Original Research Article

EVALUATION OF REPRODUCTIVE FUNCTIONS IN MALE MICE EXPOSED TO LAMBDA-CYHALOTHRIN AND SOME LOCAL SPICES

Abstract

Aim: This study was aimed at evaluating the reproductive functions in male mice exposed to lambda-cyhalothrin and local spices.

Experimental Design: A completely randomized experimental design using standard methods for analysis.

Location and Duration of Study: This study was carried out in the Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, Rivers State, Nigeria. GPS $4^{o}47'50''N$ $6^{o}58'49''E$ The study lasted for 14days.

Methodology: Forty-two male mice were randomly selected into six (6) groups A-F (n=7/group). Group B were gavage 10mg/kg/bw/day of lambda cyhalothrin (LCT) alone. Group C, D, and E were gavaged 10mg/kg/bw/day of LCT and 20mg/kg/bw/day of Tetrepleura tetraptera, Piper guineense, and Xylopia aethiopica respectively, while group F received 10mg/kg/bw/day of LCT and 20mg/kg/bw/day of combination of the three spices. All animals were allowed access to cool clean water and standard rat pellet ad libitum. Twenty-four hours to the termination of the experiment, feed was withdrawn from the animals. From each mouse, 0.5g of testis was fixed in 10% formalin *and sectioned with a Digital Microtome at 5µm thick and stained with Hematoxylin and Eosin (H &E). Photomicrographs were generated with a digital Microscope Biosphere Miller B with an image processor DN2 – Microscopy Image processing Software at X40 magnification.*

Results. Histological analysis of the testes reveal epithelium devoid of spermatogenic element in group B animals administered lambda cyhalothrin only, depicting that LCT impaired spermatogenesis in the mice. In group C the seminiferous epithelium had normal spermatogenic cell complement comparable to the control in group A. In group D, E and F there was gradual regeneration of the interstitial cells of leydig and the accompanying repopulation of the seminiferous epithelium. This reveals that Tetrapleura tetraptera seem to have a greater antioxidant and therapeutic effect against the cyhalothrin-induced reproductive

toxicity in exposed animals compared to Piper guineense and Xylopia aethiopica. Therefore, uncontrolled dietary inclusion of Piper guineense and Xylopia aethiopica is not advised as it may hinder spermatogenesis in males. Also, regulatory bodies should ensure that people and wildlife are not exposed to pesticides and insecticides at levels that may cause adverse effects by restricting the handling of such pesticides to Professionals.

Keywords: pesticides, phytochemicals, reproductive toxicity, spermatogenesis, spices,

1.0 INTRODUCTION

Man's survival has been dependent on his curiosity, his desire to examine by trial and error all aspects of his environment and to conclude which materials are medicinal, harmful or of great nutritional value. As such, different plant parts have been used as popular medicine in several countries-underdeveloped, developing and developed-as an alternative treatment for various diseases [1,2]. Several plants, roots, spices and herbs have been reported to be good sources for management against reproductive dysfunctions. Reproductive dysfunction has become a huge concern to researchers due to the fact that a healthy sexual functioning contributes significantly to one's sense of wellbeing and social life. Reports have revealed high incidence of reproductive dysfunction among the population especially the male counterparts. To achieve sexual quality, many spices, herbs, plant parts treatments are employed with little or no knowledge about their composition and possible side effect based on the dosage. Medicinal plants are rich sources of antioxidants, cost effective and potent alternative with few and transient side effects in the treatment and management of reproductive disorders, when compared with the existing conventional therapeutic drugs available in the market. It was estimated by [3] that more than two thirds of the world's population relied on plant derived drugs. Local communities have used about ten percent (10%) of all flowering plants on earth to treat various infections, although only one percent (1%) have gained recognition by modern scientists [4]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [5]. These plant-based systems will continue to play

an essential role in health care especially in rural areas around the world. Spices generally are parts of various plants cultivated for their aromatic pungent or otherwise desirable substances. They consist of rhizomes, bulbs, flower bud, fruit, seed, and leaves.

Tetrapleura tetraptera is a spice, native to West Africa. It is a gift to human race because of its medicinal properties, used as exotic spice, medicine and as a dietary supplement. *T. tetraptera* is traditionally used to treat sicknesses such as diabetes, fibroid, epilepsy, arthritis, asthma, leprosy, convulsion, fertility & sexual issues [6].

Piper guineense (Uziza) is a West African spice plant commonly called Ashanti pepper, African black pepper. The phytochemical studies of the plant revealed the presence of proteins, carbohydrates, alkaloids, steroids, glycosides, saponins, flavonoids, tannins and phenolic compounds. It also contains vitamins, minerals and fat acids [2,7].

