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Histological Changes in Rat Testicular Tissue Architecure upon Short-Term Exposure to Inhaled Dichlorvos

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Abstract

Background to the study: The use of insecticides have been encouraged in most Nigerian homes in the eradication of insects that could harm humans despite the side effects of exposure to these insecticides. The study aim was to examine the toxic effect of inhaled dichlorvos (DDVP) on the histological architecture of the testes of wistar rats on short-term exposure. ***Methods:*** Twenty (20) albino male wistar rats weighing 140g – 180g were recruited for this research and were randomly placed into four (4) groups of five (5) rats per group. Group 1 was control group, while groups 2 – 4 were given 10ml DDVP/90 ml distilled water – v/v, 20ml DDVP/80 ml distilled water – v/v and 40ml DDVP/60 ml distilled water – v/v, respectively. At completion of exposure, rats were anaesthetized with chloroform, sacrificed and their testes were harvested for routine histopathology procedure. The stained sections were morphologically evaluated and the pictures of the slides compared. Photomicrographs were obtained with the aid of Am-scope camera fitted on an Açu-scope microscope. ***Results:*** Results obtained from the control group showed that the testes presented with a normal histological make-up depicted by the presence of normal seminiferous tubules, no signs of germ cell distortions. However, experimental groups showed graded levels of degenerative changes in germinal layers of the seminiferous tubules as well as notable vacuolations. ***Conclusion:*** It can be concluded that prolonged exposure to dichlorvos inhalation could be toxic to the reproductive system of male wistar rats.

Keywords: Dichlorvos, DDVP, testes, seminiferous tubules, inhalation.

INTRODUCTION

Generally, the use of insecticides towards the destruction of insects and other pests has been encouraged towards reducing their harmful effects on human health, plant and animal growth as well as food production in industries (Van *et al*., 1996; Jitendra *et al.*, 2019). In low-income countries like Nigeria that are predominantly affected by insect-related diseases such as malaria, most of the citizens have accepted the use of these insecticides simply because they are affordable and easy to use (Essiet, 2009). However, several experimental studies have shown that continued exposure to these insecticides while they are being used could lead to adverse reproductive health effects such as degeneration of germ cells (Ray *et al*., 1992; Chattergee *et al*., 1998; Chitra *et al*., 1999; Bolognesi, 2003; Takhshid *et al*., 2012).

Dichlorvos also known as DDVP (2,2-dichlorovinyl dimethyl phosphate), is an organophosphate locally sold in parts of Nigeria as Sniper or *ota-piapia*, is a predominant pesticide used in domestic control of insects in low-income and middle-income countries (Henshaw and Iwara, 2019). Like other organophosphate insecticides, it is neurotoxic in action, and acts on the nervous system by inhibiting the enzyme acetyl cholinesterase (AchE) (Zhao *et al*., 2015; Zhang *et al*., 2015). The action between an organophosphate insecticide and the active site in the AchE protein results in the formation of a transient inter-mediate complex that partially hydrolyses, leaving a stable phosphorylated and largely non-reactive inhibited enzyme that, under normal circumstances, can be reactivated only at a very low rate (Ecobion, 1996). Therefore, this study was done to examine the toxic effect of inhaled dichlorvos on the histological architecture of the testes of wistar rats.

METHODS

Research Design

The research design was experimental which intended to investigate the dichlorvos-induced toxicity on the testes of albino wistar rats. For the purpose of achieving this, an ethical clearance was applied for and obtained (UPH/CEREMAD/REC/MM78/049) from the Research Ethics Committee of the University of Port Harcourt. Twenty (20) albino male wistar rats were recruited for this research and were randomly placed into four (4) groups of five (5) rats per group. A box-like cage measuring 40cm x 40cm x 15cm (3 in number) that was made of Perplex glass, was used as inhalation chambers for the experimental rats for groups 2 – 4. The table below shows the pattern of administration of dichlorvos (DDVP).

