Science of the Total Environment 619–620 (2018) 692–699

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Protective effect of *Nigella sativa* on 4-nonylphenol-induced nephrotoxicity in *Clarias gariepinus* (Burchell, 1822)

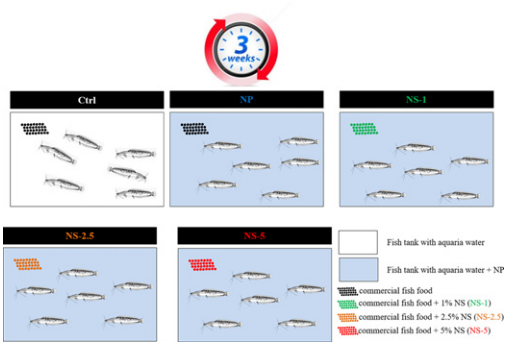
Ahmed M. Kotb [a,b], Mahmoud Abd-Elkareem [a], Nasser S. Abou Khalil [c], Alaa El-Din H. Sayed [d,e,*]

[a] Department of Anatomy and Histology, Faculty of Veterinary Medicine, Assiut University, 71516 Assiut, Egypt
[b] Institute of Anatomy and Cell Biology, University Medicine Greifswald, 17489 Greifswald, Germany
[c] Medical Physiology Department, Faculty of Medicine, Assiut University, Assiut, Egypt
[d] Zoology department, Faculty of Science, Assiut University, 71516 Assiut, Egypt
[e] Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Chiba 277-8562, Japan

HIGHLIGHTS

- 4-nonylphenol-induced nephrotoxicity in *Clarias gariepinus*
- histology, histochmistery and electron microscope were used in this study.
- *Nigella sativa* could protect the kidney against 4-Nonylphenol induced nephrotoxicity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 October 2017
Received in revised form 26 October 2017
Accepted 11 November 2017
Available online xxxx

Editor: Yolanda Picó

Keywords:
Catfish
Nephron
4-NP
Nigella sativa
Glomerulus

ABSTRACT

The aim of this study was to examine the protective effects of *Nigella sativa* (*N. sativa*) on 4-Nonylphenol-induced nephrotoxicity in *Clarias gariepinus*. 30 fishes were divided into five groups: control, 4-nonylphenol-treated, 1% *N. sativa* treated, 2.5% *N. sativa* treated, and 5% *N. sativa* treated. *N. sativa* and 4-Nonylphenol were given for 3 weeks. 4-NP and 4-NP-*N. sativa* treated fishes were compared with the control group. Kidney histology, immunochemistry, and electron microscope were assessed after 4-NP exposure. In the African catfish, 4-NP is mainly excreted through the kidney causing nephrotoxicity. Our results showed that 4-NP administration significantly disturbed the kidney structure and function. 4-NP treated fishes showed dilated glomerular vessels, fewer glomerular cells content, decreased expressions of glomerular proteins, and increased level of autophagy compared to control group ($P < 0.05$). As *N. sativa* has different immunological and pharmacological effects such as anti-apoptotic and anti-oxidant, therefore, the administration of *N. sativa* with 4-Nonylphenol significantly minimize the nephrotoxic effect of 4-NP and maintain the normal kidney structure and function. Our novel study demonstrated for the first time that *N. sativa* could protect the kidney against 4-NP induced-nephrotoxicity.

1. Introduction

Environmental pollutants with endocrine disruptor characteristics have provoked much environmental concern in the last few decades.

* Corresponding author at: Zoology department, Faculty of Science, Assiut University, 71516 Assiut, Egypt.
E-mail address: alaasayed@aun.edu.eg (A. H. Sayed).

