

## Effects of Effluent Contaminated River Water on Testicular Histology of Mice

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**Abstract:** Mature male mice were used to study the possible effects of effluent contaminated river water on reproduction using testicular histopathology. Freshly collected contaminated river water was supplied to the caged mice daily *ad libitum*. The mice were serially euthanized and testicles obtained for histology. The testes showed vacuolation and sloughing of seminiferous epithelium; this finding confirms that effluent contaminated river water contains levels of toxins sufficient enough to cause reproductive problems.

**Keywords:** Mice testicular histopathology, water pollution, endocrine disrupting compounds

### INTRODUCTION

Water bodies at present are known to be heavily polluted by chemicals which include endocrine disrupting chemicals [1, 2]; these chemicals reportedly cause adverse effects on the reproductive system of animals [3, 4]. The main source of water pollution is discharge from sewage treatment works, industries, farmlands and informal settlements close to the river [5]. In Kenya, urban draining rivers are polluted by microbial organisms [6], heavy metals [7] and pesticides [8].

Urban rivers and wastewater effluents are increasingly becoming a reliable water source for urban agriculture due to water scarcity. Thirty (30%) of Nairobi residents raise livestock and cultivate food crops along river banks [9] using untreated sewage [10]. People also scavenging in contaminated rivers for valuables to sell.

This study sought to document the effects of effluent contaminated water on the reproductive performance of the mice in order to shed an understanding on the possible dangers to man such contaminated water possess.

### MATERIALS AND METHODS

#### Animals and treatment

The current study involved twenty five (25) mature male mice purchased and kept in cages with polypropylene sides and floors, and stainless-steel grid tops (22 × 40 × 15 cm) within the animal house in the Department of Public Health Pharmacology and Toxicology (PHPT) University of Nairobi. Standard mice pellets were available *ad-libitum* and the room

conditions were maintained at an average of 60% relative humidity with approximately 12 hours light and dark cycle. An acclimatisation period of two weeks was allowed before commencement of the experiment. The mice were then randomly assigned into five groups of five animals each and their use and care was strictly in accordance with the guidelines of the Animal use and ethical Committee of the Faculty of Veterinary Medicine, University of Nairobi.

Contaminated river water was collected in the morning and supplied to the caged mice *ad-libitum*. The control group received clean tap water. After 60 days the mice were sacrificed in an anaesthesia chamber and a laparotomy performed to expose reproductive organs, the testes were dissected out and processed for histology.

#### Sample preparation

For light microscopy, the testicular samples were cleaned off blood and excess tissue trimmed; the tissue was then sectioned into smaller blocks and immediately immersed in 10 % neutral buffered formalin for 24 hours before further processing. The sections were then processed by dehydration in alcohol, clearing with xylene and embedding in paraffin wax. Five µm sections were obtained, stained and observed under a light microscope.

#### Light microscopy

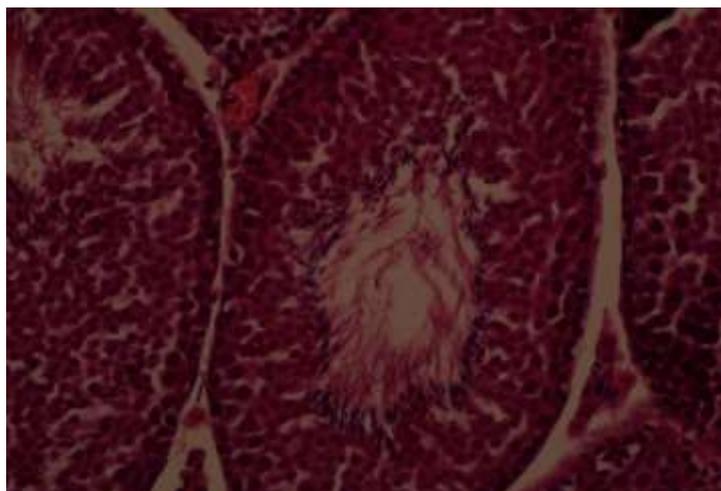
Testicular histopathological evaluation as a measure of toxicity has been used previous [11, 12]. The Haematoxylin Eosin stained sections were observed and digital images taken by a digital camera

coupled to Zeiss light microscope for histopathological analyses of seminiferous tubules.

