IMPACT OF ATRAZINE ON ACID AND ALKALINE PHOSPHATASE ACTIVITIES AND PROTECTIVE ROLE OF Spirulina IN VARIOUS ORGANS OF FISH Cyprinus carpio. Linn.

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Abstract
The aim of the study was to determine the effect of herbicide, atrazine on phosphatase activities in the various organs of fish Cyprinus carpio. The fishes were exposed to atrazine 0.5-mg/l sub lethal concentration for the period of 24, 48, 72, 96 and 120 hours. Alkaline phosphatase (ALP) and acid phosphatase (ACP) were determined in various organs (Gill, Liver and Kidney) of the fish after the experimental group II, III and IV. Atrazine (group II) and atrazine along with Spirulina exposure (group III) was significantly different on various organs. ALP and ACP values in all the organs (Gill, liver, kidney) were increased in atrazine exposure. The group III, atrazine along with Spirulina exposure were (decreased) gradually recovered. This indicates an evidence of inhibition of these enzymes in the organs by the toxicant and therefore, alteration of biochemical process in Cyprinus carpio which can be used as bio-indicators of the atrazine. Hence, the present investigation overall results shows the ACP and ALP activity were significantly (P>0.05) differences when compared with control.

Key words: ACP, ALP, Cyprinus carpio, atrazine

INTRODUCTION
Atrazine is a triazine herbicide, which is the most widely used agricultural pesticide through the world. Recently atrazine concentration was found in the farmer’s blood and urine [1]. Atrazine could cause damage the renal excretion of sodium, chloride and protein in the rainbow trout and carp [2]. Moreover, atrazine reduced plasma testosterone, olfactory sensitivity and salinity tolerance in mature male atlantic salmon [3]. Thus many European and African countries have restricted its use atrazine [4]. Several authors have investigated the effect of atrazine in fish [5, 6, 7 and 8].

Spirulina has been used as food and nutritional supplements for a long time [9]. It is generally regarded as a rich source of proteins, vitamins, essential amino acids, minerals, essential fatty acids such asΩ-linolenic acid and sulfolipids [10, 11]. Moreover in addition to omega-3 polyunsaturated fatty acids (PUFA), Spirulina has also omega-6 PUFA, phycocyanin, and other phytochemicals [12]. According to the possible applications of antigenotoxic drugs in cancer treatment, worldwide interest has converged on a wide variety of plant extract, food supplements or dietary products [13, 14, 15, 16 and 17].

The aim of this study was to evaluate the toxicity of atrazine and chelating properties of Spirulina on acid phosphatase (ACP) and alkaline phosphatase (ALP) in various organs of Gill, Liver and Kidney of Cyprinus carpio (Linn).

MATERIALS AND METHODS
Fresh water fish Cyprinus carpio were obtained from Navarathna fish farm from Pinnalur village. Experimental chemical atrazine was purchased from (TATA Atrataf 50% WP) mft by Rallis India Limited, Mumbai. The supplemented diets Spirulina were collected from Aurospriul commercial farm, from Aurovile village, near pondicherry.

The fish introduced into large cement tiles tank (4x4) disinfected with potassium permangnate and washed thoroughly prior to introduction of fish (to prevent the fungal infection). Fish were acclimatized for about 15 days before the commencement of the experiment. They were fed on commercial fish feed which given daily. LC₅₀ of atrazine was
Table 1. Variation of ALP (µg/P1/mg protein/hrs) activity of *Cyprinus carpio*, exposure to atrazine and *Spirulina* for the period of 120 hours

