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Chronic Effects of Paraquat Dichloride on Testicular Histology of *Clarias Gariepinus* Juveniles

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ABSTRACT

Paraquat dichloride is one of the most widely used herbicides reported to have toxic properties. This research was aimed at examining the histologic effects of paraquat dichloride on the juveniles of *Clarias gariepinus* exposed to varying concentrations of the toxicant. Fish samples were obtained from African Regional Aquaculture Centre (ARAC), Rivers State, Nigeria, and separated into experimental groups exposed to varying lethal concentrations of paraquat dichloride (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) for 4 days and sub-lethal (0.02, 0.04, 0.06, 0.08 and 1.0mg/l) concentrations of paraquat dichloride for 30 days. Changes in histology of the sperm cells were noted and compared with the control group (0.0mg/l). Sperm cells of samples exposed to sublethal concentrations exhibited necrosis of the tunica albuginea and degeneration of the stromal connective tissues. Tissue retraction and detachment of testicular epithelium including degeneration of spermatids with necrosis and tissue fragmentation were also observed. The results obtained showed that the use of paraquat dichloride could lead to destruction of sperm cells leading to reduction of the reproductive success of fish. Hence, appropriate monitoring of its usage should be enforced, especially in areas with close proximity to water.

Keywords: Paraquat dichloride, toxicity, testes, histology, *Clarias gariepenus.*

INTRODUCTION

Paraquat (1,1-dimethyl-4,4-bipyridininum ion) is one of the most common contact and non-selective herbicides for exterminating vegetative pests, is used for controlling terrestrial weeds and aquatic plants in several countries and its presence is reported in many water sources of the world (Gao *et al*., 2010; Ismail *et al*., 2011). It is also used for the control of several broad

leaves and grasses in plantations and other weeds in non-crop land/ urban and household settings worldwide (Aghoghovwia and Izah, 2018). It is a highly toxic weed killer. It is quick-acting and non selective, killing green plant tissues on contact. It is also toxic to human beings when swallowed and inhaled. Paraquat applied to water for aquatic weed control purposes quickly disappears due to uptake by weeds and absorption by soil, silt and particulate suspended matter.

It causes severe, acute and chronic poisoning when it is water borne (Yuan *et al*., 2004) and readily dissolves and dissociates when in an aqueous media. Lungs selectively accumulate paraquat and contain higher concentrations than cutaneous tissues, causing oedema and other lung damage leading to fibrosis. It also causes liver damage and renal failure as the kidney tries to remove absorbed paraquat (Dial and Dial, 2005). The mechanism of paraquat toxicity may be attributed to its redox potential, which involves cyclic reduction-oxidation reactions that produce ROS and depletion of nicotinamide-adenine dinucleotide phosphate hydrogen, NADPH (Tsai, 2013).

In humans, paraquat poisoning causes respiratory failure, severe central nervous system injury, and Pakinston's disease (Huang *et al.,* 2013). This is based on inhalation, ingestion and damaged skin integrity (Arivu *et al*., 2016). The exposure via damaged skin tissues could be associated to its corrosive nature (Aghoghovwia and Izah, 2018). It has been banned in the European Union countries since 2007 (Dinis-Oliveira *et al.*, 2008). However, it is still used in some countries including Nigeria. This study was therefore designed to determine the effects of paraquat on the testicular histology of *Clarias gariepenus.*

Materials and Methods

Source and Set Up of Experimental Samples

One hundred and eighty (180) juveniles of *Clarias gariepinus* were purchased from African Regional Aquaculture Centre (ARAC), Aluu, Rivers State, Nigeria. The *Clarias* species averaging 12.14 ± 1.76cm standard length and body weight of 4.45 ± 1.37g were used for the study. The fish were conveyed in a well aerated containers filled with water from the rearing ponds in the farm premises to the holding units at the Toxicology Laboratory, ARAC, where the experiment was conducted. Ten fish samples were held in containers of 20L capacity and acclimatized for two weeks in de-chlorinated water which was changed daily. The top of the

containers was covered with a net to control escape of fish. Eighteen 20L capacity containers were used in all. During this period, the fishes were fed with commercial feed containing 35% crude protein twice per day at 4% body weight.

