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Induction of apoptosis and DNA damage by 4-nonylphenol in African catfish (*Clarias gariepinus*) and the antioxidant role of *Cydonia oblonga*

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ABSTRACT

In this study, we assessed the toxic effects of sub lethal concentration (0.1 mg l^{-1}) 4-nonylphenol (4-NP) on serum biochemical parameters, liver lipid peroxidation (LPO) and antioxidant enzymes of the African catfish *Clarias gariepinus* for 14 days and the ability of the quince leaf extract to alleviate the effects of (4-NP). Fish were categorized into four groups: control, exposure to 0.1 mg l^{-1} 4-NP, exposure to 0.1 mg l^{-1} 4-NP with quince leaf extract (10 ml/30 L water), and exposure to 0.1 mg l^{-1} 4-NP with quince leaf extract (20 ml/30 L water). 4-NP exposure induced a significant ($p < 0.05$) increase in the levels of glucose, AST, ALT, creatinine, urea, uric acid, cholesterol, and G6PDH as well as, the percentages of hepatic LPO level, DNA fragmentation, and apoptotic erythrocytes ($p < 0.05$). A significant ($p < 0.05$) decrease in alkaline phosphatase (ALP), total protein, albumin, globulin, total lipids, and LDH were also recorded. Liver enzyme activities (SOD, CAT and TAC) were increased. Addition of the quince leaf extract into the water was able to reinstate the alterations in biochemical parameters, antioxidant biomarkers, apoptotic level and hepatic DNA damage induced by 4-NP.

1. Introduction

In recent times, the aquatic ecosystems have become continually contaminated with chemical pollutants from different sources. One of the most dangerous pollutants is nonylphenol ethoxylates (NPEs) which decomposed to originate a single toxic product known as 4-nonylphenol (Mekkawy et al., 2011; Rivero et al., 2008). Some investigations have studied the hematotoxic, biochemical, hormonal disruption, histopathological, embryotoxic, and genotoxic effects of 4-NP on *C. gariepinus* (Mekkawy et al., 2011; Sayed et al., 2012a, 2012b, 2013b, 2012c, 2011) which indicate that, the bioaccumulation of 4-NP in fish is the most probable cause of these effects (Soares et al., 2008). Hence, Fish hematological parameters could be ~~a~~ good biomarkers for monitoring the toxicity in aquatic ecosystems and evaluating the environmental health because they are very sensitive to pollutants (Fazio et al., 2012, 2013a, 2013b, 2013c). The estimation of the alterations in both biochemical and antioxidant parameters of fish is considered as a sensitive tool for detecting the adverse effects of pollutants (Almeida et al., 2002; Sayed et al., 2016). Increased or decreased the activities of antioxidant enzymes serves as good indicators of ~~pollutant-mediated~~ pollutant-mediated oxidative stress (Ahmad et al., 2000; Martínez-Álvarez et al., 2005; Sayeed et al., 2003).

In the past few years, interest in herbal plants has increased worldwide (Marques and Farah, 2009). The antioxidant role of quince leaf extract (*Cydonia oblonga Miller*) has been studied (Oliveira et al., 2012, 2007; Sayed et al., 2013a; Silva et al., 2004, 2008).

The most widespread freshwater fishes in Africa is the African catfish *C. gariepinus* (Nguyen and Janssen, 2002) and its economic importance extended from; about 17% of the annual fish production is represented by the Clarias fishery (Ololade and Oginni, 2010), high growth rate, high consumption rate (Adewolu et al., 2008; Karami et al., 2010), and suitable model for toxicological studies (Mahmoud et al., 2009). The aim of the present investigation was to assess whether 4-nonylphenol (4-NP) induced changes in the biochemical profile, the antioxidant status and the apoptotic level in erythrocytes of *C. gariepinus* and to investigate the probable protective effects of the quince leaf extract on 4-NP -induced toxicity.

2. Materials and methods

2.1. Fish

Healthy catfish *C. gariepinus* with average body weight 255 ± 5 g were collected from the river Nile at Assiut and were then transported to the Fish Biology Laboratory at the Zoology Department, Faculty of Science, Assiut University. The fish were kept together in 100 l

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rectangular tanks containing dechlorinated tap water and air pumps under laboratory circumstances (conductivity 270 µM cm^{-1}; pH 7.6; Dissolved oxygen 6.9 mg L^{-1}; temperature 26–27 °C; photoperiod 12:12 light: dark) for 10 weeks for acclimatization. The fish were fed on a commercial pellet diet (3% of body weight per day) and the water was changed daily to reduce impurities from metabolic wastes.

2.2. Chemicals

4- Nonylphenol (4-NP) was obtained from Sigma-Aldrich (Schnelldrof, Germany) with a purity of 99.3%. Biochemical kits of glucose, aspartic aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, creatinine, urea, uric acid, total lipids, and cholesterol were purchased from SG Mitalia Co., USA. Lactate Dehydrogenase (LDH) and Glucose-6-Phosphate Dehydrogenase (G6PDH) were determined using kits, Stanbio LDH (UV- Rate) USA and RANDOX Laboratories Ltd., PD410, United Kingdom, respectively. All other biochemical kits (LPO, SOD, CAT and TAC) were bought from Bio-Diagnostic Co., Cairo, Egypt.

