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Full Length Research Paper

Effects of 4-nonylphenol on metabolic enzymes, some ions and biochemical blood parameters of the African catfish *Clarias gariepinus* (Burchell, 1822)

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In present work, some biochemical characteristics of the catfish, *Clarias gariepinus* were studied under the effect of different sublethal doses of 4-nonylphenol (0, 0.05, 0.08 and 0.1 mgl^{-1}). Liver enzymes ALT and AST increased insignificantly at P<0.05 whereas the ALP decreased insignificantly. The activities of G6PDH showed significant increase with the increased sublethel doses of 4-nonylphenol while the activities of LDH decreased insignificantly with increase of such doses. The concentrations of serum glucose and total cholesterol significantly increased after exposure to 4-nonylphenol, although Hyperglycemia is evident. The kidney function parameters such as total serum protein and uric acid increased insignificantly after exposure to 4-nonylphenol in comparison with the control fish whereas creatinine exhibited significant increase (P<0.05). Some serum ions decreased significantly (HCO_3^- and Na^+) or insignificantly (Cu^{+2}, Cl^- and Ca^{+2}) and others increased significantly (Fe^{+2}) or insignificantly (K^+) under 4-nonylphenol stress on *C. gariepinus*. Increased serum anion gap was associated with increased hyperglycemia and insignificant hypocalcemia. Such increased gap referred to nonylphenol-induced metabolic acidosis. The adverse impact of the sublethal doses of 4-nonylphenol on the molecular structure of the protein was evident by electrophoresis. In conclusion, in addition to its tissue-specific estrogenic effects, 4-nonylphenol has non-estrogenic adverse effects on liver and kidney functions through activation of other metabolism-related genes distinct from estrogen-responsive genes.

Key words: Biochemistry, liver, kidney, anion gap, 4-Nonylphenol.

INTRODUCTION

Pollutants especially chemical ones are one of the major problems in different countries all over the world. A large number of chemicals are found to contaminate aquatic ecosystems and their animals including fish and amphibians during their adult life and sensitive stages of development (Radhaiah et al., 1987). Chemical toxicity is concentration-dependent and related to chemical persistent nature and accumulation in the aquatic ecosystem components.

Nonylphenol ethoxylate (NPE) is one of the most dangerous chemicals that are recorded in aquatic environments (Clark et al., 1992; Tsuda et al., 2000; Rivero et al., 2008). Such chemical is widely used in the production and formulation of many commercially sold products (e.g. industrial and commercial detergent, polymer resin, cosmetic products). Bacterial degradation of nonylphenol ethoxylates produces more toxic nonylphenol (NP) (Hano et al., 2009). Such compound is also estrogenic both *in vitro* and *vivo* assays (Folmar et al., 2002). Nonylphenol is not a single chemical compound but is used to refer to a family of compounds all of which have a central aromatic or benzene ring and a nine carbon chain (Cox, 1996). The stability and persistence of 4-nonylphenol (NP) in the aquatic environment is evident (Uguz et al., 2003; Sone et al., 2004; Rivero et al., 2008) and a wide variety of animals including fish, mollusks and crustaceans are affected by

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its toxicity especially due to its estrogenic-like behavior (Flouriot et al., 1995; Cox, 1996). So many countries including European Union have banned the use of NPEs in detergents and other commercial products (Environment Canada, 2002; European Union, 2002).

The adverse effects of nonylphenols and its ethoxylates increase due to their bioaccumulation in fish (Ahel et al., 1993; Cox, 1996). The bioconcentration factor (BCF) of nonylphenol in fish varies from 3 to 1300 (Ekelund et al., 1990; Ahel et al., 1993). Relatively low concentrations of nonylphenol and nonylphenol ethoxylates can cause death to fish (Staples et al., 2004; Vazquez-Duhalt et al., 2005). The environmental protection agency (EPA) stated that the freshwater life should not be affected if the one-hour average concentration of nonylphenol does not exceed 28 ppm on an average more than once every three years (Vincent and Sneddon, 2009). Lethal concentration of 0.032 ml/l nonylphenol was previously determined for *Orychroeis niloticuls* (Rivero et al., 2008) and 96 h LC_{50} for *Clarias gariepinus* exposed to NP was 3.48 mg l^{-1} (Satyanarayanan et al., 2011).

Physical and chemical blood changes are another indicator of fish pollution (Wilson and Taylor, 1993). So, blood characteristics and hematological parameters are important in diagnosing the functional status of organs of exposed animal to toxicants (Joshi et al., 2002; Adedeji et al., 2009). The complex of unspecified biochemical indicators of blood reveals the general effect of pollutants on fish and makes it possible to forecast the consequence of long-term exposure to chemical pollutants (Adedeji et al., 2009). Moreover, evaluation of blood biochemistry was considered as a useful tool for the diagnosis of diseases and assessing the physiological status of fish (Stoskopf, 1993).

