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Research Article
Spermatogenic and Histologic Evaluation of Imiprothrin in Male Reproductive System of Adult Wistar Rats

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Abstract

Objective: The present study was designed to evaluate the effects of imiprothrin exposure on the testicular histology and sperm parameters using experimental animal model. **Materials and Methods:** Weights of 20 male adult wistar rats were randomized into 4 groups (A-D) of 5 rats each. Rats in group A (control) were exposed to fresh air for 4 weeks, group B were exposed to 5 mL of imiprothrin for 3 weeks, group C were exposed to 5 mL of imiprothrin for 4 weeks and group D were exposed to 10 mL of imiprothrin for 4 weeks, via inhalation for 6 h daily, respectively. At the end of exposure duration, rats were sacrificed and the testes were excised and analyzed for histological changes. Sperm analysis including total sperm count, motility, body weight ratio, relative percentage of normal and abnormal sperms were recorded. The statistical significance between treated and control groups were analyzed by means of Student's t-test. $p<0.05$ was considered significant. One-way ANOVA and SPSS was used for further analysis. **Results:** The results obtained from this study showed a non-significant change in the gross anatomical parameters of the control group however, a significant ($p<0.05$) decrease in the testes, body weights and testis volumes in rats exposed to imiprothrin when compared to the control group. Rats exposed to imiprothrin showed significant decrease in sperm count, motility, sperm morphology and significant ($p<0.05$) increase in abnormal sperm morphology when compared to the control group. The groups of rats exposed to 5 and 10 mL via inhalation showed significant ($p<0.05$) decrease of basal seminiferous epithelial cells, marked testicular degeneration and hypospermatozoa formation compared to the control group. **Conclusion:** Imiprothrin produces testicular derangement in testicular histology and sperm parameters in adult male wistar rats.

Key words: Imiprothrin, testis, wistar rats, spermatogenic, testiculotoxic, testicular histology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Infertility has been a major medical and social pre-occupation since the dawn of human existence[1]. Infertility and exposure to household substances have been shown in several studies and many environmental xenobiotic chemicals, such as dichlorodiphenyltrichloroethane (DDT), propoxur (PPX) and polychromatic biphenyls (PCBs), doxorubicin have been discovered to have testiculotoxic effects[2-4]. The testiculotoxic effect of rhodinol and musk based incense have been established by Akingbade *et al.*[5].

However, oxidative pathway has been ascribed to insecticides induced toxicity in organs[6].

The gonad has been considered as the main target of environmental disruptions[7]. This organ has membranous structures rich in polyunsaturated fatty acid. Membrane polyunsaturated fatty acids are highly sensitive to oxidative stress manifested through lipid peroxidation, which usually result to loss of membrane integrity[8].

Studies have shown that the histopathological and biochemical effect of various types of insecticide on numerous organ such as lungs, skin and liver[9-10]. Even though there are various studies on the cytotoxic effect on different types of insecticide on several other organs, there is however a little information about the literature on the testiculotoxic implications of imiprothrin contained insecticide.

Imiprothrin is an active composition of the insecticide called MORTEIN®. Individuals are most likely to be exposed to imiprothrin dermally or by inhalation during the manufacture, formulation and application of this insecticide[11].

Malaria is a burden in most of the African countries including Nigeria and one of the control measured is the use of insecticides in common use for the control of malaria. However, imiprothrin usage has been shown to result in cytotoxicity to various organs such as lungs[12], heart[13] and liver[14-16]. Reports have shown that imiprothrin induced toxicity is mediated through oxidative pathway. There is however, a dearth of information in the literature on the testiculotoxic effect of imiprothrin.

Furthermore, because of its wide spread use in both indoor and outdoor control of insects, insecticide has enjoyed a considerable attention on account of its potential health hazards[4,17]. Many investigators have also shown that insecticides induced testicular toxicity is due mainly to oxidative stress[2,14].

Spermatozoa cell membrane is particularly susceptible to damage by oxidative lipid peroxidation because of its peculiar lipid composition[18]. Testicular oxidative stress is characterised series of morphologic biochemical changes in the injured cells. Many existing reports in the literature investigating the ability of various organs from imiprothrin induced toxicity. None however carried out studies on testicular organs and parameters in an imiprothrin induced toxic.

The present study was designed to investigate the spermatogenic and histologic evaluation of imiprothrin in male reproductive system of adult wistar rats.

