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BIOCHEMICAL BIOMARKERS FOR DOSE-DEPENDENT DAMAGE CAUSED BY 4-NONYLPHENOL IN THE JUVENILE *CLARIAS GARIEPINUS*

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The present study investigates the dose-dependent damage caused by 4-nonylphenol exposure in juvenile of *Clarias gariepinus* using some biomarkers and the effect of recovery time. Healthy juvenile *C. gariepinus* of both sexes were classified into four groups (7fish/ group; two replicates). The first one was a control group, and the other three groups were exposed for 15 days to 4-nonylphenol concentrations as 0.1 mg/L, 0.2 mg/L, and 0.3 mg/L respectively, then 15 days as a recovery period after exposure time. The parameters exhibiting significance either increased with the increase of the 4-NP doses from 0.0 in the control to 0.3 mg/L (AST, ALT, TP, Glu, Cr, and UA) or decreased with such increased doses (Alb, and Glo). In conclusion, the liver and kidney functions parameters were indicated the hepatotoxicity and nephrotoxicity of 4-NP and their alterations have decreased to a great extent after a recovery period of 15-days in a reverse order.

Keywords: 4-nonylphenol; biochemistry.

1-INTRODUCTION

Water pollution is considered to be one of the major problems worldwide especially in Egypt (El-Kowrany et al., 2016; Mekkawy et al., 2019) with regard to the fields of ecology, agriculture, veterinary, pharmaceuticals, and medicine (Khandale et al., 2018). Many toxicological studies emphasized the phenolic wastes to be the most common water pollutants generated from several industrial processes (Araujo et al., 2018; Buikema et al., 1979). These phenolic wastes led to severe ecological damage, affecting the health of aquatic habitats and species, particularly fish as a consequence of their accumulative toxic concentrations (Bai et al., 2011; Priac et al., 2017).

One of the most toxic products of those phenolic pollutants is 4-nonylphenol (4-NP) because it is highly used, soluble, and stable in the environment (Araujo et al., 2018; Priac et al., 2017; Rivero et al., 2008; Soares et al., 2008). 4-NP has been reported as having hemotoxic, hormonal disruptive, antioxidant destructive, embryotoxic and carcinogenic impacts on various teleost fish species; its resistance towards biodegradation is evident (Cionna et al., 2006; Ishibashi et al., 2006; Mekkawy et al., 2011; Popek et al., 2006; Sayed et al., 2013; Sayed et al., 2012; Sayed et al., 2011; Soares et al., 2008; Staples et al., 2004; Yang et al., 2008; Zaccaroni et al., 2009). So, many countries have banned the use of nonylphenol ethoxylates (NPEs) in detergents and other commercial products (Union, 2002). The environmental protection agency referred to sever impacts of nonylphenol (NP) on the freshwater life (Vincent and Sneddon, 2009).

Different blood cell indices including hemotoxic and biochemical ones are used as major biomarkers of systemic response to environmental stress in fishes (Abou Khalil et al., 2017; Mekkawy et al., 2011; Sayed and Soliman, 2018).

African catfish *Clarias gariepinus* is distributed throughout Africa and has economic importance (Nguyen and Janssen, 2002). Recently, it has been used as an excellent model in toxicological studies (Kotb et al., 2018; Mekkawy et al., 2019; Mekkawy et al., 2011). Although, there are so many papers on 4-nonyphenol in fish but still there is a limited knowledge about the effect of 4-nonylphenol (estrogenic and toxic pollutant), it is dose-dependent or chemical like hormone only.YESSo, the present study aims at investigating the dose-dependent damage caused by 4-NP in the juveniles of

C. garepinus through the evaluation of biochemical parameters as biomarkers.

2-MATERIALS AND METHODS

2.1 Specimens collection

Healthy juvenile *Clarias gariepinus* of both sexes with a mean weight of 108.18 ±3.5 g and a mean length of 26±0.3 cm were collected from private farm at Assiut. Fish were maintained on a natural photoperiod12 h: 12 h light-dark cycle for two months in 100L-tanks to acclimatize and to be free of any natural environment hazards. The fish were fed 3% of body weight per day, receiving a commercial pellet diet (SKRETTING Company, Egypt). The water used to raise the fish was dechlorinated and continuously aerated tap water. Avoiding stress on fish during handling, water change, and redosing every day was considered. After acclimatization, the African catfish were examined to make sure of being healthy and have no external parasites according to (AFS-FHS, 2003).–All experiments were permitted by the Committee of the Faculty of Science, Assiut University, Egypt.

