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Studies on the toxicity of 4-nonylphenol on the histopathology of liver of African catfish *Clarias gariepinus* (Burchell, 1822)

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Abstract

Endocrine-disrupting chemicals (EDCs) have the potential to disrupt the endocrine system by mimicking endogenous hormones such as estrogens and androgens. A variety of commercial products are found in river water, are estrogenic. 4-Nonylphenol (4-NP) has been found to be oestrogenic in fish and may influence the reproductive system of male fish. In the present study, the effects of three sublethal concentrations (70, 100 and 130 µg/lit) of 4-nonylphenol for 10 days on the liver were investigated in African catfish *Clarias gariepinus* (Burchell, 1822). Histological examination of the liver of fish treated with 70 µg/lit 4-nonylphenol for 10 days showed eccentric nuclei in cells, presence of number of vacuoles, increased accumulation of pyknotic nuclei whereas the liver of fish treated with 100 µg/lit 4-nonylphenol for 10 days showed, disorganization of hepatic cords, hepatocytes with eccentric nuclei, presence of number of vacuoles, presence of melanomacrophage. Similarly, the histological examination of the liver of fish treated with 130 µg/lit 4-NP for 10 days showed, disruption of normal architecture of central vein and hepatic triad, disorganization of hepatic cords, enucleated hepatocytes and dilation of sinusoids. Based on the above results, the present study indicates that 4-nonylphenol has marked effects on the liver histology of *C. gariepinus* which ultimately affects the normal metabolic activities of organism. The severity of effects of fish increases with the time of exposure.

Keywords: EDC, 4-NP, melan macrophage, hypertrophy, Kupffer cells

Introduction

Our environment, and particularly the aquatic environment, has been under emphasis within the past decades because of the large amount of chemical substances released into it. Thousands of synthetic chemical compounds are currently registered for use in industry and agriculture, and thousands of tons of these are produced annually. Irrespective of the source or original anticipated use, substantial amount of these chemicals end up in the aquatic environment due to physicochemical, hydrologic and atmospheric processes [1]. As a result more and more of our habitats are being deteriorated day by day due to such increased environmental pollution by means of various anthropogenic activities. The industrial effluents that contain toxic substances like heavy metals, pesticides and other chemicals are discharged into the water bodies. As a result, the aquatic fauna and flora are adversely affected, which lead to bioaccumulation in aquatic organisms and bio-concentration in higher vertebrates [2].

Endocrine disrupting chemicals (EDCs) or endocrine disruptors are compounds that can interfere and alter the homeostasis of the endocrine system, by modifying its response, resulting in long-term adverse effects on human and animal health or their progeny. The effects also extend to the thyroid, nervous, immune system and metabolism in general [3]. EDCs can be divided on the basis of naturally and synthetically occurring compounds. Naturally occurring compounds include hormones that normally occurring in humans or animals and plant derived metabolites. On the contrary, the second group includes various exogenous man-made substances such as synthetic hormones and drugs, industrial chemicals (organchloride pesticides, dioxins, polychloro biphenils (PCBs), polybromo biphenils (PBBs), and alkylphenols, parabens found in cosmetics and other personal care products, manufactured industrial products such as plastic additives (bisphenol A, phthalates), antifouling paints, and chemicals used in farm animal production [4, 5].

Nonylphenol (NP) is a product of industrial synthesis formed during the alkylation process of phenols. The addition of ethoxyl groups to the parent compound produces nonylphenol ethoxylates (NPE), which are used to produce industrial surfactants.

NPEs are widely used as detergents, emulsifiers, and surfactants (wetting agents) in household and industrial products such as paints, plastics, cosmetics, lubricant oils, construction materials, vulcanized rubber, and paper. They are also used in the processing of fuels, metals, petroleum, textiles, agricultural chemicals, and leather. Among them, the detergents have become one of the most commonly used household products for cleaning of domestic materials in every day of life throughout the world. The detergents are either deliberately drained into the aquatic environment such as ponds, lakes, rivers, streams, etc. or they find their way into the aquatic environment by natural sewage. NP, the predominant environmental biodegradation product of NP ethoxylates, is abundant and moderately persistent. Due to their wide usage, the occurrence of NP and NPE has been reported around the world in rivers, lakes and coastal waters [6].

