

Anti-Fertility Effect of Allethrin Based Liquid Electric Repellent on the Testes and Epididymis of Adult Male Sprague-Dawley Rat

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Abstract: *The incidence of male infertility in a population has important demographic and health implications. Effect of Allethrin on the testes and epididymis is being investigated. The aim of this research work is to determine the effect of allethrin on male reproductive system (testes and epididymis).*

A total number of twenty (20) adult male Sprague-Dawley rats of mean weight 160g were divided into four groups. Group 1, 2 and 3 animals were daily exposed to electric mortein insecticides for 30 minutes, 1 hour and 1 hour 30minutes respectively for 30 days. Group 4 rats were designated as the Control group.

The exposed animals experienced reduction in body weight, significant decrease in numbers of neutrophils, significant increase in white blood cell count and packed cell volume. Andrological parameters showed significant decrease in sperm count, sperm motility, percentage of normal sperm cells and serum testosterone level when compare with the control. Histo-architecture of the testes and epididymis showed seminiferous tubules with incomplete maturation of germinal cell layers with no spermatozoa in their lumen and some epididymal cells were also devoid of sperms. Control rats' testes and epididymis histological parameters appeared normal.

Continuous exposure to inhalation of allethrin causes reduction in body weights. Moreover, this study has revealed its anti-fertility effect on the male reproductive system.

Keywords: *Allethrin, Anti-fertility, Testes, Histo-architecture, epididymis*

1. INTRODUCTION

Pyrethroids are synthetic pesticides chemically related to the naturally occurring pesticide, pyrethrum (Bruce, 2007). Out of thousands of pyrethroids that have been made, only a few are used in the Nigeria. Allethrin is one of the type of pyrethroids that do not have cyano group. It is widely used in Nigeria households to control insects. Its uses in the textiles industry cannot be overlook as it is being used in the making of carpets, wallpapers, furniture and clothes. Allethrin is also being used in agriculture, forestry and public health services. Organism most ingest accidentally via food ingestion or inhalation via polluted air or by skin contact (Macan *et al*, 2006).

WHO in the year 2001 reported that some agents like chemotherapeutic, radiation, pharmaceutical, pesticides and insecticides have been implicated in the male reproductive dysfunction. These agents act either as direct spermatotoxins or through a steroidal pathway (WHO, 2001). Moreover, histopathological studies of the intoxicated rats treated with methomyl orally 17 mg/kg in saline daily for two months revealed variable degrees of degenerative changes in the seminiferous tubules up to total cellular destruction (Mahgoub and EI-Medany, 2006). Furthermore, Sobarzo and Bustos-Obregon in the year 2000, reported that a single injection of parathion (an organophosphate agro pesticide) to immature male mice caused a decrease in testis weight and early damage of germ cells of the mice. Quinalphos a commonly used organophosphorus insecticide reduce prostatic acid phosphatase activity and fructose content of the accessory sex glands, and plasma levels of testosterone, FSH, LH as well as relative weights of the testis and accessory sex organs (Rey *et al*, 1991). Dimethoate orally exposed to male rats increase relative weights of testis and prostate, sperm density and motility, serum and testis levels of protein and cholesterol, activity of prostatic acid phosphatase (Ngoula *et al*, 2011).

People tend to patronize Pyrethroids based insecticide these days to organophosphate based insecticide due to high intoxication of the latter, and lot of works have been done on anti-fertility

effects of organophosphate based insecticide but paucity of data as regards to pyrethroids type of insecticide. But how truly saved is pyrethroids based insecticide? Thus this work aims at evaluating the effect of allethrin based liquid electric repellent on the testes and epididymis of adult male Sprague-Dawley rat.

2. MATERIALS AND METHOD

2.1. Procurement of Allethrin

Liquid electric allethrin repellent was procured from commercially available store.

2.2. Experimental Design

A total number of twenty (20) adult male Sprague-Dawley rats of mean weight 160g were divided into four groups. Group 1, 2 and 3 animals were daily exposed to electric mortein insecticides for 30 minutes, 1 hour and 1 hour 30minutes respectively for 30 days. Group 4 rats were designated as the Control group. The animals were fed with standard rat pellet (Mosodun Ltd.Osogbo, Osun State, Nigeria) with free access to tap water. All the experimental procedures were done following the experimental guidelines of Health Research and Ethics Committee (HREC) of the Osun State University, Osogbo Campus, Osogbo, Osun State.

2.3. Animal Sacrifice and Sample Collection

The animals were sacrificed using cervical dislocation, the abdominal regions were excised and testes were removed from the scrotum sac and blotted out of fats before fixing in Boiun's fluid. Blood was collected from the apex of the heart via cardiac puncture.

