An Approach to the Interaction Between Trichodiniasis and Pollution with Benzo-a-Pyrene in Catfish (Clarias gariepinus)

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Abstract: The changes in some blood serum components of Clarias gariepinus due to exposure to Benzo-a-pyrene and infection with the external parasite Trichodina were studied. Total blood serum protein, serum albumin and globulin concentrations were significantly (P<0.05) decreased in C. gariepinus exposed to benzo-a-pyrene and in Trichodina infected fish. Albumin/globulin (A/G) ratio in C. gariepinus was neither significantly affected due to the exposure to benzo-a-pyrene nor by Trichodiniasis. Blood serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) enzymes activities, creatinine, urea and uric acid values were significantly (P<0.05) elevated in the benzo-a-pyrene exposed fish as well as in the diseased fish. Trichodina infection together with the benzo-a-pyrene exposure led to more prominent significant important (P<0.05) decrease in the fish total blood serum protein, serum albumin and globulin concentrations and revealed drastic increase in serum AST and ALT enzymes activities as well as creatinine, urea and uric acid values. Pollution due to exposure to benzo-a-pyrene adversely affects the physiological and immunological status of catfish Clarias gariepinus leading to increased susceptibility to infection with trichodina species. In conclusion, infection with Trichodina in fish exposed to pollution of benzo-a-pyrene had the highest drastic effect on the health of fish.

Key words: Benzo-a-pyrene %Trichodina %Clarias gariepinus %AST %ALT %Albumin %Globulin

INTRODUCTION

Nowadays, pollution of aquatic environments is widespread problems. The condition is mainly deriving from discharges of different types of pollutants to rivers and lakes.

In Egypt, River Nile is the main water source and was exposed to many kinds of pollutants, whether biological or chemical. Water quality of the River Nile has been affected by the discharges of agricultural drains, industrial, municipal wastes and dead animals that are thrown in several areas along the river [1, 2].

Several biotic and abiotic stress factors affect aquatic organisms. Parasite infection represents a clear biotic stress factor to the host [3]. The external protozoan parasites, especially Trichodinides represent a significant problem in fish aquacultures. Trichodina species are essentially commensals in healthy fish. The ectozoic ones use their host as convenient substrate upon which they glide and to which temporarily attach. These parasites commonly occur in fish stocks and are distributed all over the world, affecting most fish. Stress factors, especially the high organic content or pollution whatever it is predispose for more propagation on its host and converts such commensals into parasites [4, 5].

Environmental abiotic chemical pollutants such as heavy metals, pesticides and other organics, pose serious risks to many aquatic organisms. Polycyclic Aromatic Hydrocarbons (PAHs) are widely distributed in both freshwater and coastal marine ecosystems whereas, they have been found to bio-accumulate in several aquatic species. They also represent one of the most significant classes of organic pollution due to their carcinogenic and mutagenic potentials [2, 6]. Benzo-a-Pyrene (B-a-P) represents one of the most important, dangerous mutagenic and carcinogenic PAHs and has a wide distribution in the aquatic environment [7, 8]. B-a-P releases to the environment through incomplete combustion of gasoline, garbage or any animal or plant material. Also, it finds its way to the aquatic environment through runoffs, oil spill, industrial effluents and atmospheric disposition [2, 9, 10] and Many authors have...
studied the presence of B-a-P in fresh water [11, 12], especially in Egypt [13].

Because of the long co-evolution, the host and parasite populations may both live and survive together. While, man-made chemical pollutant represents a novel stress factor without any long evolutionary adaptation. Even less is known about the effects of parasites on organic chemical pollutants in aquatic organisms, the effects on toxicity of heavy metals in zebra mussels have been studied by Kraak [14]. Also, Brown and Pascoe [15] found an increased sensitivity to Cd in the acanthocephalan-infected amphipods (Gammarus pulex). However, when toxicity assays were applied, clear differences in sensitivity between the infected and uninfected clams were found [16]. Moreover, Russo and Lagadic, [17] have studied the morphological characteristics and functions of hemocytes to compare the immunological effects of biological and chemical stress in the freshwater snail Lymnaea palustris. On the other hand, pollutants may affect the parasitic infection and cause the host to be more susceptible to infection [18-22].