Many studies have investigated changes in many physiological and biochemical blood indices induced by environmental conditions and presence of contaminants (Kori-Siakpere et al.,, 2006; Maheswaran et al., 2008; Ololade and Oginni, 2010).The biochemical parameters in fish are valid for physiopathological evaluation and sensitive for detecting potential adverse effects and relatively early events of pollutant damage (Almeida et al., 2002; Matos et al., 2007; Osman et al., 2010).

The liver plays an important role in several vital functions of basic metabloishm and it is also the major organ of accumulation, biotransformation and degradation of contaminants (Matos et al., 2007). The evaluation of biochemicals in fish liver has become an important tool for monitoring environmental exposure of fish to such chemicals in experimental studies (Matos et al., 2007). Several investigations demonstrated that changes in the levels of antioxidants enzyme activities can be used as possible biomarkers in different aquatic organisms (Regoli et al., 2004; Matos et al., 2007). Such biomarkers could be used to identify possible environmental contamination before the health of aquatic organisms is seriously affected (Barnhoorn and van Vuren, 2004) and to develop water quality indices (Pickering and Pottinger, 1995). Changes in the concentrations and activities of metabolic key factors such as LDH and G6PDH enzymes (Long et al., 2003; Osman et al., 2007b; Mekkawy et al., 2010a) were used to identify heavy metal contamination in fishes. G6PDH has long been recognized as an antioxidant enzyme (Pandolfi et al., 1995; Salvemini et al., 1999) and as a biomarker of pollution induced carcinogenesis in fish (Winzer et al., 2001). The cytoplasmic enzyme LDH is widely used as marker of organ or tissue lesions in toxicology and in clinical chemistry (Das et al., 2004). In most cases of tissue damage, whether due to disease or toxic compound, the activity of LDH was reported to be significantly affected (Singh and Sharma, 1998).

Several changes in plasma ion concentrations were measured for different fish species under different types of stresses to indicate osmoregulatory disruptions (Grosell, 2006; Fernandes et al., 2007; Fast et al., 2009). Carrera et al. (2007) studied the effect of 4-nonylphenol on osmoregulation referring to its action and its ecological significance.

The African catfish *C. gariepinus* is distributed throughout Africa (Nguyen and Janssen, 2002). Such a species is of commercial importance due to its high growth rate, high stocking-density capacities, high consumer acceptability and high resistance to poor water quality and oxygen depletion (Adewolu et al., 2008; Karami et al., 2010). Some records have shown that Clarias fishery contributes about 17% of the annual fish production from all fisheries sectors (Ololade and Oginni, 2010). Moreover, the African catfish has been used in fundamental researches and considered as an excellent model for toxicological studies (Osman et al., 2007a; Mahmoud et al., 2009), since it has a well documented biology (Osman et al., 2007b; Mahmoud et al., 2009). So, the present work aimed at evaluating the adverse impacts of different sublethal doses of 4-nonylphenol on some biochemical characteristics of the African catfish, *C. gariepinus*.

MATERIALS AND METHODS

Specimen collection

Specimens of adult Catfish *C. gariepinus* were collected from the River Nile at Assiut and then were transported to Fish Biology Laboratory of Zoology Department, Faculty of Science, Assiut University. The fish [males (500 to 600) and females (1000 to 1200 g] were fed on a commercial pellet diet (3% of body weight per day) and kept together in 100 L rectangular tanks containing tap water (conductivity 2000 ls cm^{-1}; pH 7.5; oxygen saturation 88 to 95%; temperature 27 to 28°C; photoperiod 12:12 light: *C. gariepinus* dark). After two weeks of acclimatization, fishes were used for experimental setup.

Experimental setup

The adapted adult fish were classified into four groups (10 fish each): control, 0.05 mgl^{-1} 4-nonylphenol (4NP), 0.08 and 0.1 mgl^{-1} -

4NP. The time of exposure was 15 days with a daily change of 4NP. These sublethal doses of 4NP were considered according to Bennie (1999). The experimental conditions were the same as those previously mentioned with daily change of water and 4NP. 4-Nonylphenol was purchased from Sigma-Aldrich (Schnelldorf, Germany) with purity of 99.3%.

Blood sampling and biochemical parameters

After 15 days, four fish from each group were randomly selected and anesthetized to bring down the stress due to handling using tricaine methanesulfonate (0.4 g l^{-1}). Blood was collected by cardiac puncture using sterilized syringes (2 ml). The serum was then removed by subjecting the tubes to centrifugation at 3000 rpm for 5 min and then stored at –80°C until further analyses of the following blood parameters.

