PARTIAL PURIFICATION AND KINETIC CHARACTERIZATION OF RENAL CATALASE FROM CARNIVOROUS FISH, Channa striata EXPOSED TO PESTICIDES MIXTURE, DELTAMETHRIN+ENDOSULFAN

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The current work was accomplished to purify the renal catalase (CAT) from carnivorous fish, Channa striata exposed to sub-lethal dose (1/3 of LC₅₀) of deltamethrin (DM)+endosulfan(END) mixture. Fish was kept under control and experimental conditions for 14-days. Water quality characteristics like temperature, total hardness and pH were kept stabilized as 30°C, 250 mgL⁻¹ and 7.5, respectively. The biochemical technique Ammonium sulphate precipitation was used for partial purification of CAT. Kinetic characterization optimum pH and temperature was determined for CAT. Spectrophotometer was used to analyze activity of enzyme. Results of isolation showed that activity of CAT was lowest in crude extract of DM+END exposed Ch. striata renal tissue as compared to control. Similarly, specific activity of CAT was also lowest in crude extract of exposed Ch. striata renal tissue as compared to control. As a result of partial purification of renal CAT, lowest specific activity was observed in exposed Ch. striata (80.94±0.00 Umg⁻¹) than control (88.97±1.41 Umg⁻¹). The fold purification of renal CAT from exposed and control fish was calculated as 1.24±0.00 and 1.28±0.01, respectively. The percentage recovery was about 58.07±1.41 and 63.23±1.41 % for exposed and control Ch. striata, respectively. Kinetic characterization of CAT showed that the maximum activity was observed at pH 6.5 and temperature 30°C.

Keywords: Carnivorous fish, chronic exposure, insecticides mixture, catalase.

INTRODUCTION

Extensive use of pesticides in agriculture practices from last few years leads to increase in pollution of pesticides and their metabolites in aquatic ecosystems, raising serious environmental concerns (Fung and Mak, 2001). Aquatic animal’s response to these pollutants frequently induces alterations at cellular and biochemical levels, primarily structural and functional changes in the cells, organs, physiology and behavior of the individual (Parvez and Raisuddin, 2005). So, aquatic animals are unavoidably exposed to a number of pesticides which are different in structure and toxicity. Several authors have been reported the impact of individual pesticides on fish (Joseph and Raj, 2011) while the data on toxicity of pesticides mixtures is comparatively less studied. Therefore, more research is required to understand their interactions on the living organisms and to assess the risks associated with them. Among all the classes of pesticides, both organochlorine and pyrethroid insecticides are widely used, overlapping in soybean crops (Bacchetta et al., 2014). Endosulfan is a chlorinated cyclodiene, reported as highly toxic to a broad range of aquatic organisms. Deltamethrin belongs to class pyrethroids pesticides. The fish show several symptoms of stress when exposed to the deltamethrin (Datta and Kaviraj, 2003). Many classes of pesticides or their metabolites can stimulate the reactive oxygen species (ROS) formation and induce oxidative stress and cause oxidative damage in fish (Abdollahi et al., 2004; Velisek et al., 2012).

To overcome the toxicity of ROS, fish possessed an antioxidant defenses system includes superoxide dismutase, catalase and glutathione reductase (Orbea et al., 2002). Catalase is produced in peroxisome and disposed harmful free radicals, hydrogen peroxide (H₂O₂) (VijayaKumar et al., 2016). These antioxidants save the organs from oxidative injury under normal conditions. The antioxidants can serve as sensitive oxidative stress biomarkers in fish and can also detect aquatic toxicants (Ahmad et al., 2000) and provide useful information about the eco-toxicological effects of pesticides. Some other factors (pH and temperature) may also cause the modifications in enzymes activities. Most important one is the temperature. It strongly affects the metabolic rate of fish (Clarke and Fraser, 2004). The current work was performed to elucidate the toxic effect of pesticides mixture on catalase activity in renal tissue of carnivorous fish, Channa striata.
MATERIALS AND METHODS

Test Animal and Trail: Freshwater carnivorous fish, Channa striata were used for this experiment. Channa striata were collected from natural breeding grounds and live transferred to the wet laboratory at UAF. Channa striata were acclimatized to laboratory environment for few days and then transferred to 100-L glass aquaria. Each aquarium was filled with ten fishes. Abdullah et al. (2018) calculated the 96-hr LC₅₀ value (1.374 μgL⁻¹) of deltamethrin (DM) + endosulfan (END) mixture for C. striata. Sub-lethal dose (1/3rd of LC₅₀) of DM+END was used to expose the fishes for 14-days. Water quality characteristics like temperature, total hardness and pH were kept stabilized as 30°C, 250 mgL⁻¹ and 7.5, respectively.

