EFFECT OF BUTACHLOR ON ANTIOXIDANT ENZYME STATUS AND LIPID PEROXIDATION IN FRESH WATER FISH, CATLA CATLA

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ABSTRACT
The present study was undertaken to evaluate the influence of Butachlor, a widely used herbicide, on antioxidant enzyme system and lipid peroxidation formation in fresh water fish, Catla catla. Fishes were exposed to sublethal concentrations of Butachlor (1 ppm) and sacrificed 72 hrs after treatment. The effects of available usage of herbicide can be attenuated with the help of medicinal herbal treatment. Reactive oxygen species such as MDA was increased due to oxidative stress given by Butachlor. SOD, CAT and GPx levels were lowered in amount by the Butachlor. These enzyme levels were increased in the fishes fed with garlic along with control diet. Hence the exogenous supplements of this antioxidant through garlic helped in increasing amount of SOD, CAT and GPx.

KEYWORDS: Herbicide, Butachlor, Catla catla, antioxidant, lipid peroxidation.

INTRODUCTION
Indiscriminate use of these chemicals to improve agricultural production and yield may result in aquatic pollution due to rain and surface runoff. The poisoning by pesticides from agriculture fields is a serious water pollution problem and its environmental long-term effect may result in the incidence of poisoning of fish and other aquatic life forms, (Jyothi and Narayan, 1999). The major vehicle by which insecticides are distributed throughout the body is blood for vertebrates and haemolymph for invertebrates (Matsumure, 1985). The composition of blood reflects the total physiological condition of the organism. The haematological parameters of fish have been considered as diagnostic indices of pathological condition in animal (Hickey, 1976). The fish blood is being studied increasingly in research.
and environmental monitoring as a possible indicator of physiological and pathological changes in the fishery management and disease investigation (Mulcuhy, 1975). The main route of entry for any pesticide into the body of an aquatic organism such as fish is through the gills. From the gills it is transported to various parts of the body via the blood stream.

**MATERIALS AND METHODS**
The bulk sample of the fresh water fish, *Catla catla*, ranging in weight 14 gms to 17 gms and in length from 7cm to 10 cm was procured from the Tamilnadu fisheries Department, Aliyar, Tamilnadu and transported to the laboratory in well aerated polythene bag and acclimatized to the ambient laboratory temperature (28±0.2) in large tank. During the period of acclimatization they were fed every day with base diet and experimental diet. At the end of 72 hours of experimental period, blood samples are collected from the gills of fish groups and used for the analysis of the antioxidants lipid peroxidases, were estimated by the method of Yogi (1978), SOD was estimated by Kakkar et al. (1984), CAT was assayed by the method of Sinha (1972), GPx was assayed by the method of Rotruck et al. (1973).

**RESULTS AND DISCUSSION**

**Thio barbituric acid reactive substances (TBARS)**
The enzyme lipid peroxidases catalyse the lipid peroxidation reaction. During the degradation of polyunsaturated lipids, malonyldialdehyde compounds are formed which are reactive substances and cause toxic stress in cells. The production of the aldehyde is used as a biomarker to measure the level of oxidative stress in an organism.

In the present experimental study, the fishes treated with Butachlor (B) have an oxidative stress and show a increased level (3.97±0.25) of TBARS compared to the control A fishes (2.35±0.27). The increased level is significant at P<0.01 level. In the blood of fishes treated with Butachlor and fed with garlic (D), the level of TBARS (3.83±0.42) comparatively lesser than group B fishes (3.97±0.25). When group A and C were compared the group C (1.21±0.22) has decreased amount of TBARS (Table 1).

**Superoxide dismutase (SOD)**
Superoxide dismutase enzyme removes the Superoxide radicals. SOD is enzymes of defence in the detoxification of products resulting from oxidative stress. The present study shows a result of reduction in the SOD level (2.03±0.28) in the herbicide treated fishes (B) than the control A (4.77±0.15). The difference is significant at P<0.01 level.
The Butachlor treated fishes when fed with the garlic the fishes D were able to produce more of SOD (5.5±0.4) to overcome the oxidative stress than the group B fishes (2.03±0.28). The comparison between group A (4.77±0.15) and C (5.5±0.40) shows that the level of increase is significant at P<0.01 level (Table 2).

