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Endocrine Effect of Beta Cypermethrin on Female Albino Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author VCO designed and carried out the study, performed the statistical analysis and wrote the manuscript. Author HDK supervised the study, managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Pyrethroids are among the common pesticides frequently used in agriculture and in homes, and have been named among the endocrine disrupting chemicals (EDCs). Cypermethrin, a broad spectrum insecticide being extensively used for pest management and animal husbandry practices is a type II pyrethroid. Previous studies have shown that cypermethrin has adverse effect on female reproduction with no adequate information on its effect on sex hormones. The present study was highlighted to evaluate the effects of Beta cypermethrin, β-cyp, on the estrous cycle and the serum sex hormones of adult female albino rats. Twenty female albino rats were randomly assigned into four groups and they were treated by oral gavage with β-cyp, at doses of 0, 15, 30 and 50 mg/kg body weight for fourteen consecutive days. The estrous cycle was determined; and

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hormonal assays for estrogen, progesterone, LH and FSH were done using Enzyme-Linked Immunosorbent Assay (ELISA). There was no significant ($p>0.05$) change in the serum sex hormones and the estrous cycle in treated groups relative to the control. This study demonstrates that the endocrine effect of β-cyp on female albino rats is not significant. However, the altered levels of hormones recorded in this study suggests that β-cyp, could be a potential endocrine disrupting chemical (EDC) to those exposed indiscriminately to it.

Keywords: Beta cypermethrin; endocrine disrupting; estrous cycle; estrogen; progesterone; LH; FSH.

1. INTRODUCTION

The widespread use of pesticides worldwide for public health protection and agricultural pest control has resulted in severe environmental pollution and health hazards. Exposure to insecticides has been found to cause problems and outbreak of diseases in animals and human [1]. Pyrethroids commonly used as insecticides for both household and agricultural applications have been implicated in endocrine disruption in both male and female mammals [2,3,4,5].

Endocrine disrupting chemicals (EDCs) such as several pesticides are compounds that alter the normal functioning of the endocrine system of both animals and humans. EDCs act mainly by interfering with natural hormones because of their strong potential to bind to estrogen or androgen receptors [6]. Specifically, EDCs can bind to and activate various hormone receptors and then mimic the natural hormone's action (agonist action), or can also bind to these receptors without activating them. This antagonist action blocks the receptors and inhibits their action [7]. Furthermore, EDCs may interfere with the synthesis, transport, metabolism and elimination of hormones, thereby decreasing the concentration of natural hormones [7].

Beta-Cypermethrin, a type II pyrethroid insecticide, is being extensively used for pest management and animal husbandry practices due to its high efficacy against target species and relatively low mammalian toxicity [8]. Consistent with its lipophilic nature, it has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries and brains [9] leading to oxidative damage as a result of increase in reactive oxygen species [10,11]. β-cyp has been found to reduce the expression of Androgen receptor (AR), sperm production, sperm quality and testosterone levels in male mice and rats [12,8]. The Toxicity of cypermethrin to female reproductive system of mammals (rats, mice and rabbits) has been established from various studies [13,14,15,16,17]. However, there is paucity of information on the effect of this pesticide on the female sex hormones and its capability to cause endocrine disruption.

Female reproductive system especially the female pituitary-gonadal axis can be altered by exposure to pesticides and other toxic chemicals. The aim of the present study is to evaluate the effects of β-cyp on the estrous cycle and the serum sex hormones of adult female albino rats.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Beta cypermethrin (a mixture of the alpha and theta forms of the insecticide) at 95.8% purity was purchased from Haihang Industry Company, Limited, China as white to light yellow crystalline powder with CAS No: 52315-07-8 and Batch No: 20140517. The desired doses were prepared in olive oil which was purchased from the supermarket. All other chemicals were of the finest analytical grade.

2.2 Animals and Treatment

Twenty virgin mature female albino rats weighing an average of 170 g were procured from the Animal House of Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria and used for the study. The rats were acclimatized for two (2) weeks before commencing the study. They were screened and observed to exhibit regular estrous cycle. They were fed *ad libitum* with commercially sourced feed (Top Feeds Nigeria Limited) containing 16 % crude protein, and supplied with clean drinking water all through the study. Rats were randomly divided into four groups (Groups 1-4) of five animals each; with group 1 serving as the control. The rats in groups 1, 2, 3 and 4 were treated by oral gavage at the doses of 0, 15, 30 and 50 mg/kg body wt. respectively for fourteen consecutive days. Control animals (dose = 0 mg/kg) received 0.5 ml/kg of olive oil. All

administrations were started in the estrus phase. Animal's weight was taken daily and the dose adjusted accordingly.