Preparation of Test Solution

The herbicide paraquat was purchased from a commercial outlet in Port Harcourt. Out of 276g/L paraquat, stock solution of 55.2g was prepared by diluting measured volumes (i.e 199.824mls of paraquat in 20L de-chlorinated tap water for 10mg/l concentration). The de-chlorinated tap water used had the same physical and chemical properties with the one used in acclimatizing the fish. The control solutions were made up of only de-chlorinated tap water.

Test for Range Finding and Definitive Test (Pilot Study)

For each test concentration of the herbicide, smaller holding containers of 20L each were used. The test solutions and the controls were run in triplicates. For each triplicate, the test solutions and controls were prepared and labeled, thoroughly mixed and allowed to stand for about 5 minutes before use. Range finding test described by Inyang *et al.* (2018) was adopted in this study:

mls x stock solution = aquarium water(ml) x desired concentration(mg/l) ---------- equation 1

or

$N_1V_1 = N_2V_2$ (Seiyaboh *et al*., 2013) ---------- equation 2

Where N_1= Manufacturer concentration (276g),

N_2= concentration of test solution desired

V_1= Volume of the original solution added

V_2= volume of the test solution (20litres).

These were prepared by transferring 199.8mls, 399.6mls, 599.4mls, 799.8mls and 999.6mls of the original concentration of paraquat and making it up to 20L de-chlorinated tap water. The concentrations of the toxicant in the five holding tanks were prepared by adding 0.00mg/l, 144.8mls, 289.6mls, 434.4mls, 579.6mls and 724.4mls obtained from the stock solution of 55.2g into 20L of de-chlorinated tap water and converted into 0.0 (control), 0.5, 1.0, 1.5, 2.0 and 2.5mg/l. which is the final concentration for the acute toxicity test.

Determination of Sub-lethal Concentrations of Herbicide

Six 20L containers of same size were used in three replicates for the toxicants and a control. Appropriate volumes (0.2mls, 0.4mls, 0.6mls, 0.8mls and 1.0mls of stock solution were dispensed using a 5ml syringe and measuring cylinder into 20L of dechlorinated tap water in each of the tanks except the control. The juveniles were exposed to nominal concentrations of paraquat for 30 days. The concentrations used for the chronic study were 0.0 (control) 0.02, 0.04, 0.06, 0.08 and 0.1mg/l of paraquat for 30 days.

Experimental Design

The experimental design was a completely randomized design (CRD) with five treatments levels of paraquat and a control with each level having three replicates.

Histological Examination of Sperm Cells

Fishes were dissected and testes extracted and opened up, fresh sperm cells were collected from both control and herbicide-exposed fish for histological studies. The specimens were rapidly fixed in 10% phosphate buffered saline for at least 24hr. The fixed specimens were processed by passing through graded series of alcohol (70%, 95% and absolute ethanol). They were further passed through xylene and infiltrated with paraffin. From the prepared paraffin blocks, 5µm thick sections were obtained and sections were further processes as described by Culling (1974).

Results

Histological Analysis of sperm cells exposed to paraquat dichloride

The different pathological observations in the sperm cells of *C. gariepinus* exposed to paraquat dichloride are shown in (Plate 1-6). There were no pathological changes to the cells in the control (Plate 4.1). Sperm cells of fish exposed to 0.02mg/l concentration of paraquat exhibited necrosis of the tunica albuginea and degeneration of the stromal connective tissues, the testes also showed tissue retraction and detachment of testicular epithelium including degeneration of spermatids with necrosis and tissue fragmentation (Plate 2). Similarly, fish exposed to 0.04mg/l concentrations of paraquat showed tissue retraction and detachment of testicular epithelium (TRD), with degenerated and necrotic spermatids (DN) and testisticular tissue necrosis (TTN). (Plate 3). After 30 days exposure to chronic concentrations of paraquat, sperm cells of fish exposed to 0.06mg/l exhibited necrotic changes (NC) with spermatocytic cells degeneration (SD)

and vacuolation (Plate 4). Fish exposed to 0.08mg/l concentration of paraquat exhibited fragmentation of the testicular epithelium and connective tissue hypertrophy (Plate 5). Stromal cells necrosis was observed in the fish exposed to 1.0mg/l concentration of paraquat after 30 days exposure (Plate 6).

