Infertility: A product of smoke emanating from Transfluthrin coated insecticide paper (TCIP)

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Abstract
Transfluthrin is a fast-acting insecticide used in household and hygiene products, mainly against flying insects, such as mosquito and flies, and in agriculture material pests. Its uses in these areas have not been without health risk to humans and the ecosystem. Therefore, the current study was designed to investigate the effect of smoke emanating from Transfluthrin Coated Insecticide Paper (TCIP) on adult Wistar rats’ testicular functions. The rats were grouped into three. Group A was exposed to normal environmental air. Group B and C rats were exposed via whole-body inhalation to smoke emanating from 6 g and 12 g of TCIP every day for 8 weeks. The entire groups contained eight rats each. At the end of the exposure, body and organs weight, semen analysis, biochemical assay and histological examination were evaluated and determined. The results show that the exposure significantly altered the testicular cytoarchitecture, sperm quality, hormonal profile and oxidative parameters with an increase in exposure to TCIP. This study revealed that exposure to transfluthrin is detrimental to the reproductive functions of male rats.

Keywords
Transfluthrin, infertility, testis, oxidative stress, mosquito

Introduction
In recent years, cases of infertility have increased, with male aetiology gaining more attention. Initially, especially in Africa, if a couple cannot bear a child at a particular time, the focus is always on the woman. However, this has changed with civilization, the increase in reproductive science knowledge, and proper diagnostic method. Infertility in males is the male’s inability to achieve pregnancy in a fertile female.¹

Data now suggests that any change in the normal testicular morphology and physiology, endocrine function, biochemical activities or nuclei/chromosomal formation of the testicular cells can lead to either a decrease in sperm concentration and motility, testicular necrosis, testicular apoptosis, testicular atrophy or increase abnormal sperm morphology which in turn can result to the inability to achieve pregnancy in a fertile female.²³

Many drugs (pharmaceutical agents), chemicals, household materials, or radiation exposure from the mechanical machine can act either as direct spermatotoxic or through a

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Steroidal and oxidative pathway to affect the normal functioning of testicular cells.  

Household materials such as insecticide and pesticide are used in most developing counties to kill insects and pests, including mosquito. In tropical countries, the malaria situation is serious as a result of mosquito and still getting worse. Malaria threatens the lives of 40% of the world’s population. Each year, there are an estimated 300–500 million clinical cases resulting from mosquito bites. Insecticides, herbicides, and pesticides have been used to control disease-carrying insect; however, the use of these has not been without health risk to human and the ecosystem at large.

Transfluthrin (C\textsubscript{15}H\textsubscript{12}CL\textsubscript{2}F\textsubscript{4}O\textsubscript{2}) belongs to the class of insecticide called pyrethroid. It is used to kill many household insecticides like mosquito, files, ant, cockroach and the like. Transfluthrin is very active in controlling malaria by killing mosquitoes via inhalation or contact with the substance. Xenobiotic chemicals, such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), dioxin, Organophosphorus, insecticides, herbicides and some pesticides have been revealed to have been known to affecting male reproductive function. The anatomy of the testis makes it a target organ for environmental toxics. Its membrane is well vasculature and rich in polyunsaturated fatty acids, making it important to oxidative stress. Organophosphorus insecticides represent one of the most widely used classes of insecticides with a high potential for human exposure in both rural and residential areas. The exposure to smoke from Transfluthrin based mosquito coil challenge the immune system in experimental rats leading to decreased neutrophil and lymphocytes count as well as mean body weight in a study conducted in 2007. Mshelia et al. also showed that the Goldeer mosquito coil containing 0.03% transfluthrin affects the brain’s learning and memory part. It also reports increase malondialdehyde (MDA), indicating oxidative stress in the brain of rats.

Based on the literature, there is a paucity of information on the testiculotoxic effect of transfluthrin coated inflammable insecticide paper smoke on the reproductive function of Wistar rats. The study seeks to study the effect of TCIP on the reproductive function of Wistar rat, considering its histology, semen and hormonal analysis, and oxidative status.