Table 1: Rat groups and their administration of concentration levels

GROUP	CONCENTRATION LEVELS
Group 1 (negative control)	100% distilled water
Group 2	10ml DDVP/90 ml distilled water – v/v
Group 3	20 ml DDVP/80 ml distilled water – v/v
Group 4	40ml DDVP/60ml distilled water – v/v

DDVP = Dichlorvos

According to Maheshwari and Shaikh (2016), experimental animals are exposed to the test substance for a minimum of four (4) hours and are monitored for a short-term period of 14 days. Animals that die during the study will be autopsied. At the end of the study, animals are sacrificed and observed for pathological changes.

Sample Collection/Identification

Dichlorvos is the active ingredient of the insecticide, Sniper. Sniper was purchased from the Dooka Pharmacy located opposite University of Port Harcourt Teaching Hospital, Alakahia, Port Harcourt.

Acute Toxicity for Dichlorvos Inhalation

In line with studies done by Owoeye *et al*., (2012) and Ogunsola *et al*., (2019), the average lethal concentration for dichlorvos inhalation is 50 ml DDVP/50ml distilled water.

Animal Acclamitization & Handling

The animals that were used are twenty (20) male albino wistar rats weighing 140g-180g and were bred in the Animal House of the department of Pharmacology, University of Port Harcourt. They were grouped into four (4) groups of five (5) animals each and left to adapt to the environment for fourteen (14) days. They were kept in standard cages and maintained in standard laboratory condition at an average room temperature of (25 ± 2^{o}C) with relative humidity (55-64%) and light and dark conditions (12/12h). They were given standard diet and water *ad libitum*. Animal ethics and proper handling methods were closely abided. The bedding of the cages (sawdust) was changed daily and the cage also washed and disinfected weekly. The feed, Top Feeds Premier Feeds (Broiler finisher) manufactured by Premier Feed Mills Co. Ltd. (A subsidiary of Flour Mills Nig. Plc., Lagos State) were purchased at Choba, Port Harcourt. They were stabilized for one week during which they were allowed access to commercial rat feed and portable clean water ad libitum.

Histopathological Analysis

At completion of exposure, rats were anaesthetized with chloroform, sacrificed and their testes were harvested for routine histopathology procedure. The harvested testicular organs from all groups were fixed in 10% formaldehyde, and then hydrated with grades of ethanol (75%, 90%, 95% and 100%). Dehydration was then followed by clearing the samples in two changes of xylene. Samples were then impregnated in molten paraffin wax, then embedded and blocked out. Paraffin sections of 5µm thick were cut using a sledge microtome and mounted on glass slides and stained with haematoxylin and eosin (H&E) staining method. The stained sections were morphologically evaluated and the pictures of the slides compared. Photomicrographs were obtained with the aid of Am-scope camera fitted on an Açu-scope microscope.

RESULTS

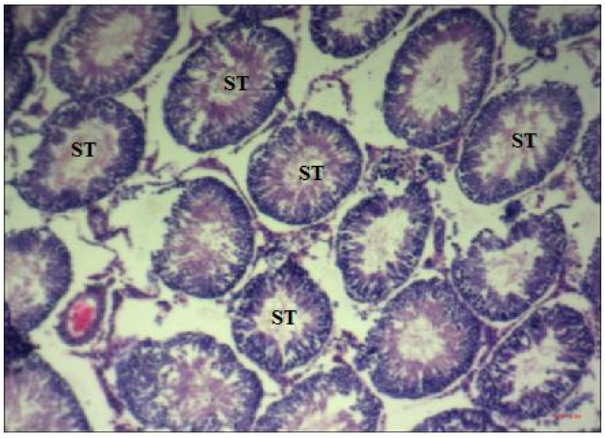

Figure 1: Photomicrograph of the control testicular tissue (x10), showing normal seminiferous tubule (ST) morphology. Germ cells are also normal

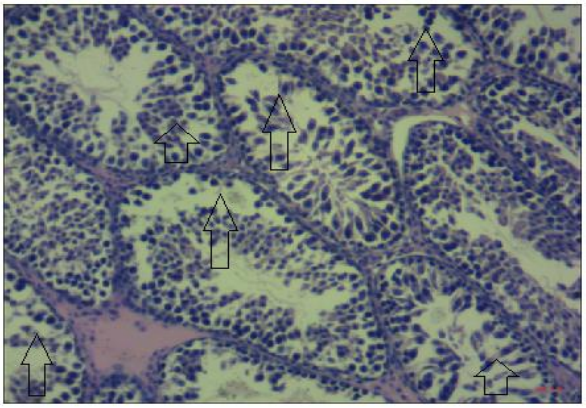

Figure 2: Group 2 testicular tissue micrograph (x40) showing minimal levels of degenerative changes in germinal layers of the seminferous tubules (arrows), which appear in the form of vacuolations, indicating arrested spermatogenesis. Also, a mild-moderate separation of basement membrane and occlusion of seminiferous tubules is observed