Although numerous investigators have tried to identify and focus attention upon specific aquatic environmental contaminants affecting fish. There is a little comprehensive research which has been done about the possible interaction between chemical pollution and parasites of fish. So, the aim of the present study was to investigate whether the fish exposed to benzo-a-pyrene are more susceptible to the experimental infection with Trichodina spp. than the non-exposed fish. Also, the study was conducted to determine the clinical signs of mass infection with trichodinia and/or pollution with Benzo-a-pyrene in catfish (Clarias gariepinus).

**MATERIALS AND METHODS**

**Fish:** In the present study, 60 Clarias gariepinus fish were obtained alive from Giza market with body weight ranged between 80-120 g. They were transported to the laboratory in large plastic water containers and maintained in the glass aquaria, acclimatized for one week before starting the experiment. Food was not provided throughout the experimental period and also before its beginning by 48 h.

**Experimental Infection with Trichodina:** Before starting the experiment all the experimental catfish were subjected to treatment with sodium chloride then after two days, five heavy infested catfishes with trichodina were put with the third and fourth groups for two days then removed for experimental infection with trichodina

**Experimental Design:** After acclimatization fish were divided into four groups. Each group contained 15 fish. The first group represents the control group, received Dimethyl Sulfoxide (DMSO only. The second group was exposed to 1/10 LC50 of Benzo-a-Pyrene (Purchased from SIGMA) (LC50 of B-a-P = 1 ppm according to Hanna et al. [23]. B-a-P was firstly dissolved in 0.5 ml Dimethyl Sulfoxide (DMSO). The third group was experimentally infected with Trichodina. The fourth group was exposed to both Trichodina infection and B-a-P.

Blood samples: Blood was collected from the caudal vein of each fish in the four groups after 5 and 10 days from the beginning of the experiment. Serum was separated from each blood sample for biochemical analysis.

Biochemical analysis: Total protein value in each fish blood serum was determined according to Cannon et al. [24]. Serum albumin concentration was measured as described by Gustafsson [25] Blood serum globulin was calculated by subtracting the concentration of albumin from that of the total protein and albumin/ globulin ratio (A/G ratio) was calculated by dividing albumin concentration over that of globulin [26]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum were determined according to Reitman and Frankel [27]. Creatinine value was determined after Rock et al. [28]. Urea concentration was measured according to Pathson and Nauch [29]. Uric acid concentration was determined according to Schultz [30].

**Statistical Analysis:** Datas were statistically analyzed using one-way analysis of variance ANOVA model of SAS. 1996 software version (6.12).

**RESULTS**

**Clinical Signs:** The clinical signs of catfish in the group 2 (benzo-a pyrene) manifested in the form of nervous manifestations, abnormal swimming, circling and erratic movement with abnormal skin, head and fins abrasions (Fig. 1- A and B ) in few cases and darkness in the others with inflamed vent (Fig 1 D), while the clinical signs of the
Fig. 1: Catfish (Clarias gariepinus) exposed to Benzo-a-pyrene Showing: A. Skin and head abrasions. C. pale anemic gills. D. inflamed vent. E. inflamed, hemorrhagic internal organs and distended gall bladder. F. silver impregnated Trichodina sp (X 40).

third group (trichodina) were flashing with some hemorrhagic spots on the skin, the clinical signs of the fourth group (benzo-a-pyrene+trichodina infection) were more severe.

Post Mortem Findings: The post mortem findings of the second group were congestion and haemorrhages in all internal organs (Fig 1 E), in addition to pale anemic gills (Fig 1 C) while in the third group, the internal organs were normal and the gills was congested. In the fourth group, the post mortem lesions were like the second one.

Assessment of Experimental Trichodina Infection: After two days all catfish of the second and fourth groups were heavily infected with trichodina species when examined with Olympus compound microscope X 40. (Fig 1 F).

Biochemical Findings: As shown in Table 1, total serum protein, serum albumin and globulin were significantly (P<0.05) decreased in Trichodina infected Clarias gariepinus, benzo-a-pyrene exposed fish and in Trichodina infected catfish exposed to benzo-a-pyrene after infection or exposure or both for 5 and 10 days comparing with control. The effect of exposure together with infection on the fish serum proteins is more drastic than either exposure or infection alone. The albumin/globuline (A/G) ratio (Table 1) was not significantly different in Trichodina infected and benzo-a-pyrene exposed catfish than control.