The liver is vital organ which play an important role in many aspects of nutrition, including lipid and carbohydrates storage etc. Any alterations in the structure of liver may be used as a useful biomarker for the evaluation of effects of environmental stressors [7]. Stressors associated alterations of hepatocytes may be found in the nucleus or cytoplasm or both [8]. Malik and Hodgson [9] reported that the liver plays a major role in complex enzymatic processes of thyroid hormones conversion. So, liver dysfunction and disease affects thyroid hormone metabolism.

Fish is known to be a source of protein rich in essential amino acids. Fish meal has been the most important food stuff used as a source of protein in aquaculture feed, because of its essential amino acid composition and palatability. The fishes are considered as one of the primary risk organisms for Endocrine Disrupting Chemicals (EDCs). Not only they are directly exposed to a wide variety of EDCs, but also sex determination in fish is known to be very labile and can be disturbed or even reversed by exogenous hormone exposure at critical developmental stages [10].

The present study was carried out with the aim to investigate the effects of three sub lethal concentrations of 4-nonylphenol on the histopathology of liver of African catfish, *C. gariepinus* (Burchell, 1822).

Materials and Methods

The fresh water African catfish, *Clarias gariepinus* (Burchell, 1822), were selected for the present study because of its availability from local market and its convenient size. It could be safely transported and maintained easily under laboratory conditions because of its air breathing habit, its hardy nature, moreover suit the experimental work.

All the fishes used during the present study were brought from the local market. The body weight of fish ranged between 250-350 gm and their length varied between 30-37cm. The fishs were maintained in glass aquaria containing 30lit of tap water,under normal conditions of light and temperature. The fishes were fed with minced goat liver every alternate day and water changed at an interval of one day. The fishs were acclimatised for one weak by keeping 6 fishes in one aquarium prior to their use in the experiment.

For the present study, the chemical 4-nonylphenol was purchased from Hi-Media where benzene used as a solvent (33.3mg 4-nonylphenol dissolved in 1ml of benzene). The four aquaria were taken, filled with 30 lit tap water and in each aquarium 6 male fishes were kept. The fishes without exposure of any toxicant in one aquarium were treated as control group and other aquaria exposed to toxicant were treated as experimental group and labelled them accordingly. The fishes exposed to three sub lethal concentrations (70, 100 and 130ug/lit) of 4-nonylphenol for 10 days were treated as experimental group.

For microscopic examination, after 10 days, surviving fishes of each group were removed and dissected. Small pieces of the liver were taken and immediately fixed in Bouins fixative. Fixed tissues were processed routinely for paraffin embedding technique. Embedded tissues were sectioned at 5μ in thickness and then stained with Eharlich hematoxylin and eosin stain (H & E) and mounted in DPX. The slides were then observed under microscope (100X and 400X).

Observations and Results

Histopathological changes in Liver

In the present study, lesions were observed in liver of experimental group fishes exposed to high dose of 4-NP for long term exposure. The occurrence and degree of alteration were positively related with the increasing dose of 4-NP, while the tissues taken from the fishes of control group does not shows any changes during the experiment.

The liver of the control group fish, *Clarias gariepinus* showed normal structure like a continuous mass of hepatic cell, hepatocytes (H) with cord like pattern interrupted by blood vessels and sinusoids. The hepatocytes were large in size, polygonal in shape with centrally located nuclei. The hepatocytes have homogenous eosinophilc cytoplasm. The sinusoids were seen as communicating channels occupied by blood cells with Kupffer cells (Kc) (Fig. 1.A)

Histological examination of the liver of fish exposed to 70 μg/lit 4-NP for 10 days showed disruption of normal liver architecture. Majority of cells showed eccentric nuclei in cells, presence of number of vacuoles, increased accumulation of pyknotic nuclei, hypertrophied Kupffer cells and disruption of hepatic triad. The severity of changes increases with increase in concentrations of 4-NP (Fig. 1. B, C, D, E, F). Histological examination of the liver of fish exposed to 100 μg/lit 4-NP for 10 days showed the disruption of normal architecture of central vein and hepatic triad, disorganization of hepatic cords, hepatocytes with eccentric nuclei, presence of number of vacuoles, increased accumulation of pyknotic nuclei, presence of melanomacrophage and disorganization of hepatic cords (Fig. 2. A, B, C, D, E, F). Histological examination of the liver of fish exposed to 130 μg/lit 4-NP for 10 days showed the disruption of normal architecture of central vein and hepatic triad, disorganization of hepatic cords, enucleated hepatocytes and dilation of sinusoids (Fig. 3. A, B, C, D).