2.2 Experimental design

Fish were classified into four groups (7fish/ group, two replicates each group). The first one was a control group and the other three groups were exposed for 15 days to 4-nonylphenol of 0.1 mg/L, 0.2 mg/L, and 0.3 mg/L respectively, then a recovery period of 15-days after exposure time. Half of the water was changed with re-dosing every day. 4-Nonylphenol (analytical standard) was obtained from Sigma–Aldrich (Tokyo, Japan).

2.3. Blood sample collection and biochemical Parameters

At the end of the exposure and recovery periods, fish from each group were randomly selected and anesthetized using ice to lessen the stress (Wilson et al., 2009) to collect blood samples from the caudal veins into non-heparinized tubes for biochemical parameters measurements.

Blood in the non-heparinized tubes was allowed to clot at 4 °C and was then separated via centrifugation at 5000 rpm at 4 °C for 20 min to isolate the serum. The analysis of biochemical parameters including glucose, aspartic aminotransferase (AST), alanine aminotransferase, albumin, total protein, globulin, creatinine, urea, and uric acid were estimated using kits (Biodiagonstic, Egypt).

2.8. Statistical analysis

The basic statistics, means, standard error, and ranges were estimated. The patterns of variations were studied by one-way ANOVA using the SPSS package (IBM-SPSS, 2012) at 0.05 and 0.001 significance level. The Tukey-HSD test and Dunnett t-tests were considered for multiple comparisons.

3. RESULTS

3.1. Biochemical parameters

The Biochemical parameters of *Clarias gariepinus* exposed to 4-NP for 15 days are presented in Table 1. The parameters exhibiting significance either increased with the increase of 4-NP doses from 0.0 in the control to 0.3 mg/L (AST, ALT, TP, Glu, Cr, and UA) or decreased with such increased doses (Alb and Glo). Similar patterns of significant variations toward increase or decrease were recorded after recovery for 15-days (Table 1). Such situation referred to the fact that the 15-day recovery period was insufficient to remove the impacts of 4-NP doses in concern.

Table (1): Effect of different doses of 4-nonylphenol on the biochemical parameters, mean ± SE (range) of the African catfish *Clarias gariepinus* after exposure to 4-nonylphenol for 15 days and recovery for 15 days.