<table>
<thead>
<tr>
<th>ORGANS</th>
<th>GROUPS</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I CONTROL</td>
<td>8.215 ± 0.57</td>
<td>8.34 ± 0.58</td>
<td>8.42 ± 0.58</td>
<td>8.42 ± 0.58</td>
<td>8.29 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>II ATRAZINE</td>
<td>6.134** ± 0.30</td>
<td>5.19** ±0.25</td>
<td>5.04 **± 0.25</td>
<td>4.25 **± 0.22</td>
<td>4.32** ± 0.21</td>
</tr>
<tr>
<td></td>
<td>III ATR + SPI</td>
<td>5.19** ± 0.31</td>
<td>6.32 **± 0.37</td>
<td>6.26 **± 0.37</td>
<td>7.12* ± 0.42</td>
<td>7.43 NS ± 0.44</td>
</tr>
<tr>
<td></td>
<td>IV SPIRULINA</td>
<td>8.39 NS ± 0.58</td>
<td>8.49 NS ± 0.59</td>
<td>8.65 NS ± 0.60</td>
<td>8.79 NS ± 0.61</td>
<td>8.96 NS ± 0.64</td>
</tr>
</tbody>
</table>

|        | I CONTROL | 0.635 ± 0.04 | 0.639 ± 0.04 | 0.642 ± 0.04 | 0.653 ± 0.04 | 0.679 ± 0.04 |
|        | II ATRAZINE | 0.412 **± 0.02 | 0.404** ±0.02 | 0.385 **± 0.01 | 0.363 **± 0.01 | 0.342 **± 0.01 |
|        | III ATR + SPI | 0.529** ± 0.03 | 0.535 **± 0.03 | 0.551** ± 0.03 | 0.576** ± 0.03 | 0.612* ± 0.05 |
|        | IV SPIRULINA | 0.642** ± 0.04 | 0.653 NS ± 0.04 | 0.672 NS ± 0.04 | 0.712 NS ± 0.04 | 0.739* ± 0.05 |

|        | I CONTROL | 1.408 ± 0.09 | 1.410 ± 0.09 | 1.413 ± 0.09 | 1.435 ± 0.10 | 1.426 ± 0.09 |
|        | II ATRAZINE | 1.212* ± 0.06 | 1.265 NS ± 0.06 | 1.201* ± 0.06 | 1.965** ± 0.09 | 1.618* ± 0.08 |
|        | III ATR + SPI | 1.515** ± 0.09 | 1.519** ±0.09 | 1.626** ±0.09 | 1.645** ± 0.08 | 1.715** ± 0.10 |
|        | IV SPIRULINA | 0.739 NS ± 0.05 | 0.815 NS ± 0.05 | 0.829 **± 0.05 | 0.846 *± 0.05 | 0.892** ± 0.06 |

** Significant at 5% levels
* Significant at 1% levels
NS – Non Significant

calculated by the log – dose / Profit regression line was recorded. The test fishes were grouped in four groups are, Group I control, Group II atrazine, Group III Atrazine + *Spirulina*, Group IV *Spirulina*, in each groups for sublethal concentration of atrazine for a period of 120 hours. Simultaneously a control was maintained to compare to toxicant values. A Group of 6 fishes were exposed to each groups and exposure period. At the same time 6 fishes, used control, were kept in clean trough.

Enzymes were assayed spectrophotometrically. The method of [18] was used for ALP analysis while that of [19] was used for ACP analysis. The data were subjected to analysis of students’ t test.

**RESULTS**

The level of ACP in tissues of *Cyprinus carpio* were increased concentration of atrazine (Table 1) and there were significant differing mean ACP values when compared to control fish. On the other hand ACP group III atrazine along with *Spirulina* exposure were gradually recovered (P>0.05). But the group IV *Spirulina* supplement values was not significant for (P<0.01) the period of 24, 48, 72, 96 and 120 hours respectively.
Table 2. Variations of ACP (µg/P1/mg protein/hrs) activity of *Cyprinus carpio*, exposure to atrazine and *Spirulina* for the period of 120 hours