Creatinine (Cr), Uric acid, Aspartic Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), glucose, cholesterol and total protein were determined by kits of SGMitalia Company. Enzyme activities of Lactate Dehydrogenase (LDH) and Glucose-6-Phosphate Dehydrogenase (G6PDH) were determined in serum with spectrophotometer (Micro lab 200 vital scientific Dieren–The Netherlands) at a wavelength of 340 nm and at 37°C using kits, Stanbio LDH (UV- Rate) procedure No. 0940 USA for the quantitative determination of Lactate Dehydrogenase (Kachmar and Moss, 1976) and RANDOX Laboratories Ltd., PD410, United Kingdom BT294QY for the quantitative determination of Glucose-6-Phosphate Dehydrogenase, (Kornberg, 1955). The concentrations of Hco_3^-, Na^+, K^+, Cl^-, Cu^{+2}, Fe^{+2} and Ca^{+2} were measured in serum using an atomic absorption spectrophotometer (GBC Model 300).

Protein analysis by polyacrylamide gel electrophoresis (SDS-PAGE)

Serum of each treatment in addition to control were suspended in 1.0 ml lysing buffer (100 ml 0.625 M (0.706 g/100 ml) Tris-HCl (pH 6.8) - 2% SDS (w/v) - 10% glycerol (v/v) - 5% 2- mercaptoethanol (v/v)). Heated at 100°C for 5 min, centrifuged at 10,000 rpm for 30 min and 50 µl of each extracted protein treatment was used for protein analysis using SDS-PAGE according to Laemmli (1970) in the first dimension. The low molecular weight standards (Pierce, USA) were run concurrently and the protein molecular mass was determined using Gel-Pro Analyzer package (Media Cybernetica, 1993 to 1997)

Statistical analysis

The basic statistics, means, standard errors and ranges of the measured parameters were estimated. The patterns of variation due to 4-nonylphenol doses were studied by one way analysis of variance using SPSS package (SPSS, 1998) at the 0.05 significance level. Levene's test of equality of error variance of the dependent variables was applied, with rejection of the null hypothesis for raw, log-transformed and SQRT-transformed data. So, the homogeneity of variance was assumed for raw data. The Tukey-HSD test was considered for multiple comparisons.

Ethical statement

All experiments were carried out in accordance to the Egyptian laws and University guidelines for the care of experimental animals. All procedures of the current experiment have been approved by the committee of the Faculty of Science of Assiut University, Egypt.

RESULTS

Biochemical blood parameters

Changes in the biochemical parameters in experimental fish are shown in Table 1. Liver enzymes ALT and AST increased insignificantly at $P<0.05$ whereas the ALP decreased insignificantly. The activities of G6PDH showed significant increase with the increased sublethel doses of nonylphenol while the activities of LDH decreased insignificantly with the increase of such doses. The concentrations of serum glucose and total cholesterol significantly increased after exposure to 4-nonylphenol, although Hyperglycemia is evident.

The kidney function parameters such as total serum protein and uric acid increased insignificantly after exposure to 4-nonylphenol in comparison with the control fish whereas creatinine exhibited significant increase ($P<0.05$). Under the effect of 4-nonylphenol, HCo_3^- and Na^+ decreased significantly whereas Cl^-, Cu^{+2} and Ca^{+2} decreased insignificantly. K^+ increased insignificantly whereas Fe^{+2} exhibited significant increase. So, nonylphenol affects the transport of chloride and calcium ions and hence osmoregulation in fish gills. The serum anion gap (Table 1) increased with increase 4-nonylphenol doses referring to metabolic acidosis and renal tubule dysfunction. But such increase in anion gap did not implicated in fish mortality of *C. gariepinus*. The increase in anion gap, creatinine and glucose may suggest a shock response under stress of higher doses of 4-nonyphenol.

Electrophoresis of serum protein

Under 4-nonylphenol stress, the protein fraction patterns (Figures 1 and 2 and Tables 2 and 3) show variability in the number, molecular weight and percentage of protein fractions of serum of *C. gariepinus* in comparison with the control. Few sex-related variations are evident especially under stress. Two protein fractions (r3 and r4) were recorded in the control male and female and disappeared under stress. Nonylphenol stress lead to the appearance of other protein fraction which not recorded in the control fish (r7, r8, r12 and r16). Protein fraction, r6 is the only fraction which persists in the control and treated fishes with highest percentage (32.75 to 47.75%) of the total protein in each individual. Based on presence/absence criterion, similarity between treated and control fish can be expressed in clusters (Figure 2). There are two main clusters: one includes the control sex and the other includes the treated fishes. The later main clusters are divided into three subclusters. Such variability in protein fractions reflects the variation in gene activity (activation-inactivation mechanism) and protein synthesis under nonylphenol stress. Protein recorded fractions suggest that the albumin and globulins fractions were affected by this pollutant.

Table 1. Effect of different doses of 4-nonylphenol on the biochemical parameters (mean ± SD, range) of the African catfish *Clarias gariepinus*.

