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A study on the toxicity of 4-nonylphenol on the histopathology of testes of African catfish *Clarias gariepinus (*Burchell, 1822)

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Abstract
In the present study, the effects of long term exposure (5 and 10 days) of 100 µg/lit 4-NP (nonyphenol) on the testis were investigated in African catfish *Clarias gariepinus* (Burchell, 1822). Histological examination of the testis of fish treated with 100 µg/lit 4-NP for 5 days showed the disintegration of cysts, separation of cells within the cysts, hypertrophy of sertoli cells and vaccuolation in testis. Histological examination of the testis of fish exposed to 100 µg/lit for 10 days showed alteration in structure of the primary spermatocytes. The structure of the spermatocytes changed from spherical to sickle shaped. Hypertrophy of sertoli cell, severe destruction of germ cells (spermatogonia), and vacuole formation was also seen. The study indicated that 4-nonylphenol had marked effects on the histology of testis of *C. gariepinus.* The severity of effects of fish increased with the time of exposure and it was noticed that there were marked structural changes in the testis exposed to 4-Nonylphenol for long term exposure.

Keywords: Endocrine disruption, Hypertrophy, 4-nonylphenol, Sertoli cells, Vaccuolation, Spermatocytes

INTRODUCTION

The pollution of aquatic environment has received considerable attention in recent years owing to the toxicity of chemicals. Chemical pollution appears to be due to increase of industrialization. A large number of chemicals can contaminate aquatic environments and their animals including fish and amphibians during their adult life and sensitive stages of development (Radhaiah *et al.,* 1987). These chemicals affect the organism by either interfering in their development during their developmental stages or in the physiology of adults altering their normal homeostasis. Their toxicity appears because of their persistence in the environment and their accumulation in the biota tissue (Mekkawy *et al.,* 2011). There is growing concern worldwide, especially in developing countries, about a group of xenobiotics, which is known to disrupt the endocrine system of organism. These groups of chemicals are called as endocrine disrupting chemicals (USEPA, 2000).

Endocrine disruptors can interfere with the production, release, metabolism, and elimination of or can mimic the occurrence of natural hormones (Tabb and Blumberg, 2006; Matthiessen *et al.,*2006).The pollution of the aquatic environment caused by the discharge of non-ionic surface active substances such as alkylphenols (APs) and their biodegradation products nonylphenol (NP) and octylphenol (OP) has attracted the attention of scientists due to their estrogenic and toxic effects on living organisms (Jobling *et al.,* 2003; Weber *et al .,* 2002; Liney *et al.,* 2005). Nonylphenol ethoxylate (NPE) has been found in aquatic environments, particularly in river water (Rivero *et al.,* 2008). This compound is widely used in the manufacture of non-ionic surfactants, lubricants, stabilizer polymers, antioxidants, alkylphenol chemicals, detergents, paints, anaerobic treated sewage sludge, polystyrene tubes, insecticides and herbicides. In the aquatic environment NPE breaks down to 4- nonylphenol (NP), which is more stable and persistent (Guenther *et al.,* 2006; Rivero *et al.,*2008).

NP has been reported as estrogenic, toxic and carcinogenic effects in various teleost fish species, birds and mammals, and enhanced resistance towards biodegradation, potential ability to bio-accumulate in a aquatic organisms (Cionna

et al., 2006; Ishibashi *et al.,* 2006; Popek *et al.,* 2006; Vetillard and Bailhache,2006; Yang *et al.,* 2008; Zaccaroni *et al.,* 2009).It is also recorded to accumulate in the internal organs of many fish and avian species, reaching its concentration 10-100 times higher. It is of course the public and scientific issue because it passes down to humans though the food chain.

NP profoundly impairs testicular function as evidenced by reduced testis size (Chitra *et al.,* 2002), low circulating testosterone, disturbed testicular structure and suppressed spermatogenesis (Tan *et al*., 2003; Cardinali *et al*., 2004).NP administration increased ROS level and lipid peroxidation and depressed the activity of antioxidant enzymes such assuperoxide dismutase and glutathione reductase in rat testis (Chitra and Mathur, 2004).Several studies has been carried out to evaluate the adverse effects of Nonylphenol on gonad histopathology, vitellogenin, estrogen receptor and aromatase expression in different species of fishes such as *Xiphophoru shelleri* (Kwark *et al*., 2001), *Xiphophorus maculates* (Magliulo *et al*., 2002), *Danio rerio* (Lin and Janz, 2006),Rampel *et al.,* (2006), *Gobicocypris rarus* (Zha *et al*., 2007) and *Salmo salar* (Arukwe and Roe, 2008).

