Effect of Triazophos on Protein Metabolism in the Fish, *Channa punctatus* (Bloch)

Abdul Naveed and C. Janaiah
Department of Zoology, Panchsheel College of Education, Nirmal - 504106, Adilabad, A.P. India
Department of Zoology, Kakatiya University, Warangal -506 009, A.P. India

Abstract: On treatment with sublethal dose of triazophos exposure i.e., 24, 48, 72 and 96 h on protein metabolism of fish *Channa punctatus* were observed. The harmful toxic effect of triazophos, is an Organo Phosphorus (OP), is investigated by measuring key enzymes in protein metabolism, Viz. catalases, Super Oxide Dismutase (SOD) and Xanthine Oxidase (XOD) and these antioxidant enzymes were altered. In the present investigations the activity of catalases reduced under toxicity because that triazophos initiates the enhancement of super oxide radicals. The inhibition of SOD activity in the fish reveals the impairment of antioxidant defense mechanism and also reduction in molecular oxygen and normal metabolism step. The decreased activity of XOD leads to increase in cellular damage through photolytic activity or non availability of iron under stress condition.

Key words: Antioxidant enzymes, catalase, *Channa punctatus*, Triazophos, SOD, XOD

INTRODUCTION

Triazophos (O, O - diethyl, 0.1 phenyl, 1H, 1, 2, 4, Triazol 3yl - phosphorothio) is an organophosphate, widely used singly or in mixture as an insecticide to control the pests on the paddy and on cotton fields. Triazophos, as in other organophosphate compounds, is known to produce toxic effects by inhibiting of the acetyl cholinesterase activity. Organophosphate (OP) pesticides are widely used because of their biodegradability (BookHout and Monroe, 1977). Basically the most prevalent xenobiotics arising out of agricultural and industrial activities are pesticides and heavy metals where the stress response mechanisms have been widely addressed in vertebrates in general and fish in particular. Oxygen availability is a limiting growth factor and chronic hypoxia or hyperoxia may be an important environmental stressor influencing fish growth (Wilhel et al., 2005). However, biological systems protect themselves against free radicals (and others derived from oxygen) aggression converting these free radicals in oxygen by oxidation phenomena or reduction through antioxidant system. Univalently reduced $O_2^-$ are reduced to uncharged $H_2O_2$ either spontaneously or by Superoxide dismutase (Halliwell and Guteridge, 1985). Superoxide dismutase (SOD) and Catalase (CAT) have been detected in a wide variety of mammalian cells. These enzymes play an important role in protecting the cell against the potentially toxic effects of environmental pollutants (Kuthan et al., 1986). Superoxide dismutase catalyzes the dismutation of the superoxide ion ($O_2^-$) to hydrogen peroxide and oxygen molecule during oxidative energy processes. The reaction diminishes the destructive oxidative processes in cells. The levels of antioxidant enzyme have been extensively used as an early warning indicator of like pollution (Lin et al., 2001). Fish have been proposed as indicators for monitoring land-based pollution because they may concentrate indicative pollutants in their tissue, directly from water through respiration and also through their diet. Fish are frequently subjected to proxidant effects of different pollutants often present in the aquatic environment. In the present study on attempt has been made to observe the effect of triazophos on certain enzymes of protein metabolism in *C. punctatus*.

MATERIALS AND METHODS

The freshwater fish *Channa punctatus* were collected from the lake of Nirmal, Adilabad district of Andhra Pradesh. The fish were stored in cement tank (6x3x3 feet) containing 60 L-dechlorinated tap water for 3 week for acclimatization under laboratory conditions. Water was changed every 24 h. Commercial fish food was supplied to fish during acclimatization period. Dead fish (if any) were removed from the cement tank as soon as possible to avoid water fouling. Adult fish of nearly similar (weight 50±1.30 g) and (length 25.5±1.21 cm) were selected for experiments. Stock solution of the pesticide was prepared in acetone. Acclimatized fish were treated with 90% pure technical grade triazophos. The IUPAC name of

Corresponding Author: Abdul Naveed, Department of Zoology, Panchsheel College of Education, Nirmal - 504106. Adilabad, A.P. India
triazophos pesticide is (O, O-diethyl, 0.1 phenyl, 1H, 1, 2, 4, Triazol 3yl-phosphorothio). According to (Bayne et al., 1977), LC50 (40% E.C) 48h value of triazophos for fish C. punctatus is 0.019 ppm. Six tubs were set up for each concentration and each tub contained 6 fish in 6L dichlorinated tap water. Water temperature was kept at 22±1.0°C during whole experimental period. Qualities of experimental water PH = 7.2-7.3; dissolved oxygen = 9.2 mg/L; free carbon dioxide =10.0 mg/L; alkalinity = 69 mg/L were measured according to the method of (APHA/ AWWA/WEF, 1998) Control groups were kept in dechlorinated tap water without any treatment. Fish were not fed before 24 h and during the experiment. After 24, 48, 72 and 96 h of exposure, fish were removed from tubs and washed with freshwater. Fishes of both treated as well as control groups were killed by a severe blow on the head.

