

Cypermethrin-Induced Anti-Fertility and the Ameliorating Effect of Lycopene Supplementation in Female Rats

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Abstract: *This study aimed at evaluating the potential anti-fertility amelioration of Lycopene co-administered with Cypermethrin (CYP) in female Sprague-Dawley rats. Twenty eight sexually matured female rats were divided into seven groups (A-G). Group A-control, B given CYP only, C and D, given CYP+5,000 and 10,000mg/kg/bw/day of processed *S. lycopersicum*, E and F, CYP+ 5,000 and 10,000mg/kg/bw/day of Fresh *S. lycopersicum*, while G were given 10mg/kg/bw/day of pure Lycopene (LYP) for 70 days. Each exposed female was introduced to unexposed male for mating. Body weight of female rats recorded throughout this period while upon delivery, other fertility indices were recorded. Result showed no significant difference $P > 0.05$ in the maternal weight and a significant difference between the litter size of the control and treatment groups $P < 0.05$. Dams in control group delivered live pups, 25% in grps B-G, 50% in grp D and 0% in grp E. Other female rats later dissected had no pups, dead or alive. This shows that CYP is an endocrine disrupting chemical induced pseudo-pregnancy and fetotoxicity. Lycopene showed no protective effect on Cypermethrin-induced reproductive toxicity in the female rats*

Keywords: *antioxidant, birth weight, fertility, litter size, pyrethroids.*

Date of Submission: 07-03-2018

Date of acceptance: 23-03-2018

I. Introduction

Reproduction is a delicate biological process involving both genetic and environmental factors contributing to its success. The issue of secondary infertility has become a global health issue while researchers around the world seek for solutions. Several reports have strongly linked the exposure of a group of Endocrine disrupting chemicals (EDCs) to the increase in reproductive dysfunction suffered by humans extrapolating from animal models [1,2,3,4,5]. Endocrine disruptors such as pesticides interfere with reproductive hormones even at very low concentrations. Cypermethrin is synthetic pyrethroid widely used as a pesticide in agriculture, forestry, public and animal health programmes [6]. It is one of the EDCs that target the reproductive system by damaging the sperm cells and oocytes, altering the DNA structure, sex ratio, age at puberty and onset of menopause, miscarriages, premature birth and pregnancy outcomes. [7,8,9]. The administration of 10mg Cypermethrin to adult female mice for 4 weeks has been reported to increase the number of dead pups in treated females [10]. The use of plant products to address health issues is increasing rapidly due to the fact that many plants possess antioxidant properties without the attendant side effects. Lycopene is one of the most potent singlet oxygen quencher of all carotenoids. It is abundant in red fruits, tomatoes, apricots, grape fruits, pawpaw, watermelon. Lycopene supplementation studies conducted in both human and animals focused on male reproduction in alleviating lipid peroxidation in male infertility, decrease DNA damage, increased general immunity, sperm quality, count and motility. [4,11,12,13, 14]. There is a paucity of information on the effect of EDCs in female reproductive system. This study was therefore, carried out to investigate the effect of cypermethrin and co-administration of lycopene on fertility and fecundity.

II. 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, Nkpolu- Oroworukwo, Port Harcourt, Rivers State [Coordinates: 4°47'50" N 6° 58'49" E].

2.2 Experimental animals and management

Twenty-eight sexually matured female Sprague-Dawley rats (183.07±24.29) were bred at the Reproductive Physiology and Genetics Animal House from parents obtained from the Department of Biochemistry, University of Port Harcourt, Nigeria. The rats were housed individually in plastic cages under standard conditions (12 hL:12hD,) and acclimated for two weeks prior to the commencement of the experiment. All animals were fed standard rodent pellet and cool clean water *ad libitum*. All experiments were 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 served as control, Group B received Cypermethrin Emulsifiable Concentrate (EC) diluted to 30 mg/kg/bw/day with canola oil. Group (C and D) were administered 5,000 and 10,000 mg/kg/bw/day of processed *Solanum lycopersicum* dissolved in distilled water as well as 30mg/kg/bw/day of Cypermethrin. Group (E and F) 5,000 and 10,000mg/kg/bw/day of fresh *Solanum lycopersicum*, as well as, 30mg/kg/bw/day. Group G received 10mg/kg/bw/day of pure Lycopene. All treatments were administered 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

2.4 Data collection

Data was collected on body weight, litter size, percent pregnant females, percent delivery and litter weight, percent fetal loss.