https://doi.org/10.1016/j.scitotenv.2017.11.131
0048-9697/

The most common group of environmental endocrine disrupters is the alkylphenol polyethoxylates, which are widely used as nonionic surfactants and antioxidants in detergents, pesticides, herbicides, paints, cosmetics, and plastic ware (Nimrod and Benson, 1996). 4-Nonylphenol (4-NP) is the most important derivative of the biodegradation process of alkylphenol polyethoxylates (Uguz et al., 2003). 4-NP exists in varying concentrations in rivers, lakes, tap water, wastewater, ground water, treated drinking water, and sediments (Chaube et al., 2013; Jie et al., 2017; Uguz et al., 2003; Yu et al., 2009). The non-biodegradable nature, prolonged persistence in the environment, and its biomagnification through the food chain directs much attention towards 4-NP as a major global hazardous aquatic pollutant (Rivero et al., 2008). The uptake rate of 4-NP varies in different organs. The highest concentration of 4-NP was found in the brain while the lowest was in muscles. Other organs like gill, liver, kidney, ovary and plasma showed intermediate accumulation ranges (Gautam et al., 2015).

It has been shown previously that 4-NP has estrogenic, carcinogenic, hepatotoxic, and nephrotoxic effects (Abd-Elkareem et al., 2017; Blom et al., 1998; Rivero et al., 2008; Sharma and Chadha, 2017a; Sharma and Chadha, 2017b; Uguz et al., 2003). 4-NP mechanism of action is still not fully clear, but it may have toxic effects through different mechanisms. Furthermore, it has been shown that 4-NP could induce vascular smooth muscle relaxation (Hsieh et al., 2009) which could be an explanation for the vasodilatation effect which has been noticed in some cases of 4-NP toxicity (Sharma and Chadha, 2017b). Furthermore, it was shown that 4-NP could induce RBC's apoptosis which leading to accumulation of cell depresses and free heme inside the tissues (Mekkawy et al., 2011). Since Melano-Macrophage Center (MMC) is important for eliminating destroyed cellular and also considered to be an important hallmark for increasing autophagy (Agius and Roberts, 2003), it was shown that 4-NP induced a marked increase in MMC (De Vico et al., 2008; Passantino et al., 2005), as well as a marked induction of autophagy (Duan et al., 2017).

In addition, its ability to cause endocrine, enzymes, and oxidative stress in fish (Sayed and Ismail, 2017; Sayed et al., 2011; Sayed et al., 2016), it has been observed that 4-NP toxicity was accompanied with an elevation of blood parameters of kidney diseases like uric acid, creatinine, and glucose (Sayed and Hamed, 2017). Furthermore, it has been shown that the high dose of *N. sativa* might cause damage to the kidney tissue which determined through the analysis of different blood parameters as well as histological examination of kidney tissue (Dollah et al., 2013).

N. sativa (NS) is one of the most popular, safe, non-detrimental, cytoprotective,and widely used as medicinal plant all over the world. NS is a flowering plant with dark-crescent shaped seeds and belongs to the family Ranunculaceae which commonly grows in North Africa and Asia. The NS seeds have been used along many centuries as a medicinal plant and as a food additive (Ahmad et al., 2013; Ijaz et al., 2017; Shahid et al., 2017).

Furthermore, it has been shown that NS could protect against nephrotoxicity induced by Cyclosporine (Shahid et al., 2017), Gentamicin (Yaman and Balikci, 2010), Morphin (Jalili et al., 2017), Bromobenzene (Hamed et al., 2013), and 4-NP (Abou Khalil et al., 2017). The mechanism of NS protective effect is not fully understood, but may be related to its antioxidant, anti-inflammatory, hypotensive, hypolipidemic, antioxidant, hypoglycemic, and anticytotoxic effects (Adam et al., 2016; Amin and Hosseinzadeh, 2016; Gholamnezhad et al., 2016; Mohammed and Arias, 2016). In addition, the protective effect of NS could be also related to its autophagy inhibitory effect and its vasomodulation mechanism (Malik et al., 2017; Racoma et al., 2013). On the other hand, NS has a clear effect to lower the kidney functions parameters in some cases of nephrotoxicity (Dollah et al., 2013; Mousavi, 2015).