**Materials and methods**

Insecticide paper (Rambo\textsuperscript{®} Bayer Cropscience, Gongoni Company Limited) was purchased from a retail outlet in Ogbomosho, Oyo State, Nigeria. This insecticide paper weighed 6 g and contained 0.45% of transfluthrin, 2.5% of Essential Oil and 97.05% of inert ingredients.

**Animals**

Twenty-four (24) male Wistar rats were obtained from an animal breeding stock at the University of Ibadan, Ibadan, Oyo State. They were housed under standard laboratory conditions with a 12 h daylight cycle and have access to feed and water *ad libitum*. They were acclimatized to laboratory conditions for 2 weeks before grouping for the experiments. Group A was exposed to normal environmental air. Group B and C rats were exposed via whole-body inhalation to smoke emanating from 6 g (burns for 4 h), and 12 g (burns for 8 h) of transfluthrin coated insecticide paper, every day for 8 weeks respectively. The entire groups contain eight rats each. The cages were divided with the aluminium wire mesh in order to allow a small space for the TCIP by allowing the smoke to penetrate and well saturated the cage, allowing maximum exposure to the rats to the smoke emanating from the TCIP. They were exposed to average of 4 h to 8 h a day for period of 8 weeks, as an effort to imitate normal time that a man sleeps in a day.

After the 8 weeks of exposure, the animals were weighed and anaesthetized with 80 mg/kg of body weight of ketamine to harvest the organ. The testis was harvested for histopathological examination. The blood samples were collected into the heparinized bottle and centrifuged at 4000 Xg for 10 min using a bench centrifuge. The plasma was evaluated for biochemical analysis.

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 laboratory animals in Biomedical Research as promulgated by the Canadian Council of Animal Care (CCAC, 1985). Furthermore, the experimental animal 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.

**Histological procedures**

Testes were excised, blotted and fixed in Bouin’s fluid overnight, dehydrated and embedded in paraffin. The paraffin-embedded samples were sectioned at 5 μm thickness. Haematoxylin and eosin (H&E) staining technique was used. The slides were examined using a light microscope and captured using a Leica Scanner.

**Biochemical analysis**

Biochemical analysis of plasma glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA) and testicular reduced glutathione (GSH), superoxide dismutase (SOD) were measured by standardized enzymatic colorimetric methods using an assay kit from Randox Laboratory Ltd. (Co. Antrim, UK) and hypophysial-gonadotropic hormones (Follicle-stimulating hormone; FSH, Luteinizing hormone; LH and testosterone; TT) were measured with enzyme-linked immunosorbent assay (ELISA) kits from Fortress diagnostics Ltd (Antrim, UK).
Sperm motility and progressive motility

As Mohammad-Reza described, the caudal parts of the epididymis were separated from the testis. They were placed in a beaker containing 1 ml buffered physiological saline solution, after which section was incised with a pair of sharp scissors and left for a few minutes to liberate its spermatozoa into the saline solution. Semen drops were placed on the slide, and two drops of warm 2.9% sodium citrate were added. The slide was covered with a coverslip and examined under the microscope using the \( \times 400 \) magnification objective.

**Total sperm count**

This was determined by using the new, improved Neubauer counting chamber (haemocytometer). One drop of the diluted sperm suspension was transferred to each counting chamber of the haemocytometer and allowed to stand for 5 min. The chamber was placed under a binocular light microscope using an adjustable light source. The chamber’s ruled part was then focused, and the spermatozoa were counted in four 16-celled squares. The sperm concentration was calculated and expressed as \( [x] \times 10^6/\text{ml} \), where \( x \) is the number of spermatozoa in a 16-celled square as described by Yokoi and Mayi.

**Sperm morphology**

Evaluations of sperm cells morphology were done with the aid of the light microscope at \( \times 400 \) magnification. Caudal sperm was taken from the original dilution for motility and diluted 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada). Five hundred sperms from the sample were scored for morphological abnormalities. Spermatozoa with rudimentary tail, round head and detached head were considered morphologically abnormal.

