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Modulatory effects of green tea extract against the hepatotoxic effects of 4-nonylphenol in catfish (*Clarias gariepinus*)

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ABSTRACT

The antioxidant role of the green tea (*Camellia sinensis)* extract (GTE) was examined to remedy the toxic effects of (0.2 mg l^{-1}) 4-nonylphenol(4-NP). Biochemical parameters, antioxidant enzymes, liver lipid peroxidation (LPO), DNA fragmentation, and apoptosis as well as histopathology of liver of African catfish *Clarias gariepinus* were considered. Catfishes were divided into four groups: first group (control), second group (0.2 mg l^{-1} of 4-NP), third group (0.2 mg $l^{-1}$of 4-NP +100 mg GTE l^{-1}water), and fourth group (0.2 mg l^{-1} of 4-NP +200 mg GTE l^{-1}water). The results showed that significant increments of serum glucose, AST, ALT, total protein, total lipids, cholesterol, G6PDH, and cortisol. Meanwhile, serum acetylcholinesterase, ALP, and LDH were significantly reduced. In addition, antioxidant enzymes (SOD, CAT, GST, and TAC) levels were reduced in 4-NP treated fish compared to control. Also, there were significant increments in hepatic LPO, DNA fragmentation, and apoptotic erythrocytes in 4-NP treated fish compared to control. Liver of 4-NP treated fish showed some histopathological alterations such as, vacuolization in hepatocytes, congestion in central vein, infiltration of mononuclear inflammatory cells, and necrosis as well as depletion of glycogen content of liver. Addition of green tea extract into the water restored the alterations in most of those biomarkers induced by 4-NP. We concluded that, GTE has a protective role against hepatic failure, depletion of antioxidant defense, and genotoxicity induced 4-NP in *C. gariepinus*.

1. Introduction

4-Nonylphenol (4-NP) is regarded as an environmental endocrine disruptor, utilized as important raw materials for cleansers, emulsifiers, and wetting agents in industry and is likewise found in paints, pesticides, and family toiletries (Jie et al., 2017). Most of 4-nonylphenol (4-NP) studies revealed several effects on the liver of fish (Abdulla Bin-Dohaish el, 2012; Asifa and Chitra, 2016; Kaptaner and Unal, 2011; Midhila and Chitra, 2015; Sayed et al., 2012a, 2012b; Uguz et al., 2003).

Due to the importance of the liver in lipid and carbohydrates metabolism, its structure was used as bioindicator for previously exposure to contaminants (Hinton and Lauren, 1990). The biochemical and antioxidant alterations in fish were used as biomarkers for detecting the harmful effects of environmental stressors (Sayed et al., 2016). Also, up regulation or down regulation of antioxidant enzymes gives a good indication for oxidative stress caused by pollutants (Martínez-Álvarez et al., 2005; Sayed and Ismail, 2017).

Histopathological and histochemical modifications were used for environmental pollutants exposure studies indicated changes in body homeostasis (Hinton et al., 1992; Sayed et al., 2012b). On the other hand, genotoxicity surveillance of fish is useful for either conservation genetics of species or evaluation the well-being condition of fish (Sharma and Chadha, 2017). Recently, the modulations of 4-NPtoxicity by phytochemicals were investigated (Abou Khalil et al., 2017; Sayed and Hamed, 2017; Sayed and Ismail, 2017; Sayed et al., 2016).

Green tea extract (GTE) consists of sundry polyphenolic ingredients with antioxidant properties such as the flavanol (catechins), epigallocatechin-3-gallate (EGCG), and epicatechin-3-gallate (ECG). Green tea ingredients have antioxidants properties through oxidizer agents interfering, pro-oxidant enzymes inhibition, and antioxidant enzymes activation as well as anti-inflammatory agents (Tipoe et al., 2007) giving them the potential to affect human health and disease (Senanayake, 2013). Green tea extract (GTE) previously reported to remedy hepatotoxicity induced by D-galactosamine (Lin et al., 2009), cadmium chloride (Vinoth Kumar et al., 2010), cyromazine and chlorpyrifos (Heikal et al., 2013), mercury (Shirai et al., 2013), ethanol (Lodhi et al., 2014), ketamine (El-Fattah and Ismail, 2015), copper

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nanoparticles (Ibrahim et al., 2015), and cypermethrin (Zahra et al., 2016).

African catfish (*Clarias gariepinus*) has been used in toxicological studies as excellent animal model since it has a well-documented biology (Mahmoud et al., 2009; Mekkawy et al., 2011; Sayed and Soliman, 2017). This study aims to investigate the effects of high dose of 4-NP on the serum and liver parameters of this species as well as to assess the modulatory effect of green tea extract on hepatooxidative damage induced by 4-NP using histological, biochemical, antioxidants, and histochemical biomarkers.

2. Materials and methods

2.1. Fish

Healthy male catfish *C. gariepinus* with average body weight 423 ± 89.5 g were used from Fish Biology Laboratory, Zoology Department, Faculty of Science, Assiut University. The fish were kept together in 100 l rectangular tanks containing dechlorinated tap water and air pumps under laboratory circumstances for 12 weeks for acclimatization. The fish were fed 5% of body weight per day commercial pellet diet and the water was changed daily to reduce impurities from metabolic wastes.

