



Protective Role of Lycopene on Hormonal Profile and Posttesticular Functions of Male Rat Exposed to Sublethal Doses of Cypermethrin

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Authors' contributions

This work was carried out in collaboration between both authors. Author EEO designed the study, supervised the experiment while author AOO carried out the experiment and wrote the first draft of the manuscript. Both authors read and jointly approved the final manuscript.

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ABSTRACT

Aim: The aim of the study is to evaluate the Protective role of Lycopene on hormonal profile and posttesticular functions of male rats exposed to sublethal doses of Cypermethrin.

Study Design: The study was a completely randomized design employing relevant statistical tools for analysis and interpretation.

Place and Duration of Study: The study was carried out in the Reproductive Physiology and Genetics Research Laboratory of the Department of Applied and Environmental Biology, Rivers State University, Rivers State. The experiment lasted for 35 days.

Methodology: For the sperm morphology assay, sperm reserves and hormonal profiling, semen samples were drawn from the caudal epididymis with a syringe and placed on a clean glass slide. A drop of freshly prepared eosin-Y was added to make a thin smear and examined under the microscope for morphological abnormalities. A portion of the testis and epididymidis was homogenized separately with sharp pointed scissors in normal (physiological) saline. The suspension was mixed and strained through a double layer of sterile cheese cloth into graduated

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test tubes. All the samples were covered and stored for 24 hours at 4°C. A dilution was made for counting in Neubauer haemocytometer. The hormonal concentration was determined using the Randox Monza Laboratories assay kit from Co-Atrim, United Kingdom.

Results: Results of oral administration of Cypermethrin and co-administration of lycopene in rats showed Group G co-administered pure Lycopene had the lowest sperm head abnormalities of 3.5%, group B administered Cypermethrin only had the highest head abnormalities of 44.7%. Similarly, Group G, co-administered pure Lycopene had the lowest percentage of tail abnormalities of 10.1% while Group B had the highest percentage of sperm tail abnormalities of 32.4%. There was a significant ($p < 0.001$) decrease in concentration of all androgens considered in group B administered Cypermethrin only.

Conclusion: Exposure to Cypermethrin only as in group B disrupted the production of all androgens considered, increased the percentage of abnormal spermatozoa, reduced sperm motility, viability and sperm reserves. However, results recorded from the co-administration of *Solanum lycopersicum* and pure lycopene, in groups C-G indicate the protective role of this potent antioxidant on spermatogenesis and hormonal profile.

Keywords: Androgens; cypermethrin; lycopene; spermatozoa; sperm reserves.

1. INTRODUCTION

Pesticides have been used as the best means of pest control and eradication over the years. Regulatory restrictions on organophosphate and organochlorides pesticides have led to an increase in the use of Pyrethroids pesticides bringing about a wide spread concern to researchers. It is noteworthy that exposure to insecticides increase the induction of free radical with deleterious effects in germ cells of animals and adverse effect on human fertility [1] including disruption of spermatogenesis, decline in the concentration of sperm, motility of sperm, normal sperm morphology and altering the functioning of various reproductive hormones such as testosterone [2,3,4,5]. Although pyrethroids are considered safe and of low mammalian toxicity, reports have shown the various adverse effects of Cypermethrin, an endocrine disruptor, by induction of reproductive dysfunction in males with indicators such as, abnormal sperm cell morphology, decrease in testicular enzymes, inhibition of testosterone synthesis and decrease in fecundity [6,7,8,9]. Epidemiological data revealed an increase in male reproductive function disorder over the past 50 years suggesting a correlative relationship with increasing amount of all forms of endocrine disrupting chemicals in the environment [10]. The global average sperm count in man has also dropped by half from 113 to 66 million within the past 50 years while sperm morphology abnormalities increased [11,12]. Androgens are the most important hormones in normal male reproduction, estrogens also play a crucial role due to the presence of large quantities of estrogen in

the rete testis fluid and spermatic vein of numerous mammals; so the balance between androgens and estrogens are important for normal spermatogenesis [13,14,15, 16,17].