Many therapeutic effects such as treatment of cough, bronchitis, rheumatism, dysentery and infertility are already being found when using the aerial part of *Xylopia aethiopica* [8]. Previously phytochemical investigation of *X. aethiopica* yielded alkaloids, saponins, tannins, terpenes, flavonoids and glycosides [8,9].

Several research studies have indicated that sperm counts have been in decline for decades and scientists say modern lifestyles and contacts with chemicals are a contributing factor. Exposure to pesticides is just one of the reasons for this decline. Pesticides are one of the most potentially harmful chemicals released into the environment without guided regulations. Pyrethroids may be considered safe with low toxicity to mammalian cells but reports have shown different deleterious effects of deltamethrin, [10,11], Cypermethrin [12, 13], lambda cyhalothrin [14,15,16], which are endocrine disruptors, by induction of reproductive dysfunction in males.

The increasing incidence of male infertility necessitate scientific research into plant and plant parts with fertility enhancing potentials. The available antioxidants are expensive and not readily available to the common man, hence this study was undertaken to evaluate the possible antioxidant capability of *Tetrapleura tetraptera, Piper guineense and Xylopia aethiopica* for reproductive dysfunction in rats exposed to lambda cyhalothrin.

2.0 MATERIALS AND METHODS

2.1 Experimental Location

The study was carried out in the animal house of the Department of Animal & Environmental Biology of the Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Rivers State with GPS coordinate *4°47'50"N 6°58'49"E.*

2.2 Experimental animal care and management

Forty-two sexually matured Swiss male mice of mean weight 20.57±3.35g were bought from animal farm at Oyigbo Rivers State, Nigeria and were transported to the Animal house Department of Animal & Environmental Biology, Rivers State University, Port Harcourt Rivers State. They were allowed to acclimatize for 7days before the commencement of the experiment. The rats were fed with standard pellet and clean water *ad libitum*. All experiments were conducted according to the institutional protocols of animal care at Rivers State University, Port Harcourt, and the standard procedure for ethical treatment of laboratory animals.

2.3 Test materials

Lambda cyhalothrin was purchased from a chemical store around the study area. The spices were also purchased from a local market around the study area. They were identified and confirmed in Department of Plant science and Biotechnology, Rivers State University Port Harcourt. The seeds were sun dried and healthy seeds selected for. The selected seeds were milled into powdery form and stored.

2.3 Experimental design

The experimental animals were separated into 6 groups (A-F) of seven mice each (n=7). Group A was the negative control and so received no test chemical or spices. Group B animals were gavage 10 mg/kg/bw/day of lambda cyhalothrin only, Group C animals received 10 mg/kg/bw/day of lambda cyhalothrin and 20 mg/kg/bw/day of *T. tetraptera*, Group D received 10 mg/kg/bw/day of lambda-cyhalothrin and 20 mg/kg/bw/day of *P. guineense*, Group E received 10 mg/kg/bw/day of lambda-

cyhalothrin and 20 mg/kg/bw/day of *X. aethiopica*. Group F received 10 mg/kg/bw/day of lambda-cyhalothrin and 20 mg/kg/bw/day of combination of the spices.

Feed was withdrawn from the animals 24 h to the termination of the experiment. Each mouse was dissected and testis removed for histopathological analysis.

2.4 Histopathological evaluation of testis

For each mouse, 0.5 g of the testis was fixed in 10% formalin and sectioned with a digital microtome at 5 µm thick. Histological sections mounted on slides were stained with Hematoxylin and Eosin (H&E). Photomicrographs were generated with a digital Microscope Biosphere Miller B with an image processor DN2-Microscopy Image Processing Software at 40x magnification (Orlu, 2014).

3.0 RESULTS

The seminiferous epithelium of the swiss mice exposed to lambda cyhalothrin and some local spices are shown in fig 1-6.

The normal architecture of seminiferous tubules and spermatogenic elements of animals in group A (control) are shown in (Fig 1). Figure 2 represents the transverse section of testis in group B showing deformed seminiferous epithelium with both mitotic and meiotic elements being degenerated leaving spaces without spermatogenic elements. Figure 3 shows visible regeneration of the interstitial cells of Leydig and increase in both mitotic and meiotic spermatogenic elements in group administered 10 mg/kg/bw/day lambda cyhalothrin and 20 mg/kg/bw/day of *T. tetraptera*. The seminiferous epithelium of animals in group D and E exposed to 10 mg/kg/bw of cyhalothrin and 20 mg/kg/bw/day of *P. guineense*, *X. aethiopica* showed gradual regeneration with fewer deformed seminiferous tubules seen compared to the epithelium in group B is shown in (Figures 4 and 5). Figure 6 shows sections of seminiferous epithelium in group F with lumen gradually filled with maturing spermatozoa and some vacuolated sections still visible.