Biochemical parameter	Groups			
	Control mean±SD (Max-Min)	0.05 mg^{-1} 4-nonylphenol mean±SD (Max-Min)	0.08 mg^{-1} 4-nonylphenol mean±SD (Max-Min)	0.1 mg^{-1} 4-nonylphenol mean±SD (Max-Min)
AST (IU l^{-1})	34.41±2.16 (32.12-36.81)^{a}	35.15±1.78 (33.18-36.91)^{a}	35.68±1.58 (33.71-37.11)^{a}	36.97±0.71 (36.1-37.8)^{a}
ALT(IU l^{-1})	16.97±1.14 (15.8-18.1) ^{a}	17.65±0.8 (16.4-18.5)^{a}	18.27±1.09 (17.2-19.8) ^{a}	18.87±0.77 (17.8-19.6)^{a}
ALP (IU l^{-1})	47±3.65 (43-51) a	45.75±2.98 (42-49) ^{a}	43.75±2.75 (41-47) ^{a}	41.25±2.87 (38-45)^{a}
LDH (IU l^{-1})	66.78±3.37 (62.14-70.05) ^{a}	64.9±3.18 (62.16-68.12)^{a}	63.36±2.15 (61.12-66.01)^{a}	61.13±2.57 (58.11-64.11)^{a}
G6PDH (g/dl)	65.49±1.11 (64.12-66.78) ^{a}	66.64±1.26 (65.14-68.11)^{a}	67.88±0.95 (67.12-69.12)^{ab}	69.9±1.7 (68.15-72.18)^{b}
Glucose (Mg/dl)	72.9± 3.2 (68.6-75.8)^{a}	74.92±4.34 (68.6-78.4)^{ab}	79.4±0.9 (78.2-80.4)^{bc}	81.7± 0.78(80.8-82.6)^{c}
Total protein (Mg/dl)	4.1±0.316 (3.8-4.5) ^{a}	4.37±0.4 (3.9-4.8) ^{a}	4.6±0.57 (3.9-5.2) ^{a}	4.97±0.38 (4.6-5.5)^{a}
Total cholesterol (Mg/dl)	213±1.81 (211-215) ^{a}	217.75±2.87 (214-221)^{ab}	225.5±6.66 (220-235)^{bc}	232.25± 8.18 (221-240)^{c}
Creatinine (Mg/dl)	0.367±0.022 (0.34-0.39) ^{a}	0.3925±0.012 (0.38-0.41)^{ab}	0.457±0.0 4 (0.41-0.51)^{b}	0.467±0.058 (0.4-0.54)^{b}
Uric acid (Mg/dl)	22.67±0.74 (21.8-23.6) ^{a}	22.9±0.245 (22.6-23.1)^{a}	23.5±0.74 (22.8-24.5)^{a}	25.27±0.61 (24.6-25.8)^{a}
HCO^{-} (mEq l^{-1})	19.75±1.5 (18-21)^{a}	18.75±0.957 (18-20)^{ab}	16.25±1.7 (14-18)^{bc}	13.75±1.7 (12-16)^{c}
Na^{+} (mEq l^{-1})	126.25±3.09 (122-129)^{a}	124±2.16 (121-126) ^{ab}	120.5±1.29 (119-122)^{bc}	117.25±3.59(112-120)^{c}
K^{+} (mEq l^{-1})	4.10±0.18 (3.9-4.3) ^{a}	4.37±0.368 (4.02-4.89) ^{a}	4.53±0.31 (4.15-4.91)^{a}	4.78±0.41 (4.18-5.11)^{a}
Cl^{-} (mEq l^{-1})	95.75±3.3 (92-99) ^{a}	93.25±3.59 (90-98)^{a}	91.75±1.7 (90-94)^{a}	90.25±1.25 (89-92)^{a}
Cu^{+2} (µg/ml)	108.89±8.85 (103.14-122.1)^{a}	106.36±9.31 (100.12-120.1)^{a}	105.47±9.06 (100.21-119.01)^{a}	99.105±1.12 (98.11-100.12)^{a}
Anion gap (mEq l^{-1})**	10.8±0.12 (7.8-12.8)a	12.0±0.18 (10-14)^{ab}	12.5±0.18 (12-13)^{bc}	13.2±0.12 (13-15)^{c}
Fe^{+2} (µg/ml)	15.65±0..59 (14.8-16.2) ^{a}	16.42±0.28 (16.1-16.8)^{ab}	16.77±0.784 (15.9-17.8)^{ab}	17.35±0.51 (16.9-17.8)^{b}
Ca^{+2} (µg/ml)	52.63±1.93 (50.12-54.16) ^{a}	52.86±2.75 (50.11-56.12) ^{a}	50.36±1.17 (49.21-52.01)^{a}	48.84±1.45 (47.14-50.11)^{c}

* Different letters indicates there is a significant difference at ($p \leq 0.05$; ** Anion gap= $Na^{+}+K^{+}-Cl^{-}-HCO^{-}$.