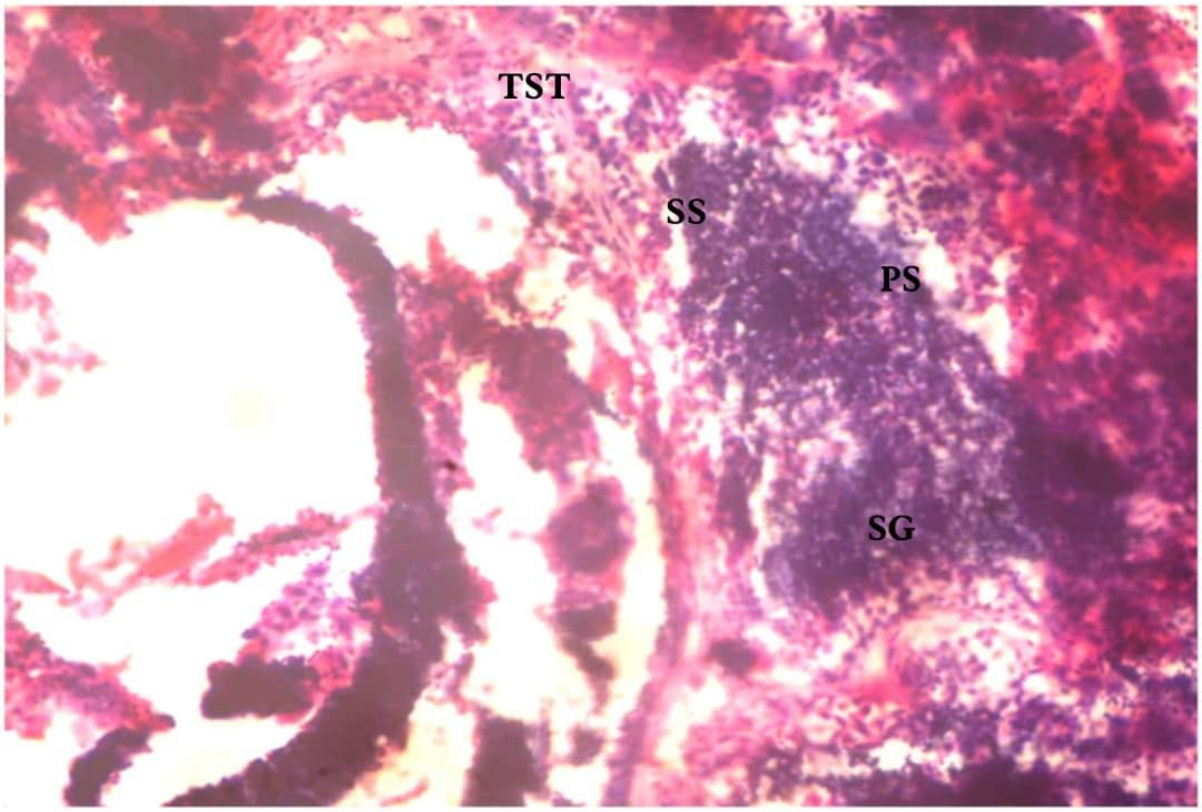

Plate 1: Photomicrograph section of Normal Control Fish testes at (0.00mg/l) showing spermatogonia (SG), primary (PS) and secondary spermatocytes (SP) covered with testicular supporting tissues. (TST), (H&E, x100).

