

PYRETHROIDS EXPOSURE: IMPLICATIONS FOR TESTICULAR DYSFUNCTION IN RATS

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ABSTRACT

Pyrethroids are synthetic pesticides which at low dose of exposure are harmless to humans but at environmentally high concentrations they cause debilitating effects in humans and animals alike. Lack of regulation of the insecticide applications in agriculture and among Nigerian households is therefore a cause for health concern. The reproductive effects of these chemicals are not precisely known. Twenty healthy, sexually active male Wistar rats were randomly divided into four groups. Three groups were exposed to the insecticide in sprayed puffs from the aerosolized insecticide for 15, 30 and 45 seconds/day respectively in air – tight plastic housing for 60 days while one group served as the untreated control. All animals were euthanized via cervical dislocation; the testes were excised and fixed in Bouin's fluid for routine histological studies using haematoxylin and eosin. The cauda epididymis was also excised for semen quality evaluation. Reduced body weight, alteration of testicular microstructure and significant increase in the proportion of abnormal and non-motile sperm cells were observed in animals exposed to pyrethroid. Exposure to pyrethroid insecticide may lead to body weight loss accompanied with testicular dysfunction possibly leading to sterility in the rats.

KEY WORDS: Pyrethroids, Testes, Wistar rats, Reproduction, Insecticide, Sterility.

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INTRODUCTION

Pyrethrum is a naturally occurring mixture of chemicals found in certain chrysanthemum flowers of *Chrysanthemum cinerariaefolium* and *Chrysanthemum cinereum*. Pyrethrum was first recognized as having insecticidal properties around 1800 in Asia and was used to kill ticks and various insects such as fleas and mosquitos [1,2]. Six individual chemicals called

pyrethrins are known to have active insecticidal properties in the pyrethrum extract. The pyrethrins are pyrethrin (I & II), cinerin (I & II), jasmolin (I & II) which are often used as household insecticides to control insects on pets or livestock [3,4,5,2]. Pyrethroids are synthetic chemicals that are very similar in structure to the pyrethrins, but are often more toxic to insects, as well as to mammals, and last longer

in the environment than pyrethrins [1,5]. More than 1,000 synthetic pyrethroids have been developed. Pyrethrins and pyrethroids are often combined commercially with other chemicals called synergists, which enhance the insecticidal activity of the pyrethrins and pyrethroids. The synergists prevent some enzymes from breaking down the pyrethrins and pyrethroids, thus increasing their toxicity [1,5].

Pyrethroid insecticides are widely used in agriculture and public health to control insects, weeds, animals, and vectors of disease. Although the use of pesticides is of benefit in general, but abuse of the pesticides is harmful due to their potential toxicity to humans and animals [6]. Though humans can metabolize pyrethroid insecticide, the chemical is known to be toxic to humans when exposed to large quantities or for over long periods of time [7]. Eil and Nisula, [8] reported that six synthetic pyrethroids bind with androgen (a male sex hormone) receptors, and alter normal androgen function. Other studies suggest that some pyrethroid or their metabolites severely affect the semen quality [9] of exposed animals and may also possess endocrine disrupting properties [10]. Permethrin, a pyrethroid insecticide has also been reported to reduce male reproductive hormone levels, sperm number and motility in humans [11] and animal models [12]. Similarly, Turki *et al.*, [13] has ascribed cypermethrin-induced oxidative stress in treated animals to severe alteration to testicular enzymes and significant reduction in testicular weight. Baygon™ [14] is a pyrethroid domestic insecticide [14,15] commercially available to the Nigerian population without apparent government regulation of its application.

MATERIALS AND METHODS

Animals: Wistar rats were obtained from Ladoko Akintola University of Technology, Nigeria Animal House and their treatments complied with the institution's guidelines and principles for humane treatments of animals.

A total of twenty healthy, sexually active male Wistar rats weighing between 150 – 180 g were randomly divided into four groups, namely control and three treated groups, (n= 5). They were housed individually in plastic cages and

acclimatized under a 12 hours light 12 hours dark photoperiod, relative humidity was approximately 30- 40% and temperature was maintained at 25±2°C. The three treated groups were exposed to spray of insecticides in sprayed puffs from the aerosolized insecticide (Baygon™) for 15, 30, 45 seconds/day respectively in air – tight plastic containers for 60 days.

