 days, adversely affected the AChE and ACP in different organs of freshwater fish, Ctenopharyngodon idella and these alterations might be attributed to increased autolysis in the tissues due to cytotoxicity.

In the present study, brain AChE is the most inhibited than all the tissues. This might be due to the pesticide activity on the brain. Since, the compound is neurotoxic hence the activity levels of AChE were inhibited. This study is in agreement with the earlier reports of Ray and Gosh, (2006) [40], Wang et al., 2009) [41], R. Jindal and M. Kaur, 2014) [42]. The elevation in alkaline phosphatase suggests an increase in the lysosomal mobilization and cell necrosis due to pesticide toxicity. Elevation of ACP activity in brain was reported earlier in stress-exposed Labeo rohita, 2000b) [43]. Sub-acute studies with monocrotophos showed increased activities of ACP content in plasma, which are conventional indicators of liver injury (Jyothi and Narayan, 2000) [36].

But profenofos intoxication caused remarkable elevation in the activity levels of ACP in all the test tissues it is significant in contrast to the pyrethroid effect on acid phosphatase, the results were in agreement with the study of Patil V.N.et al., 2014) [43]. The acid phosphatase (ACP) activity in the liver tissue of Ctenopharyngodon idella after profenofos exposure was found to be higher when compared to that in control fishes. Increase in acid phosphatase can be interpreted as a shift of the tissues emphasis on energy breakdown pathway from normal ATPase system to phosphatase system. In the present investigation it is found that, significant alterations in the enzyme contents in all the tissues as compared to control were observed (Santha Kumar et al., 2000a; Patil V.N. et al., 2014) [44, 43].

The parameter measured could be biomarker of toxicological effects of the pesticide on the fish and help in the diagnosis of the pollution and control on indiscriminate use of the pesticide is suggested. The results demonstrated that of organ phosphorus pesticides have the capacity to act based on the concentration of the toxicant. Thus, the effect of the increasing concentration of pesticide profenofos exhibited in increasing alterations in the biological, behavioural and biochemical aspects of the test species. This is consistent with a priori assumption of this concept, which is dose dependent to contribute to the overall effect on the test species Ctenopharyngodon idella.

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