MATERIALS AND METHODS

Insecticide: A commonly used active composition of insecticide 'MORTEIN'® containing 0.10% w/w imiprothrin, 0.09% w/w parallethrin, 0.05% w/w cypermethrin and other inert ingredients were purchased from Tolu Pakad Pharmacy and stores, Ado, Ekiti State Nigeria on the month of May, 2016.

Other chemicals and reagents used: Sodium bicarbonate, Haematoxylin and Eosin were product of BDH Chemicals, Ltd., Poole England. Absolute Alcohol Xylene, Chloroform, Normal Saline were obtained from the chemistry department of Afe Babalola University Ado-Ekiti, Ekiti State, Nigeria.

Animals: Twenty male wistar rats (10-12 weeks old) weighing 190-250 g were obtained from the Animal House of Federal Polytechnic Ado-Ekiti, Ekiti State Nigeria. An approval was sought and obtained from the departmental ethical committee on animal use. The rats were allowed to acclimatize for 2 weeks and were fed with pelletised chows and water *ad libitum* and the experimental work was carried out in the Animal House of Afe Babalola University Ado-Ekiti, Ekiti State, Nigeria around June, 2016.

Relatively constant environmental condition were maintained with proper aeration and good source of light (12 h light/12 h dark and 24±2°C). Food and water were provided *ad libitum*. The weighing and observations were conducted before the rats were exposed to Imiprothrin respectively. The weights of the animals were estimated at procurement, during acclimatization, at commencement of the experiments and twice within a week throughout the duration of the experiment, using an electronic analytical and precision balance (BA210S, d = 0.0001 g) (Satorius GA, Goettingen, Germany).

Experimental procedures involving the animals and their care were conducted in conformity with international, national and institutional guidelines for the care of laboratory animals in Biomedical Research and Use of Laboratory Animals in Biomedical Research as promulgated by the Canadian Council of Animal Care[19]. Further, the animal experimental

Table 1: Animal groupings and Imiprothrin exposure

Groups	Exposure
A	Animals in this group served as the control and were exposed to fresh air for 4 weeks
B	Rats in this group were exposed to 5 mL of imiprothrin via inhalation for 6 h (9 am-3 pm) every day, for a period of 3 weeks
C	Rats in this group were exposed to 5 mL of imiprothrin via inhalation for 6 h (9 am-3 pm) every day, for a period of 4 weeks[22]
D	Rats in this group were exposed to 10 mL of imiprothrin via inhalation for 6 h (9 am-3 pm) every day, for a period of 4 weeks

models used conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals[20].

Experiment design: Four groups of rats (A, B, C and D) consisting of 5 animals each (Table 1) was housed separately in four undisturbed cages of size 5 m^3 with cross ventilation[21-22].

Animal sacrifice and sample collection: The rats at the time of sacrifice were first weighed and then anaesthetized by placing them in a closed jar containing cotton wool soaked in chloroform. The abdominal cavity was opened up through a midline abdominopelvic incision to expose the reproductive organs. Then the testes and epididymis were harvested. The weight of the testes of each animal was evaluated .The testes were weighed with an electronic analytical and precision balance (BA 210S, d = 0.0001-Sartoriusen GA, Goettingen, Germany). The volume of each testis was measured by water displacement method. The testes of each rat were measured and the average value obtained for the parameters was regarded as one observation.

Determination of epididymal sperm parameters

Progressive sperm motility: It was done immediately after the semen collection. Semen was squeezed from the caudal epididymis onto a pre-warmed microscope slide (27°C) and two drops of warm 2.9% sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope using X400 magnification. Ten fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Therefore, the motility of 100 sperms was assessed randomly. Sperms were labelled as motile, sluggish or immotile. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e., 100)[23].

Epididymal sperm concentration: Spermatozoa in the right epididymis was counted by a modified method of Yokoi and Mayi[24]. Briefly, the epididymis was minced with anatomic scissors in 5 mL physiologic saline, placed in a rocker for 10 min and allowed to incubate at room temperature for 2 min. After incubation, the supernatant fluid was diluted 1:100 with solution containing 5 g sodium bicarbonate and 1 mL formalin (40%). Total sperm number was determined by using the new improved Neuber's counting chamber (haemocytometer). Approximately 10 µL of the diluted sperm suspension was transferred to each counting chamber of the haemocytometer and was allowed to stand for 5 min. This chamber was then placed under a binocular light microscope using an adjustable light source. The ruled part of the chamber was then focused and the number of spermatozoa counted in five 16-celled squares. The sperm concentration was calculated, multiplied by 5 and expressed as $[X] \times 10^6$ mL^{-1}, where [X] is the number of spermatozoa in a 16-celled square.