2.5 Mating Procedure

At the end of the seventy (70) days administration, twenty-eight (28) females were introduced to twenty-eight (28) males of the same age and species that were not previously exposed to a pesticide. They were paired in the ratio 1male: 1female for mating purposes.

Twenty-Four hours after the introduction of the females to the males, vaginal swap was taken and examined under a microscope to ensure that mating had taken place. The gestation length was fixed at 21-24 days, after which female rats that did not deliver within the period were opened up. Upon delivery, the gestation length, body weight, litter size, litter weight was determined and the pups were checked for deformities.

2.6 Statistical analysis

Data were subjected to analysis of variance using the SPSS 20 software. All data in tables and figures are presented as the mean±S.D. and the significance level was set at $p < 0.05$.

III. Results

The mean weight of all the rats in each treatment group throughout the period of experiment is shown in Figure 1 (a-g.) There was a gradual increase in the body weight of all the animals from week 1 to week 9. Upon the introduction of the male at week 9 and following mating, there appeared to be a sharp increase in the body weight which was sustained from the tenth week to the termination of the experiment. However, the weights of all animals in the treatment group did not statistically differ from that of the control ($P > 0.05$).

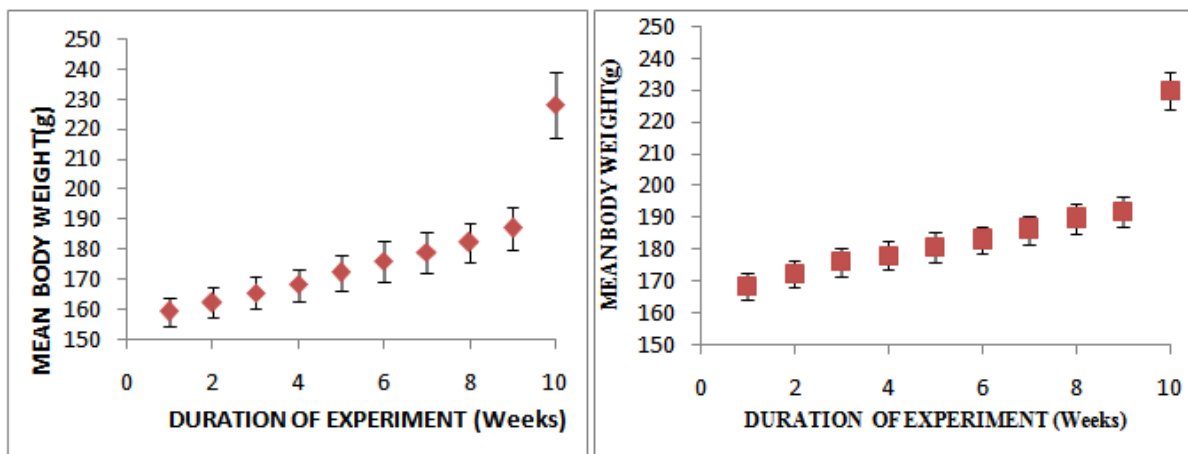


Fig.1a: Body weights of Animals in the control group (grp A) Fig 1b: Body weight of Animals administered Cypermethrin only (grp B)

