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Histopathological changes on testes of guppy fish *Poecilia reticulata* Peters (1859) exposed to vydate

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Key words: Guppy fish, Testes, Pesticide, *Poecilia reticulata*, Vydate.

Abstract

The present study was aimed to identify the histopathological changes of the testicle in the Guppy fish resulting from exposure to the vydate pesticide; the investigation conducted on 60 fish of Guppy males collected from local markets, fish were divided into 4 groups, each one included 15 fish, group 1 has exposed for (0.3 mg/L) of vydatepesticide, group 2 Exposed for (0.5mg/L), group 3 exposed for (0.8 mg/L), and total control. Then the groups were sacrificed at (2, 4 & 6) weeks and the samples of testes were collected and fixed with Bouin's solution and processed with routine paraffin technique and tissue sections were stained with H&E stain. The results showed that the testes of the control group were surrounded by a connective tissue capsule which contained melanocytes. The testicular parenchyma composed of groups of seminiferous tubules which separated from each other by interstitial tissue which contains Lyedie cells. The germinal epithelium showed variable stages of spermatogenesis started with primordium germ cells with divided to give rise spermatogonia, primary spermatocytes, spermatid and spermatozoa. In treated groups, at two weeks exposure to vydate showed mild vascular congestion of the interstitial tissue, blood vessels and shrinkage of the Lyedie cells; while at four weeks exposure the signs revealed advanced changes within seminiferous tubules showed degenerative changes and necrosis and there were appearance of giant cells among developing germ cells. The study concluded that the changes are increased with increasing both the concentration and the duration of exposure, as evidenced by our current study.

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Introduction

Pesticides are defined as chemicals used to reduce the effects of organisms such as insects, grasses and rodents (Valavanidis *et al.*, 2006; Candioti *et al.*, 2010; Choung *et al.*, 2013). Pesticides are classified as insecticide, fungicide, herbicide and rodenticide. the use of insecticides in large amounts in agriculture not only accumulates in soil and water, but also leads to the poisoning of wildlife, fish and other aquatic invertebrates, thus, pests insecticides are contaminated to the seas, lakes and rivers in different ways to the degree of disturbance Insisting that insecticides become part of the food chain, bioaccumulation of these substances will create various environmental problems (Yeh *et al.*, 2005; Gunes and Yerli, 2011; Li *et al.*, 2011).Carbamate pesticides are of great importance in agriculture because of their wide range of chemical biochemical activity, in addition, they contain a wide range of compounds if they contain pesticides, fungi and herbicides, but insecticides are the most widely used. Carbamates are low toxic to humans (Wolfe *et al.*, 1978). It works to inhibit acetylcholine esterase, so it is toxic to humans. The most important pests that the vydate insecticide works against are mites, leaf perforates, aphids, beetles, and other economically important nematodes, in addition, the insecticide is used to treat the main pests that affect vegetable and fruit crops, vydate inhibits the enzyme acetylcholine esterase (AchE), which works on the transfer of nerve impulses Nerve impulse transmission (Harvey and Reisen, 1978; Material Safety data sheet, 2012).

Theaim of our investigation to identify the histopathological changes of the testicle in the Guppy fish resulting from exposure to the vydate pesticide.

Materials and methods

Sampling

Sixty-two adult Guppy male fish were collected at a length of 2.5-2 cm from local markets; the method of Wasu *et al.* (2009) was used to reach the lethal dose of half the number of the Guppyfish. Five aquarium measured (20 × 20× 40cm) were equipped, each contained 16 fish. The fish were exposed to different concentrations and duration 96 hours of vydate insecticide (Table 1).

Experimental Design

Fish were randomly divided into four groups, each group included 15 male Guppy fish, group 1 exposed to (0.3 mg / L), group 2 exposed to (0.5 mg / L), group 3 exposed to (0.8 mg / L) as well as control group. The fish were sacrificed and then dissected after 2, 4, and 6 weeks. They were then fixed with Bouin's solution and then processed with paraffin technique and tissue blocks were sectioned at 5-7µm and the sections has stained Hematoxylin and Eosin stain (Bancroft and Stevens, 2012).

Results

The mean lethal concentration of the vydate pesticide was 96 hours on Guppy fish (4.8 mg / L), as shown in Table 1.

Table 1. The Guppy fish are exposed to the vydate pesticide for 96 hours.

Conc. Of vydate	Log conc.	Fish exposed	Fish dead	Mortality %
1.2	0.079	18	7	38.88%
2.4	0.380	18	6	33.33%
3.6	0.556	18	7	38.88%
4.8	0.681	18	9	50%
6	0.778	18	12	66%

Histopathological changes

Control group: The results showed that the testes of control group were surrounded by connective tissue capsule which contained melanocyte that gives a characteristic of most of the ovoviviparous fish called the testicular capsule. The testicular parenchyma composed of groups of seminiferous tubules. The seminiferous tubules were separated from each other

by interstitial tissue which contains Lyedric cells; spermatogenesis contains the various stages of sperm formation. The germinal epithelium showed variable stages of spermatogenesis started with primordium germ cells with divided to give rise spermatogonia is the mother of sperm that develops from primary spermatocytes into spermatid and spermatozoa (Fig.1).