Sperm morphology: The sperm cells were evaluated with the aid of light microscope at X400 magnification. Caudal sperm were taken from the original dilution for motility and diluted 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada). Five hundred sperm from the sample were scored for morphological abnormalities[25]. Briefly, in wet preparations using phase-contrast optics, spermatozoa were categorized. In this study a spermatozoon was considered abnormal morphologically if it had one or more of the following features: rudimentary tail, round head and detached head and was expressed as a percentage of morphologically normal sperm.

Tissue preparation for light microscopy: This was done as described by Ojewale *et al.*[26]. Briefly, the testes of each animal were fixed in bouin's fluid for 48 h for histological examination. The already-fixed tissues in bouin's fluid, after whole body perfusions were transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 h, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through 3 changes of absolute alcohol for an hour each and then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C.

Three changes of molten paraffin wax at 1 h interval were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were

orientated perpendicular to the long axes of the testes. These sections were designated "vertical sections". Serial sections of 5 µm thick were obtained from a solid block of tissue, fixed on clean slides to which Mayer's egg albumin had been coated to cement the sections to the slides properly and were stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene the sections were oven-dried between 35 and 40°C. The slides were viewed under a research microscope connected to a computer monitor for qualitative and quantitative evaluations.

Statistical analysis: All data were expressed as Mean±SD of number of experiments (n = 5). The level of homogeneity among the groups was tested using Students' t-test (one-way ANOVA) as done by Snedecor and Cochran[27]. Where heterogeneity occurred, the groups were separated using Duncan's multiple range test (DMRT). A value of $p<0.05$ was considered to indicate a significant difference between groups[28]. Analysis of data was analyzed using both electronic calculator and Statistical Package for Social Sciences (SPSS)/PC computer program (version 19.0 SPSS, Cary, NC, USA).

RESULTS

Behavioural observation in rats after exposure to imiprothrin: When the rats were first exposed to imiprothrin, they appeared excited and crowded in the corners of the cage, attempting to escape. After inhalation of the imiprothrin the rats had become more difficult to handle being more aggressive, frequently congregate, cluster to each other and started biting one another, although, this adverse response of the animals seemed to diminish gradually.

There was non-significant decrease in testis weight, testis weight/body weight ratio and testis volume in group B when compared to the control equivalent, whereas statistically significant ($p<0.05$) decrease was observed in group C and D compare to the control counterpart (Table 2).

Sperm count: The group of rats exposed to 5 mL of imiprothrin for 3 weeks showed non-significant decrease in sperm concentration ($131.2\pm2.7\times10^6$ mL^{-1}) compared to the control group ($142.5\pm3.0\times10^6$ mL^{-1}), group C provoked significantly ($p<0.05$) decreased sperm concentration ($98.8\pm1.9\times10^6$ mL^{-1}) and 10 mL of imiprothrin for 4 weeks exposed group showed marked oligospermia ($75.9\pm1.5\times10^6$ mL^{-1}) with their sperm concentration being significantly lower ($p<0.05$) compared to the control group as indicated in Table 3 below.

Sperm motility: Although the sperm motility of group B showed a lower non-significantly (72.4±4.9%) compared to the control group (98.9±6.7%). However, the group C and D still had significantly lower ($p<0.05$) (64.8±3.6%) and (58.3±2.3%) value compared to the control counterpart as indicated in Table 3 below.

Sperm morphology: Five millilitres of imiprothrin for 3 weeks showed evidence of non-significantly decrease in normal sperm morphology (75.6±4.8%) and non-significantly increased in abnormal sperm morphology (24.8±6.3%) compared to the control group (84.9±6.7, 20.2±5.8%), respectively. The group C however, showed a significant ($p<0.05$) decrease in normal sperm morphology (68.4±2.6%) and a significant ($p<0.05$) increase in abnormal sperm morphology (29.7±1.8%) when compared to the control group. Moreover, the rats exposed to 10 mL of imiprothrin for 4 weeks also had significant ($p<0.05$) (53.8±1.9%) decrease in normal sperm morphology and a significant increase in abnormal sperm morphology ($p<0.05$) (46.52±3.9%) when compared to the control group as indicated in Table 3 below.