**Statistical analysis**

All quantitative data were expressed as mean \( \pm \) SD of the number of experiments \( (n = 8) \). The groups’ homogeneity level was tested using Analysis of Variance (ANOVA) as done by Snedecor and Cochran. A value of \( p < 0.05 \) was considered to indicate a significant difference between groups. Data analysis was done using both an electronic calculator and the Statistical Package for Social Sciences (SPSS)/PC program (version 16.0 SPSS).

**Results**

**Effect of transfluthrin coated insecticide paper (TCIP) on body and organ weight**

As presented in Table 1, the experimental rats’ body weight gain (group B and C) were significantly different compared with the control at \( p < 0.05 \). At 12 g exposure to TCIP, the testicular weight was significantly reduced. Simultaneously, that of the epididymis of both groups B and C were lower concerning the control (\( p < 0.05 \)).

**Effect of transfluthrin coated insecticide paper (TCIP) on sperm parameters**

Significant reductions in sperm motility (both Groups B and C), caudal epididymal sperm count (Groups C), and normal sperm cells (both Groups B and C) were observed in rats exposed to TCIIP compared to control rats (Figures 1 to 3).

**Sperm motility**

In Table 2, the values of SOD, GPx, CAT and GSH from group C rats were significantly reduced with the significant increase in MDA value Four times that of Group A.
However, for Group B, the parameters were reduced, but CAT and GSH were significant at $p < 0.05$. Similarly, the level of lipid peroxidation (MDA value) for group B was high significantly ($p < 0.05$).

Effect of Transfluthrin Coated Insecticide Paper (TCIP) on levels of serum testosterone (T), Luteinizing hormone (LH) and Follicle-stimulating hormone (FSH)

The Group C rats’ hormonal profile shows a significant reduction in all evaluated parameters (T, LH and FSH) compared with the control group. Whereas the group B rats had a slight reduction in Testosterone value and a significant reduction in LH and FSH values concerning group A at $P < 0.05$ (Figures 4 to 6).

**Effect of transfluthrin coated insecticide paper (TCIP) on testicular histology profiles**

As shown in Figure 7, group A (Control) had a normal testicular histology characterized by intact; germinal epithelium with all spermatogenic cells, interstitium with Leydig cell and lumen containing numerous spermatozoa. The photomicrographs of Group B and C shows serve distortion of testicular histo-architecture characterized by vacuolization, widening of interstitium with fluid accumulation, depletion and degeneration of germinal cell, sloughing and tubules devoid of spermatozoa (Figures 8 and 9).

**Discussion**

Vector control is the most important to dipping malaria at the community level, but for an individual, personal protection against mosquito bite is the first line of defence.7,21 This explains the drastic increase in the use of several insecticide forms; liquid, coil, paper such as transfluthrin coated insecticide paper (TCIP), to kill mosquito.22 However, the present study exposed the Wistar rats for the average of 4 to 8 h daily imitating daily exposure of man to mosquito coil.

In this study, smoke emanating from both grams of TCIP reduces the body and organ weight of rat. This is in agreement with the report of Garba et al.12 and Ahmed et al.23 involving the exposure of rat to smoke from mosquito coil. Reduction in body weight and testicular weight is one of the pieces of evidence that suggest, to a certain degree, the toxicity of any substance as seen in this study with transfluthrin. The reduction in body weight may be due to a reduction in food consumption, while that of the organ weight might be due to cell degeneration in the testis and epididymis. Also, both might be due to TCIP decreasing the anabolic effect of testosterone responsible for body building and germinal cell production.24–26 This hypothesis agrees with our finding on hormonal analysis and testicular histology. Tissue degeneration is a significant pathology seen in this study. This increases with an increase in the concentration of exposure. The experimental rat testis showed vacuolization within the germinal epithelium, widening of interstitium with fluid accumulation, depletion and degeneration of germinal cell, sloughing and tubules devoid of spermatozoa. Several studies have reported similar finding after exposure of the animal to the different