<table>
<thead>
<tr>
<th>ORGANS</th>
<th>GROUPS</th>
<th>Hours of Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>GILL</td>
<td>I CONTROL</td>
<td>1.25 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>II ATRAZINE</td>
<td>5.32 **± 0.26</td>
</tr>
<tr>
<td></td>
<td>III ATR + SPI</td>
<td>3.12 **± 0.18</td>
</tr>
<tr>
<td></td>
<td>IV SPIRULINA</td>
<td>1.40 *± 0.09</td>
</tr>
<tr>
<td>LIVER</td>
<td>I CONTROL</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>II ATRAZINE</td>
<td>0.78 **± 0.03</td>
</tr>
<tr>
<td></td>
<td>III ATR + SPI</td>
<td>0.61 **± 0.03</td>
</tr>
<tr>
<td></td>
<td>IV SPIRULINA</td>
<td>0.42 NS ± 0.02</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>I CONTROL</td>
<td>1.28 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>II ATRAZINE</td>
<td>4.12 **± 0.20</td>
</tr>
<tr>
<td></td>
<td>III ATR + SPI</td>
<td>3.48 **± 0.20</td>
</tr>
<tr>
<td></td>
<td>IV SPIRULINA</td>
<td>1.39 NS ± 0.09</td>
</tr>
</tbody>
</table>

** Significant at 5% levels  
*  Significant at 1% levels  
NS – Non-Significant

The results show significant reduction of ALP activity in liver *Cyprinus carpio*. The group II atrazine sub-lethal concentration was decreased (P>0.05) when compared with control values. The observed values group III decreased when compared with group II. The group IV *Spirulina* supplemented values nearly control values in 24, 48, 72, 96 and 120 hours respectively (P<0.01). The activity of liver tissue of *Cyprinus carpio*, the observed values of ACP in atrazine (group II) exposure was significantly increased when compared with control values (P>0.05). The investigation of ACP activity of atrazine along with *spirulina* were gradually recovered (P>0.05). But the group IV *Spirulina* supplemented values significantly lower than the controls (P<0.01).

**DISCUSSION**

In the present study, to examined the response of fresh water fish *Cyprinus carpio* to the protective effect of *Spirulina* against atrazine toxicity. The phosphatases (ACP and ALP) are important biomarkers because they are involved in adaptive cellular response to the potential cytotoxicity and genotoxicity of pollutants [20]. The increase in some of the concentration may be as results of liver damage or arrested bone growth [21, 22]. According to [23],
phosphate plays an important role in the transport of metabolise across membranes. The rate of transport appeared to be more pronounced in the gill and kidney in comparison to the liver, considering the level of ALP activity in these organs. This may be due to the strategic roles of this organ play in the managements of toxicant and their metabolic waste.

ALP is microsomal enzyme, which is involved in membrane transport because of its high concentration in vertebrate’s kidney and its action on a number of phosphomonoesterase [24]. Decline in ALP activity may result from fall in the rate of synthesis of glycogen caused by lowered metabolic demands and electrolytic imbalance due to tissue overhydration [25].

Decrease in ALP may reflect a change in endoplasmic mass known to occur in the cell membrane [24]. Since it also function in the conversion of energy compounds NADPH to NAP [26]. Therefore, decline ALP activity could result in biosynthesis shift and energy metabolism pathway of the exposed organism [27]. Results from the present work indicate this may happen in wild fish exposed to atrazine.

*Spirulina* is a naturally digestable food that helps to protect the immune system, lower cholesterol and facilitate minerals absorption. *Spirulina* is rich in chlorophyll, protein, beta-carotene and nucleic acid. This amazing whole food detoxifies the liver and kidneys, build and enrich the blood, cleanse the arteries, enhances intestinal flora and inhibits the growth of fungi, bacteria and other microorganism. [28]. *Spirulina* reduced hepatic damage due to drug abuse fertilizers and heavy metal exposures, inflammatory response [29, 30].

The enzymatic responses to sub-lethal atrazine toxicity in *Cyprinus carpio* seem to be concentration – dependent and their usefulness as biomarkers appeared to be related to the organ studied.

**CONCLUSION**

We conclude that phosphatase levels the different organ (Gill, Liver, Kidney) not represent good indices of the effect of atrazine in *Cyprinus carpio*. However, the activities of ALP and ACP in the organs (Gill, Liver, Kidney) of fish potential useful bio-indicators in the *spirulina* is reducing the herbicide atrazine toxicity.

**AKNOWLEDGEMENT**

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