Table 2: Effect of imiprothrin on gross anatomical parameters of wistar rats treatment

Treatment groups	Initial body weight (g)	Final body weight (g)	Body weight diff. (g)	Testis weight (g)	Testis volume (mL)	Testis weight/ body weight ratio
A	210.0±2.2	230.0±1.2	20.0	1.30±3.1	1.25±0.3	0.006
B	228.0±4.9	200.0±1.5	28.0*	1.20±0.6	1.14±0.2	0.006
C	235.2±3.0	181.0±2.0	57.0*	1.90±0.7*	1.090±0.4*	0.005*
D	242.0±5.4	180.0±4.4	62.0*	0.62±0.5*	0.650±0.3*	0.003*

*$p<0.05$ significantly different from control. Values are expressed as Mean±SD for n = 5 in each group

Table 3: Effect of imiprothrin on the sperm parameters of male wistar rats

Treatment groups	Sperm count ($\times10^6$ mL^{-1})	Sperm motility (%)	Sperm normal (%)	Morphology abnormal (%)
A	142.5±3.0	98.7±6.7	84.9±6.7	20.20±5.8
B	131.2±2.7	72.4±4.9	75.6±4.8	24.80±6.3
C	98.8±1.9*	64.8±3.6*	68.4±2.6*	29.70±1.8
D	75.9±1.5*	58.3±2.3*	53.8±1.9*	46.52±3.9*

*$p<0.05$ significantly different from control, Value are expressed as Mean±SD for n = 5 in each group

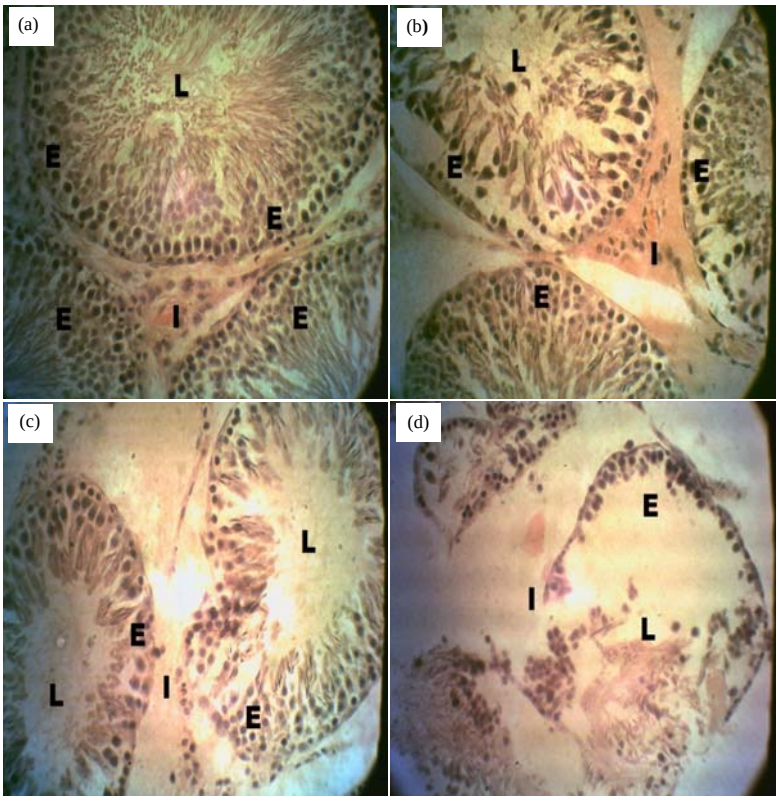

Fig.1(a-d): (a) Testes of rats in the control (A) group showed normal features with successive stages of transformation, (b) Structural changes obtained included minimal damage in the testicular interstitium, (c) Showed degeneration in testicular architecture and (d) Showed total degeneration of testicular interstitium
Stain: Haematoxylin and Eosin, Magnification: ×400, E: Seminiferous epithelium, L: Lumen of seminiferous tubule, I: Testicular interstitium

Effect of imiprothrin on the histological profiles of the testis: In Fig. 1a, the testes of rats in the control (A) group showed normal features with successive stages of transformation of the seminiferous epithelium into spermatozoa with no sign of atrophy or toxicity with respect to spermatocyte, germ cell, leydig cells or sertoli cells.