Superoxide dismutase (SOD: EC. 1.15.1.1) activities were measured by the method of (Beachamp and Fridovich, 1971). The tissues were homogenized (20% w/v) in potassium phosphate buffer pH (7.5) containing 1% poly Vinyl Pyrolydone, and centrifuged at 16000 rpm for 15 min. Values are expressed as micro moles of formazan formed / mg protein.

Catalases (CAT: EC. 1.11.1.6) activities were measured by the method of (Beers and Sizer, 1952). The (10% W/v) tissue homogenate was prepared in 50 moles of phosphate buffer (pH 7.0) and centrifuged at 16000 rpm for 45 min and again the supernatant was centrifuged at 10500 rpm for 1 h and resulting supernatant was used as the enzyme source. The reaction mixture was incubated in spectrophotometer for 45 min to achieve temperature equilibration and to establish blank rate if any. The enzyme activity was expressed as micromoles H2O2 decomposed/mg protein/min.

Xanthine oxidase (XOD: CE. 1.17.3.2) activities measured by the method of (Worthington, 2004). The assay mixture contained 1.9 mL of phosphate buffer (pH 7.5), 1.0 mL of hypoxanthine and 0.1 mL of enzyme source. The reaction was initiated by the addition of enzyme source. Blank contains all the assay mixture except 1.0 mL reagent grade water as a substitute to the substrate. The rate is proportional to enzyme concentration within limits of 0.01 to 0.02 units per test. The activity was expressed as micro moles of Urate formed/mg protein/min.

Data exhibited homogeneous variances, so comparisons among different treatments were made by one-way analysis of variance, followed by Manu-Whitney U-test. All the tests were made with the software statistical. Minimum significance level was 95% (p<0.05).

RESULTS

The activity of SOD observed in the tissue of liver, brain and kidney tissue of Channa punctatus during sublethal concentration of triazophos for 24, 48, 72 and 96 h of exposure periods. The SOD activity significantly decreased in all the tissues during the toxic exposure periods.

The activity of Catalases (CAT) is observed in the tissue of liver, brain and kidney tissue of Channa punctatus during sublethal concentration of triazophos for 24, 48, 72 and 96 h of exposure periods.

Table 1: Effect of (XOD, SOD, CAT) in Liver tissue of Channa punctatus (Bloch) intoxicated with Triazophos

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>3.46±0.27</td>
<td>3.21±0.19*</td>
<td>2.97±0.38*</td>
<td>2.46±0.76</td>
<td>2.03±0.46</td>
</tr>
<tr>
<td>PC = -7.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalases</td>
<td>0.172±0.014</td>
<td>0.164±0.017*</td>
<td>0.150±0.012*</td>
<td>1.46±0.26</td>
<td>0.139±0.015</td>
</tr>
<tr>
<td>PC = -4.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>0.41±0.036</td>
<td>0.350±0.02*</td>
<td>0.271±0.01</td>
<td>0.186±0.003</td>
<td>0.149±0.002</td>
</tr>
<tr>
<td>PC = -14.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value is mean SD of 6(Six) observations; All values are statistically significant from control at 5% level (p<0.05); PC denotes percent change over control; *: Not significant; Units as per Table 3

Table 2: Effect of (XOD, SOD, CAT) in Brain tissue of Channa punctatus (Bloch) intoxicated with Triazophos

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>2.94±0.39</td>
<td>2.68±0.11*</td>
<td>2.43±0.26</td>
<td>2.07±0.21</td>
<td>1.93±0.41</td>
</tr>
<tr>
<td>PC = -8.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalases</td>
<td>0.145±0.018</td>
<td>0.136±0.013*</td>
<td>0.131±0.016*</td>
<td>0.128±0.011*</td>
<td>0.124±0.015*</td>
</tr>
<tr>
<td>PC = -6.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>0.563±0.046</td>
<td>0.475±0.015</td>
<td>0.420±0.021</td>
<td>0.379±0.017</td>
<td>0.327±0.053</td>
</tr>
<tr>
<td>PC = -15.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value is mean SD of 6(Six) observations; All values are statistically significant from control at 5% level (p<0.05); PC denotes percent change over control; *: Not significant; Units as per Table 3
Table 3: Effect of (XOD, SOD, CAT) in Kidney tissue of *Channa punctatus* (Bloch) intoxicated with Triazophos