Fig 1:Transverse section of testis from the control group. @magx40

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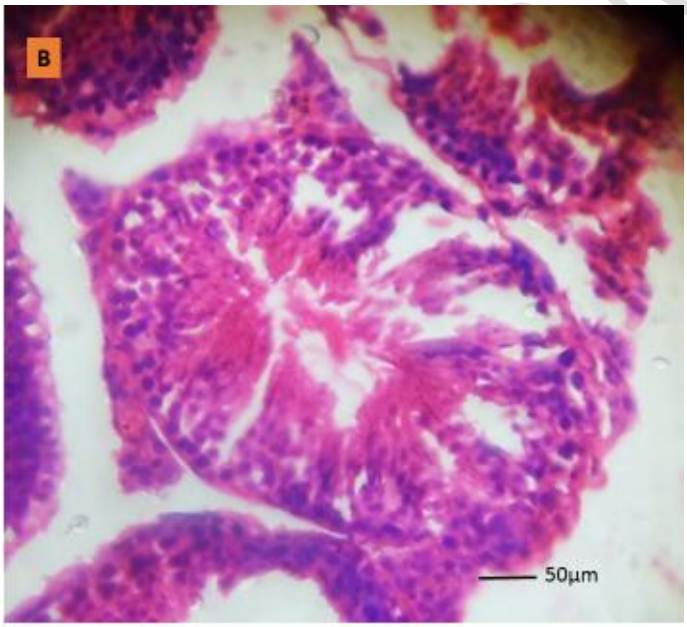

Fig 2:T/S of testis in mice exposed to LCT alone. @magx40

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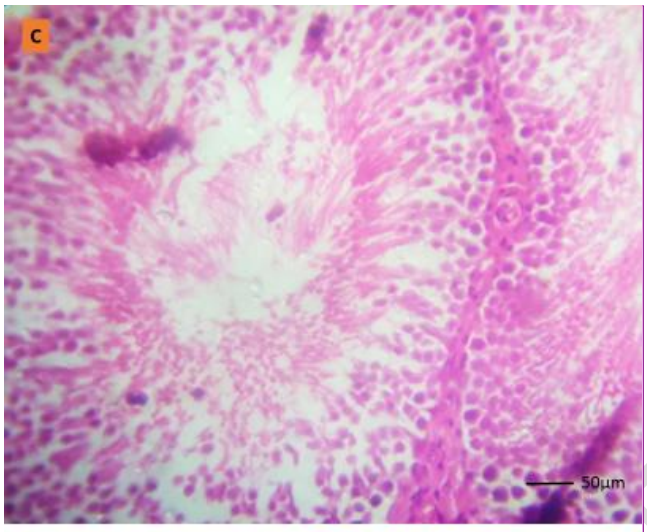

Fig 3 :T/S of testis in mice exposed to LCT and *T. tetraptera*. @magx40

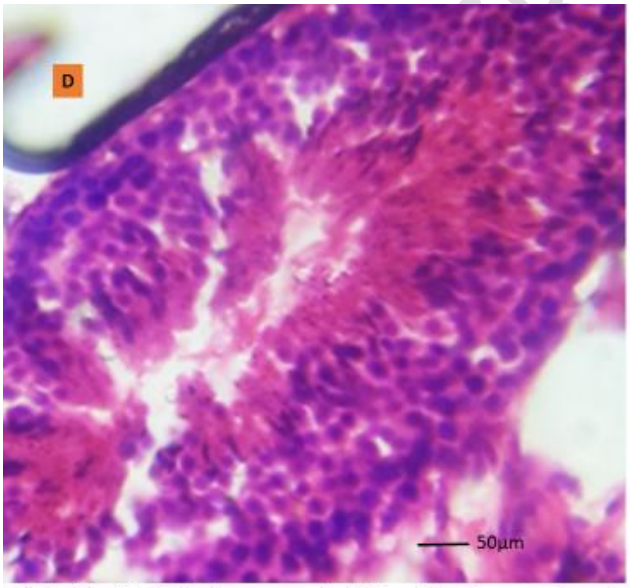

Fig 4: T/S of testis in mice exposed to LCT and *P. guineense*. @magx40

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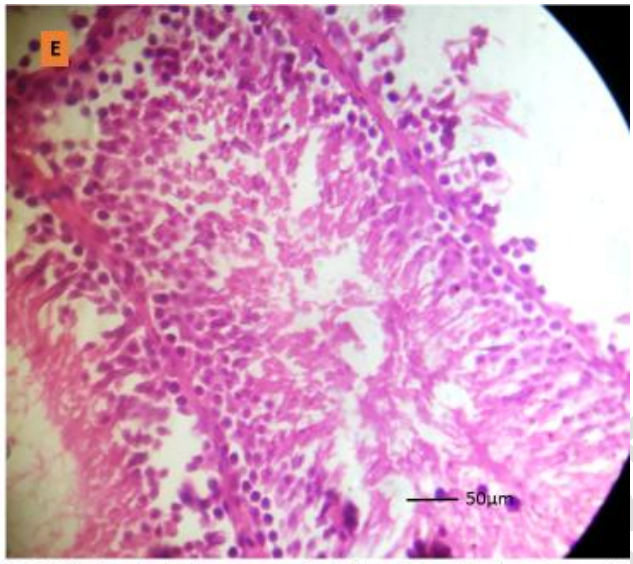