In Group B (Fig. 1b), the structural changes obtained included minimal damage in the testicular interstitium, the outline of the seminiferous tubules moderately reduced. It is also observable truly that the concentration of spermatozoa in the center of seminiferous tubules has been reduced minimally.

The seminiferous tubules of the rat in Group C (Fig. 1c) showed degeneration in testicular architecture. There was reduction in testicular interstitium and were detached from the seminiferous tubules, isolating them from each other. The diameter of the lumen was reduced, characterized by slight vacuolization of the interstitium and reduced spermatozoa.

The rats in Group D (Fig. 1d) showed total degeneration of testicular interstitium, significant reduction in the diameter of seminiferous tubules. Degenerated necrotic cells was observed in the seminiferous tubules, also they were observable disorganized germinal epithelium in most of the seminiferous tubules.

DISCUSSION

In this study, it was observed that the control group of animal models had a non-significant increase in gross anatomical parameters. The improved values of body weight of the control animals could mean that they were still in their active growth phase during the study[29].

The findings from this study showed a significant decrease in the testes and body weights and testis volumes in rats exposed to imiprothrin ($p<0.05$) when compared to the control group, this is in concordance with the report which also investigated insecticide exposure in animal models[17]. The decrease in body and testicular weight of the animals that

were exposed to imiprothrin in this experiment are also in conformity with previous reports of considerable decrease in body and testicular weight as a result of seminiferous tubular derangements[3,7,29-30].

Furthermore, the observed decrease in body weights of rats in this study may also be due to a diminished food consumption caused by developed anorexia or decreased utilization of food[31].

The histological evidences in this study showed degenerative changes characterized by vacuolization of the interstitium, reduced luminal spermatozoa and devoid spermatozoa in cross section of the seminiferous tubules of rats exposed to various concentration of imiprothrin (5 mL for 3 weeks, 5 mL for 4 weeks and 10 mL for 4 weeks). This is in conformity with several other previous reports on male infertility experiments in animal models involving cytotoxic chemicals[17,32].

Studies assessing sperm parameters utilize the caudal epididymis[11,33]. In this study, the imiprothrin exposed rats showed significant reductions in spermatozoa concentration, sperm motility, normal sperm morphology and increase in normal sperm morphology when compared to the control groups. These results are found to be consistent with several other reports on insecticide exposure[4,17]. Previous studies have also shown that insecticide exposure in animal models has led to decrease testicular sperm count, increase in percentage number of abnormal sperm and decrease in normal sperm morphology[17,22]. Imiprothrin induced testicular toxicity is due mainly to oxidative stress[6].

The significant increase in the percentage of morphologically abnormal sperm cells in the imiprothrin exposed rats could be due to the ability of imiprothrin to either interfere with the spermatogenic processes in the seminiferous tubules, epididymal functions and anterior pituitary secretions of gonadotropins which may result in alteration of spermatogenesis[34].

In addition the significant decrease in sperm motility of the exposed rats with various concentrations suggested that imiprothrin was able to permeate the blood testis barrier with a resultant alteration in the micro-environment of the seminiferous tubules, since it has been reported that the decrease in sperm motility caused by chemical agents was due to their ability to permeate the blood testes barrier[35].

Insecticide spray is a popular and cost-effective method of personal protection against insect bite. Given the uncontrolled manner in which this product is used, it is recommended that further studies been done to ascertain the level of toxicity in higher animal models including humans.

CONCLUSION AND FUTURE RECOMMENDATION

The results of this study showed that imiprothrin causes the changes in gross anatomical parameters, deranged sperm parameters and testis histology degeneration on the wistar rat's testis. Despite these well-established toxic effects of imiprothrin on the rat testis, there is a need for further investigations in humans to determine its lethal dose.

SIGNIFICANCE STATEMENTS

This study discovers the use of inhalation smoke after the process of spermatogenesis and sperm parameters and indicates the detrimental effects of these inhalation materials on male reproductive system and can be beneficial for malaria induced testicular damage rats. This study will help the researcher to unveil the critical area of testicular damage that caused by the incense materials that many researchers were not able to explore. Thus, a new theory on the incense materials may be arrived at.

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