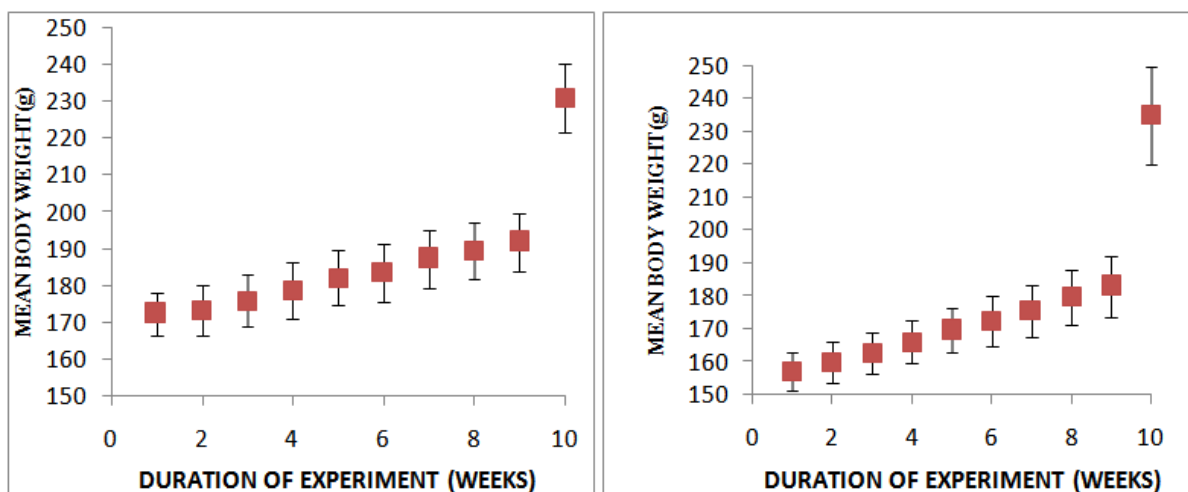


Fig 1c: Body weight of animals administered Cypermethrin & 5,000mg/kg/bw/day of *P. Solanum lycopersicum* (grp C) 10,000mg/kg/bw/day of *P. Solanum lycopersicum* (grp D)

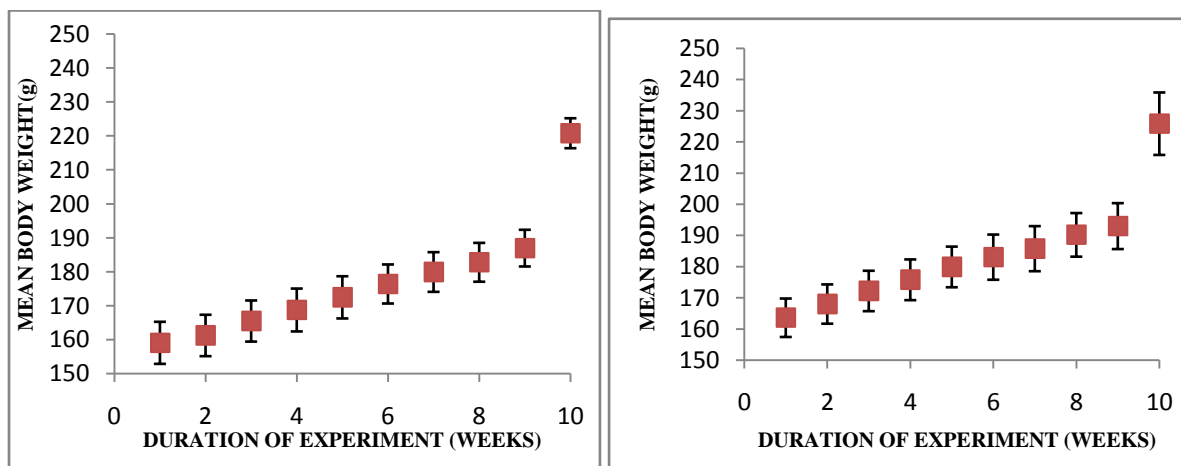


Fig. 1e: Body weight of animals administered Cypermethrin & 5,000mg/kg/bw/day of *F. Solanum lycopersicum* (grp E) 10,000mg/kg/bw/day of *F. Solanum lycopersicum* (grp F)