In males, urinary pyrethroid metabolites are correlated with a decrease in sperm count, a decrease mobility of sperm, an increase in abnormal sperm morphology as well as an increase in DNA damage, which may result in decreased fertility and pregnancy [18,19]. It has been reported that there was a significant reduction in the levels of luteinizing hormone, testosterone of male rats fed 40 mg/kg/day of Cypermethrin for 35 days. [20] also reported a decrease in testosterone, serum protein, increase in Transaminases and alkaline phosphatase in rats administered tetramethrin, Sumuthrin and deltamethrin. However, [2] observed a significant reduction in the extragonadal sperm counts, motility, testosterone production, and plasma testosterone levels of mice exposed to cis-Permethrin in a dose-dependent manner. With chronic exposure to endocrine disrupting chemicals (EDCs), there is need for exogenous antioxidant to compliment the endogenous antioxidant that will reduce reproductive stress.

2. MATERIALS AND METHODS

2.1 Experimental Location

The study was carried out in the Reproductive Physiology and Genetics Research Laboratory of the Department of Applied and Environmental Biology, Rivers State University, Port Harcourt

Nkpolu-Oroworukwo Rivers State (Coordinates 4°47'50"N 6°58'49"E).

2.2 Experimental Animals

Twenty-eight sexually matured female Sprague-Dawley rats (mean weight 236±35.6g) were obtained from the Department of Biochemistry, University of Port Harcourt, Nigeria. The rats were housed individually in plastic cages under standard conditions (12hL:12hD) and acclimated for two weeks prior to the commencement of the experiment. All animals were fed with standard rodent pellet and cool clean water *ad libitum*. The experiment was conducted according to the institutional animal care protocols at the Rivers State University, Nigeria and followed approved guidelines for the ethical treatment of experimental animals.

2.3 Experimental Design and Procedure

Twenty eight adult female Sprague-Dawley rats were assigned to seven groups (A-G) of 4 (four) rats each. Group (A) received neither Cypermethrin nor lycopene and so acted as control, Group B received Cypermethrin Emulsifiable Concentrate (EC) diluted to 30 mg/kg/bw with canola oil. Group (C and D) 5,000 and 10,000 mg/kg/bw/day of processed *Solanum lycopersicum* dissolved in distilled water, Group (E and F) 5,000 and 10,000 mg/kg/bw/day of blended *Solanum lycopersicum*. Group G received 10 mg/kg/bw/day of pure lycopene capsule. All the groups were exposed to their treatment by oral gavage for 70 days. All animals were observed daily for behavioral changes; signs of intoxication, mortality, morbidity as well as food and water intake. Animals were weighed twice a week and the average weight per week recorded to the nearest 0.01 g.

2.3.1 Sperm morphology assay

Semen samples were drawn from the caudal epididymis with a syringe and placed on a clean glass slide. A drop of freshly prepared eosin-Y was added to make a thin smear. Each slide was examined under the microscope for morphological abnormalities. This was observed with a digital microscope Biosphere miller B with an imageprocessor, DN-2 microscopy image processing software at X40 magnification. A total of 100 spermatozoa were observed and scored for abnormalities. The sperm abnormalities were classified under head and tail abnormalities.

2.3.2 Gonadal and extragonadal sperm reserves

The testes and epididymis were removed, freed of their *tunica albuginea* and weighed. A portion of the testis and epididymis was homogenized separately with sharp pointed scissors in normal (physiological) saline. The suspension was mixed and strained through a double layer of sterile cheese cloth into graduated test tubes. All the samples were covered and stored for 24 hours at 4°C. A dilution was made for counting in Neubauer haemocytometer. All sperm reserves were expressed in billions [21,22].

2.3.3 Determination of hormonal profile

The hormonal concentration was determined using the Randox Monza Laboratories assay kit from Co-Attrim, United Kingdom. The hormones considered include Progesterone, Testosterone, Follicle stimulating Hormone, Estradiol and Luteinizing Hormone [23].

3. RESULTS

3.1 Analysis of Sperm Morphology Biomarker

The effect of oral administration of Cypermethrin and co-administration of Lycopene on spermatozoa morphology is shown in Plate 1a-g. Various sperm abnormalities were observed including double headed sperm, folded tails, folded sperm, cut tail, coiled tail, very short hook sperm, detached head, pin head, wrong angle hook.

Table 1 shows the summary of spermatozoa morphology abnormalities in Sprague-Dawley rats co-administered Cypermethrin and Lycopene.

Group G co-administered pure Lycopene had the lowest sperm head abnormalities of 3.5%, followed by groups C and D co-administered processed *S. lycopersicum* with 8.5%. Groups E and F, co-administered fresh *S. lycopersicum* recorded 12.6% sperm head abnormalities, same as the control. However, group B administered Cypermethrin only had the highest head abnormalities of 44.7%. Similarly, Group G, co-administered pure Lycopene had the lowest percentage of tail abnormalities of 10.1% while Group B had the highest percentage of sperm tail abnormalities of 32.4%.