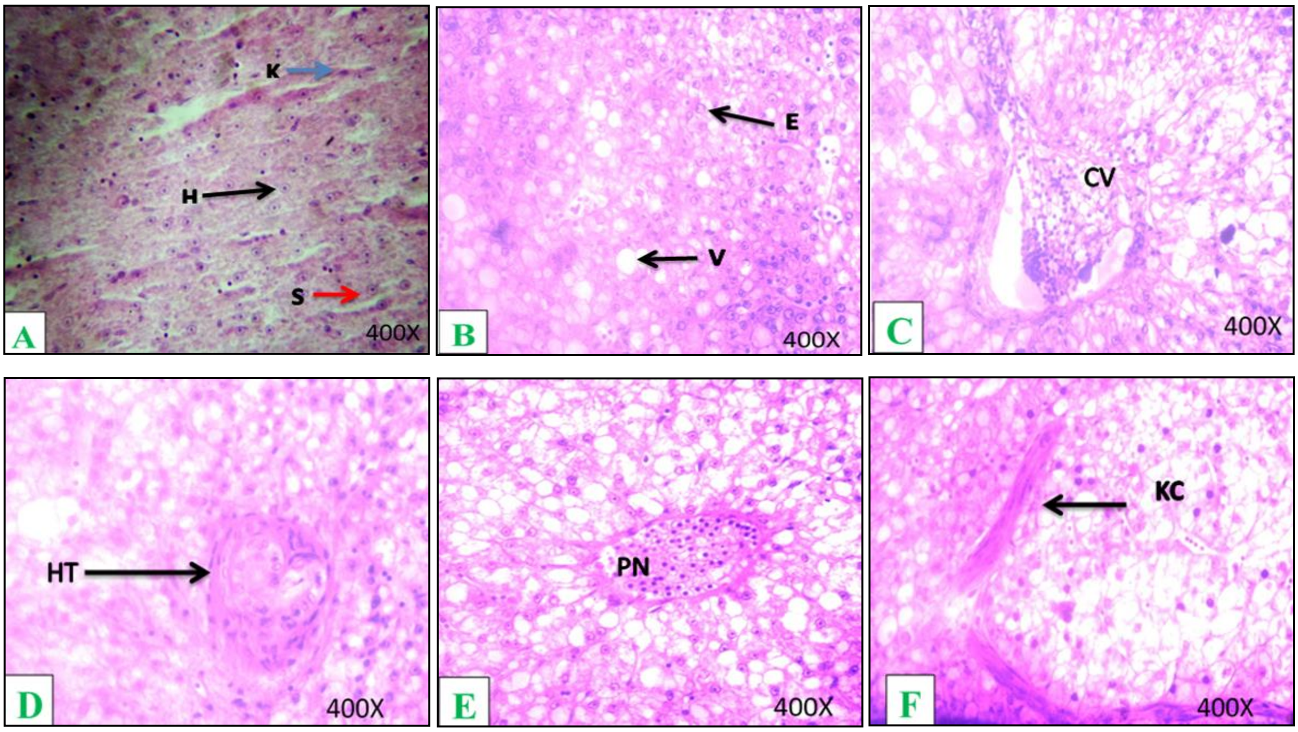

Fig 1: Microphotograph showing changes in liver of control fish *Clarias gariepinus*, hepatocytes, blood sinusoid, and Kupffer cells (1A). HE (A-400X).

Microphotograph showing histopathological changes in liver of experimental fish *Clarias gariepinus* (B, C, D, E, F) exposed to 70µg/lit 4-NP for 10 days showing,
(B) Hepatocytes with eccentric nucleus with vacuoles. HE (400X).
(C) Disruption of normal architecture of central vein. HE (400X).
(D) Disruption of hepatic triad. HE (400X).
(E) Increased accumulation of pyknotic nucleus. HE (400X).
(F) Hypertrophy of kupffer cell. HE (400X).