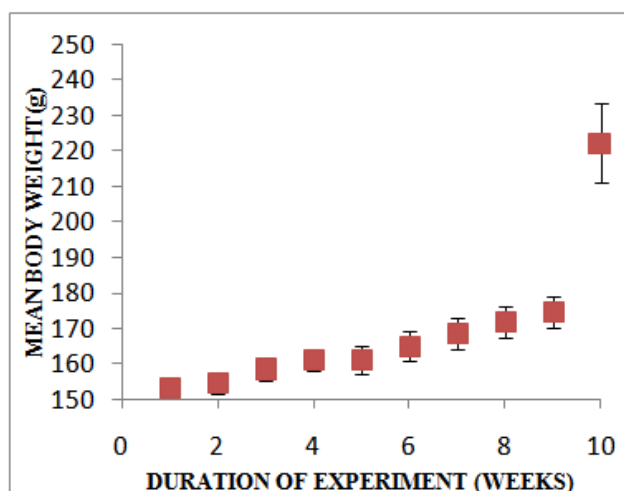


Fig. 1g: Body weight of animals administered Cypermethrin & 10mg/kg/bw/day of Pure Lycopene (grp G)

Fig.1(a-g):Effect of co-administration of Cypermethrin and Lycopene on the bodyweight of female Sprague-Dawley rats

Fig. 2(a-c) shows the F₁ generation of the pregnant females in the control and treatment groups. All pups in the treatment group appeared normal with no morphological abnormalities when compared with those in the control group. Fig.2. (d-g) shows some of the female rats especially from group E that showed all signs of pregnancy but did not deliver after the gestation period and when dissected 10 days after the expiration of the gestation period no fetus dead or alive were found.



**Fig 2a.PUPS from control group of exposed female rats, Litter size 6
group D of exposed female rats , Litter size 4**



Fig 2b: PUPS from



Fig 2c: PUPS from group C of exposed female rats , Litter size 2



Fig 2d: Exposed Female Rat (grp B) whose pregnancy exceeded 22 days +10, when opened no pups were found dead or alive (see arrows)



Fig 2e: Exposed Female Rat (grp C & D) whose pregnancy exceeded 22 days +10 when opened no pups were found dead or alive



Fig 2f: Exposed Female Rat (grp C & D) whose pregnancy exceeded 22 days +10 when opened no pups were found dead or alive



Fig 2g: Exposed Female Rat (B-G) whose pregnancy exceeded 22 days +10 when opened no pups were found dead or alive

Fig.2(a-g):Effect of co-administration of Cypermethrin and Lycopene on fertility and fecundity of female Sprague-Dawley rats

Table 1 shows the initial, final and percentage change in body weight of animals in the control and treatment groups. The percentage change in body weight was highest in group D and G with values (31.15% and 30.66% respectively), not significantly different from group A (control) with 30.3%. There was a systematic decrease in body weight in groups B, C, E and F without statistical significance (see Table 1). The numbers of pregnant females are also presented in Table 1. In group A, the rats experienced 100% fertility with a total litter size of 24 and a litter weight of 134.88g. Groups B, C, F and G exhibited 25% fertility with total litter sizes of 4, 2, 6, and 3 and litter weights of 22.54, 9.71, 34.57, and 16.56g respectively. Group D had 50% fertility with a total litter size of 4 and a litter weight of 34.57g. None of the female rats in group E conceived, thereby having 0% fertility and none delivered.

Table 1: Fertility indices of female Sprague_Dawley rats co-administered Cypermethrin and Lycopene. *values are in mean±SD. * key: NFB(No of female bred), DEL(delivery), LS(litter size), TLW(total litter