The present study was carried out with the aim to investigate the effects of long term exposure of 100µg/lit 4-NP on the histopathology of testis of African catfish, *C. gariepinus.*

MATERIALS AND METHODS

The fresh water African catfish, *C. gariepinus* (Burchell, 1822), were selected for the present study because of its availability from the fish 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 Gokulpeth 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 three 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 two aquaria exposed to toxicant were treated as experimentalgroup and labelled them accordingly. The fishes exposed to 4-nonylphenol by adding 100µg/lit in the two aqaria for 5 and 10 days were treated as experimental group.

For histological examination, after 5 and 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 (200X and 400X).

RESULTS AND DISCUSSION

Histopathological changes in the testis: Testes of *C. gariepinus* are paired organs found in the abdominal region and each is enclosed in a peripheral connective tissue sheath. Testes consist of many seminiferous tubules lined with spermatogonic epithelium which gives rise to successive stages of spermatogenesis. In the present study, the experiment conducted during resting phase (Dec-Jan) of reproductive cycle of the *C. gariepinus*therefore, the histological examination of the testis of the control group fish showed three spermatogenic stages primary spermatogonia (PSG), secondary spermatogonia (SSG) and primary spermatocytes (PSC) and Sertoli cells (Fig. A).

Primary Spermatogonia (PSG) were large in size located along the periphery of the lobules. The PSG were prominent with conspicuous cells boundaries and each has a large spherical nucleus. Secondary Spermatogonia (SSG) were slightly smaller than PSG. The nuclei were darkly stained and amount of cytoplasm was comparatively less. Primary spermatocytes (PSC) were present in the forms of cysts and each group contains many primary spermatocytes in most cases they were slightly larger than secondary spermatocytes and have deeply stained nuclei.

Histological examination of the testis of fish exposed to 100 µg/lit 4-NP for 5 days showed the disintegration of cysts, separation of cells within the cysts, hypertrophy of Sertoli cells and vaccuolation in testis (Fig. B and C). Histological examination of the testis of fish exposed to 100 µg/lit for 10 days showed alteration in structure of the primary spermatocytes. The structure of the spermatocytes changes from spherical to sickle shaped. Hypertrophy of Sertoli cell, severe destruction of germ cells (spermatogonia), and vacuole formation was also seen (Fig. D, E and F).

The histological examination of the testis of *C. gariepinus* revealed severe effects on the testicular structure of the seminiferous tubules exposed to nonylphenol treatment. Exposure of testis to nonylphenol resulted in the reduction of number of