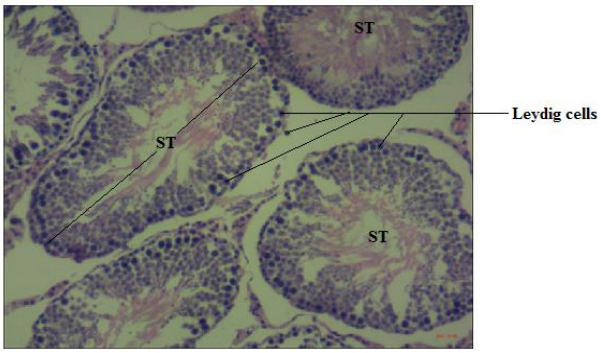

Figure 3: Group 3 testicular tissue micrograph (x40) normal architecture of seminiferous tubules, along with the visible presence of germ (Leydig) cells, although they are highly distorted

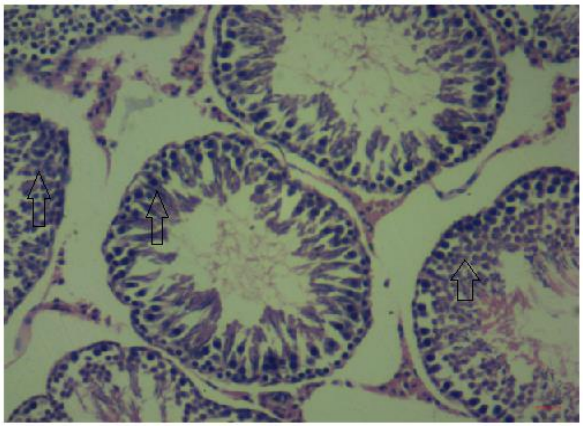

Figure 4: Group 4 testicular tissue micrograph (x40) histological architecture of scattered seminiferous tubules, mild signs of distortion of germ (Leydig) cells

DISCUSSIONS

The current study was undertaken to analyse critically the histopathological effect of inhaled exposure to dichlorvos (Sniper) on the testicular tissue architecture of adult wistar rats.

Results obtained from the control group showed that the testes presented with a normal histological make-up depicted by the presence of normal seminiferous tubules, no signs of germ cell distortions. These observations were in line with studies done by Russell *et al*., (1996) and El-Deeb *et al*., (2007).

However, this study noticed in the experimental groups that the exposure to dichlorvos brought about significant histological changes in their testicular architecture compared to that of the control group. Testes of group 2 rats showed minimal levels of degenerative changes in germinal layers of the seminferous tubules which appeared in the form of vacuolations, possibly suggesting arrested spermatogenesis. Also, a mild separation of basement membrane and occlusion of seminiferous tubules was observed.

Subsequently, moderate to severe toxic observations were also seen in the other experimental groups that were exposed to higher concentrations of dichlorvos. El-Deeb *et al*., (2007) in a similar study noted that the testis of the chlorpyrifos-treated rats shows severe degree of degenerative changes in germinal layers of seminiferous tubules with enlargement of blood vessels. As explained by Thomas (2001), these changes could be as a result of the toxicity of organophosphates on seminiferous tubules and their testicular blood vessels (vascular stasis), and possible impediment in hormonal mechanisms of either gonadal or the hypothalamic-hypophyseal axis.

CONCLUSION

It can be concluded that prolonged exposure to dichlorvos inhalation could be toxic to the reproductive system of male wistar rats.

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Competing Interests: There is no conflicting or competing interest.

AUTHORS' CONTRIBUTIONS

The first author designed the study, wrote the study protocol and the first draft of the manuscript. The second author managed the literature search, did the study analyses and statistical analysis. Both authors read and approved the final manuscript.

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