Abbreviations used:
CV- Central vein, HT- Hepatic triad, S- Sinusoid, H- Hepatocytes, KC- Kupffer cells, En- Eccentric nucleus and PN- Pyknotic nucleus

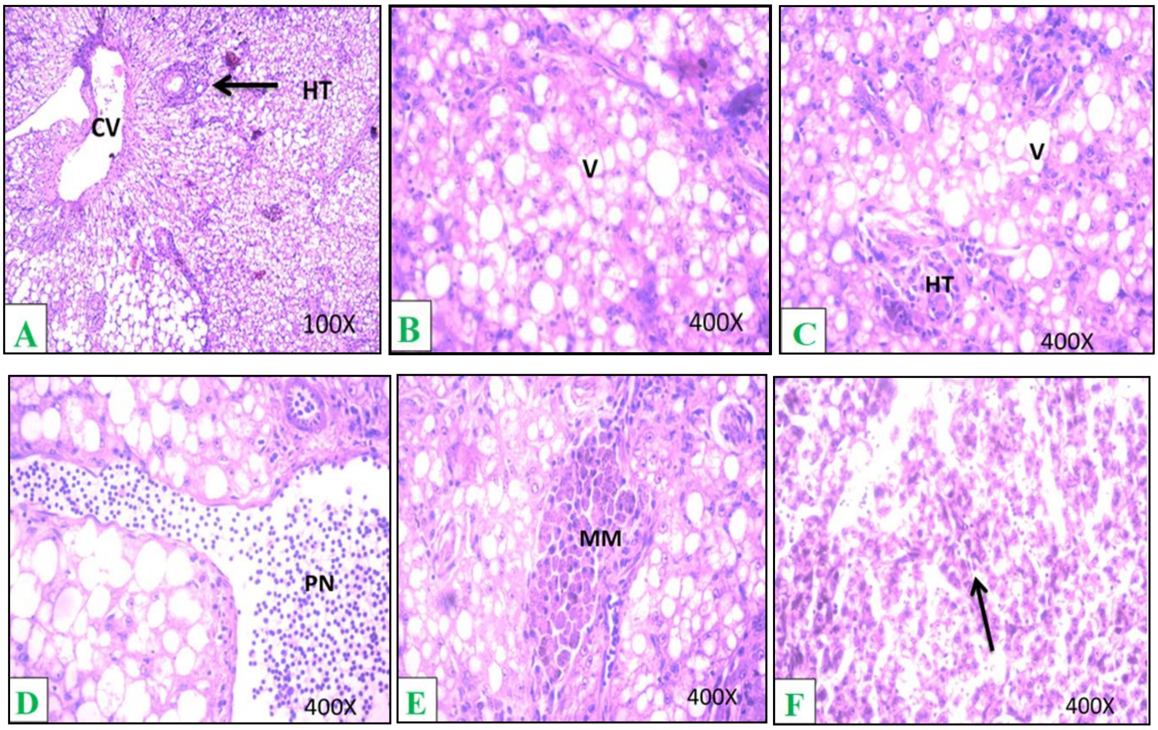

Fig 2: Microphotograph showing histopathological changes in liver of experimental fish *Clarias gariepinus* (A, B, C, D, E, F) exposed to 100µg/lit 4-NP for 10 days showing,

(A) Disruption of normal architecture of central vein. HE (400X).
(B) Presence of number of vacuoles. HE (400X).
(C) Disruption of hepatic triad. HE (400X).
(D) Increased accumulation of pyknotic nucleus. HE (400X).
(E) Presence of Melan macrophage. HE (400X).
(F) Arrow showing disorganization of hepatic cords. HE (400X)

Abbreviations used:
CV- Central vein, HT- Hepatic triad, S- Sinusoid, H- Hepatocytes, KC- Kupffer cells, PN- Pyknotic nucleus, V- Vacuoles and MM- Melan macrophage