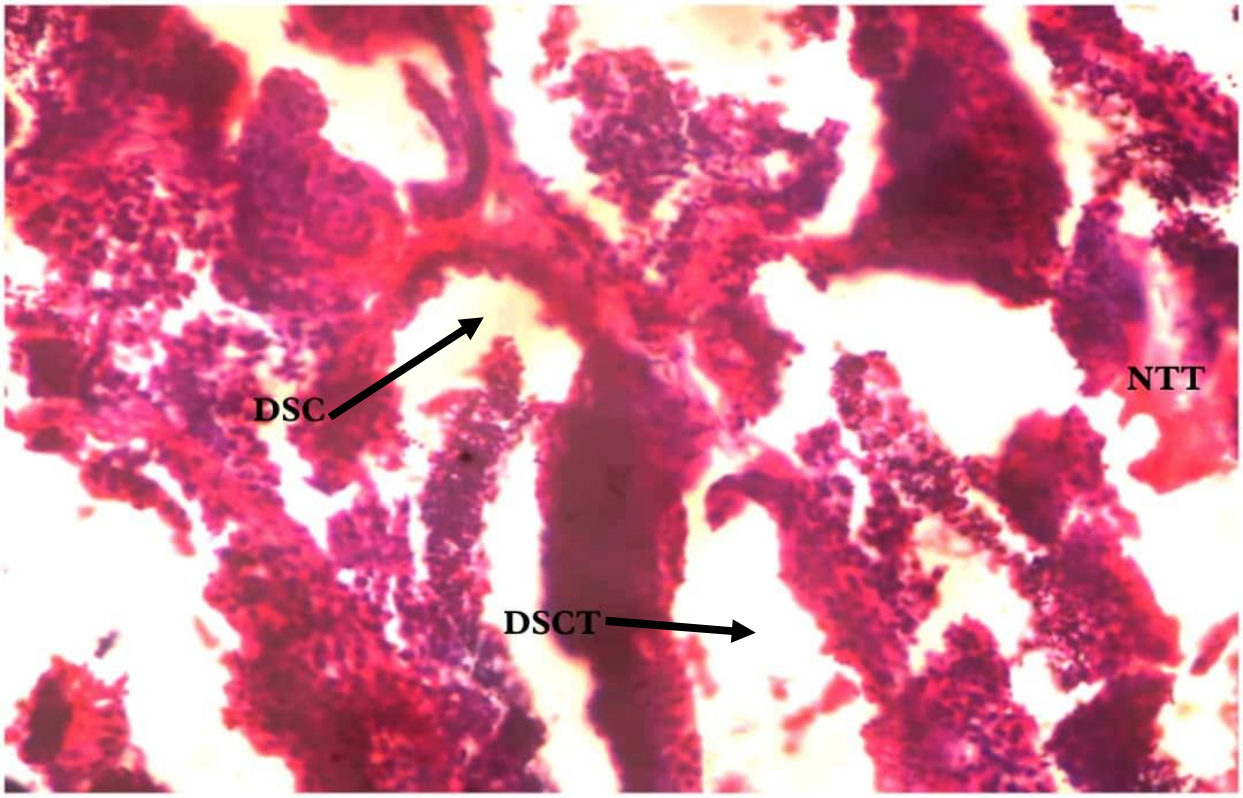

Plate 2: Photomicrograph Section of fish testes at 0.02mg/l concentration of paraquat showing dimunitive, degenerated spermatic cells with nuclear pyknosis (DSC) necrotic tunica albuginea (NTT) and Degenerative stromal connective tissues, (DSCT), (H&E X100).

Plate 3: Photomicrograph section of Testicular alterations at 0.04mg/l concentration of paraquat showing tissue retraction and detachment of testicular epithelium (TRD), with degenerated and necrotic spermatids (DN) with testicular tissue necrosis (TTN).