**Catalase (CAT)**

Catalase is an enzyme that catalyses decomposition of hydrogen peroxide to oxygen and water and is present in all aerobic cells. The control and experimental diet fed fishes (C) shows an increased level (82.68±1.79) of enzyme than control group (A) (80.11±1.05). The enzyme level is very much reduced in the stressed group of fishes (B) (49.17±4.79) than the control (A) (80.11±1.05) group. The level of significance in variation is P<0.01 level. When the stressed fishes were fed with garlic (D) the amount of the enzyme increased (52.29±5.96) than group B (49.17±4.79) (Table 3).

**Glutathione peroxidase (GPx)**

Glutathione peroxidase is an enzyme which catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides. The GPx level was very much reduced in the herbicide stressed fishes (B) (8.15±0.80) than in the control group (A) (12.14±0.63). The difference is highly significant (P<0.001). The stressed group when fed with garlic (D) they were able to increase the GPx to a considerable level (8.15±0.80 to 8.72±0.22) (Table 4).

**Anti-oxidantal effects of Allium Sativum**

The result shows a significant increase in the amount of antioxidant such as SOD, CAT, GPx and decrease in the intermediate by products of lipid peroxidation. It has long been considered that *A. sativum* has several beneficial effects for human and animals exhibiting antimicrobial antioxidant and antihypertensive properties. The garlic supplemented diet improved lysozymes activity in juvenile hybrid Tilapia and enhanced its immune ability (Diegene Ndong et al 2006). Garlic extract has been shown to reduce cholesterol levels and increase blood coagulation time (Bordia et al. 1975). According to Rahman (2003), the protective effect of garlic is associated with its antioxidant properties.

According to Diab et al. (2002) using garlic in fish forming has become popular for enhancing the activity of non-specific defence systems and conferring protection against diseases. Garlic decrease both total cholesterol and low density lipoprotein in addition to reducing blood pressure.
Garlic extracts exerts antioxidant action by scavenging reactive oxygen species (ROS), enhancing the cellular antioxidant enzymes such as SOD, CAT and GPx and increasing glutathione in the cells (Metwalley A. et al., 2009). In this present investigation also, the influence of A. sativum on the Butachlor stressed Catla catla was clearly visible.

**Effects of herbicide and garlic on MDA (TBARS)**

In the present study, the Butachlor induced fishes fed with garlic (group D), showed a comparatively decreased level of MDA than the group B fishes which were not fed with garlic. Hence it is clear that garlic has played its role on the reduction of MDA in group D fishes. The intake of garlic as an exogenous antioxidant supplier in the presence of Butachlor exposure enhances the activities of endogenous antioxidant enzymes, thus preventing a possible involvement of ROS (MDA) in the inhibition of lipidperoxidation.

**Effects of herbicide and garlic on Superoxide dismutase (SOD)**

SODs are among the first line of defence in the detoxification of the products resulting from oxidative stress. Treatment with SOD decreases ROS generation and oxidative stress. SOD activity in blood serum and tissues homogenates showed significant increase in *Tilapia nilotica* fish groups fed on diets contained garlic compared to the control group (Metwalley, 2009).

In the present investigation, the group D fishes shows significant increase in SOD level than the group B fishes because of the difference in the diet given. Hence it is clear that the oxidative stress produced by the Butachlor induced free radial formation has reduced the antioxidant enzyme such as SOD. This stress has been reduced to a significant level in the group D fishes due to the diet containing garlic. This show garlic administered with diet has played its role in the elevation of antioxidant in group D fishes. The results are in correlation with the results of Pal et al. (2009) who exogenously administered the antioxidants to the albino rats. Moreover similar results were show in the use of garlic in fish forming enhanced the activity of non-specific defence systems in *Oreochromis nilaticus* (Diab et al., 2002).
Table 1. Estimation of Thio barbituric acid reactive substances (TBARS) in the blood of *Catla catla*.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>AMOUNT OF TBARS (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control fishes fed with control diet (A)</td>
<td>2.35 ±0.27</td>
</tr>
<tr>
<td>Butachlor induced fishes fed with control diet (B)</td>
<td>3.97±0.25** (+68.93)</td>
</tr>
<tr>
<td>Control fishes fed with experimental diet (C)</td>
<td>1.21±0.22** (-48.51)</td>
</tr>
<tr>
<td>Butachlor induced with experimental diet (D)</td>
<td>3.83±0.42NS (+62.97)</td>
</tr>
</tbody>
</table>

The Each value is mean ± SD of five observations.

Signs + or – indicates increase or decrease over control.

* indicates significant (P<0.05).