As represented in Table 2, serum ALT and AST enzymes activities were significantly (P<0.05) elevated in C. gariepinus exposed to benzo-a-pyrene, Trichodina infected C. gariepinus and Trichodina infected catfish exposed to benzo-a-pyrene after 5 and 10 days as compared with the control group. The enzyme activity in either infected or non infected catfish exposed to benzo-a-pyrene was significantly (P<0.05) higher than infected catfish.

Creatinine, urea and uric acid values in serum (Table 2) were significantly (P<0.05) higher in C. gariepinus exposed to benzo-a-pyrene, Trichodina infected fish and Trichodina infected fish exposed to benzo-a-pyrene after 5 and 10 days than the control group. The exposure of infected and non infected fish to benzo-a-pyrene for 5 or 10 days significantly (P<0.05) elevated the concentration of creatinine, urea and uric acid in the serum as comparing with the infected fish without exposure as shown in Table 2.
DISCUSSION

Benzo-a-pyrene is one of the most important, dangerous mutagenic and carcinogenic polycyclic aromatic hydrocarbons (PAHs) formed when gasoline, garbage or any animal or plant materials are incompletely burned. The aim of the present study was to determine the effect of pollution with benzo-a-pyrene on catfish (Clarias gariepinus), susceptibility of infection with trichodina in polluted fish and interaction between both. The clinical signs were nervous manifestation abnormal swimming, circling and abnormal skin pigmentation which may be attributed to the direct damaging effect of benzo-a-pyrene on nervous system affecting the nervous control, this explanation may be supported by the finding of Tu et al. [31]. The pale gills and congestion with hemorrhages of internal organs may be due to the anemic and inflammatory effect of pollution with benzo-a-pyrene. The clinical signs and post mortem lesions nearly agree with the findings of Hose et al.[32], Irwin et al.[34], Laycock et al.[8], Marzouk et al.[23] and Hanna et al.[33]. They also reported that the ecological significance of such morphological abnormalities revealed a decrease in feeding and inability to escape predators which finally leading to reduced survivability. They also added that the continuous production of mutagenic and carcinogenic pollutants resulting in anemia and impaired ability to respond to different environment stress factors and diseases. Regarding the clinical signs which appear after the experimental infection with Trichodina, including flashing and congestion of gills these finding may be due to severe irritation of trichodina during their gildings movement, this findings coincide with those of findings of Vera et al.[5], Osman [35], Vera et al.[5] and Ozer and Ozturk [36]. The present study revealed that the Clarias gariepinus were more susceptible to infection by Trichodina spp. during the first two days after exposure to Benzo-a-pyrene than the non exposed fish. This is in accordance with Jokinen et al. [37] who demonstrated that the immune functions are impaired in Clarias gariepinus originating from the contaminated water. Also, in previous studies, a similar explanation was suggested for the increase of the ciliate Ichthyophthirius multifiliis infection [21] and the increase in Dactylogyrus species communities [38] in roach from contaminated Lake Vatia. Moreover, Barker et al. [20] reported that the prevalence and intensity of metacercariae of Cryptocotyl lingua on the fins and gills of winter founder (Pleuronectes americanus) in water adjacent to a pulp and paper mill.

Table 1: Serum proteins concentrations (g/dl) and A/G ratio in Clarias gariepinus exposed to benzo-a-pyrene and infected with Trichodina