2.4. Sperm Count Assay

Sperm motility was assessed by the method described by (Melissa, 2002). The spermatozoa were counted by hemocytometer using the improved Neubauer (Deep 1/10 mm, LABART, Germany) chamber as described by (Melissa, 2002).

2.5. Sperm Morphology and Motility Assay

Sperm live/dead ratio and motility was determined using 1% Eosin and 5% Nigrosin in 3% sodium citrate dehydrate solution according to the method described by (Melissa, 2002).

2.6. Testosterone Assay

This was carried out with the use of Testosterone ELISA Kit obtained from Monobind Inc. Lake forest, CA, U.S.A (Product code 3725-300). The essential reagents used for this assay were biotinylated antibody, enzyme-antigen conjugate and a serum native antigen, upon mixing all these reagents, a competitive reaction set-in between the native antigen and enzyme antigen conjugate for a limited number of antibody binding sites. The amount of testosterone that is able to bind to the testosterone antiserum will be inversely proportional to the concentration of testosterone in the well. The absorbance in each well at 450nm was read in a microplate reader (using a reference wavelength of 620-630nm to minimize well imperfections).

2.7. Histological Examination

Routine histological processing using Haematoxylin and Eosin staining method was carried out. The testes were fixed in 10% formal saline, dehydrated in ascending grades of alcohol, cleared in xylene, quickly dipped into molten paraffin wax before finally embedded in molten paraffin wax in order to form hard block. The hard block containing the tissue was then sectioned by the rotary microtome at 4µm thickness. The sections were then floated in water bath at 40⁰c and transferred to a glass slide and stained with heamatoxylin and eosinstains. The slides were then viewed under light microscope at ×100 and ×400 magnification and photomicrographs were taken in at both magnifications.

3. STATISTICAL ANALYSIS

One way ANOVA and student t-test were used to analyse the data using Graph Pad Prism v.6. The results were presented as Mean ± SEM with significant level at *P*-value <0.05 while the histological examination, Sperm analysis and Serum testosterone level were carefully studied and analysed to establish any correlation between the groups.

4. RESULTS

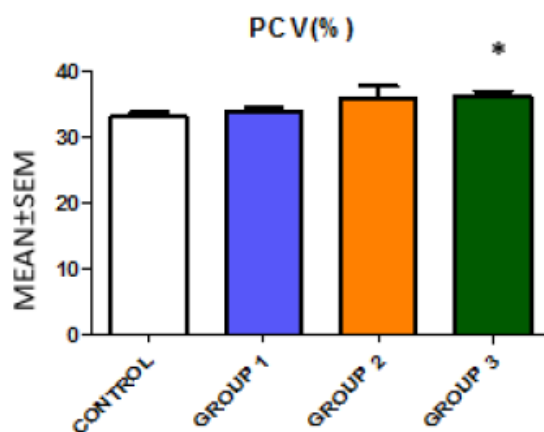


Figure1. Showed comparison between the control group and treated groups packed cell volume after treatment with allethrin. Group 3 rats (1hour 30minutes) showed slight increase in packed cell volume rate (* $P < 0.05$), while group 1 (30 minutes) and group 2 (1 hour) rats showed no significant change statistically.

*: p -value < 0.05

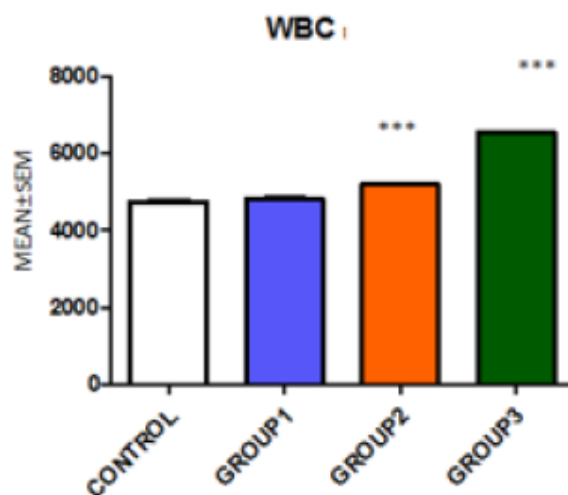


Figure2. Showed comparison between the control and treated groups white blood cell count after treatment with allethrin. Group 2 rats (1hour) and group 3 rats (1hour 30minutes) have high significant increase in white blood cell count (***) $P < 0.0001$ while group 1 rats (30 minutes) showed no significant change statistically.