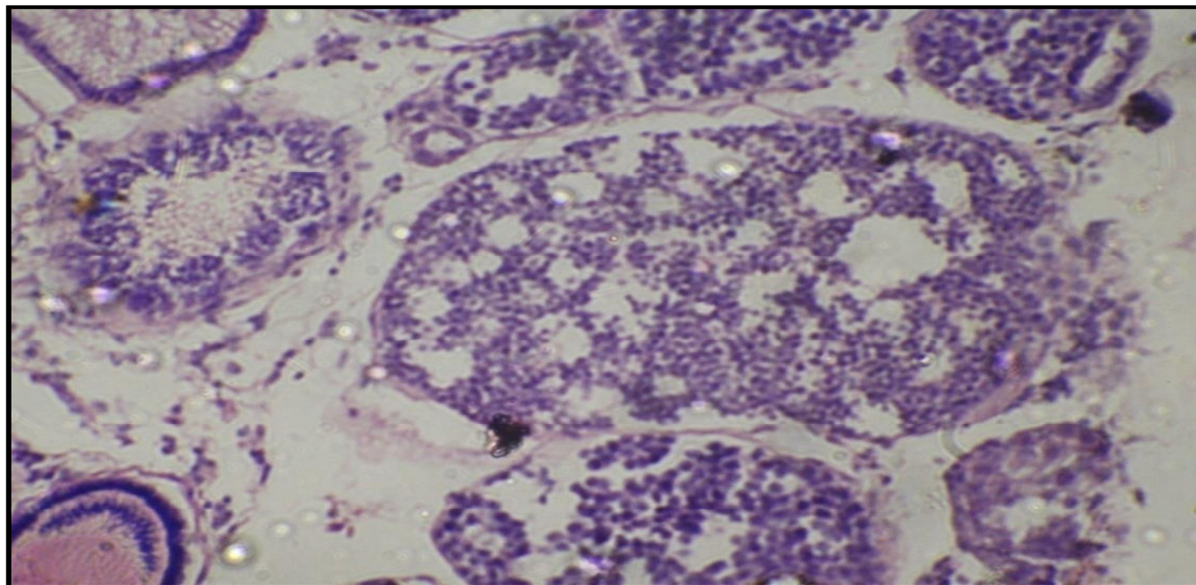


Fig. 1. Cross section of the guppy fish testes at control showing SPG: Spermatogonia, SPC: Spermatocyte, SPT: Spermatid, SE: Sertoli cell, LY: Lyedric cell, ST: Seminiferous tubule, Interstitial tissue (), () Blood vessel, (H&E).

Treated groups for two weeks: The first signs which seen under light microscopy that represented by disarrangement of the interstitial tissue associated with vascular congestion, marked shrinkage, and of the Lyedric cells in compared to those in the control group has emerged. One of the conditions associated toxic effects was the congestion which begins with the partial sloughing of germinal epithelium from the basal membrane which seen in treatment group of (0.5mg/L).

These sings were seen in all treated groups with concentrations of (0.8, 0.5) mg / l and for all exposure periods except for the treated group at concentration(0.3 mg / L) and for 2 weeks of exposure which revealed no pathological changes (Fig.2).

Treated groups for four weeks: at this period, section revealed the transmission of the damage caused by the pesticide into the spermatozoa. Degeneration was observed in some germ cells and necrosis in seminiferous tubules especially in seminiferous tubules those shows active spermatogenesis containing the developing sperm cells primary spermatocytes and spermatid.

The characteristic cause that accompanied this period of exposure was the appearance of giant cells among the developing sperm cells (Fig.3).

Treated groups for six weeks: In this period, all groups exposed to the three concentrations (0.3, 0.5, 0.8 mg/L) were showed advanced sighs of degeneration, necrosis and sloughing of germinal

epithelium and the effects were generalized in most spermatozoa with an increase in the rate of decreased sperm population (Fig.4).

Discussion

The organisms' sensitivity to the pollutants varies depending on the size of their bodies. Larger living

organisms dispose of pollutants faster than small-scale organisms, so it is necessary to identify the lethal medium concentration of half the numbers of laboratory animals for different species of fish (Al-Azawi, 2010).