Sample collection and processing: After treatment, the animals were dissected and the testes and epididymis were harvested immediately after exsanguination. The testes were fixed in Bouin's fluid and processed by the method for paraffin embedment and stained with Hematoxylin and Eosin (H & E). The slides were evaluated for pathological changes under light microscope.

Semen Analysis: The epididymis was placed in normal saline for evaluation of sperm quality (sperm count, sperm motility and sperm morphology). The concentration of spermatozoa was determined by the haemocytometer method [16] and were also evaluated using the improved Neubauer chamber (Deep 1/10 mm, LABART, Germany). The histomorphometry (i.e. cross sectional area, lumen diameter and germinal epithelium diameter) was evaluated using Image J software (USA) from the photomicrographs of the testes.

Statistical Analysis: Data collected were analyzed using two-way analysis of variance (ANOVA) followed by Tukey's (HSD) multiple comparison test with the aid of SPSS (V20; USA). Data were presented as means ± SEM (standard error of mean). ≤ 0.05 was considered statistically significant. All graphs were drawn using the GraphPad Prism v.6 (GraphPad Software Inc., USA).

RESULTS

Reduced body weight was observed among the treated animals when compared to untreated controls, which appeared to be exposure period dependent (Figure 1). The control animals showed round/oval shaped seminiferous tubule (ST), abundant spermatozoa in the lumen with intact interstitial cells within the interstitial space. Animals exposed to pyrethroid for 15 seconds showed scanty spermatozoa within the lumen and degenerated basement membrane (Figure 2). Animals exposed to pyrethroid for 30

and 45 seconds respectively showed elongated and abnormal arrangement of seminiferous tubule with scanty spermatozoa within the lumen and reduced spermatogenic cells. Wide interstitial space with few Leydig cells were also noticed (Figure 3).

Fig. 1: Body weight change of rats exposed to pyrethroid insecticide.

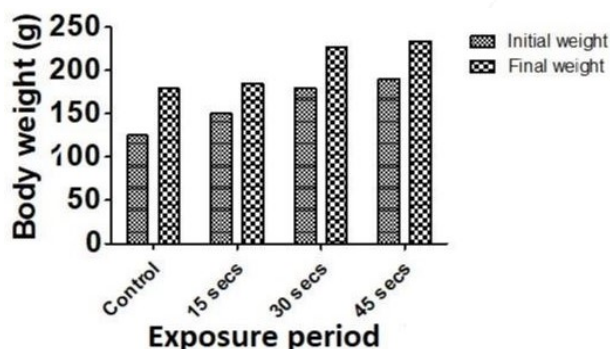


Fig. 2: Photomicrograph representation of testicular structure in control and animals exposed to pyrethroid for 15 seconds. Stain – H&E – x100 & 400. ST- seminiferous tubule, MS- matured spermatozoa in the lumen, SG- spermatogenic cells, IS- interstitial space, LC- Leydig cells.

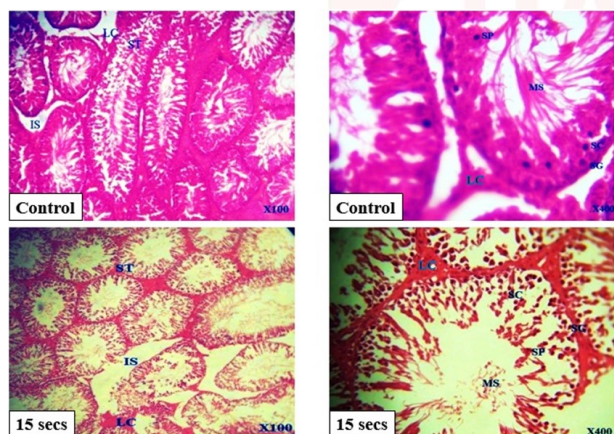


Fig. 3: Photomicrograph representation of testicular structure in animals exposed to pyrethroid for 30 and 45 seconds (Group C and D). Stain – H&E – x100 & 400. ST- seminiferous tubule, MS- matured spermatozoa in the lumen, SG- spermatogenic cells, IS- interstitial space, LC – Leydig cells.