2.3. Quince preparation and extraction

Healthy quince leaves were collected from quince trees cultivated on a farm of the Faculty of Agriculture, Assiut University in June (before fruit ripening). The quince leaf extract was prepared as described in Osman et al. (2010). The methanolic leaf extract was analyzed and described in details by Osman et al. (2010).

2.4. Experimental design

The adapted catfish were categorized into four groups (9 fish per each); the first group was the control, second group was exposed to (0.1 mg L^{-1}) 4- nonylphenol (4-NP), the third group was exposed to (0.1 mg L^{-1}) 4-NP with the adding of quince leaf extract (10 ml/30 L water) and the fourth group exposed to (0.1 mg L^{-1}) 4-NP with the adding of quince leaf extract (20 ml/30 L water), there were three replicates for each group. The concentration of 4-NP exposure (0.1 mg L^{-1}) was chosen with the toxic observed value (Mekkawy et al., 2011). The conditions of the experiment were at that of acclimatization with changing all the tap water and concentration of 4-NP every day.

2.5. Biochemical analysis

At the end of the experiment, six fish from each group were collected and anesthetized with 100 mg L^{-1} benzocaine solution (Neiffer and Stamper, 2009). Blood samples were collected from the caudal vessels of fish and allowed to clot in clean, dry centrifuge tubes at room temperature, then centrifuged at 5000 rpm, at 4 °C for 20 min and the serum were separated for the analysis of biochemical parameters (glucose, aspartic aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, creatinine, urea, uric acid, total lipids, LDH, cholesterol, and G6PDH).

2.6. Liver LPO, antioxidant biomarkers, and DNA fragmentation measurements

Liver tissue was stored at −80 °C for liver antioxidant biomarkers (LPO, SOD, CAT and TAC), lipid peroxidation, and DNA fragmentation measurements, Samples of liver tissues were homogenized in cold phosphate buffer saline (0.1 M pH 7.4) using a Potter–Elvejhem glass/Teflon Homogenizer. Then, the homogenate was filtered and centrifuged for 10 min at 4 °C with a velocity of 1600 rpm; the supernatant was stored at −20 °C until analysis. LPO level was detected using the supernatant (20%) according to the method of Ohkawa et al. (1979). The activity of SOD was determined by the method described by Nishikimi et al. (1972). Determination of CAT activity, according to the method of Aebi (1984) and total antioxidant capacity (TAC) was assessed according to Koracevic et al. (2001).

DNA Fragmentation was determined by the procedure of Kurita-Ochiai et al. (1999) using a spectrophotometer (Micro lab 200 vital scientific Dieren, The Netherlands) at 575 or 600 nm against the reagent blank. The percentage of fragmented DNA was estimated by the following formula: % of fragmented DNA = fragmented DNA /(fragmented + intact DNA) ×100.

2.7. Apoptosis detection

Blood smears were prepared after completion of the desired exposure. The smears were fixed in absolute methanol for 10 s after drying at room temperature and then stained with Acridine Orange stain (Life Technologies, Carlsbad, USA) to detect erythrocytes apoptosis. The modified protocol of (Darzynkiewicz, 1990) was used to detect the apoptosis in RBCs as described in details by Sayed (2016). Cells were observed under Zeiss Axioplan2 fluorescence microscope (×200) provided with a digital 3 CCD color video camera (Sony, AVT-Horn).

2.8. Statistical analyses

All values are presented as mean ± SE. The data obtained from the experiment were subjected to one-way analysis of variance (ANOVA) test using (SPSS 2004), version19. Means were tested using the least significant difference (LSD) test to compare the blood biochemistry values between control and treated groups. In all cases, $P < 0.05$ was the accepted significance level.

2.9. Ethical statement

Experimental setup and fish handling were approved by the Research, Ethical Committee of the Faculty of Science, Assuit University, Assuit, Egypt.

3. Results

The effects of (4-NP) subacute intoxication, as well as the protective effects of the quince leaf extract on serum biochemical profile of the African catfish, are shown in Table 1. The glucose level of blood of the African catfish showed a significant ($P < 0.05$) elevation in fish exposed to NP compared with control (Table 1). Glucose was increased in the 3rd group exposed to 4-NP +10 ml quince extract compared to the control. Serum liver enzymes activity of AST and ALT were significantly ($P < 0.05$) increased with NP toxicity, 4-NP +10 ml quince extract and 4-NP +20 ml quince extract and such increment was higher in the group exposed to 4-NP when compared to that in the 3rd and 4th groups (Table 1). ALP was significantly ($P < 0.05$) decreased in *C. gariepinus* exposed to 4-NP when compared with control (Table 1). Adding the quince extract (10 ml) into the water along with 4-NP could significantly up-regulate ALP compared to the intoxicated group; while quince extract (20 ml) increased ALP level significantly compared to the 4-NP-intoxicated group. A significant ($P < 0.05$) decrease was recorded in serum total protein, albumin and globulin of the group exposed to NP only compared to the control (Table 1). Interestingly, adding quince extract into the water along with 4-NP could significantly enhance the levels of total protein, albumin, and globulin, particularly; the 4th group which received the high dose of quince extract (20 ml/30 L water).

Concurrently, the results of serum renal products indicated that creatinine and urea were significantly ($P < 0.05$) higher in the 2nd group exposed to 4-NP only compared to the control (Table 1). In contrary, these parameters reversed nearly to the control values in the blood of fish exposed to 4-NP with quince extract. Whereas, mean values of uric acid exhibited a marked ($P < 0.05$) increase in all the