<table>
<thead>
<tr>
<th>Parameter Group</th>
<th>Total protein 5 days</th>
<th>Total protein 10 days</th>
<th>Albumin 5 days</th>
<th>Albumin 10 days</th>
<th>Globuline 5 days</th>
<th>Globuline 10 days</th>
<th>A/G ratio 5 days</th>
<th>A/G ratio 10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.06± 0.23 A</td>
<td>4.98± 0.20 A</td>
<td>2.32± 0.15 A</td>
<td>2.30± 0.10 A</td>
<td>2.74± 0.10 A</td>
<td>2.68± 0.10 A</td>
<td>0.95± 0.03 A</td>
<td>0.96± 0.03 A</td>
</tr>
<tr>
<td>Benzo-a-pyrene</td>
<td>3.85± 0.19 a</td>
<td>3.64± 0.20 aB</td>
<td>1.80± 0.07 a</td>
<td>1.68± 0.09 aB</td>
<td>2.05± 0.09 a</td>
<td>1.96± 0.09 a</td>
<td>0.87± 0.03 A</td>
<td>0.89± 0.04 A</td>
</tr>
<tr>
<td>Trichodina</td>
<td>4.15± 0.21 aB</td>
<td>3.95± 0.18 aC</td>
<td>1.87± 0.12 aB</td>
<td>1.80± 0.12 aC</td>
<td>2.28± 0.09 aB</td>
<td>2.15± 0.08 aC</td>
<td>0.90± 0.02 A</td>
<td>0.93± 0.03 A</td>
</tr>
<tr>
<td>Benzo-a-pyrene and Trichodina</td>
<td>3.40± 0.15 ab</td>
<td>2.90± 0.16 abc</td>
<td>1.54± 0.06 ab</td>
<td>1.39± 0.06 abc</td>
<td>1.86± 0.07 ab</td>
<td>1.51± 0.06 abc</td>
<td>0.83± 0.04 A</td>
<td>0.82± 0.04 A</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E; n=5.

The small letters a, b and c represents significant changes against capital letters A, B and C in the same column respectively by LSD using ANOVA at p=0.05.

Table 2: Some biochemical changes in the blood serum of Clarias gariepinus exposed to benzo-a-pyrene and infected with Trichodina

<table>
<thead>
<tr>
<th>Parameter Group</th>
<th>ALT (U/dl) 5 day</th>
<th>ALT (U/dl) 10 day</th>
<th>AST (U/dl) 5 day</th>
<th>AST (U/dl) 10 day</th>
<th>Creatinine (mg/dl) 5 day</th>
<th>Creatinine (mg/dl) 10 day</th>
<th>Urea (mg/dl) 5 day</th>
<th>Urea (mg/dl) 10 day</th>
<th>Uric acid (mg/dl) 5 day</th>
<th>Uric acid (mg/dl) 10 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34.75 ± 2.56 A</td>
<td>35.00 ± 2.40 A</td>
<td>71.50 ± 3.35 A</td>
<td>72.17 ± 3.58 A</td>
<td>0.85 ± 0.03 A</td>
<td>0.90 ± 0.03 A</td>
<td>31.80 ± 1.78 A</td>
<td>32.15 ± 1.56 A</td>
<td>3.10 ± 0.12 A</td>
<td>3.21 ± 0.23 A</td>
</tr>
<tr>
<td>Benzo-a-pyrene</td>
<td>61.23 ± 3.34 Ab</td>
<td>64.60 ± 4.04 Ab</td>
<td>121.58 ± 7.42 B</td>
<td>134.04 ± 8.27 B</td>
<td>1.25 ± 0.04 Ab</td>
<td>1.32 ± 0.05 Ab</td>
<td>43.16 ± 1.67 Ab</td>
<td>46.23 ± 2.03 Ab</td>
<td>4.35 ± 0.15 Ab</td>
<td>4.75 ± 0.18 Ab</td>
</tr>
<tr>
<td>Trichodina</td>
<td>44.28 ± 2.08 AbC</td>
<td>45.76 ± 2.45 AbC</td>
<td>93.83 ± 5.60 AbC</td>
<td>96.37 ± 5.57 AbC</td>
<td>1.02 ± 0.04 AbC</td>
<td>1.05 ± 0.04 AbC</td>
<td>37.85 ± 1.20 AbC</td>
<td>39.42 ± 1.63 AbC</td>
<td>3.57 ± 0.13 AbC</td>
<td>3.81 ± 0.05 AbC</td>
</tr>
<tr>
<td>Benzo-a-pyrene and Trichodina</td>
<td>68.08 ± 3.69ac</td>
<td>72.36 ± 3.64ac</td>
<td>132.60 ± 6.65ac</td>
<td>144.48 ± 6.72ac</td>
<td>1.36 ± 0.05ac</td>
<td>1.40 ± 0.05ac</td>
<td>45.71 ± 1.77ac</td>
<td>49.51 ± 2.34ac</td>
<td>4.55 ± 0.21ac</td>
<td>5.24 ± 0.22ac</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E; n=5.