Isolation of tissue Enzyme Extract: Renal tissue was homogenized in phosphate buffer of pH 6.5 by the ratio of 4:1 (w/v). Homogenate was filter and filtrate was centrifuged in refrigerator centrifugal machine at 10,000 rpm for 15 minutes.

Partial Purification of CAT: Zia et al. (2007) method was applied to partially purify the crude CAT. The purification technique ammonium sulfate precipitation included Salting in and Salting out steps.

Salting In Method: Crude extract was saturated with ammonium sulfate (60%) by dissolving 42 g in 100 mL of enzyme and refrigerated for 4 hrs at 4°C. After 4 hrs it was centrifuged for 15 minutes at 10,000 rpm and 4°C to obtain the supernatants and residues.

Salting Out Method: The obtained supernatant was saturated up to 80% ammonium sulfate by adding 56 g/100 mL of enzyme and kept it at 4°C over night. The sample was centrifuged at 10,000 rpm for 15 minutes to obtain the supernatant and residue. One ml of phosphate buffer (pH 6.5) was added in residue.

Residue Desalting: Precipitated enzyme sample obtained through salting out process was dialyzed against low ionic strength phosphate buffer (pH 7.4).

Enzyme Assay: Chance and Mehaly (1977) protocol was followed to check the CAT activity.

Determination of Protein Contents: Biuret method proposed by Gornall et al. (1949) was used to evaluate the protein contents of sample.

Specific activity of renal CAT: After partial purification specific activity was decreased in pesticides mixture exposed fish (80.94±0.00 U mg⁻¹) as compared to control (88.97±1.41 U mg⁻¹). Figure 2 showed that specific activity increased at every step of partial purification.

Kinetic Characterization of CAT

Determination of Optimum pH and Temperature: To get the optimum pH purified CAT was tested against different pH ranging from 4-12. Optimum temperature was obtained by assaying the CAT against various ranges form 5-50°C by keeping the pH constant at which purified CAT had maximum activity.

Data Analyses: Data obtained from this experiment analyzed by applying ANOVA to see the statistical differences (Steel et al., 1997). MS excel was used to draw the graph.

RESULTS

Activity of renal CAT: The inferences of this study showed that the crude CAT activity in renal tissue of DM+END exposed fish was decreased (125.33±0.71 U mL⁻¹) when compared with control (145.66±0.71 U mL⁻¹). However, after desalted CAT activity remained lower in exposed fish as 32.03±0.71 U mL⁻¹ in comparison of control (28.88±1.36 U mL⁻¹). Figure 1 showed that at each step of purification the activity was decreased.

Specific activity of renal CAT: After partial purification specific activity was decreased in pesticides mixture exposed fish (80.94±0.00 U mg⁻¹) as compared to control (88.97±1.41 U mg⁻¹). Figure 2 showed that specific activity increased at every step of partial purification.
Catalase activity in carnivorous fish

Figure 2. Specific activity of renal CAT of *C. striata* at each step of partial purification.

*CAT Fold purification and Yield/Percent recovery:* This parameter identifies how many folds an enzyme purified at every step of purification. It was noted that at last step highest fold purification of renal CAT was calculated as 1.28±0.01 and 1.24±0.00 for control and exposed fish, respectively. The yield recovery was decreased at every step of purification and percentage recovery was about 58.07±1.41 and 63.23±1.41 % for exposed and control fish, respectively (Table 1).

**Kinetic Characterization**

*Optimum pH and Temperature:* Generally, activity of enzymes increased as the pH and temperature increased up to a certain limit. Similarly, in case of present study initial activity was increased, and maximum activity was observed at 6.5 pH and 30°C temperature after that CAT activity decreased (Fig.3 and 4).