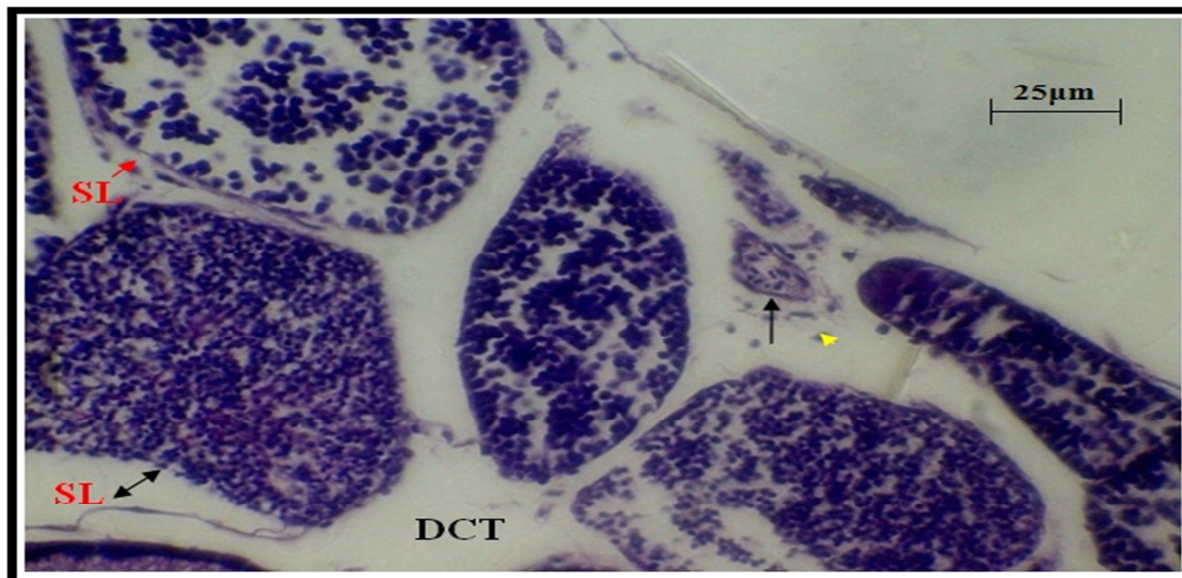


Fig. 2. Cross section of guppy fish testes at 2 weeks of vydate treatment 0.5 mg/L Showing DCT: Disorder of connective tissue, Atrophy lydic cell (◄), SL: Sloughing, congestion (↔) (H&E).

The toxicity of chemical compounds is inversely proportional to the size and age of the fish (Barry *et al.*, 1995). The results of the current study showed the toxicity of the vydate herbicide in fish, where the medium lethal concentration method (LC₅₀) was used to measure the toxicity of the pesticide for 96 hours. The LC₅₀-96h value of the vydate pesticide was 4.8 mg/L in Guppy fish.

The pesticides, as well as the contaminants, affect the proliferative tissue, especially the testicles, by causing damage to spermatozoa and interstitial tissue damage, thus destroying the Lydic cells which responsible for the production of the testosterone hormone or affecting Sertoli cell that feed and support sperm and thus lead to testicular atrophy and thus inhibit spermatogenesis partially or completely (Mathews, 1939; Swarup and Srivastava, 1979). Bagchi *et al.* (1990) reported that the weakness of the

interstitial cells is the result of the inhibition of the toxins of the steroidal origin enzyme. Other cases described in the present study are the vascular congestion in the interstitial tissue may be due the effect of the pesticide on the walls of blood vessels to lead to the vasodilatation (Microcirculation of the micro vascular system that represents veins and lichens) as a response to the occurrence inflammation leading to the vasodilation of arteriole sores caused by nerve stimulation, thus will lead to flow large amount of blood consequently pressure on the precapillary sphincter (a muscle that surrounds the area between the vein and the arteries), this muscle spread according to the amount exchanged between the two regions and the accumulation of blood inside the capillaries and precisely in the area of capillary poetry bed, and thus lead to a high bloodstream within the dilated vein. In the end, the expansion of capillaries and relaxation in the arteries stimulates chemical

media (Robbiss and Kumar, 1987). The changes induced by the vydate herbicide were the case of the sloughing of germ cells from the basement membrane surrounding the sperm. The sloughing may be attributed to the effect of the pesticide on the

basement membrane on which the germinal cells are attached by hemidesmosomes to isolation of cells towards the center of the tubules and leading to decrease in sperm cell size (Donald and Zackary, 2012).