DISCUSSION

Nonylphenol (NP) is a more toxic persistent degradation product of nonylphenol ethoxylates (Jigang et al., 2010). It is suspected to be endocrine-disrupting chemicals. Nonylphenol was found to have tissue-specific estrogenic adverse effects and non-estrogenic effects on different organ functions of aquatic animals, especially fishes (Madigou et al., 2001; Marin et al., 2008). In the present work, different non-estrogenic adverse effects of nonylphenol were recorded on the biochemical characteristic of the African Catfish, *C. gariepinus* including liver function enzymes, osmoregulation and other metabolic parameters; such effects were dose-dependent. Mekkawy et al. (2011) reported dose-dependent 4-nonylphenol-induced changes in some blood parameters of such species including apoptosis, erythrocyte morphological alterations, micronucleus formation and blood counts. The present authors also reported nonylphenol hormonal effects on the thyroid and reproductive hormones and reproduction tissues.

Different studies referred to the common effects of variable types of stress including xenobiotics on the liver function and metabolic enzymes (Mekkawy and Lashien, 2003; Öner et al., 2008; Adedeji et al., 2009, Mekkawy et al., 2010b; Osman et al., 2010). In different fish species including *C. gariepinus*, ALT and AST enzymes activity were found to increase in response to pesticides (Adedeji et al., 2009), heavy metals (Öner et al., 2008; Mekkawy and Lashien, 2003; Mekkawy et al., 2010b) and nonylphenols (Bhattacharya et al., 2005; Bhattacharya et al., 2008); such response was dose-dependent. In present work, the sublethal concentrations of 4NP caused insignificant increase of these enzymes

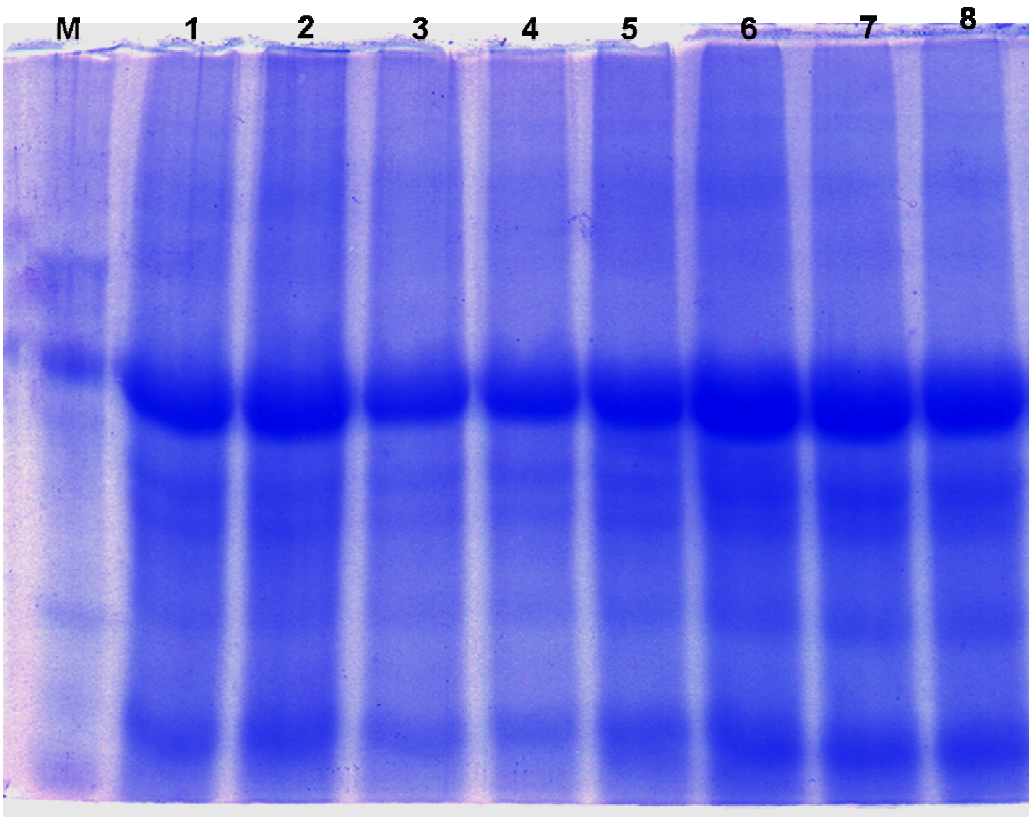

Figure 1. Protein fractions identified in serum of male and female catfish, *C. gariepinus* under different doses of 4-nonylphenol in comparison with control. M; unstained protein marker (200 kda), 1; male control, 2; female control, 3; male treated with 0.05 mgl^{-1} of 4-nonylphenol for 15 days, 4; female treated with 0.05 mgl^{-1} of 4-nonylphenol for 15 days, 5; male treated with 0.08 mgl^{-1} of 4-nonylphenol for 15 days, 6; female treated with 0.08 mgl^{-1} of 4-nonylphenol for 15 days, 7; male treated with 0.1 mgl^{-1} of 4-nonylphenol for 15 days, 8; female treated with 0.1 mgl^{-1} of 4-nonylphenol for 15 days.