DISCUSSION

Catalase (CAT) is a peroxisomal antioxidant, which scavenges the hydrogen peroxide and transfers them into less reactive species (oxygen and water) thereby preventing lipid peroxidation. In present work renal CAT activity and specific activity in pesticides mixture exposed fish was lower than control. Similarly, deltamethrin exposure caused reduction in CAT level in fish (Atif et al., 2005; Sharma and Ansari, 2013). Exposure of deltamethrin caused inhibition in CAT activity in kidney tissues of *C. punctatus* (Sayeed et al., 2003). Vineela and Reddy (2014) also reported a gradual decrease in kidney CAT activity of *Catla catla* exposed to organochlorine pesticides (Lihocin). Low level of CAT was observed in kidney of African catfish (Hamed, 2016). According to Vasantharaja et al. (2012) sub-lethal dose of cypermethrin inhibited the CAT level in kidney of *Cirrhinus mrigala*. Tripathi and Singh (2013) also confirmed the decrease in CAT activity of *C. punctatus* due to toxicity of alphamethrin. Some other pyrethroids including cyfluthrin (Sepici-Dincel et al., 2009), deltamethrin (Yonar and Sakin, 2011), cypermethrin and λ-cyhalothrin (Kumaret et al., 2012), fenvalerate (Prusty et al., 2013) and cypermethrin (Parthasarathy and Joseph, 2013) also decreased the CAT activity in different fish species. Abdelkhalek et al. (2015) recorded a significant decrease in CAT kidney tissue of tilapia fish upon exposure to

<p>| Table 1. Partial purification of renalcatalase in <em>C. striata</em> by using ammonium sulfate precipitation |
|-------------------------------------------------|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Precipitation</th>
<th>Protein(mg mL⁻¹)</th>
<th>Fold purification</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steps</td>
<td>Control</td>
<td>DM+END</td>
<td>Control</td>
</tr>
<tr>
<td>Crude Extract</td>
<td>3.06±0.01A</td>
<td>2.75±0.01A</td>
<td>1.00±0.02D</td>
</tr>
<tr>
<td>Salting In</td>
<td>2.48±0.01B</td>
<td>2.24±0.03B</td>
<td>1.05±0.01C</td>
</tr>
<tr>
<td>Salting Out</td>
<td>1.23±0.01C</td>
<td>0.88±0.01C</td>
<td>1.18±0.01Ba</td>
</tr>
<tr>
<td>Desalting</td>
<td>0.36±0.01D</td>
<td>0.35±0.01D</td>
<td>1.28±0.01Aa</td>
</tr>
</tbody>
</table>

Small alphabet superscripts show the difference between treatments within the same row while capital alphabet shows significant (*P*<0.05) difference among different steps of purification within the same column.

Figure 3. Effect of different pH on renal CAT activity (U/mL) of *C. striata* under control and exposed conditions.
deltamethrin. According to Suneeth (2014) exposure of endosulfan an organochlorine and fenvalerate a synthetic pyrethroid significantly reduced the CAT activity in {Labeo rohita} when compared to control. {Oreochromis niloticus} showed the inhibition in renal CAT activity when exposed to toxicants (Ahmed et al., 2016).

The unit of catalase in one milligram of protein is known its specific activity and it is most important indication of CAT purification. In present study specific activity of CAT enhanced at every step of purification. Similarly, specific activity and fold purification of purified CAT from {Pyrobaculum caldidentis} was calculated as 23,500 U/mg of protein and 91, respectively (Amo et al., 2002). Specific activity of CAT was observed as1011.84 Umg⁻¹ for metal stressed fish (Ahmed et al., 2016)

In present research optimum pH and temperature for renal CAT was observed as 6.5 and 30°C, respectively. Similarly, Ahmed et al. (2016) also noted the optimum pH and temperature for CAT purified from tilapia as 7.0 and 25°C, respectively. Vetrano et al. (2005) purified catalase form mammalian sources and noted that optimum pH lie in range of 7-9.

**Conclusion:** This work concluded that exposure of organic compounds can change the antioxidant enzymes response in fish. The catalase purification from renal tissue is convenient and simple technique and can be used for cross laboratory comparisons. Thus, catalase can serve as a valuable biomarker of aquatic pollution. This purified technique has successfully applied in all those areas where agriculture activities are intensive and water contamination has become a most important ecological concern. Results also concluded that the catalase activity changed in renal tissues of fish at different pH and temperature. This may suggest that catalase respond to pH and temperature may be a physiological adaptation under various environments.

**REFERENCES**


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the Urdaibai and Plentzia estuaries (Bay of Biscay). Aquat. Toxicol. 58:75-98.


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