Fig 5 :T/S of testis in mice exposed to LCT and *X. aethiopica*. @magx40

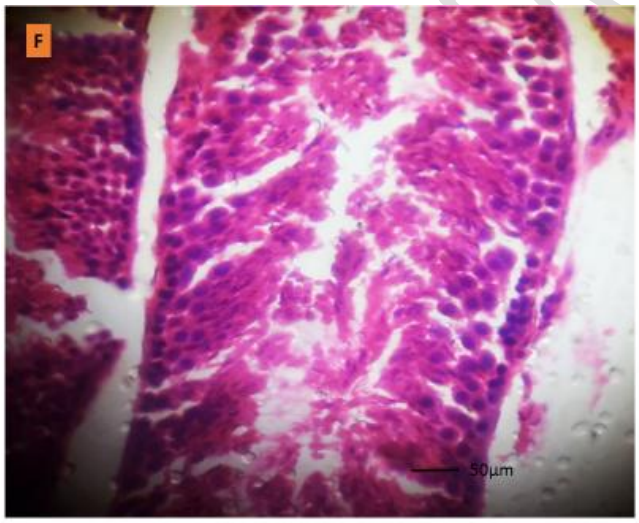

Fig 6 :T/S of testis in mice exposed to LCT + combination of the spices. @magx40

4.0 DISCUSSION

Reproductive dysfunction in males is one of the common causes of social frustration especially in developing countries. Male infertility has been linked to exposure to various environmental toxicant such as pesticides [11, 12,13, 14] reported that pesticides as well as insecticides that exhibit anti-androgenic activity could be responsible for the increased incidence in various male infertility and other sexual disorders.

From this study, histopathological analysis of the testes showed epithelium devoid of spermatogenic element in the group of animals administered lambda-cyhalothrin only, indicative of the potential of Cyhalothrin to induce reproductive toxicity. In group C the seminiferous epithelium had normal spermatogenic cell complement comparable to the control in group A. In groups D, E, F there was gradual regeneration of the interstitial cells of leydig and the accompanying repopulation of the seminiferous epithelium. The lumen gradually filled with maturing spermatozoa with some vacuolated sections still visible. This reveals that *T. tetraptera* seem to have a greater antioxidant and therapeutic effect against the Cyhalothrin-induced reproductive toxicity in exposed animals compared to *P. guineense* and *X. aethiopica*.

Other researchers have reported that cyhalothrin is considered as an endocrine disruptor [10,15,]. [16] revealed seminiferous tubules and epithelium devoid of spermatogenic elements in mice exposed to lambda cyhalothrin only while treatment with *Citrullus lanatus* and *Annona muricata* regenerated the epithelium. Also [14] reported oxidative damage of testes and testicular histopathological alterations in rats exposed to LCT while caffeic acid reduced the deleterious effects of lambda cyhalothrin on male fertility.

[17] reported that the levels of luteinizing hormone, testosterone of male rats ingested with 40mg/kg/day of cypermethrin significantly reduced while histopathological analysis of the testes revealed congested blood vessels, marked hemorrhage, significant accumulation of connective tissues around the seminiferous tubules which contained large number of immature spermatids.

The prevention of cypermethrin-induced reproductive toxicity in rat by resveratrol and reported weight loss of testis and epididymis, reduction in testicular sperm head counts, sperm motility and live sperm counts, as well as, increased sperm abnormalities was assessed [15]. Other researchers also have shown the protective role of lycopene, curcumin and CoQ10 against reproductive toxicity [12,18,19,20].

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Obulor and Orlu [16],

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In conclusion, the spice *T. tetraptera* seems to possess a greater antioxidant and therapeutic effect against the cyhalothrin -induced reproductive toxicity in exposed animals compared to *P. guineense* and *X. aethiopica*. It is hereby recommended that uncontrolled dietary inclusion of *P. guineense and X. aethiopica is not advised as it may* hinder spermatogenesis in males. Also, regulatory bodies should ensure that people and wildlife are not exposed to pesticides and insecticides at levels that may cause adverse effects by restricting the handling of such pesticides to professionals.

ETHICAL APPROVAL

The experiment was conducted according to the institutional animal care protocols at the Rivers State University Nkpolu-Oroworukwo, Port Harcourt, Rivers state, Nigeria and followed approved guidelines for the ethical treatment of experimental animals.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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