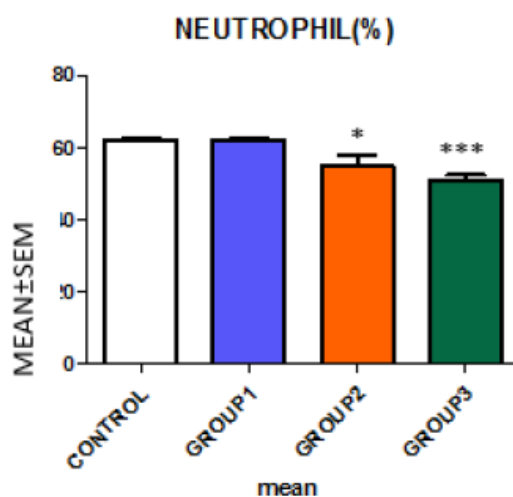


Figure3. Showed comparison between the control group and treated groups number of neutrophils after treatment with allethrin. Group 1 rats (30 minutes) do not show any significant change in their neutrophils, group 2 rats (1 hour) showed slight decrease in their numbers of neutrophils (* $P < 0.05$) while group rats (1hour 30minutes) showed high significant decrease in their number of neutrophils (***) $P < 0.0001$.

SERUM TESTOSTERONE CONCENTRATION (ng/ml)

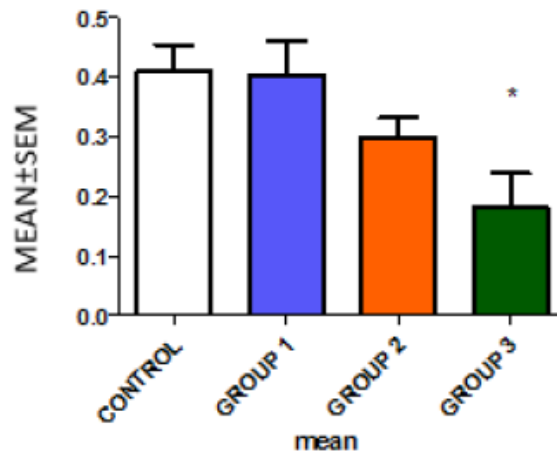


Figure4. Showed comparison between the control group and treated groups serum testosterone after treatment with allethrin. Group 3 rats (1hour 30minutes) showed slight decrease in serum testosterone (* $P < 0.05$)

*: p value < 0.05

SPERM COUNT

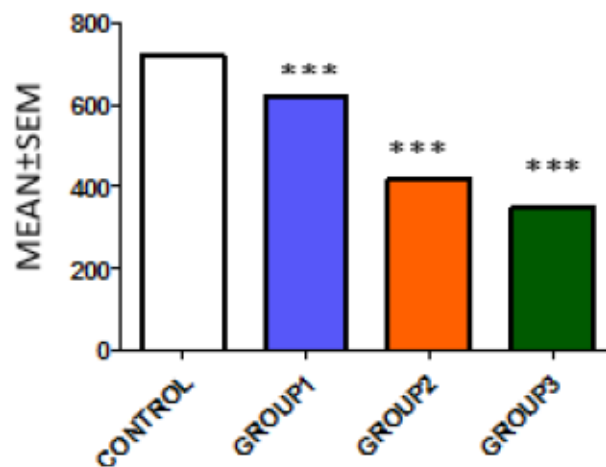


Figure5. Showing reduction across all groups sperm count when compared to the control group (** $p < 0.0001$).

SPERM MORPHOLOGY

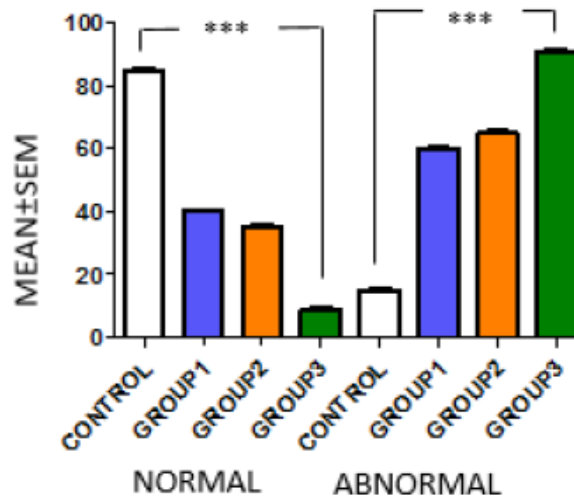


Figure6. showed progressive decrease in amount of normal sperm and progressive increase in number of normal sperm from group 1 to group 3 when comparing with the control group (** $p < 0.0001$).