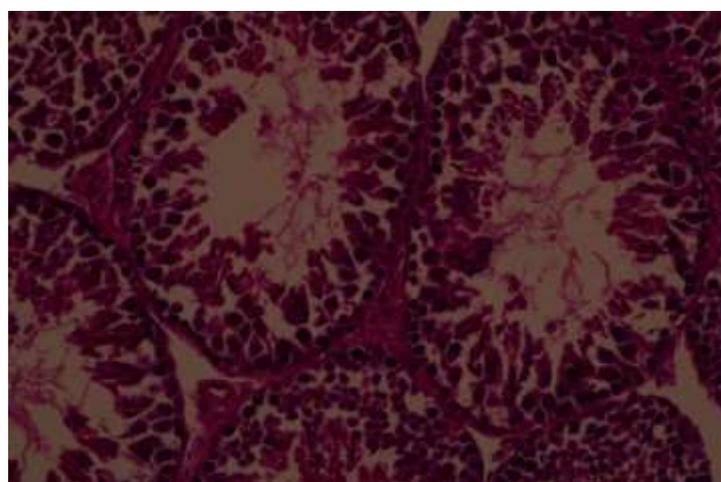
## RESULTS

In the present study, a survival rate of 100% was recorded. Normal testicular architecture was observed in the control group (Figure 1). The seminiferous epithelium was well developed with active spermatogenesis.

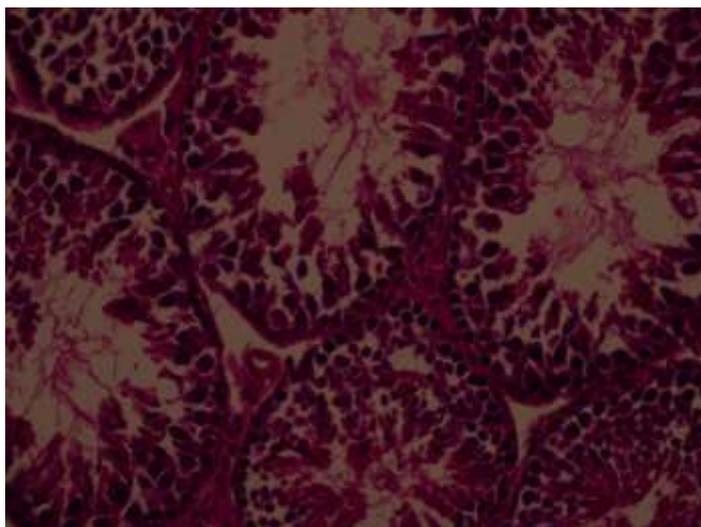
In the experimental group, the normal layered architecture of the seminiferous tubule was lost with some tubules showing disorganisation of germ cells. This was due to the sloughing off and displacement of germ cells in the apical tubular epithelium to the lumen (Figure 4 and 5). There was a reduction in the seminiferous epithelium due to germ cell desquamation (Figure 2); the seminiferous epithelium thus appeared depopulated with a less compact arrangement between the epithelial cells and an increase in the intraepithelial empty spaces (Figure 2).