activity. Satyanarayanan et al. (2011) working on the same species stated that ALT and AST increased with increase of NP doses till 0.5 and 0.75 mgl^{-1} then decreased with 1 mgl^{-1}. Increase in the levels of ALT and AST has been shown to reflect liver damage (Bhattacharya et al., 2005; Bhattacharya et al., 2008) but to a limited action. The 4-nonylphenol-induced ALP enzyme exhibited insignificant decrease. The alkaline phosphatases are a group of enzymes mainly associated with the liver and bone, but are also found in cells of intestine and kidney. Accordingly, 4-nonyphenol can affect indirectly to some extent different ALP-related metabolic processes. The increase in serum ALT and AST and decrease in ALP under some heavy metal stress on *Oreochromis niloticus* were recorded by Öner et al. (2008). The pesticide Diazinone induced a signify-cant decrease in blood alkaline phosphatase and acid phosphatase of *C. gariepinus* whereas, ALT and AST were comparable in the control and treated fishes (Adedeji et al., 2009). In other fish species these enzymes increased with stresses (Bhattacharya et al., 2005, 2008).

The blood glucose, LDH and G6PDH have been used as bioindicators of stress in the catfish *C. gariepinus* (Osman et al., 2007b, 2010; Sayed et al., 2007; Adedeji et al., 2009; Mekkawy et al., 2010a; Satyanarayanan et al., 2011) and other different fish species (Mekkawy and Lashien, 2003; Mekkawy et al., 2010b). In present study, the blood glucose level increased significantly ($P<0.05$) in fish exposed to 4-nonylphenol as compared to the control fish. Similar increased level of glucose was recorded in blood of fishes exposed to ultraviolet radiation (Sayed et al., 2007; Mekkawy et al., 2010a), heavy metals (Mekkawy and Lashien, 2003; Osman et al., 2007b; Mekkawy et al., 2010b) and other pollutants (Poléo and Hyherød, 2003; Rosety-Rodriguez et al., 2005; Adedeji et al., 2009). The chemical pollutants modulate the metabolism of carbohydrates, causing hyperglycemia by stimulating the glycogenolysis in fish (Levesque et al., 2002; Osman et al., 2010).

The literatures in concern with the adverse effects of 4-nonylphenol or its allies on the G6PDH and LDH enzymes activity of fishes are rare (Satyanarayanan et al., 2011) but there are many studies about the effects of