2.3 Sample Collection

The vaginal smear was collected daily in the morning using the pipette smear technique. The tip of a Pasteur pipette containing few drops of normal saline (0.9% NaCl) was inserted into each rat's vagina. The fluid was used to flush cells from the vaginal lining after which the resulting suspension was placed on a clean glass slide and examined under light microscope. The phases of estrous cycle were confirmed depending on the different characteristic cells.

The animals were anaesthetized under chloroform at the end of the experiment. Blood samples were collected from the retro orbital plexuses by inserting a microhaematocrit capillary tube into the medial canthus of the eye until the bony orbit was contacted; the tube was gently rotated and withdrawn slightly to allow the blood to flow through the capillary tube into the sterile plain bottles. The Collected blood was allowed to stand for 30-45 min in order to coagulate and then centrifuged for 15 min at 3000 rev/min to obtain the serum for hormone analysis. The serum was then tipped into a separate vial, placed in microcentrifuge tubes, capped and stored at -20°C until analysis. The serum was later subjected to hormonal assay by ELISA method for assessment of estrogen, progesterone, LH and FSH levels.

2.4 Statistical Analysis

Statistical analysis was done using SPSS 21. Values were expressed as mean ± SEM and data were analysed using the one-way ANOVA followed by the Tukey post-test. The significance level was set at $p<0.05$.

3. RESULTS

Two out of the five animals in group 4 died in the course of the treatment after showing marked weight loss and persistent diestrus hence the group was not included in the analyses. The remaining animals in that group recorded weight loss and persistent diestrus.

Although there was no significant difference ($p>0.05$) in the phases of the estrous cycle of treated groups when compared with control, the result indicated that the proestrus, estrus and metestrus phases of the cycle decreased across the treated groups when compared with the control while the diestrus phase increased across the treated groups when compared with the control (Fig. 1).

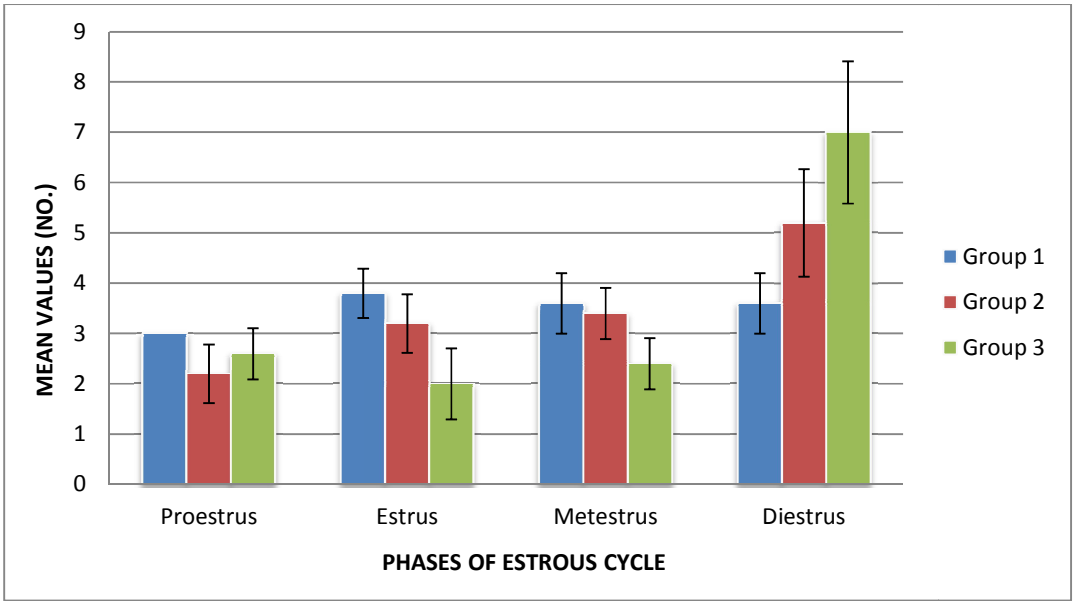

Fig. 1. Effect of beta-cypermethrin on estrous cycle

Furthermore, the results showed that beta-cypermethrin had no significant effect on the female steroid hormones - estrogen, progesterone, LH and FSH ($p>0.05$). However, Estrogen, LH and FSH levels decreased across the treated groups when compared with the control while the progesterone level increased across the treated groups when compared with the control (Figs. 2-5). Both progesterone and estrogen levels increased and decreased respectively in a dose dependent manner across the groups.