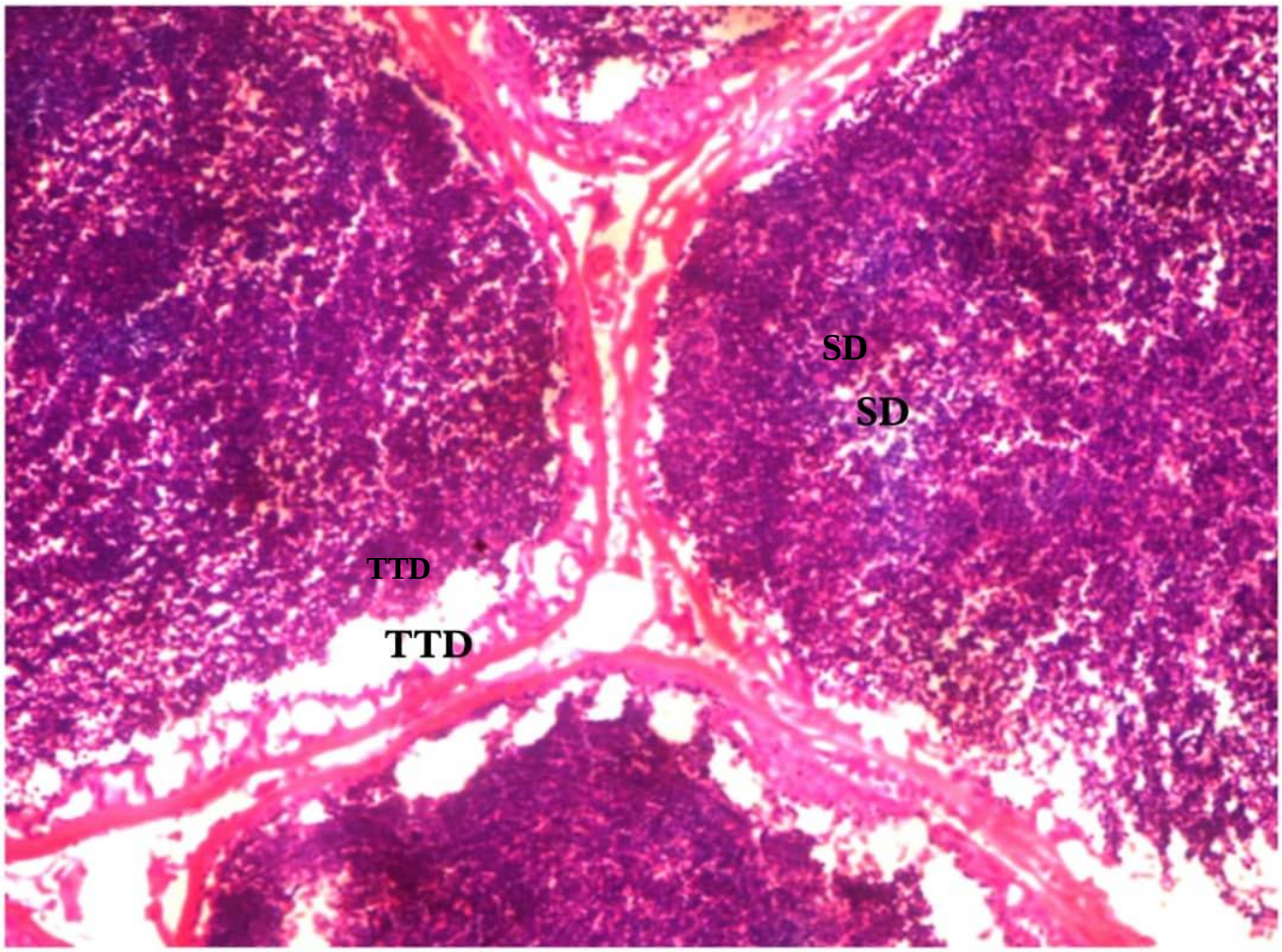

Plate 4: Photomicrograph sectioned testisticular tissue degeneration (TTD) produced by detachment and fragmentation of basement membrane (arrows, inset) delimiting seminiferous lobules at 0.06mg/l concentration of paraquat after 30 days exposure. The scattered nuclei from spermatozoa are showed. Necrotic changes (NC) are observed with spermatocytic cells degeneration (SD) and vacuolation, (H&E x100).