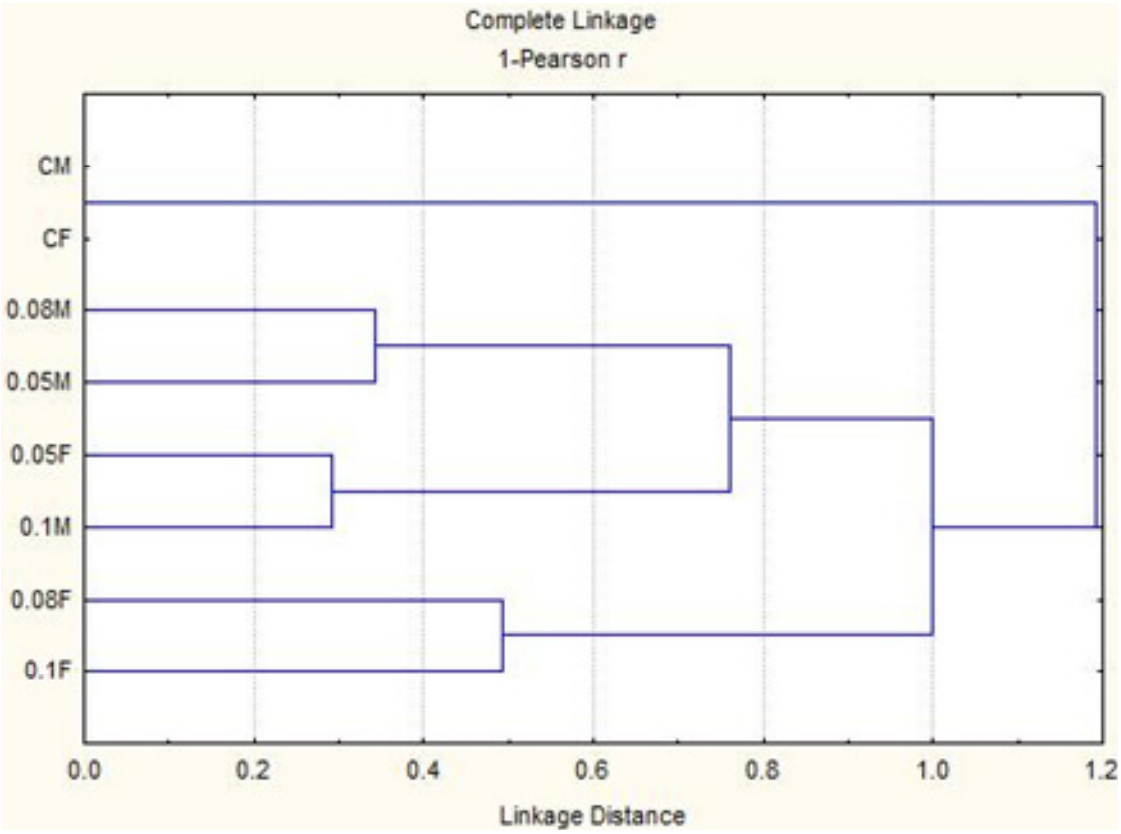

Figure 2. Clustering of electrophoretic protein patterns on the basis of band presence-absence criterion to illustrate the relationship between the control and 4-nonyphenol treated sexes of *C. gariepinus*.

other chemical pollutants on these enzymes (Mekkawy et al., 2010a). The stress-induced LDH and G6PDH exhibited variable patterns toward increase, decrease or no trend (Pandey et al., 2003; Rosety-Rodriquez et al., 2005; Adedeji et al., 2009; Mekkawy et al., 2010a; Satyanarayanan et al., 2011). In present work, LDH exhibited insignificant decrease with increase of 4NP doses while G6PDH level increased significantly. Satyanarayanan et al., (2011) reported that LDH increased with increase of NP to higher value (159.09±2.84) at 0.5 mg l^{-1} NP then decreased to 52.18± 0.95 at 1 mgl^{-1} NP. Leopold et al., (2003) reported that the increase in G6PDH activity represents protection against elevated levels of reactive oxygen species in cells exposed to an oxidative stress through the increased NADH production. LDH is generally associated with cellular metabolic activity and so, its inhibition may be due to ion imbalance or plasma membrane damage (Sastry and Gupta, 1980) and may also due to formation of enzyme inhibitor complex (Singh and Sharma, 1998; Rajanna et al., 1999).

Increase in the blood cholesterol concentration is used as an indicator of liver dysfunction because homeostasis of lipids is one of the principle liver functions (Kaplan et al., 1988). Many studies are in concern with such parameters to estimate the nature and degree of stress in fishes (Poléo and Hyherød, 2003; Mekkawy and Lashien, 2003; Rosety-Rodriguez et al., 2005; Osman et al., 2007b; Sayed et al., 2007; Mekkawy et al., 2010a). In present work, the blood cholesterol level was significantly ($P<0.05$) increased in *C. gariepinus* exposed to 4-nonylphenol as compared with control fish. The increase of cholesterol as a response to pollution could be due to fact that excess energy reserves are required by organisms to mediate the effects of stress (Lee et al., 1983).

Total protein, creatinine and uric acid are biomarkers for muscle and purine metabolism, liver damage and kidney acid (Goss and Wood, 1988; Sayed et al., 2007; Hadi et al., 2009; Mekkawy et al., 2010a). The protein make up of an organism is of important diagnostic significance (Shalaby et al., 2006; Hadi et al., 2009) because of protein's involvement in enzymes, hormones and antibodies as well as osmotic pressure balance and maintaining acid-base balance. Total protein level may increase, decrease or exhibit no significant trend (Sayed et al., 2007; Mekkawy et al., 2010a, b). In present study, 4-nonylphenol-induced total protein level significantly increased with dose increase referring to limited effect on protein-related metabolic processes. The rise in the creatinine of *C. gariepinus* referred to 4NP-induced effects on muscle metabolism whereas the insignificant

Table 2. Protein fractions (%) identified in serum of *C. gariepinus* under the effect of different doses of 4-nonylphenol in comparison with the control.

Lanes	Dose							
	Control		0.08 mgl^{-1} 4-nonylphenol		0.05 mg^{-1} 4-nonylphenol		0.1 mg^{-1} 4-nonylphenol	
	Male	Female	Male	Female	Male	Female	Male	Female
Protein fractions				%				
r1								
r2								
r3	6.57	6.32						
r4	6.20	7.40						
r5								
r6	35.22	32.75	46.89	47.75	39.39	44.22	44.38	42.14
r7					7.34			
r8			12.24	16.18	8.92	5.05		
r9	10.56	12.29		9.85		13.10	18.73	16.91
r10	5.07	9.32	8.01		9.21	6.43	6.91	
r11	7.08	4.39	4.81	6.27	7.65			7.36
r12				8.84				
r13	8.05	9.32	11.84		9.66	10.18	9.45	12.83
r14	6.17	3.21						
r15	15.07	14.99	16.20	11.11	17.83	21.02		20.76
r16							20.52	
r17								

Table 3. Molecular weight (kda) identification of protein fractions in serum of *C. gariepinus* under the effect of different doses of 4-nonylphenol in comparison with the control.