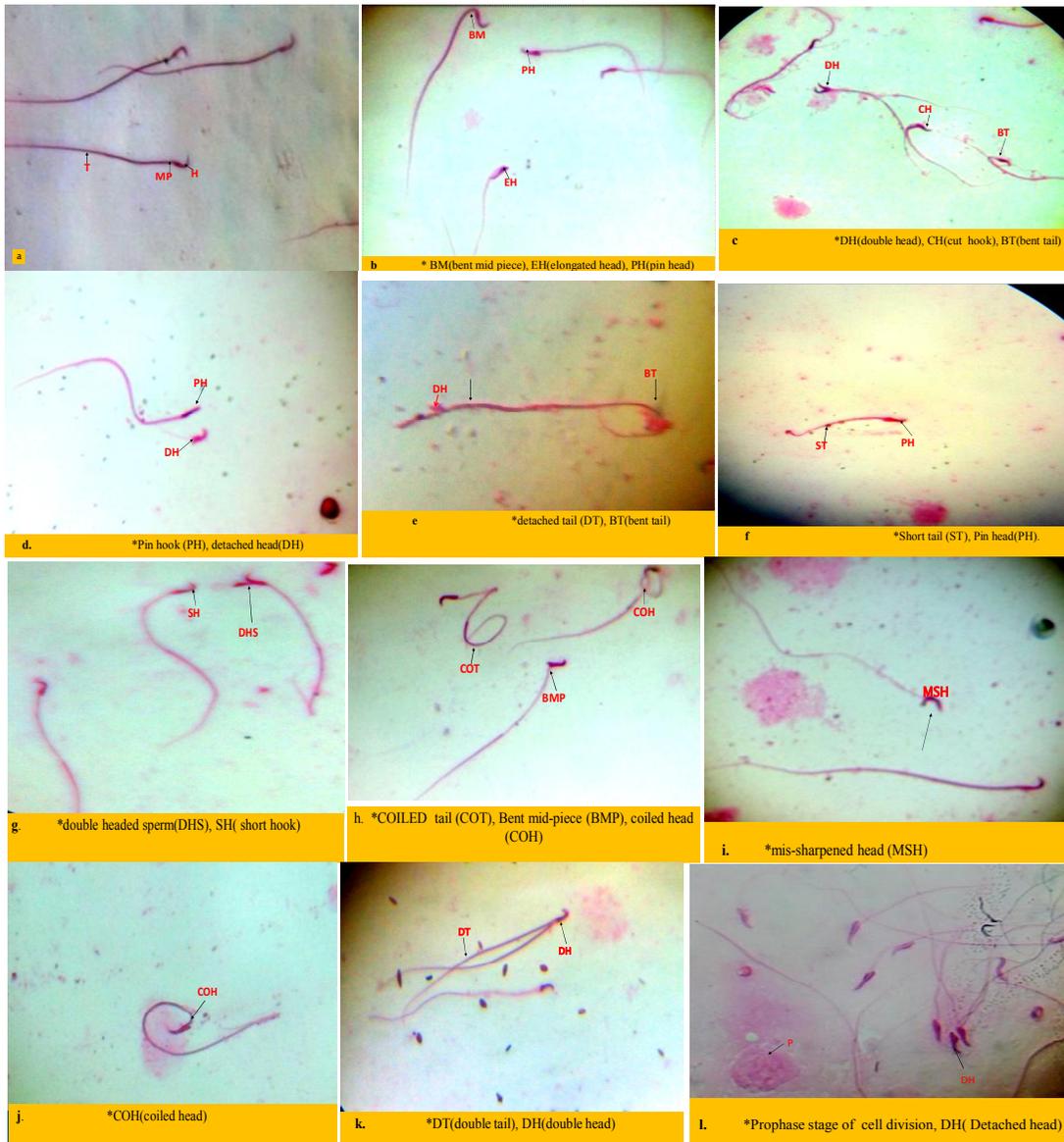


Plate 1. Spermatozoa morphology of rats exposed to CYP and LYP

a= normal sperm morphology from the control group b-l= Spermatozoa morphology abnormalities observed from groups B-G

Table 1. Summary of spermatozoa abnormalities in SD rats co-administered CYP and LYP