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>3.01±0.13</td>
<td>2.71±0.19*</td>
<td>2.63±0.36*</td>
<td>2.17±0.41</td>
<td>2.10±0.36</td>
</tr>
<tr>
<td>Values are expressed in micro moles formazan formed/mg of protein/h</td>
<td></td>
<td>PC = -9.96</td>
<td>PC = -12.62</td>
<td>PC = -27.90</td>
<td>PC = -30.23</td>
</tr>
<tr>
<td>Catalase</td>
<td>0.136±0.015</td>
<td>0.130±0.019*</td>
<td>0.125±0.026*</td>
<td>0.120±0.036*</td>
<td>0.115±0.045*</td>
</tr>
<tr>
<td>Values are expressed in micro moles formazan formed/mg of protein/h</td>
<td></td>
<td>PC = -4.41</td>
<td>PC = -8.08</td>
<td>PC = -11.76</td>
<td>PC = -15.44</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>0.684±0.019</td>
<td>0.630±0.01*</td>
<td>0.572±0.071</td>
<td>0.519±0.016</td>
<td>0.492±0.061</td>
</tr>
<tr>
<td>Values are expressed in micro moles formazan formed/mg of protein/h</td>
<td></td>
<td>PC = -7.89</td>
<td>PC = -16.37</td>
<td>PC = -24.12</td>
<td>PC = -28.07</td>
</tr>
</tbody>
</table>

Each value is mean SD of 6(Six) observations; All values are statistically significant from control at 5% level (p<0.05); PC denotes percent change over control; *: Not significant

Fig. 1: Activity of Super Oxide Dismutase (SOD) in different tissues of *Channa punctatus* under sublethal concentration of triazophos during 24, 48, 72 and 96 h. Of exposure period

Fig. 2: Activity of Xanthine Oxidase (XOD) in different tissues of *Channa punctatus* under sublethal concentration of triazophos during 24, 48, 72 and 96 h. Of exposure period
Control
24 h
48 h
72 h
96 h
Liver
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Micro moles of H₂O₂ for mg proteint/h
Fig. 3: Activity of catalase in different tissues of Channa punctatus under sublethal concentration of triazophos during 24, 48, 72 and 96 h of exposure period.

activity significantly decreased in all the tissues during the toxic exposure periods.

The activity of XOD is observed in the tissue of liver, brain and kidney tissue of Channa punctatus during sublethal concentration of triazophos for 24, 48, 72 and 96 h of exposure periods. The XOD activity significantly decreased in all the tissues during the toxic exposure periods.

The activity of SOD, XOD and Catalases in the tissues of liver, brain and kidney tissues of Channa punctatus during sublethal concentration of triazophos for 24, 48, 72 and 96 h of exposure periods. SOD, XOD and CAT activities in all the tissues significantly decreased after prolonged exposure periods. All the results are presented in the Table 1, 2, 3 and Fig. 1, 2, 3.

**DISCUSSION**

Insecticides are extensively used in agriculturally developing countries to protect the crop from harmful pests. SOD enzyme present in all eukaryotes contained copper or Zinc which played an important role in the catalyses of Superoxide radicals to form hydrogen peroxide, further it is converted in to H₂O by Catalases (Nelson and Cox, 2005). Svadlenka *et al.* (1975) reported that SOD is a link in the biological defense mechanism through disposition of endogenous cytotoxic O₂⁻, which are deleterious to structural proteins of plasma membrane. The decreased activity of SOD in erythrocytes of calves was observed by (Patra and Swarup, 2000). It is observed that the pesticides produce oxidative stress by inhibiting the activity of SOD. According to (Nelson and Cox, 2005; Sathyanarayana, 2005) catalases play an important role in protection of cell from the hydrogen peroxide toxicity. Gaetani *et al.* (1994) reported that catalases consist of four protein sub units containing haem group and it acts as antioxidant enzyme. Mostly these catalases are found in peroxisomes. In C. punctatus the activity of catalases reduced under toxicity of pesticides, because pesticides initiate the enhancement of superoxide radicals that can inhibit the catalasas activities (Yu, 1994). XOD is an important enzyme that converts hypoxanthine to xanthine, subsequent to uric acid. This enzyme contains FAD, molybdenum and Iron are exclusively found in liver, intestine and little amount in other tissues of animals (Sathyanarayana, 2005; Hille and Nishino, 1995; Hellesten, 1994) also reported XOD played a vital role in conversion of toxic ammonia into nontoxic uric acid. Xanthine oxidase produces hydrogen peroxide which is very harmful to the animal, and then it converts into H₂O and O₂. Further, the uric acid may act as an antioxidant and free radical scavenger protects the cells from oxidative damage. (Sheehan *et al*., 2001; Guskov *et al*., 2002). The decrease in XOD activity leads to increase in cellular damage and may be due to non availability of Iron to the fish during toxic period. It is proposed that measurement of antioxidant in fish tissues may prove to be useful in biomonitoring of exposure to aquatic pollutants.

**CONCLUSION**

It is observed that in the C. punctatus the decreased SOD indicates the triazophos produces oxidative stress by inhibiting activity of SOD. The decreased catalase activity reduces peroxidative damage in the tissues in response to the levels of antioxidant were modulated and decreased XOD activity in C. punctatus reported that triazophos...
induces peroxidative damage in liver, kidney and brain which led to modulation of antioxidant may prove to be useful in biomonitoring of exposure to aquatic pollutants.

ACKNOWLEDGEMENT

We thank Abdul Shukur; founder Panchsheel College of Education, Nirmal for support and providing lab facilities for the present study. Author’s also thankful to the management of Maxwell Scientific Organization for financing the publication.

REFERENCES