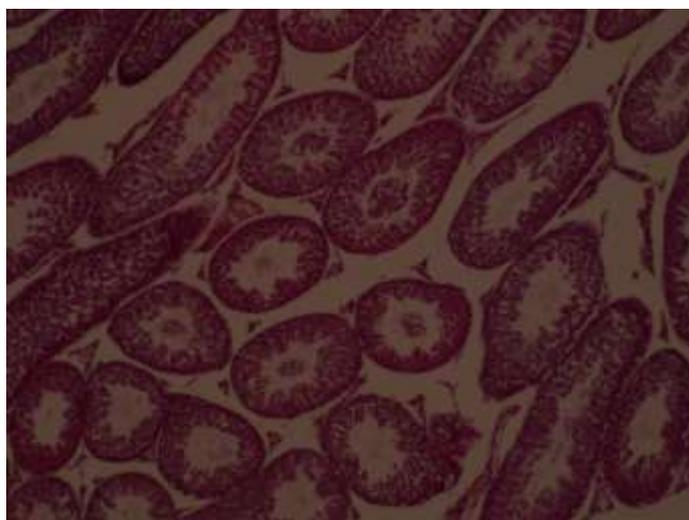
**Fig 1: Normal architecture of seminiferous tubular epithelium with developing germ cells (Magnification×400 H&E stain).**



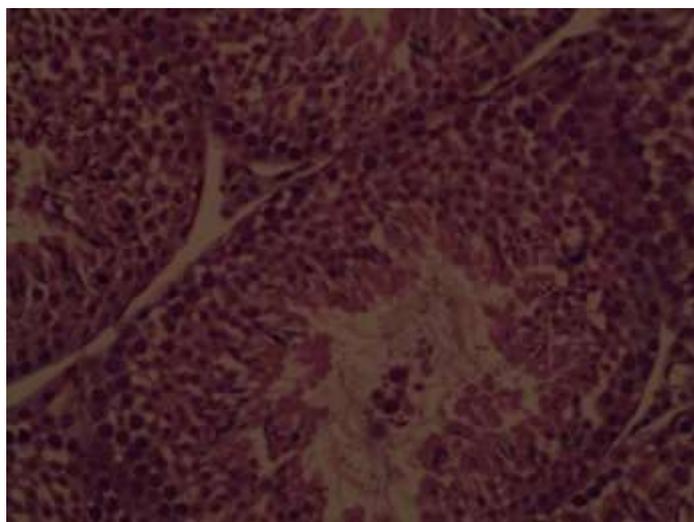
**Fig 2: Histological section of the testis from polluted river water treated mice showing increased intraepithelial spaces and decline in the number of germ cells due to sloughing (Magnification×400 H&E stain)**



**Fig 3: Histological section of the testis from polluted river water treated mice showing depletion of the seminiferous epithelium due to sloughing of germ cells (Magnification×400 H&E stain)**



**Fig 4: Histological section of the testis from polluted river water treated mice showing accumulation of the sloughed germ cells in the lumen of the seminiferous epithelium (Magnification ×100 H &E stain)**



**Fig 5: Histological section of the testis from polluted river water treated mice showing accumulation of the sloughed germ cells in the lumen of the seminiferous epithelium (Magnification ×400 H &E stain)**

## DISCUSSION

The seminiferous tubules appeared disorganised with sloughing off and displacement of germ cells in the tubular lumen (Figure 4 and 5), similar observation were made by Adamkovicova *et al.*; [12] after administration of cadmium to male rats. There was also a decrease in the epithelial height which indicated that cell associations in the germ epithelium were disturbed due to toxicity; these findings agree with earlier reports on exposure of male rats to diazinon [13, 14]. The germ cell sloughing may reflect functional damage to the Sertoli cell which is normally charged with anchoring germ cells [14], the loss of germ cell attachment and their subsequent displacement caused an increase in the intraepithelial spaces in the treated mice (Figure 2) compared to controls (Figure 1). Such changes are reported to occur following disruption of endogenous hormonal function [14]. Our results further agrees with the observations by Hess and Nakai [15], Kumar and Nagar [16] who found Vacuolation and sloughing of germ cells after administration of Pyrethroids and Deltametrin to male rats respectively.

In this study “real live” exposure to environmental pollutants was achieved in order to replicate the effects seen on environmental exposure experience by animals, such exposure is known to allow substances that would not produce any effects by themselves to produce significant effects even at low concentrations [17]. This observation agrees with our previous report on reproductive perturbation in boars exposed to wastewater [18].

The current study demonstrate that contaminated water contains sufficient pollutants able to cause significant testicular histopathological changes similar to these observed with known endocrine disrupting chemicals.

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