Lanes	Dose								
	Marker	Control		0.08 mg^{-1} 4-nonylphenol		0.05 mg^{-1} 4-nonylphenol		0.1 mg^{-1} 4-nonylphenol	
		Male	Female	Male	Female	Male	Female	Male	Female
Protein fractions					Molecular weight (kda)				
r1	200								
r2	150								
r3	120	118.98	119.59						
r4	100	105.1	102.65						
r5	85								
r6		76.449	75.607	77.29	78.131	77.29	76.168	75.607	76.168
r7	70					70.701			
r8	60			57.101	61.667	57.826	59.565		

Table 3. Cont.

r9		55.652	56.377		52.899		55.362	54.348	55.362
r10	50	48.974	50.435	51.159		51.159	50.725	47.692	
r11		38.974	37.692	38.205	42.051	40			38.205
r12	30				27.586				
r13	25	24.667	26.379	25.517		24.667	25.862	23.867	24.333
r14	20	19.638	20	20.333					
r15	15	14.348	15.362	14.493	14.855	15.217	14.348		13.768
r16								13.116	
r17	10								

increase in uric acid may be due the limited effect of 4NP on purine metabolism; renal tubules may also be damaged. Hadi et al. (2009) reported that the increase of creatinine level might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrates metabolism.

Ions are very important for any organism because the involved in most biological processes such as respiration, muscle contraction, absorption, nerve impulses transmission, osmoregulation, acid-base balance and execration in fish. Different studies considered the adverse effects of different types of stress on these biological processes in fishes (Părt et al., 1985; Burkhardt-Holm et al., 2000; Hughes et al., 2000; McCormick et al., 2005). In present work, some ions decreased significantly (HCO_3^- and Na^+) or insignificantly (Cu^{+2}, Cl^- and Ca^{+2}) and others increased significantly (Fe^{+2}) or insignificantly (K^+) under 4-nonylphenol stress on *C. gariepinus*. Părt et al. (1985) reported increased ion permeability and sodium efflux of gill epithelial cells of rainbow trout due to the toxicity of nonylphenol-ethoxylate. Accordingly, McCormick et al. (2005) indicated that estrogenic compounds may cause a general shift toward increased capacity for ion uptake (Na^+, Cl^- and Ca^{+2}). Calcium seems to play an important response to direct as well as indirect effects of nonylphenol (Burkhardt-Holm et al., 2000). Such Nonylphenol could cause cell death through inhibition of the calcium pump in the endoplasmic reticulum (Hughes et al., 2000). Histopathological alterations in the liver of rainbow trout as necrosis, cell number decrease and vacuolation were mediated by inhibition of calcium pump after exposure to NP (Uguz et al., 2003).

The serum anion gap in fishes was considered by many authors to describe the degree of stress on the kidney and accumulation of organic acids (Converse et al., 1994). In present work, the increased serum anion gap in *C. gariepinus* referred to metabolic acidosis and renal tubule dysfunction. But the increase in anion gap did not implicated in fish mortality of that species exposed to 4-nonyphenol. The increase in anion gap, creatinine and glucose may suggest a shock response under stress of higher doses of 4-nonyphenol. Such increased anion gap was associated with insignificant hypocalcemia.

Iron is necessary for the formation of some proteins, hemoglobins, myoglobins and cytochromes (Arain et al., 2006). Also, it is necessary for oxygen transport, cellular respiration and peroxide deactivation. In present work, increased Fe^+ was recorded under 4-nonylphenol stress in comparison with the control fish.

Serum protein of fishes is representing by variable fractions including prealbumin, albumin and globulins fractions (Hussein and Mekkawy, 2001). Electrophoresis (or SDS-PAGE) of serum protein and identification of their fractions by molecular weight, number and concentration are valid in postulating stress-induced variations (Hanson and Tabita, 2003; Mekkawy et al., 2010a). Such variations reflect the activation and inactivation of genes under pollutant stress (Mekkawy and Lashin, 2003; Mekkawy et al., 2010a). In present work, the 4-nonylphenol doses reflect variability in band patterns, fraction molecular weight and fraction appearance and disappearance. Only one protein fraction persist with the increased doses of 4-nonylphenol. Moreover, sexual dimorphism was evident in the control and treated fish.

Interestingly, In spite of the tissue-specific estrogenic effects that have reported for 4-nonylphenol, it showed non-estrogenic adverse effects on liver and kidney functions of the studied

species, *C. gariepinus.*

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