Groups	H. ABN	%H. ABN	T. ABN	%T. ABN
A	14.33±0.58 ^c	12.6	20.00±1.00 ^c	12.6
B	50.67±2.08 ^d	44.7	51.33±1.16 ^d	32.4
C	9.67±1.53 ^b	8.5	17.33±0.58 ^{bc}	10.9
D	5.67±1.16 ^a	5	14.33±1.15 ^a	9
E	14.33±0.58 ^c	12.6	21.33±0.57 ^c	13.4
F	14.67±1.53 ^c	12.9	18.33±0.58 ^{bc}	11.6
G	4.00±1.00 ^a	3.5	16.05±1.01 ^b	10.1

*Values are Mean ± SD. Values with different superscripts are significantly different (P< 0.001)
H (Head), T (Tail), ABN (abnormalities)

3.2 Gonadal and Extragonadal Sperm Reserves

The mean gonadal sperm reserves (GSR) and extragonadal reserves (ESR) of the experimental and control groups are presented in Table 2. The mean GSR in the control group and in group administered Cypermethrin only was $4.47 \pm 0.46 \times 10^6$ and $2.42 \pm 0.40 \times 10^6$ respectively was non-significantly ($p > 0.05$) different. In groups C, D, E and F co-administered processed *S. lycopersicum* and fresh *S. lycopersicum* at 5000 and 1000 mg/kg/bw/day, there was no significant difference ($p > 0.05$) between the treatment groups. However, it was noted that group G had the highest value which was significantly different ($p < 0.05$) from other treatment groups.

There was numerically higher sperm storage in the epididymides of groups A, D, E, F, G than B administered Cypermethrin only and group C administered 5000 mg/kg/bw/day of fresh *S. lycopersicum*.

3.3 Effect of Lycopene on Hormonal Profile of Male SD Rats Exposed to Sub-lethal Doses of Cypermethrin

The effect of oral administration of Cypermethrin and Lycopene on the level of hormones in male Sprague-Dawley rats is shown in Fig. 1. A significant decrease ($p < 0.05$) was observed in the concentration of Testosterone from $1.63 \pm 0.15 \mu\text{g/ml}$ in control to $1.23 \pm 0.15 \mu\text{g/ml}$ in group B Cypermethrin only. With the co-administration of processed *S. lycopersicum*, testosterone level increased from $1.23 \pm 0.15 \mu\text{g/ml}$ in group B to $1.73 \pm 0.35 \mu\text{g/ml}$ and $1.90 \pm 0.30 \mu\text{g/ml}$ at 5,000 and 10,000 mg/kg/bw/day respectively. With co-administration of fresh *S. lycopersicum*, the hormone level dropped to $1.43 \pm 0.05 \mu\text{g/ml}$ and $1.50 \pm 0.10 \mu\text{g/ml}$. Both values were not significantly different ($p > 0.05$) from the control. Co-administration of pure Lycopene resulted in significant ($p < 0.001$) increase in the testosterone level higher than that observed in the control (Fig. 1a).

There was no significant difference ($p < 0.001$) between the treatment groups in the level of estradiol (E_2). The values obtained ranged from $18.4 \pm 0.12 \text{ pg/ml}$ in group B (administered Cypermethrin only) $19.98 \pm 0.23 \text{ pg/ml}$ in group D (co-administered 10,000 mg/kg/bw/day of processed *S. lycopersicum*) (Fig. 1b).

Luteinizing hormone (LH) in the control group was not significantly different ($p > 0.001$) from the group that received either processed, fresh *S. lycopersicum* or pure Lycopene, but it was significantly ($p < 0.001$) lower in group B (co-administered Cypermethrin) compared with the control (Fig. 1c). Progesterone concentration ranged from $1.65 \pm 0.08 \mu\text{g/ml}$ in the control group to $1.69 \pm 0.25 \mu\text{g/ml}$ in group co-administered processed *S. lycopersicum*. All values observed in treatment groups were significantly ($p < 0.001$) higher than the control (Fig. 1d).

Fig. 1e shows significant difference ($p < 0.001$) in the concentration of Follicle stimulating hormone (FSH) in all treatment groups with values ranging from $0.18 \pm 0.51 \text{ IU/L}$ in the control to $0.12 \pm 0.01 \text{ IU/L}$ in group B, administered Cypermethrin only.

4. DISCUSSION

Sperm morphology is an important criterion in determining semen quality and viability as it is indicative of the functionality of the epididymis during spermatozoa transport following spermiation into latter stages of spermatogenesis. Maturation process and acquisition of motility are post-testicular processes which occur in the epididymis.