The small letters a, b and c represents significant changes against capital letters A, B and C in the same column respectively by LSD using ANOVA at p=0.05.
were higher than in the clean reference area. Other studies have shown that increased prevalence of protozoan ciliates is a sensitive indicator of several pollutants [18, 22] and this was also experimentally shown by Lehtinen [39]. A number of studies have suggested immuno-suppression among freshwater, marine and estuarine fishes in highly contaminated areas, which may represent a main cause of pollutant associated diseases. Confirming this also, both increase and decrease in the prevalence of ectoparasitic monogeneans which have been noted in fish exposed to various pollutants [18, 40]. It seems, therefore, that the effluents influence the parasite communities both through the immunological response and condition of the host and also affect the parasites themselves.

In the present study the exposure of *Clarias gariepinus* for 5 and 10 days led to significant decrease in total serum protein, albumin and globulin. The infection of the fish with Trichodina also led to significant decrease in these parameters. The exposure to benzo-a-pyrene in the presence of Trichodina infection caused more drastic decline in the concentration of total protein, albumin and globulin in the blood serum of the catfish. A/G ratio in *C. gariepinus* was neither significantly affected by the exposure to benzo-a-pyrene nor by the infection with Trichodina. The decrease in serum proteins may be due to some degree of liver dysfunction under the conditions of pollution or disease. Under the stress conditions the gills become leaky to water and ions leading to disturbance in osmoregulatory balance [41]. So the decrease in serum total protein, albumin and globuline may be due to haemodilution. Also, this result was in agree with that of Marzouk *et al.* [8] who revealed total hypoproteinaemia and hypoglobulinaemia in B-a-P exposed *Oreochromis niloticus* fish. The A/G ratio is an index used to track relative changes in the composition of serum or plasma [42]. The non changed A/G ratio value indicates that the albumin and globulin were nearly equally decreased. Then the osmoregulatory imbalance and haemodilution were more likely the cause of the decline in blood serum total protein, albumin and globulin levels in benzo-a-pyrene exposed and *Trichodina* diseased catfish. Moreover, the necrosis of the hepatic tissue may result in decrease of protein synthesis [43].

Concerning the blood serum ALT and AST enzymes activities in *Clarias gariepinus*, shows that the exposure of catfish to benzo-a-pyrene and Trichodina infection as well as the exposure of fish to benzo-a-pyrene beside the *Trichodina* infection significantly stimulated the activities of both enzymes after 5 and 10 days against control. The exposure of fish to benzo-a-pyrene in the presence or absence of Trichodina infection is significantly more powerful in stimulating ALT and AST enzymes activities than Trichodina infection alone. The increase of serum AST and ALT activities in fish exposed to benzo-a-pyrene and Trichodina infected fish may be due to hepatic cells injury or increased synthesis of the enzymes by the liver as described by Yang and Chen [44] in stressed fish. Also [8] revealed significant increase in ALT and AST after exposure of *Oreochromis niloticus* to B-a-P.

Benzo-a-pyrene intoxication, *Trichodina* disease and benzo-a-pyrene intoxification accompanying *Trichodina* disease, significantly increased creatinine, urea and uric acid contents in the blood serum of *C. gariepinus* after 5 and 10 days comparing with control. The significantly highest increase in serum creatinine, urea and uric acid levels taken place in catfish exposed to benzo-a-pyrene in the presence of Trichodina infection, while the least significant increase occurred in diseased fish without exposure. The increased levels of serum creatinine in the current study may be induced by glomerular insufficiency, increased muscle tissue catabolism or impairment of carbohydrate metabolism [45]. The urea is excreted in fish mainly through the gills. So in the present study the elevation in the urea level may be due to gill dysfunction [46]. Elevated serum uric acid levels may be due to disturbance in the kidney function [47].

It was concluded that the alternations of the blood serum parameters in the benzo-a-pyrene exposed *Clarias gariepinus* and Trichodina species infected fish are clear manifestation of adverse effects of benzo-a-pyrene and Trichodina on the vital organs and tissues. It is obvious that the benzo-a-pyrene pollution was more highly dangerous than the external parasitism, Trichodiniasis. However the occurrence of pollution with benzo-a-pyrene affect severely the physiological and immunological status of catfish *Claris gariepinus* increasing susceptibility to infection with trichodina species. The presence of parasite infection (Trichodiniasis) with pollution of benzo-a-pyrene had the highest drastic effect on the health of fish.

**REFERENCES**


289