| Table 2. Effect of transfluthrin coated insecticide paper (TCIP) on oxidative parameters. |
|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
|                         | SOD (μmol/ml/mg protein) | GPx (μmol/ml/mg protein) | CAT (μmol/ml/mg protein) | GSH (μmol/ml/mg protein) | MDA (μmol/ml)            |
|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Group A                 | 5.02 ± 1.37             | 1.59 ± 0.60             | 11.03 ± 2.20            | 0.8 ± 0.57              | 1.32 ± 0.43             |
| Group B                 | 3.89 ± 1.19             | 0.56 ± 0.23             | 9.01 ± 3.15*            | 0.52 ± 1.00*            | 2.82 ± 0.80*            |
| Group C                 | 3.13 ± 1.08*            | 0.38 ± 0.05*            | 7.87 ± 2.55*            | 0.37 ± 1.10*            | 4.68 ± 1.02*            |

*P < 0.05 significantly different from control. Values are expressed as mean ± SD for $n = 8$ in each group.
Elbetieha et al. reported testicular atrophy, degeneration and haemorrhage in the interstitium. Our study’s derangement is mainly due to increased oxidative stress, which is collaborated our result on the oxidative parameter (Figure 2). The increase of MDA value to about four times that of control is indicative of lipid peroxidation. 

Exposure to TCIP led to the reduction of an enzymatic antioxidant such as GSH, SOD, CAT and GPx in our result. These are in accordance with Atessahin et al., Mshelia et al., and Jewo et al.

Exposure to the exogenous substance that can increase this ROS generation can affect testicular architecture and sperm production, as seen in our results (Figures 1 to 3 and 7 to 9). We documented a significant reduction in sperm count.
count, motility and increased abnormal sperm cell. Transfluthrin, just like other pyrethroids, has been described to affect sperm production and quality through the oxidative pathway. This agrees with the result of Mathur et al. and Akunna et al. The fact that sperm cell has a high amount of polyunsaturated fatty acid in their membrane and their production involve repeated cell division with the expression of genes, and high mitochondria activities make it susceptible to oxidative stress thus can result in cell death, more abnormal cell and low motile cell as seen in our study.

Sperm production is extraordinarily vulnerable to testicular inflammation, which causes impairment to the germinal epithelium and increases apoptosis of germinal cells. Our histology result gives support to the effect of inflammation on spermatogenesis, suggesting a direct association between TCIP and testicular inflammation. Increased inflammation is associated with increased oxidative stress, which itself impairs sperm function.

Further study to look into the effect of TCIP showed that Transfluthrin might affect the hypothalamo-pituitary-hormonal regulation pathway. Our result shows a significant reduction of testosterone, LH and FSH in the group exposed to a high concentration, with only testosterone not significant in the low exposure. These findings are consistent with the data reported by Duan et al., Farombi et al., Friedmann, Schrader. It has been reported that decreased testosterone levels in rats, resulting in increased germ cell apoptosis after treatment with Gossypol acetic acid (GAA). In hypothalamo-pituitary hormonal regulation pathway, testosterone is vital in the maturation of sperm cell. This is regulated by LH, which is produced in the anterior pituitary gland.

Similarly, for spermatogenesis to be sustained, FSH is vital in the maintenance of the germ cell. Exposure to TCIP might have distorted this activity, causing the low TT, which in turn caused germinal cell degeneration, apoptosis and lumen devoid of the matured sperm cell, as seen in our histological finding. This agrees with another study reporting reduced male reproductive hormone. This reduction in hormonal profile may also be due to oxidative stress, which may have suppressed or alter the receptor site for gonadotropin-releasing hormone on gonadotrophic cell, thus reducing the amount or preventing gonadotropin secretion.

**Conclusion**

Our results strongly provide sufficient scientific evidence of the destructive impact of the smoke emanating from TCIP on the testes in male Wistar rats. These exposures cause significant degeneration of testicular histarchitecture, increase oxidative status, and alter male hormonal profile, affecting spermatogenesis’s quality and quantity.

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