Though Regulatory authorities such as Environmental Protection Agency (EPA), World health Organization (WHO) emphasize on the importance of the abnormalities in sperm head and tail in many reproductive toxicity studies [24,25], there is dearth of information on Cypermethrin effect on sperm cell morphology. In this study, several abnormalities in sperm cells especially in group B administered Cypermethrin only were observed (Plate 1b-g) indicating that Cypermethrin-induced reproductive stress, decreased sperm concentration, motility and normal sperm morphology. Supporting this finding [26] reported the proportion of abnormal sperm cells increasing with increasing doses of Cypermethrin. In the light of this, the result of this study reveals that *Solanum lycopersicum* and pure Lycopene significantly ($p < 0.01$) reduced the number of abnormal sperm and so can ameliorate the effect of Cypermethrin-induced reproductive stress. Gonadal sperm reserves is a reflection or tool for the assessment of sperm production. It also indicates the impact of an external toxicant, age and perhaps the amount of degeneration that occurs during spermatogenesis while extragonadal reserves

shows the efficiency of the storage potential of the epididymides indicating the concentration of matured spermatozoa that has acquired motility while passing through the epididymides. From the study there was reduction in the gonadal and extragonadal sperm reserves when compared to the values observed with the co-administration of *Solanum lycopersicum* and pure Lycopene indicating the effective role of Lycopene in spermatogenesis.

Concise efforts have been channeled toward the study of the adverse effect of Endocrine disrupting chemicals with anti- androgenic activity [5,16,17,19,27]. Pesticides as well as some insecticides that exhibit anti- androgenic activity may be responsible for the increased incidence of male and female infertility, sexual disorders including low sperm count, reduced sperm motility and viability, abnormal sperm cell morphology (Table 1). Many of the chemicals in the environment are endocrine disruptors and act in antagonism, binding to the androgen receptors and mimic vital hormones thereby preventing the transcription of androgen-dependent genes. Reduction in hormones especially androgens, including testosterone inhibit sexual development and maturation as well as, impair optimal reproductive organ functions during spermatogenesis. The findings in this study agree with previous reports on several pyrethroid pesticides [17,28].

Hormones play a vital role in the maintenance of reproductive functions. Testosterone (TET), which is the main androgen, is responsible for the initiation and maintenance of spermatogenesis in mammals while the Luteinizing Hormone (LH) stimulates the release of testosterone from the Leydig cells [13,15,29], therefore its reduction impairs the process of spermatogenesis leading to fewer spermatogenic

elements and thus lower concentration of spermatozoa [1].

The Follicle Stimulating Hormone (FSH) regulates the production of spermatozoa in Sertoli cells. Pesticides and other endocrine disrupting chemicals disrupt the normal functions of the Hypothalamic-Pituitary-gonadal axis leading to reduction in androgens and increase in Estrogens with the net effect of feminization of males and causing infertility in both males and female mammals.

In this study, administration of Cypermethrin only as in group B resulted in a significant ($p < 0.001$) reduction in the concentration of Testosterone, Progesterone, Follicle Stimulating and Luteinizing hormone. The mechanism of action of Cypermethrin is mimicking the action of natural pyrethrins, which are anti-androgenic and the disruption of the transcription of androgen-dependent genes reflected in the reduction of the production of testosterone. Therefore, the result of emanating from the groups administered Cypermethrin alone in this study, agrees with the study of [30] who reported a competitive interaction of pyrethroids with human androgen receptors thereby drastically reducing testosterone levels in the exposed individuals.

Worthy of note, however, was the observation that the concentration of androgens including Testosterone, FSH, LH and Progesterone were significantly ($p < 0.001$) elevated with co-administration of *Solanum lycopersicum* at 5000 and 10000 mg/kg/bw/day, as well as, pure Lycopene (Fig. 1). The elevation in concentration indicates the role of Lycopene as an antioxidant in ameliorating the oxidative stress produced by increased release of free radicals by Cypermethrin.