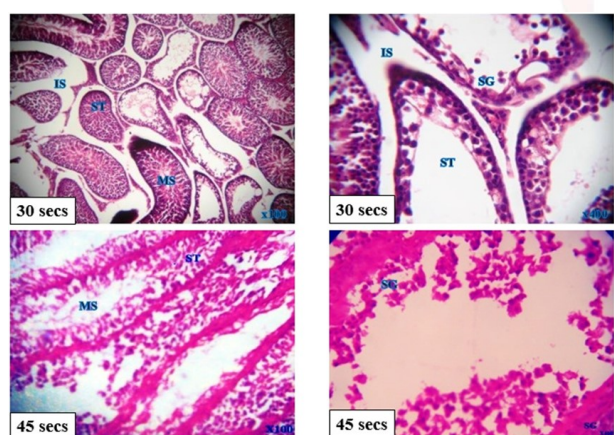


Table 1: Semen Analysis.

Groups	Sperm count (x10 ⁶)	Percentage motile Sperm (%)	Percentage non-motile Sperm (%)	Percentage normal Sperm (%)	Percentage abnormal Sperm (%)
Control	36.4 ± 5.79	50.0 ± 4.49	50.0 ± 4.51	68.0 ± 2.12	32.0 ± 2.12
15 secs	35.1 ± 2.49	38.0 ± 3.77	62.0 ± 3.77	42.0 ± 2.12*	58.0 ± 1.16*
30 secs	31.8 ± 6.86	28.0 ± 3.77*	72.0 ± 3.77*	38.0 ± 2.12*	62.0 ± 2.12*
45 secs	23.6 ± 1.71*	22.0 ± 2.12*	78.0 ± 2.12*	32.0 ± 2.12*	68.0 ± 2.12*

*Statistical significantly difference compared to the control rats.

Table 2: Testicular Morphometry; seminiferous tubule (ST), cross sectional area (CSA), lumen diameter (LD), germinal epithelium diameter (GED).

Groups	ST. CSA	ST. LD	GED
Control	4.79 ± 1.16	1.59 ± 0.08	0.95 ± 0.07
15 secs	1.48 ± 0.11*	1.12 ± 0.07	0.50 ± 0.03*
30 secs	1.38 ± 0.21*	1.01 ± 0.18	0.61 ± 0.16
45 secs	13.4 ± 0.86*	4.13 ± 0.43*	0.63 ± 0.09

*Statistical significant difference compared to the control rats.

DISCUSSION

The observed reduction in the body weight among the treated animals could be attributed to the reduced food intake associated with the endocrine disruption effect of the pyrethroids [17]. Atrophic and abnormal elongation of the seminiferous tubule, irregularly distant arrangement of the seminiferous tubule with scanty spermatozoa within the lumen and atrophic germinal epithelium as well as the widening interstitial spaces with few or no testosterone producing Leydig cells that were observed among the exposed rats appear to support the report of Rajawat and co-workers [18] as debilitating effects of pyrethroids on testicular structure. Pyrethroid exposure resulted in reduced total sperm cells and abnormal rise in the percentage of non-motile and abnormal sperm cells as reported in the work of Oda and El-Maddawy [19], that feeding rats with deltamethrin for 60 days caused significant adverse effects on reproduction apparatus in rats. Sperm motility refers to the ability of spermatocytes to move spontaneously. Sperm motility is aided by a normal morphology of the sperm cell. The sperm cell is made up of the head, mid-piece and the tail. Sperm morphology refers to the structure and form of the spermatocytes. Our study may have also confirmed the report of Tyler *et al.* [9]. The evident testicular histopathological

changes and drastic reduction in the evaluated semen quality might be due to induction of oxidative stress [13] and alterations in the testicular androgenic activities of animals exposed to pyrethroids.

This hypothesis may be supported by the findings of various scientists who reported a reduction in the testosterone concentration in animals exposed to pyrethroids [19,8], coupled with the fact that testosterone producing Leydig cells were degenerated in the treated animals (20). The study may have demonstrated endocrine disruption effect of the pyrethroid insecticide specifically by being anti-androgenic.

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Conflicts of Interests: None

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