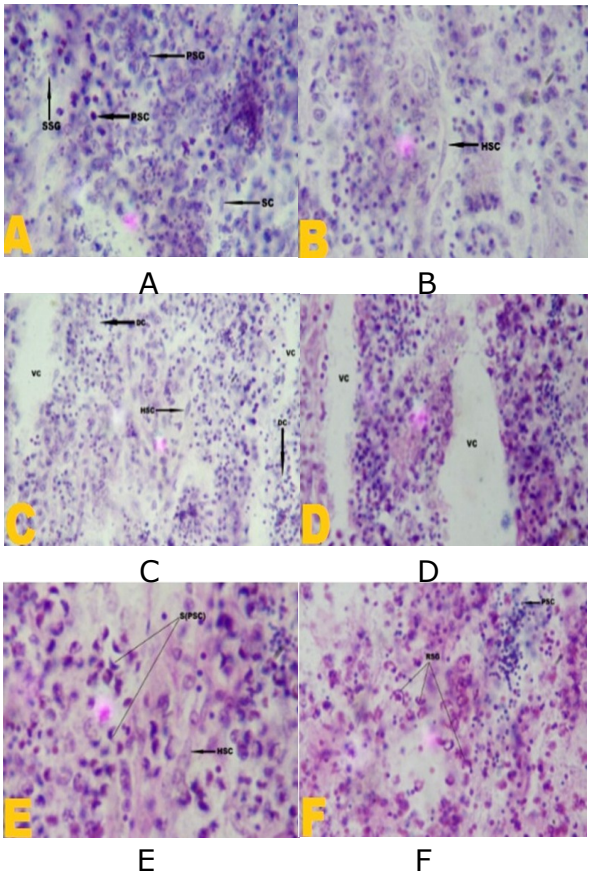

Fig. A. *Showing primary spermatogonia (PSG), secondary spermatogonia (SSG) and primary spermatocytes (PSC) and Sertoli cells of testis from control group fish C. gariepinus (400X).***Fig. B.** *Showing the disintegration of cysts, separation of cells within the cysts, hypertrophy of Sertoli cells in the testis of experimental group fish C. gariepinus exposed to 100µg/lit 4-NP for 5 days (400X).***Fig. C.** *Showing the vacuolization in the testis of experimental group fish C. gariepinus exposed to 100µg/lit 4-NP for 5 days (400X).***Fig. D.** *Showing the severe vacuolization in the testis of experimental group fish C. gariepinus exposed to 100µg/lit 4-NP for 10 days (400X).***Fig. E.** *Showing the alteration in structure of the primary spermatocytes in the testis of experimental group fish C. gariepinus exposed to 100µg/lit 4-NP for 10 days (400X).***Fig. F.** *Showing the destruction of germ cells in the testis of experimental group fish C. gariepinus exposed to 100µg/lit 4-NP for 10 days (400X).***Abbreviations used:PSG-** Primary spermatogonia**, SSG**- Secondary spermatogonia**, PSC-** Primary spermatocytes, **SE-** Sertoli cells, **HSC-** Hypertrophied Sertoli cells, **DC-** Disintegration of cysts, **VC -** Vaccuolation, **S(PSC)-** Sickle shape primary sper-

cyst, disintegration of cysts, separation of germ cells within the cyst, hypertrophy of the Sertoli cells. Similar effects have previously been seen in platyfish (*Xiphophorus maculatus*) exposed to nonlyphenol and 17 b-estradiol (Kinnberg *et al.,* 2000), in adult guppies exposed to estrogenic and antiandrogenic compounds (Kinnberg and Toft, 2003).

A possible explanation for the above changes in the testis structure is an effect on the Sertoli cells. Several roles have been attributed to the Sertoli cells, including formation of cysts in which spermatogenesis takes place, spermatozeugmata morphology regulation of spermatogenesis and spermiation, germ cells support and nutrition, phagocytosis of residual bodies, inhibin secretion. Nonylphenol acts on Sertoli cell and it may responsible for causing such adverse effects on the testis.

In the present study, testis shows the vaccuolation, structural changes in the germ cells such as sickle shaped germ cells and the rupturing of the germ cells. Similar observations were made by the Shalaby and Migeed, (2012) where they studied the impact of environmental contaminant on the testis of *Oreochromis* niloticus. Kumar *et al.,* (2007) also reported the presence of large number of inter and intra tubular vacuoles, condensation of spermatogonic cells in the testis of *Heteropneustus fossilis* after exposure of liner alkyl benzene sulphonate. Vergillo *et al.,* (2012) reported disorganization of the cysts' arrangement, vacuolization of germ cells and sperm aggregation in the testis of *Gymnotus carapo* following 24 h of Hg exposure.

The mechanism where oestrogens and oestrogenic chemicals such as nonylphenol cause inhibitory or degeneratory effects on testicular development and structure is still unknown. A possible explanation is a direct effect on the Sertoli cells that may result in the changes described earlier. Such a direct effect on Sertoli cells is supported by the fact that oestrogen receptors are found in Sertoli cells in mammals (Nakhla *et al.,* 1984) and preliminary evidences suggest that they are also found in the dog fish, *Squalus acanthias* (Dubois and Callard, 1989). Nonylphenol may act indirectly via the hypothalamus- pituitary axis to alter gonadotropin synthesis and secretion or it may also act directly on the testis. Altered gonadotropin secretion resulting in disruption of sex steroid production, can have secondary effects on the testicular cells e.g. Sertoli cells, which are dependent upon the correct hormone level for normal functioning.

Direct effects on the testis can be either cytotoxic when the disruption is caused by damage to the testis cells in general or endocrine, in which the functioning of specific cells e.g. Sertoli cells are disrupted due to endocrine malfunction (Kime, 1999). Oestrogenic chemicals may also exert their effects directly on the testis via inhibition of androgen synthesis (Tradeau *et al.,* 1993). Recently, an oestrogen receptor with affinity for nonylphenol has been identified in the testis of the Atlantic croaker *Micropogonias undulates* (Loomis and Thomas, 1999). The nonylphenol induced effects on the testicular structure observed in the present

study could be mediated via such testicular oestrogen receptors.

The severe effects on the testis reported here occurred at 100ug/l for long term exposure of nonlyphenol. It is possible that similar effects may also be observed after long term exposure to lower concentration. Nonylphenol is strongly lipophilic (Ahel and Giger, 1993) and tends to accumulate in aquatic organisms. Other oestrogenic chemicals in the environment may have the potential to produce effects similar to those of nonylphenol. Indeed a combination of several oestrogenic chemicals may have additive effects (Sumpter and Jobling, 1995). Thus it is quite possible that nonylphenol either by itself or through its contribution to the pool of environmental oestrogens, may have adverse effects on the reproductive system of male fish.

Conclusion

The present study can be concluded that the 4-nonylphenol is toxic to the fish. It shows very drastic effects on the histological structure of fish and its severity depends on the duration of exposure. The severe effects observed in the present study directly or indirectly shows the reduction in the germ cells and reproductive fitness which leads to the infertility in the fish.

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