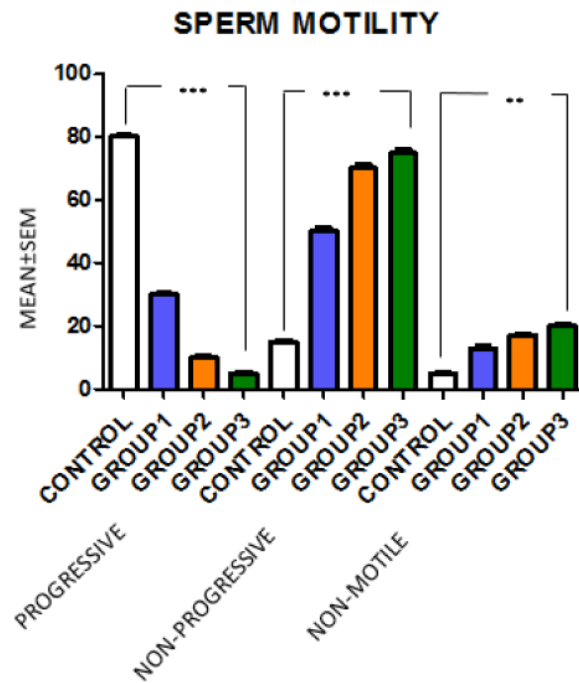
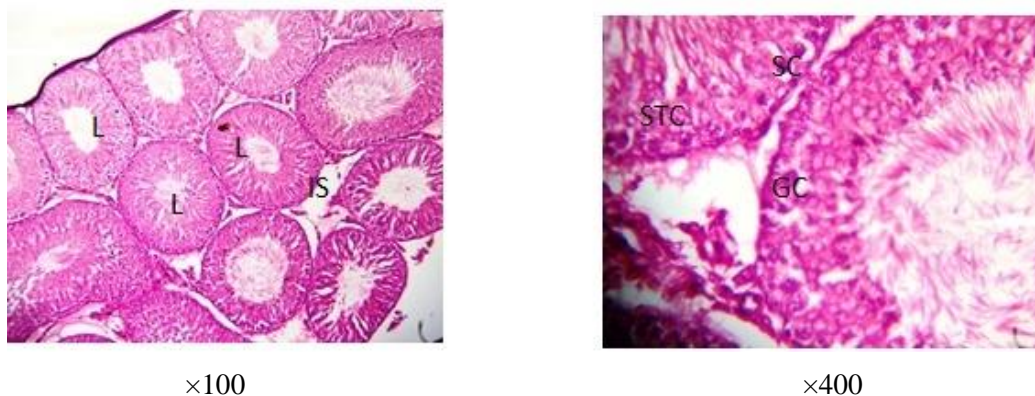


Figure7. showing reduction in sperm motility across all groups from group 1 to group 3 while comparing with the control group (** $p < 0.0001$).

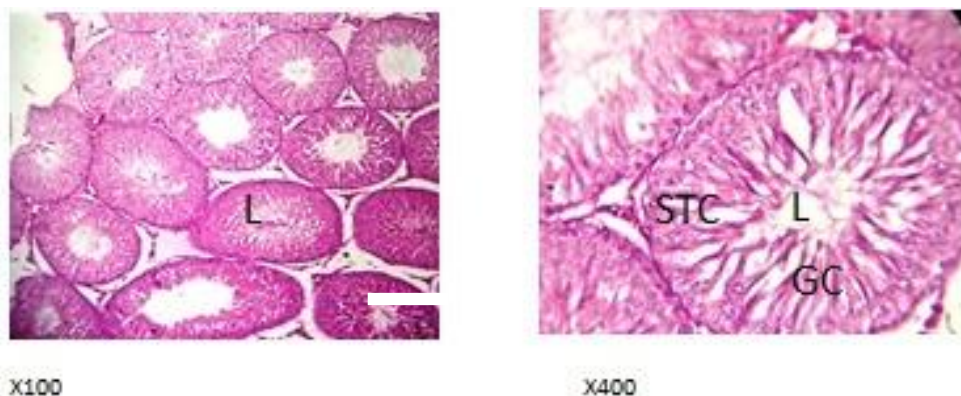
4.1. Photomicrographs

Figure8.



The longitudinal section of the control group testicular section stained by haematoxylin and eosin showed normal seminiferous tubules with normal spermatogonia cell (SC). Sertoli cells (STC) appeared normal and showed normal germ cell layer (GC) with normal maturation stages, the lumen (L) appear normal with presence of spermatozoa and the interstitial spaces (IS) and Leydig cells (LC) also appeared normal.

Figure9.



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There was marked reduction in serum testosterone concentration for long time allethrin exposed group, this observation is in accord with the work of Meeker *et al*, 2008 that noticed that exposure to pyrethroid insecticides will cause reduction in serum testosterone concentration.

The seminiferous tubules of the rats exposed over a long time as seen in group 3 rats in this work showed maturation arrest, it was also observed by Shala by *et al*, 2010 that over exposure to pyrethroid insecticide for a long time induced testicular lesions characterized by moderate to severe degenerative changes of seminiferous tubules and incomplete arrest of spermatogenesis.

In conclusion, over exposure to allethrin has negative effect on male fertility, steps should be taken to avoid individuals from been over exposed to allethrin or pyrethroid insecticides.

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