Table 2. Effect of co-administration of CYP and LYP on GSR and ESR in SD rats

Groups	GSR (X10 ⁶)	ESR (X10 ⁶)
A	4.47 ± 0.46	0.79 ± 0.57
B	2.42 ± 0.40	0.22 ± 0.14
C	3.78 ± 0.53	0.38 ± 0.64
D	4.04 ± 0.12	0.63 ± 0.14
E	2.59 ± 0.14	0.55 ± 0.56
F	3.63 ± 2.11	0.65 ± 0.73
G	4.51 ± 0.91	0.60 ± 0.49
F-value	1.92	1.31
SIG	0.21	0.36

*Values are Mean ± SD; GSR =Gonadal Sperm Reserves; ESR= Extragonadal Sperm Reserves

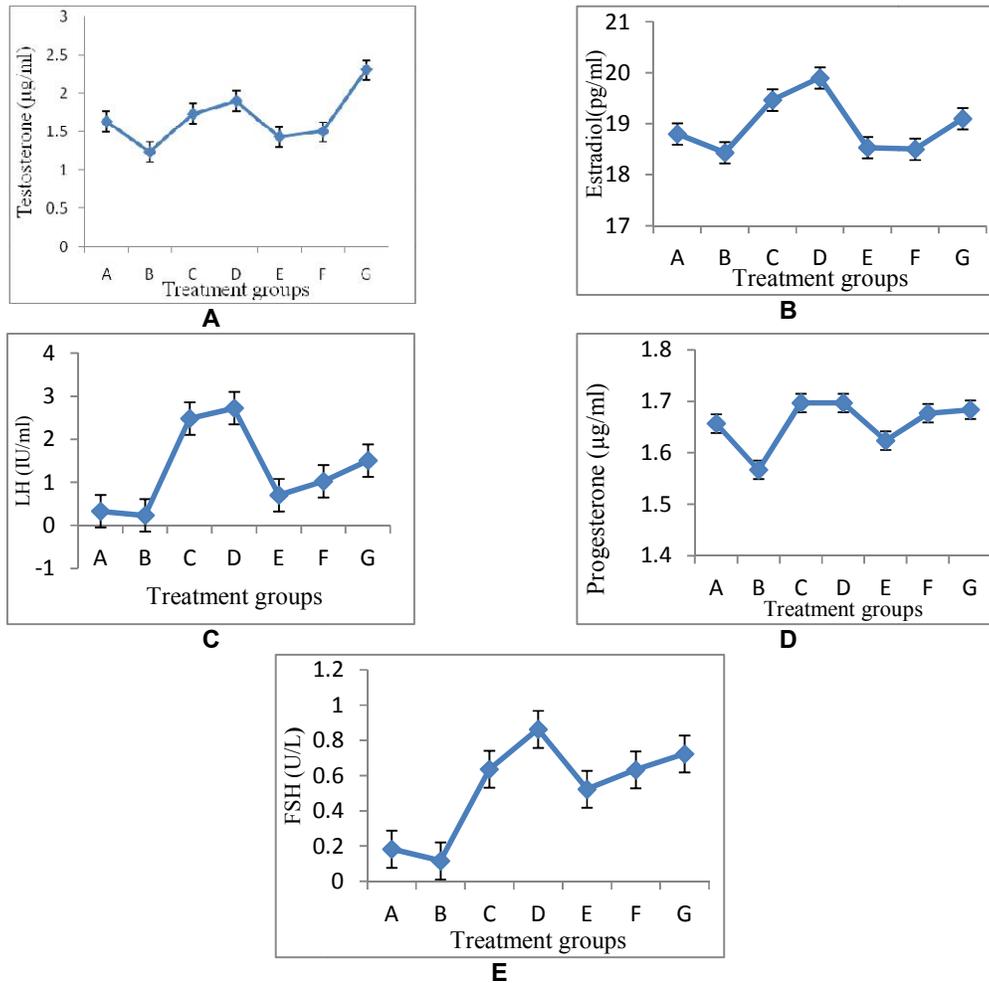


Fig. 1. Hormonal levels in sprague-dawley rats administered cypermethrin and lycopene
 Key: A= Testosterone levels (TET), B= Estradiol levels (E_2), C= Leutinizing hormone (LH), D= Progesterone levels (PROG), E=Follicle Stimulating Hormone (FSH)

5. CONCLUSION

Exposure to Cypermethrin only disrupted the production of all androgens considered, increased the percentage of abnormal spermatozoa, reduced sperm motility, viability and Sperm reserves in this study. However, with the co-administration of lycopene, the levels of androgens increase, percentage of abnormal sperm cell morphology reduced and the sperm reserves increased. Thus in terms of lycopene efficacy, it was concluded that pure lycopene had the highest ameliorative effect followed by processed and fresh fruits of *Solanum lycopersicum* respectively. This findings have important implications in hormonal profile and reproductive risk assessment of those inadvertently exposed to Cypermethrin.

ETHICAL APPROVAL

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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