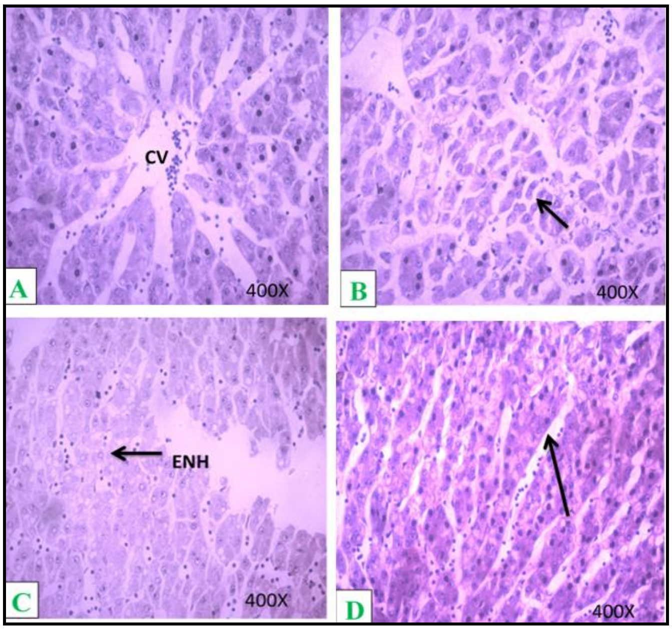

Fig 3: Microphotograph showing histopathological changes in liver of experimental fish *Clarias gariepinus* (A, B, C, D) exposed to 130µg/lit 4-NP for 10 days showing,

(A) Disruption of normal architecture of central vein and hepatic triad. HE (400X).
(B) Arrow showing the disorganization of hepatic cords. HE (400X).
(C) Enucleated hepatocytes.HE (400X).
(D) Arrow showing dilation of sinusoids. HE (400X).

Abbreviations used:
CV- Central vein and ENH- Enucleated hepatocytes

Discussion

It has been reported that Nonylphenol like estradiol (E2) is estrogenic [11] and affects the histology of developing immune and endocrine organs and those in direct contact with aquatic environment [12, 13]. During the present study histological examination of liver tissues of *C. gariepinus* exposed sub-lethal concentrations of 4-NP for 10 days exposure showed severe and variable effects. These include disorganization of the hepatic cords, enucleated hepatocytes and cells with eccentric nucleus. Similar effects were observed by [14, 15] who reported a severe disorganization of hepatic cords, rupture of hepatocytes and eccentric nucleus. The dissociation between hepatocytes and disorganization of hepatic cords in the liver, probably related to cell necrosis and degeneration of structural proteins in the hepatocyte's membrane [16, 17].

The present study showed that NP activates the phagocytic activity of the sinusoidal cells by increasing the number of Kupffer cells. Similar findings were reported by [18, 19] who observed increase in the Kupffer cells in fish exposed to different environmental toxicants. This might be a result of increased autophagy throughout the hepatic tissue to help in removing the accumulated NP and its metabolites where lysosomes are involved in intracellular break down into small metabolic products. The Kupffer cells hyperplasia might be correlated with the amount of injury to the hepatic tissue induced by NP intoxication and represent a defense mechanism of detoxification and might be contributed to hepatic stress [20].

In the present study, liver showed the presence of vacuoles and melanomacrophages. The other sections showed abnormalities, such as extensive pycnosis, dilation of sinusoids, congestion of central vein, ruptured membrane of central vein and the accumulation of eosinophilic material in the central vein.

Vaccuolation of the hepatocytes in the fishes exposed to different xenobiotics have also been reported by many workers [21, 22, 23, 24, 25, 26] observed that the formation of vacuoles is a defense mechanism by which the cell collects harmful and degraded substances to prevent interference with its viable activity whereas [27] noted that interference with fatty acid metabolism leads to accumulation of neutral fat, resulting in equal-edged and non-membranous empty vacuoles. The neutral fat is dissolved by organic solvents during tissue preparation leaving unstained empty vacuoles.

A number of fish diseases are associated with melanomacrophage aggregates as phagocytic cells [28]. The widespread of this pathologic condition in the present study may be due the loss of energy in the detoxification process, increase in metabolic bi-products causes aggregation of more melanomacrophage [29]. These findings are similar to those reported by [30, 31, 32].

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