** indicates significant (P<0.01).

Not significant – NS.

Table 2. Estimation of Superoxide dismutase (SOD) in the blood of *Catla catla*.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>AMOUNT OF SOD (unit/min/mg Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control fishes fed with control diet (A)</td>
<td>4.77±0.15</td>
</tr>
<tr>
<td>Butachlor induced fishes fed with control diet (B)</td>
<td>2.03 ±0.28** (-57.44)</td>
</tr>
<tr>
<td>Control fishes fed with experimental diet (C)</td>
<td>5.5±0.40** (+15.30)</td>
</tr>
<tr>
<td>Butachlor induced with experimental diet (D)</td>
<td>3.13±0.33** (-34.38)</td>
</tr>
</tbody>
</table>

The Each value is mean ± SD of five observations.

Signs + or – indicates increase or decrease over control.

* indicates significant (P<0.05).

** indicates significant (P<0.01).

Not significant – NS.

Table 3. Estimation of Catalase activity (CAT) in the blood of *Catla catla*.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>AMOUNT OF CAT (µmol/min/mg Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control fishes fed with control diet (A)</td>
<td>80.11±1.05</td>
</tr>
<tr>
<td>Butachlor induced fishes fed with control diet (B)</td>
<td>49.17±4.79** (-38.62)</td>
</tr>
<tr>
<td>Control fishes fed with experimental diet (C)</td>
<td>82.68±1.79** (-3.208)</td>
</tr>
<tr>
<td>Butachlor induced with experimental diet (D)</td>
<td>52.29±5.96NS (-34.72)</td>
</tr>
</tbody>
</table>
The Each value is mean ± SD of five observations.
Signs + or – indicates increase or decrease over control.
* indicates significant (P<0.05).
** indicates significant (P<0.01).
Not significant – NS.

Table 4. Estimation of Glutathione peroxidases (GPx) in the blood of *Catla catla*.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>AMOUNT OF GPX (µmol/min/mg/Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control fishes fed with control diet (A)</td>
<td>12.14±0.63</td>
</tr>
<tr>
<td>Butachlor induced fishes fed with control diet (B)</td>
<td>8.15±0.80** (-28.21)</td>
</tr>
<tr>
<td>Control fishes fed with experimental diet (C)</td>
<td>11.47 ±0.39** (-5.52)</td>
</tr>
<tr>
<td>Butachlor induced with experimental diet (D)</td>
<td>8.72±0.22NS (-28.17)</td>
</tr>
</tbody>
</table>

The Each value is mean ± SD of five observations.
Signs + or – indicates increase or decrease over control.
* indicates significant (P<0.05).
** indicates significant (P<0.01).
Not significant – NS.

Effects of herbicide and garlic on Catalase activity (CAT)

It is shown in the table 3 that the amount of CAT is very much reduced in the Butachlor treated fishes (B) than the control (A) fishes. The oxidative stress given by the herbicide is more in the enzyme catalase. The CAT activities are similar to the SOD activities. The activities of antioxidant enzymes SOD and CAT in the blood cells of albino rats treated with endosulfan were found significantly decreased (P>0.001) compared to the vehicle controls (Pal et al.2009). Garlic in diet has the power to increase the CAT level in the fishes (Group C and D) which were fed with experimental diet. Similar results were produced by Metwalley (2009) in *Oreochromis niloticus* fed with garlic.

Effects of herbicide and garlic on glutathione peroxidase (GPx)

The present experimental shows (Table 4) the amount GPx in the control and experimental groups of *Catla catla* explaining that the stress given to the experimental fishes has reduced the GPx level significantly (group A and B). The results carry support from various authors. African cat fish *Clarius garipinus* induced by heavy metals shows reduction in the
glutathione systems (Farombi, 2008). The experimental fishes fed with garlic have raised the level of GPx considerably. The difference between the control (A) and Butachlor induced fishes (B) were attenuated by the garlic diet to the group C and D fishes.

Hence it is understood, that in the present investigation also the garlic has influenced the *Catla catla* fish to increase the GPx level to overcome the stress given by the herbicide. The present findings suggest that the herbicide Butachlor induces oxidation stress and toxicity in the fish *Catla catla*. It has been mediated through the formation of free radicals and ROS. In the lipid peroxidation, the amount of ROS such as MDA or TBARS has increased to a significant level. Moreover, the stress also reduces the antioxidant enzymes such as SOD, CAT and GPx levels in *Catla catla*.

REFERENCE