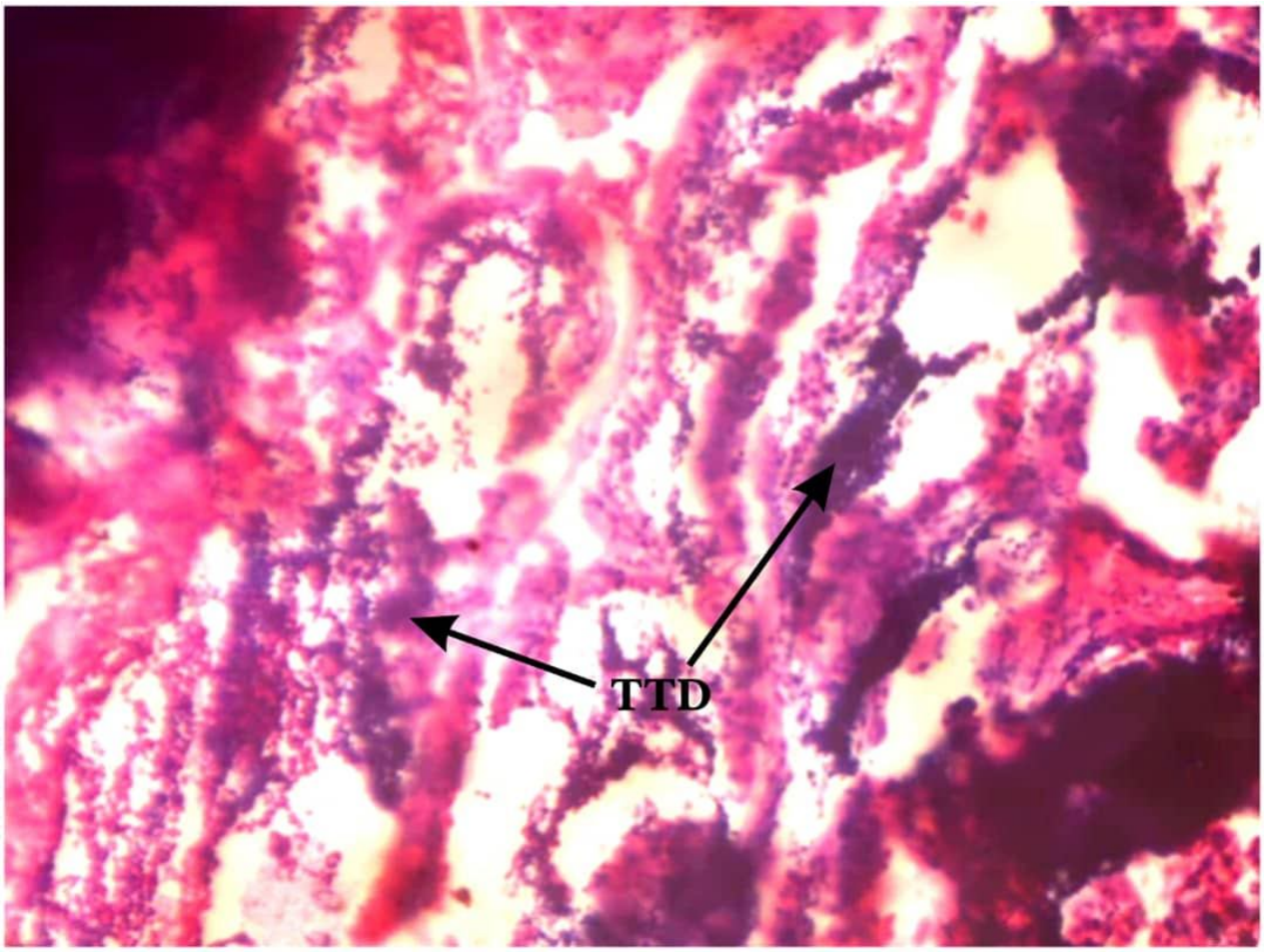

Plate 5: Photomicrograph section of fish testes showing testicular cells and tissue (TTD) degeneration at 0.08mg/l concentration of paraquat after 30 days exposure. There is fragmentation of the testicular epithelium and connective tissue hypertrophy. (H and E X100)