Treatments / Parameters	Control	0.1 mg/L 4-NP	0.2 mg/L 4-NP	0.3 mg/L 4-NP
	M±SE (Min-Max)	**M±SE (Min-Max)**	**M±SE (Min-Max)**	**M±SE (Min-Max)**
Exposure				
Alb (mg/dl)	1.82 ± 0.03[a] (10.8 - 11.9)	1.57 ± 0.03 [b] (1.5 - 1.64)	1.48 ± 0.026 [bc] (1.45 -1.56)	1.35 ± 0.0500[c] (1.23 - 1.47)
Glo (g/dl)	3.22 ± 0.06 [a] (3.11 - 3.4)	2.82 ± 0.009 [b] (2.8 - 2.84)	2.69 ± 0.087[b] (2.45 - 2.84)	2.54 ± 0.118[b] (2.35 - 2.89)
Alb / Glo	56.53 ±1.85[a] (51.47-60.12)	55.752±1.027[a] (53.57-57.74)	55.28±2.03[a] (51.76-59.18)	53.65±4.134[a] (42.56-62.55)
AST (µ/l)	33.39 ± 0.62[a] (32.14 - 35.14)	35.78 ± 0.759 [b] (34.14 - 37.7)	38.027 ± 0.28 [c] (37.2 - 38.5)	39.06 ± 0.45[c] (38.14 - 39.9)
ALT (µ/l)	17.13 ± 0.3 [a] (16.25 - 17.54)	20.755 ± 0.61 [b] (19.22 - 22.15)	24.075 ± 0.74 [c] (22.2 - 25.8)	27.25 ± 0.55[d] (25.8 - 28.5)
TP (mg/dl)	4.05 ± 0.11[a] (3.8 - 4.3)	4.48 ± 0.14 [ab] (4.21 - 4.8)	4.83 ± 0.032 [b] (4.78 - 4.93)	5.0425 ± 0.23[b] (4.35 - 5.4)
Glu (mg/dl)	69.08 ± 2.15 [a] (65.4 - 75.33)	89.725 ± 0.94[b] (87.9 - 92.3)	94.72 ± 3.09 [b] (85.6 - 98.6)	96.875 ± 0.68[b] (95.7 - 98.5)
Cr (g/dl)	0.347 ±0.012[a] (0.32 - 0.38)	0.445 ± 0.009 [b] (0.42 - 0.46)	0.47 ± 0.008 [b] (0.45 - 0.49)	0.53675 ± 0.005[c] (0.522 - 0.55)
UA (mg/dl)	2.507 ± 0.02 [a] (2.46 - 2.55)	2.645 ± 0.06[b] (2.55 - 2.81)	2.92 ± 0.026 [c] (2.87 - 2.98)	3.3 ± 0.028[c] (3.22 - 3.34)
Recovery				
Alb (mg/dl)	1.82 ± 0.025 [a] (1.78 - 1.88)	1.64 ± 0.01 [b] (1.6 - 1.68)	1.57 ± 0.02 [b] (1.54 - 1.65)	1.43 ± 0.011[c] (1.42 - 1.47)
Glo (g/dl)	3.27 ± 0.047 [a] (3.2 - 3.40)	2.93 ± 0.01 [b] (2.9 - 2.98)	2.65 ± 0.06 [c] (2.5 - 2.8)	2.59 ± 0.027[c] (2.55 - 2.65)
Alb / Glo	55.69±1.2[a] (52.35-57.8)	56.083±0.446[a] (55.17-57.19)	59.32±1.417[a] (55.357-61.6)	55.37±1.0035[a] (53.58-57.647)
AST (µ/l)	33.93 ± 0.48 [a] (33.14 - 35.22)	35.49 ± 0.37 [b] (34.5 - 36.4)	37.07 ± 0.26 [c] (36.4 - 37.5)	38.55 ± 0.29 [d] (37.8 -39.2)
ALT (µ/l)	17.18 ± 0.132 [a] (16.8 - 17.42)	18.57 ± 0.25 [b] (17.9 - 19.2)	22.55 ± 0.22 [c] (22.2 - 23.2)	38.55 ± 0.29[d] (37.8 -39.2)
TP (mg/dl)	3.87 ± 0.118 [a] (3.7 - 4.2)	4.24 ± 0.02[b] (94.2 - 4.3)	4.52 ± 0.05 [c] (4.36 - 4.62)	4.80 ± 0.06 [d] (4.65 - 4.95)
Glu (mg/dl)	68.37 ± 1.46 [a] (65.4 - 72.40)	78.12 ± 0.85 [b] (75.9 - 79.9)	88.15 ± 0.35 [c] (87.5 - 88.9)	93.37 ± 0.69[d] (92.4 - 95.4)
Cr (g/dl)	0.34 ± 0.012 [a] (0.32 - 0.38)	0.38 ± 0.004 [a] (0.3 - 0.39)	0.45 ± 0.01 [b] (0.42 - 0.47)	0.53 ± 0.007[c] (0.52 - 0.55)
UA (mg/dl)	2.51 ± 0.05 [a] (0.32 - 0.38)	2.81 ± 0.06 [b] (2.6 - 2.98)	2.97 ± 0.007 [b] (2.9 - 2.98)	3.15 ± 0.025[c] (3.11 - 3.2)

Values with the same letters within a parameter are not significantly different at 0.05 level (horizontal comparison).
Alb , Albumin ; Gol , Globulin ; Alb/ Gol , Albumin / Globulin ; AST , Aspartate aminotransferase ; ALT , Alanine aminotransferase ; TP , Total Protein , Glu, Glucose ; Cr , Creatinine ; UA , Uric acid and NP,4-nonylphenol.