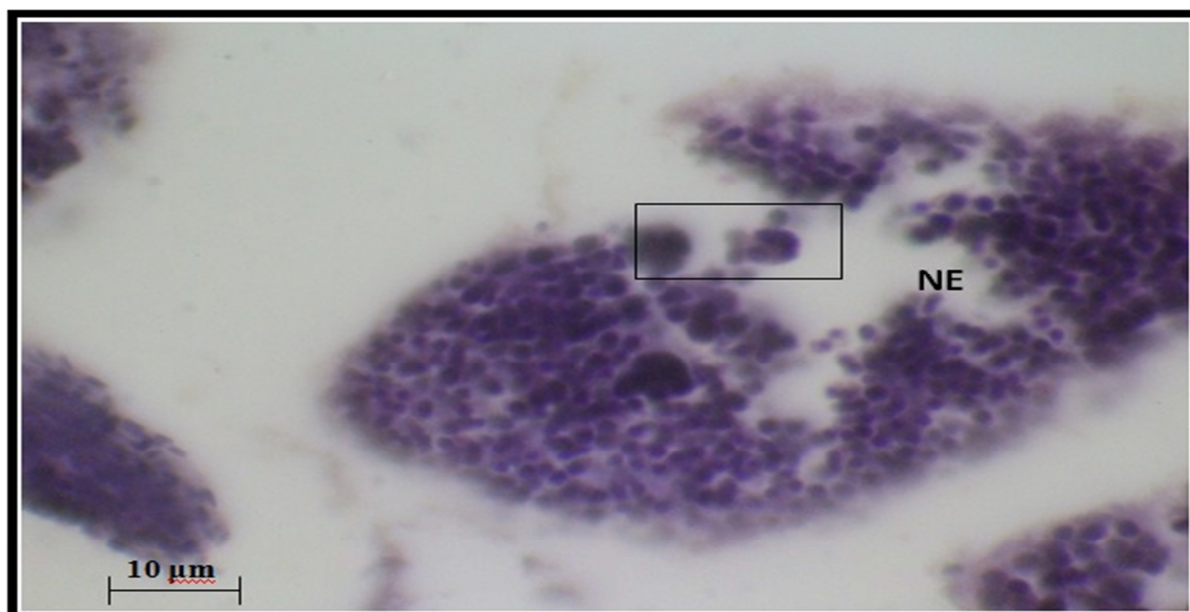



Fig.3. Cross section of guppy fish testes at 4weeks of vydate treatment 0.3 mg/L Showing NE: Necrosis, Giant cells ()(H&E).

The infiltration of giant cells or syncytial cell may indicate a defect in the process of division where the nucleus is divided into several consecutive divisions while the cytoplasm is not divided (Kadim *et al.*,

1972), or due to the accumulation of some cells in the testicle tissue (Young *et al.*, 2011) in which the fabric is to resist the damage that causes the pesticide.

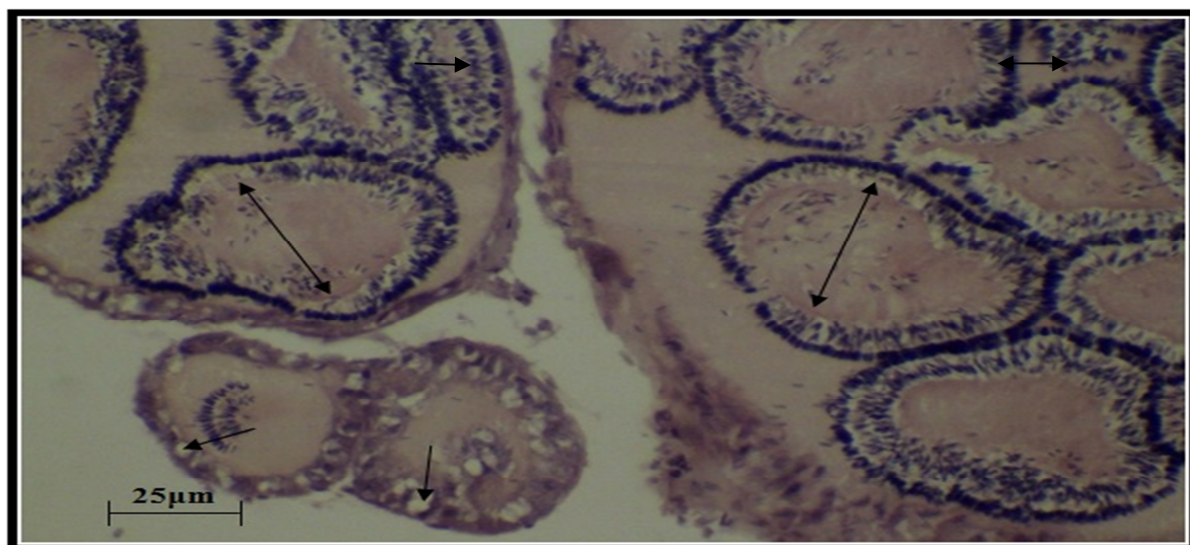




Fig. 4. Cross section of guppy fish testes at 6weeks of vydate treatment 0.3mg/ L showing degeneration (), decreased number of spermatozoa () (H&E).

Conclusion

The present study concluded that the vydate pesticide has some irreversible retroactive pathological changes on the testes organ.

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