2.2. Chemicals

4- Nonylphenol (4-NP) with purity 99.3% was obtained from Sigma–Aldrich (Schnelldrof, Germany). Stock solution from 4-NP as maximum solubility in water (7 mg L^{-1}) was prepared and then diluted concentrations were used. Biochemical analysis kits of glucose, aspartic aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, total lipids, cortisol, and cholesterol were purchased from SG Mitalia Co., USA. Lactate Dehydrogenase (LDH), acetylcholinesterase (AchE), and Glucose-6-Phosphate Dehydrogenase (G6PDH) were measured using kits of Stanbio LDH (UV- Rate) USA, RANDOX Laboratories Ltd., and PD410, United Kingdom, respectively. All other biochemical kits (LPO, SOD, GST, CAT and TAC) were purchased from Bio-Diagnostic Co., Cairo, Egypt.

2.3. Green tea extract (GTE)

Green tea decaffeinated extract was purchased from Myprotein, UK. The nutritional composition as amount per serving (750 mg) is (std. to 98% polyphenols, 45% EGCG, and other ingredients: vegetable cellulose (capsule), rice flour, stearic acid, silica). Freshly prepared stock solution from GTE as 1000 mg L^{-1} was used for preparation of different concentrations from GTE.

2.4. Experimental design

After acclimatization to lab conditions, fish were categorized into four groups (6 fish per each); control, 0.2 mg L^{-1} 4-NP, 0.2 mg L^{-1} 4-NP with the adding of GTE (100 mg L^{-1}water), and 0.2 mg L^{-1} 4-NP with the adding of GTE (200 mg L^{-1} water), exposure was for two weeks and there were three replicates for each group. The concentration of 4-NP (0.2 mg L^{-1}) was chosen within the toxic observed value which recorded in many food products (Loyo-Rosales et al., 2004; Mekkawy et al., 2011). The conditions of the experiment were as that of acclimatization (conductivity 258.75 μM cm^{-1}; pH 7.38; dissolved oxygen 6.85 mg L^{-1}; temperature 28.45 °C; photoperiod 12:12 light: dark) with changing half of the water and redosing of the 4-NP concentration every day.

2.5. Apoptosis detection

According to Darzynkiewicz (1990), the apoptosis detection in RBCs was done. Briefly, the blood smears were fixed in absolute methanol for 10 s after drying at room temperature and then stained with Acridine Orange (Life Technologies, Carlsbad, USA). Cells were observed under Zeiss Axioplan2 fluorescence microscope (×200) provided with a digital 3 CCD color video camera (Sony, AVT-Horn, Japan).

2.6. Biochemical and enzymes analysis

Six fish/group at the end of the experiment were collected and anesthetized with 100 mg L^{-1} benzocaine solution (Neiffer and Stamper, 2009). Blood samples from the caudal veins were collected, then allowed to clot at room temperature, then centrifuged at 5000 rpm at 4 °C for 20 min and serum were collected for the biochemical and enzymes parameters analysis (glucose, aspartic aminotransferase (AST)), alanine aminotransferase (ALT), total protein, total lipids, cholesterol, alkaline phosphatase (ALP), acetylcholinesterase (AchE), cortisol, glutathione S-transferase (GST), LDH, and G6PDH.

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

Liver tissue was stored at −80 °C for liver antioxidant biomarkers (SOD, CAT and TAC), lipid peroxidation, and DNA fragmentation measurements. Samples of liver tissues were homogenized in phosphate buffer (0.1 M pH 7.4) using a Potter–Elvejhem glass Homogenizer. Homogenate filtration and centrifuged for 10 min at 4 °C at 1600 rpm; the supernatant was used for analysis. LPO level, SOD activity, CAT activity, TAC activity, DNA fragmentation were assessed to the method of Ohkawa et al. (1979), Nishikimi et al. (1972), Aebi (1984), Koracevic et al. (2001), and Kurita-Ochiai et al. (1999) respectively.

2.8. Histopathology and histochemistry

A small fresh piece of liver was carefully collected for light microscopy. Tissues were rinsed and fixed in 10% neutral buffered formalin for at least 48 h, dehydrated in graded ethanol, and cleared in xylene prior to embedding in paraffin blocks. Dewaxed Section (5 μm) were stained with Harris's hematoxylin and eosin stain (H&E), while general carbohydrates were stained with PAS reaction (Drury and Wallington, 1980). Sections were visualized and studied using an Olympus microscope (BX50F4, Olympus Optical Co., LTP, Japan).

2.9. Statistical analyses

Means and standard divisions were estimated using the SPSS package (SPSS, 1998). One-way analysis of variance at the 0.05 significance level was considered. The LSD test was considered for multiple comparisons.

2.10. 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

3.1. Biochemical parameters

Catfish treated with 4-NP displayed significantly elevation ($P < 0.05$) in level of serum glucose in comparison to control. Addition of GTE (100 mg L^{-1} water) caused insignificantly decrease in serum glucose in comparison to 4-NP treated fish. Whilst, addition of GTE (200 mg L^{-1} water) caused significantly decrease in serum glucose compared to 4-NP treated fish (Table 1).

4-NP caused significantly elevations ($P < 0.05$) in levels of AST,