4. DISCUSSION

Various scientific efforts were exerted to discover different valid and innovated biomarkers through the use of fish exposed to a wide range of contaminants in different aquatic ecological and toxicological studies (Alkaladi et al., 2015; Hou et al., 2013; Kalbassi et al., 2011; McShan et al., 2014; Sayed et al., 2018; Sayed and Soliman, 2018). Nonylphenol as one category of these contaminants that was found to be more toxic for fishes (Araujo et al., 2018; Mekkawy et al., 2011). (Mekkawy et al., 2011) studied the toxicity levels of 4-nonylphenol on the adult African catfish *Clarias gariepinus*. Such findings were emphasized in the present study on the juveniles of that species in a dose-dependent response of biochemical parameters. Generally, 4-NP is an endocrine-disrupting chemical compound (Sayed et al., 2012; Zaccaroni et al., 2009). McCormick et al. (2005) and Taylor et al. (2011) had stressed that NP is estrogenic-like chemical compound which may disturb biochemical and physiological processes. It has been stated that xenoestrogen increased plasma LH levels in Atlantic croaker (Khan and Thomas, 1998).

4-NP has been stated to be toxic in different types of fish at concentrations from 17 to 3000 µg/L (Abou Khalil et al., 2017; Kotb et al., 2018; Mekkawy et al., 2011; Sayed et al., 2019). A dose-dependent increased damage caused by 4-NP was evident in the current study and other works (Al-Sharif, 2012; Mekkawy et al., 2011; Sayed and Ismail, 2017; Sharma and Chadha, 2017). Similar findings were recorded under the stress of carbosulphan (Nwani et al., 2011), 2,4-dicholorophenoxy acetic acid (Ateeq et al., 2005), UVA (Sayed et al., 2013; Sayed et al., 2016), and arsenic (Sayed et al., 2015).

Different researches referred to the variability of pollutant effects on the biochemical indicators; such as AST, ALT, TP, Glu, Cr, and UA with an increase, decrease or no effect (Alkaladi et al., 2015; Imani et al., 2015; Joo et al., 2018; Rajkumar et al., 2016 ; Sayed and Authman, 2018; Shaluei et al., 2013). The pattern of adverse impacts in blood parameters may depend on fish species, age, stage of reproductive cycle and disease (Luskora, 1997) in addition to many environmental factors (Pandey, 1977) and seasonal conditions (Joshi and Tondon, 1977 ; Khan, 1977).

Different toxicological studies attracted the attention to liver and kidney function indices to be important biomarkers in fishes (Abdel-khalek et al., 2017; Sayed and Hamed, 2017). The present work and those of Mekkawy et al. (2011) emphasized this statement with regard to *Clarias gariepinus* in a 4-NP-dose dependent manner. Satyanarayanan et al. (2011) working on the same species stated that ALT and AST increased with the increase of NP doses till 0.5 and 0.75 mgl\L then decreased with higher doses. In other fish species, ALT and AST enzymes activity increased in response to pesticides (Adedeji et al., 2009), heavy metals (Mekkawy et al., 2010b; Öner et al., 2008), and nonylphenols (Bhattacharya et al., 2008; Mekkawy et al., 2011); such response was dose-dependent. The elevated levels of ALT and AST in fish were interpreted to be attributed to the generated reactive oxygen species affecting the permeability of hepatocytes via cellular damage resulting in an outflow of these enzymes (Owolabi and Omotosho, 2017). Elevations of kidney function indices such as uric acid and creatinine were associated with 4NP toxicity (Sayed and Hamed, 2017). Hadi et al. (2009) reported that the increase of creatinine level might be stimulated by glomerular insufficiency, increased muscle tissue catabolism, or the impairment of carbohydrates metabolism. Kidney dysfunction and nephrotoxicity are results of oxidative stress (Narra et al., 2017).