4-NP in the African catfish (*C. gariepinus*) is metabolized by many tissues and excreted through the kidney to outside the body (Lu et al., 2012; Sharma and Chadha, 2017b), therefore we used these fishes as a model to study 4-NP nephrotoxic effect (Sayed et al., 2012b). The African catfish (*C. gariepinus*) is an omnivore fish and is the most widespread African freshwater fish. It used as an excellent candidate for aquaculture as well as in fundamental research (Nguyen and Janssen, 2002). It has been used as animal model for toxicology after chemicals exposure (Mekkawy et al., 2011; Sayed and Ismail, 2017; Sayed et al., 2016), and UVA exposure (Mahmoud et al., 2009; Mekkawy et al., 2010; Sayed et al., 2013).

Up to our knowledge, no previous studies highlighted the role of NS to protect against 4-NP induced nephrotoxicity in *C. gariepinus*. In addition, using natural therapeutic agents like NS to fight and treat nephrotoxicity in fish is more useful, safer, and economic for fish, environment, and consumers than other chemical compounds. Therefore, the aim of our novel present study is to focus on the potential protective influences of dietary NS on 4-NP induced nephrotoxicity in *C. gariepinus* and to shed the light on the possible mechanism of action of NS through histological, immunohistochemical, and ultrastructure evaluations of the kidney.

2. Materials and methods

2.1. Experimental animals

Thirty male adults (351.0605 ± 3) fishes (*C. gariepinus*) were used from the Fish Biology and pollution lab of Faculty of Science, Assiut University. Fishes were maintained in aerated recirculating tank containing experimental media water at 28 °C at 12 h light/12 h dark cycles. Fishes were fed with a commercial pellet twice daily, 5% of body weight and the water was changed daily to reduce impurities from metabolic wastes. The whole experimental period extended to 3 weeks. Following the acclimatization period, the African catfish were examined to be free of external parasites and healthy according to AFS-FHS (2003).

Fishes were equally divided into five groups; six fishes in each group (Supplementary Fig. 1). Untreated control group (Ctrl) received a commercial pellet as previously described by the current authors (Abou Khalil et al., 2017). 4-Nonylphenol (Sigma-Aldrich with a purity of 99.3%) treated group (4-NP); maintained with 0.1 mg L^{-1} 4-NP dissolved in aquaria water (Mekkawy et al., 2011). 1% *N. sativa* treated group (NS-1) maintained with 0.1 mg L^{-1} 4-NP dissolved in aquaria water and fed with a commercial pellet contains 1% *Nigella sativa*. 2.5% *N. sativa* treated group (NS-2.5) maintained with 0.1 mg L^{-1} 4-NP dissolved in aquaria water and fed with a commercial pellet contains 2.5% *Nigella sativa*. 5% *N. sativa* treated group (NS-5) maintained with 0.1 mg L^{-1} 4-NP dissolved in aquaria water and fed with a commercial pellet contains 5% *Nigella sativa*. Fish were sacrificed by decapitation.

2.2. Immunohistology

After 3 weeks of 4-NP exposure, kidneys of scarified fishes were collected and fixed in 10% neutral buffered formalin for paraffin tissue sections. Fixed kidneys were dehydrated in ascending grades of ethanol, cleared in methyl benzoate, and then embedded in paraffin wax. Paraffin blocks were sectioned at 5 μm thick. Sections were stained with Haematoxylin and Eosin (H&E) (Sigma Aldrich, Germany), Crossmon's Trichrome (Sigma Aldrich, Germany), and Perl's Prussian blue (Sigma Aldrich, Germany). The following antibodies were used according to our previously published protocol (Kotb et al., 2016): anti Nephrin (Santa Cruz, Germany), anti LC3 (Santa Cruz, Germany), anti mTOR (Proteintech, USA). As secondary antibodies, Cy3 conjugated anti-rabbit and Cy2-conjugated anti-mouse antibodies (Santa Cruz, Germany) were used. DAPI (Santa Cruz, Germany) was used to visualize nuclei. Image J 1.48v software was used for histological sections analysis.

2.3. Semithin sections and transmission electron microscopic (EM) preparations

Small pieces (2 mm thick) of kidneys of newly scarified fishes were fixed in 2.5% glutaraldehyde in phosphate buffer (PH 7.2) for 24 h. The