| GR PS | initial weight (g) | final weight (g) | % Δ in weight | N F B | % F/ PF | % DEL | LS | TLW | % Pseudo-pregnancy |
|-------|--------------------|------------------|---------------|-------|---------|-------|----|-------------|--------------------|
| A | 172.64±9.3 | 228.89±12.2 | 30.30 | 4 | 100 | 100 | 24 | 134.88±0.64 | 0 |
| B | 168.58±8.1 | 223.04±27.1 | 24.42 | 4 | 25 | 25 | 4 | 22.54±0.43 | 75 |
| C | 172.23±11.6 | 226.91±21.6 | 24.14 | 4 | 25 | 25 | 2 | 9.71±0.74 | 75 |
| D | 157.23±11.6 | 228.36±17.5 | 31.15 | 4 | 50 | 50 | 4 | 23.46±0.43 | 50 |
| E | 159.09±12.4 | 225.71±8.2 | 29.51 | 4 | 0 | 0 | 0 | 0 | 100 |
| F | 163.61±12.3 | 226.43±17.3 | 27.74 | 4 | 25 | 25 | 6 | 34.57±0.81 | 75 |
| G | 153.24±5.4 | 220.98±18.6 | 30.66 | 4 | 25 | 25 | 3 | 16.56±0.43 | 75 |

weight)

A=control; B administered cypermethrin only; C= CYP+5000mg/kg/bw/day processed *S.lycopersicum*; D =CYP+10,000mg/kg/bw/day processed *S.lycopersicum*; E = CYP+5000mg/kg/bw/day fresh *S.lycopersicum*; F=CYP+10,000mg/kg/bw/day fresh *S.lycopersicum*; G= Cypermethrin + pure lycopene@10mg/kg/bw/day.

IV. Discussion

All the rats in the treatment group and control survived with no visible signs of changes in appearance throughout the period of the study. However, they were restless, appeared to be distressed, gasping for air, itching of paws following administration of Cypermethrin only in group B. This supports the earlier finding of toxic effects of Cypermethrin when exposed to either target or non-target organisms [15]. The onset of signs of poisoning was characterized by salivation, tremors and increased startle response in experimental animals [16].

The maternal weight and litter weight of pups exposed to Cypermethrin and co-administered Lycopene at concentrations used in this study showed no significant difference between the treated and control groups. This is indicative that the Cypermethrin at the dose used may not be a systemic toxin since body weight loss is a demonstration of accumulated stress exposure. However, the litter size significantly decreased among the treatment groups as compared to the control. This collaborates with the finding of [17] who observed reduced litter size in dams exposed to Cypermethrin.

The percentage decrease in fertility as compared to control (see Table 1) may be a confirmation that Cypermethrin is an endocrine disrupting chemical with ability to distort hormonal profile of the female rats resulting in prolonged estrus, inhibits implantation to some extent. Cypermethrin has been reported to possess the ability to cross the placenta barrier within the first 10 days of conception, distorting the physiological functioning of the fetuses. This can affect the litter weight and size where its toxicity permits the growth and differentiation of the fetuses. This is similar to the findings of [3,10] where treated males and female mice with cypermethrin 10mg/kg/day prior to mating experienced a deleterious effect on neonatal and juvenile behavior in the offsprings, decrease in fertility, litter size, pup viability [17,18]. Moreover, treatment of rats with deltamethrin and 10mg/kg/bw/day of Cypermethrin during gestation produced embryonic death and an increase in the number of dead pups [10] which was also observed in female rats exposed to Cypermethrin and co-administered Lycopene. In this study, all the female rats exposed to Cypermethrin and co-administered 5,000mg/kg/bw/day of fresh *Solanum lycopersicum* and those shown in Fig d-f appeared to be pregnant but when

opened, no pups dead or alive were found. This is an indication of the induction of a state of Pseudo-pregnancy. In conclusion, despite the co-administration of lycopene to ameliorate cypermethrin-induced reproductivetoxicity in females, there appeared to be no improvement in the fertility indices and fecundity of rats used in this study., Perhaps, Cypermethrin is also fetotoxic as none of the pups delivered lived up to 10 days post natal. Further studies will be necessary to determine the possible effect of pyrethroids on the reproductive system of the females and an urgent search for an indigenous antioxidant that could help ameliorate the toxic effect of these pyrethroids.

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AdetutuOlubunmi Godwin-Obulor "Cypermethrin-Induced Anti-Fertility and the Ameliorating Effect of Lycopene Supplementation in Female Rats." *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)* 11.3 (2018): 57-63.