Serum albumin, globulin, and glucose were used as biomarkers of fish general health status (Authman et al., 2013; Sayed and Moneeb, 2015).

Under toxicity, such parameters exhibit increase, decrease, or no change (Sharmin et al., 2016). In the present study, the albumin, globulin, and consequently albumin/globulin ratio in the 4-NP-exposed groups were significantly ($P < 0.05$) reduced, which indicates liver dysfunction as mentioned by Abdel-khalek et al. (2017), Narra et al. (2017), Sayed and Hamed (2017), Sayed et al. (2017), and Khalil et al. (2017). In the current study, the blood glucose level increased significantly in fish exposed to 4-nonylphenol with the increase of doses. Similar increased level of glucose was reported in fishes exposed to ultraviolet radiation (Mekkawy et al., 2010a; Sayed et al., 2007), heavy metals (Mekkawy et al., 2010b; Osman et al., 2007), and other pollutants (Adedeji et al., 2009; Mekkawy et al., 2019). Total protein levels may increase, decrease, or exhibit no significant trend (Mekkawy et al., 2010a; Mekkawy et al., 2010b; Sayed et al., 2007). The present results showed an increase in total protein in a dose-dependent mode. Authman et al. (2013) stated that the increased total protein detoxifies the toxicant and overcomes stress. Sayed et al. (2011) and Chen et al. (2017) reported a similarly significant increase in total protein in fish exposed to pollutants. Such pollutants disturb or even destroy the total protein influential balance, antioxidant capability, immune response, and the general body health of fish.

In conclusion, the liver and kidney functions parameters were indicated the hepatotoxicity and nephrotoxicity of 4-NP and their alterations have decreased to a great extent after a recovery period of 15-days in a reverse order.

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التأثيرات البيوكيميائية المعتمد على جرعات ال٤نونيل فينول لصغار القرموط الافريقى.

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تبحث هذه الدراسة في الضرر المعتمد على الجرعة الناتج عن التعرض لـ ٤-نونيلفينول في صغار القرموط الافريقي كلاريس جريبنس باستخدام بعض المؤشرات الحيوية وتأثير وقت الاسترداد. تم تقسيم هذه الصغار من كلا الجنسين الي اربع مجموعات (سبعه في كل مجموعة و تم تكرارها مرتين). و كانت المجموعة الأولى هي المقياس ، وتعرضت المجموعات الثلاث الأخرى لمدة ١٥ يومًا لتركيزات ٤-نونيلفينول عند ٠.١ ملجم / لتر ، ٠.٢ ملجم / لتر ، و ٠.٣ ملجم / لتر على التوالي ، ثم ١٥ يومًا كفترة تعافي بعد فترة التعرض. و بقياس بعض المؤشرات الحيويه و ، الكميائيه في الدم مثل (الألبومين ، الجلوبيولين ، نسبة الالبيومين الى الجلوبيولين الكبد، الجلوكوز ، البروتين الكلي ، الكرياتينين ، وحمض اليوريك) بعد وانزيمات التعرض لـ ٤ -نونيلفينول لمدة ١٥ يومًا اظهر وجود زيادة تعتمد على الجرعة في كلا من انزيمات الكبد، الجلوكوز ، البروتين الكلي ، الكرياتينين ، وحمض اليوريك) ونقصان يعتمد ايضا على الجرعة في كلا من الالبيومين و الجلوبيولين. خلصنا إلى أنه معلمات وظائف الكبد والكلى اوضحت السمية الكبدية والسمية الكلوية لـ ٤ -نونيلفينول وانخفضت تغيراتها إلى حد كبير بعد فترة الشفاء لمدة ١٥ يومًا بترتيب عكسي.