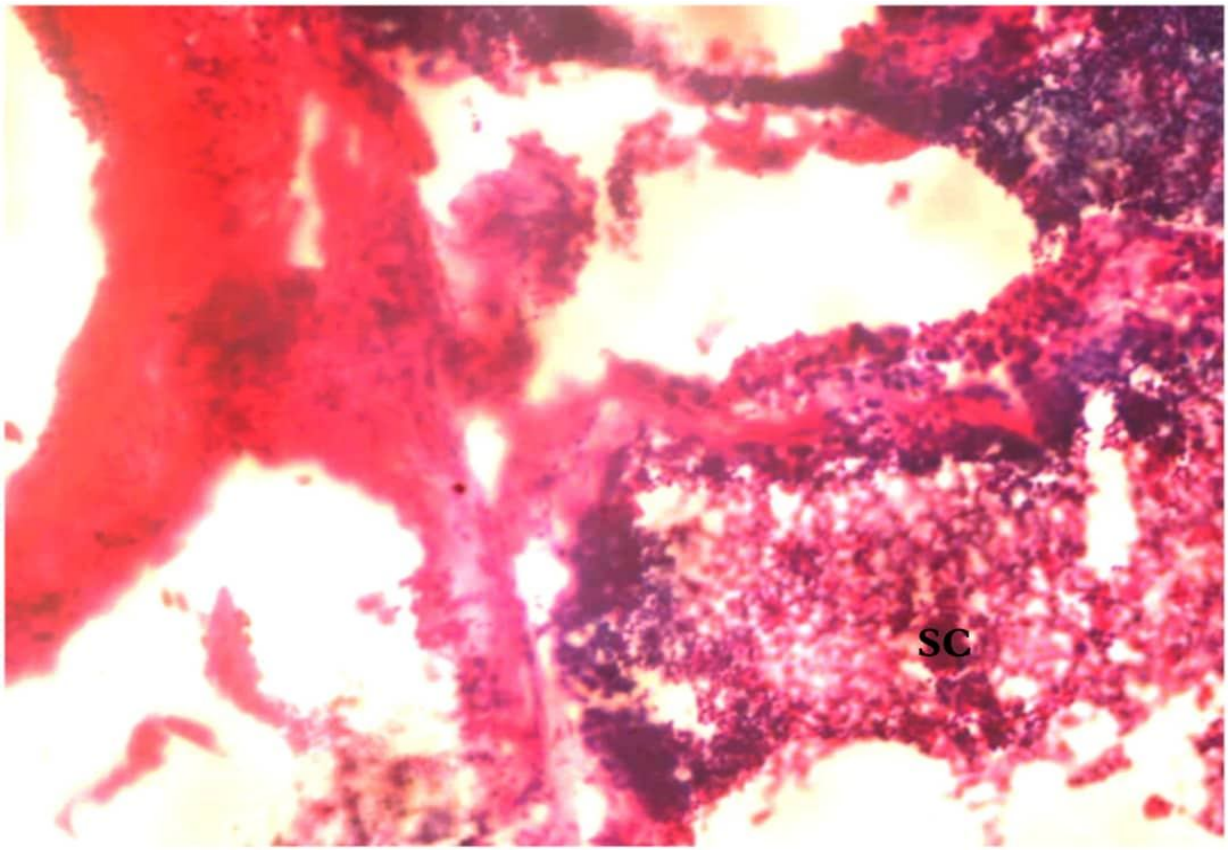

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Plate 6: Photomicrograph section of testicular tissues from Abormal testicular cells 1.0mg/l showing spermatocytes (SC) and stromal cells necrosis. H&E x100.

Discussion

Histological effects of chronic concentrations of paraquat on fish sperm cells

This study shows that chronic concentrations of paraquat are toxic to *C. gariepinus*. This is in agreement with the findings of previous researchers (Doherty *et al*., 2011; Ayoola, 2008; Okayi *et al*., 2010) who observed the toxic effects of herbicides on catfish. Paraquat is a harmful herbicide that can be considered to adversely influence reproductive activities with endogenous hormonal disruption (Dutta and Maxwell, 2003). Paraquat also has direct effect on gonads causing the production of low quality gametes thereby affecting sexual activity and spawning.

Significant reduction levels of reproductive steroids in Atlantic salmon (*Salmo salar*) and zebra fish (*Danio rerio*) after exposure to sub lethal doses of paraquat were reported by (Mlambo *et al*., 2009). In mammals, paraquat has destructive effects on enzymes involved in spermatogenesis

(Ducolomb *et al*., 2009). The observation of this experiment agrees with studies that have shown several adverse effects of toxic pollutants on testis structure and spermatogenesis process in rats and fish (Yamaguchi *et al*., 2007; Jorsaraei *et al*., 2010; Orlu and Gabriel, 2011). Qualitative analysis of the histological sections indicated that at least one degenerative effect was seen in each section. With the increase of dose exposure, the adverse effects on testes were more distinctive and damage tissues with necrotic areas were observed. The lumen structure was disordered in some parts. Histopathological alterations observed in this study are nearly similar to those found in Mozambique tilapia (*Oreochromis mossambicus*) (Mlambo *et al*. (2009) and black goby (*Gobius niger*) (Louiz *et al*. (2009) affected by organic pollutants like DDT. Large numbers and great sized cells were related to control groups that can show paraquat dose-dependent inhibitory effects on the spermatogenesis. In control groups, germ cells were larger and were commonly recognized and also clusters of spermatozoa were obviously seen in the lumens.

Conclusion and Recommendation

This study has demonstrated the negative impacts of paraquat dichloride on the testes of *Clarias gariepinus*. We recommend that all major regulatory agencies through which herbicides are registered should have information on toxicity to non-target organisms.

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