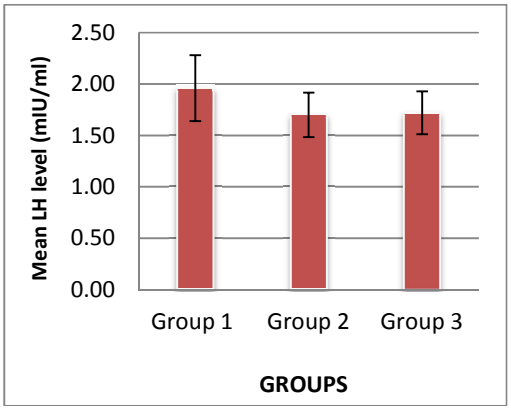

Fig. 2. Effect of beta-cypermethrin on mean LH level

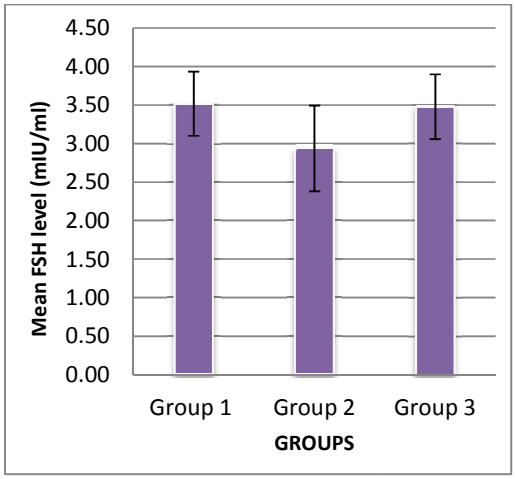

Fig. 3. Effect of beta-cypermethrin on mean FSH level

4. DISCUSSION

Sex hormones in females are known to regulate the estrous cycle as well as other reproductive functions and characteristics. These sex hormones (estrogen and progesterone) are primarily produced in the ovaries under the influence of FSH and LH from the anterior pituitary through a feedback mechanism. Some chemicals such as pesticides that disrupt hormonal synthesis, secretion or function are often called endocrine disruptors. Pyrethroids are known to act as endocrine disruptors as they can interact competitively with androgen receptors and sex hormone binding globulin (SHBG) causing disruption of the endocrine system or by mimicking the effect of the female hormone, estrogen [18,19]. Wissem et al. [7] has shown that Endocrine disrupting chemicals may interfere with the synthesis, transport, metabolism and elimination of hormones, thereby decreasing the concentration of natural hormones.

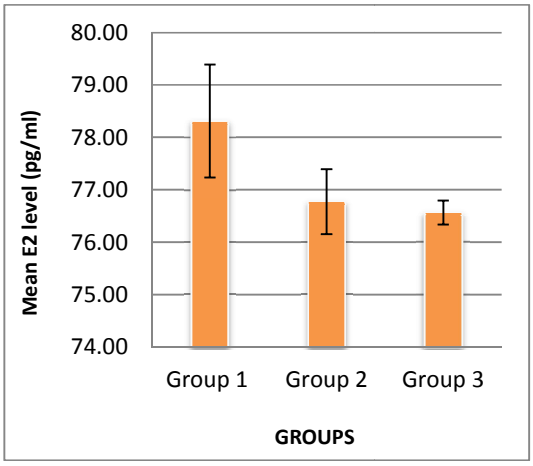

Fig. 4. Effect of beta-cypermethrin on mean estrogen (E2) level

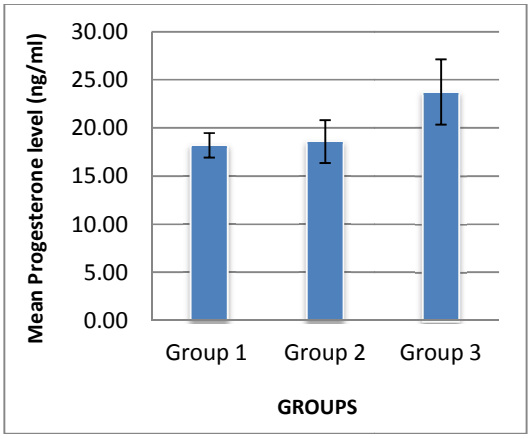

Fig. 5. Effect of beta-cypermethrin on mean progesterone level

The result from this study indicated a non significant decrease in serum LH, FSH and estrogen levels and a non significant increase in serum progesterone level in adult female rats when treated with beta cypermethrin. Since all the phases of the estrous cycle are controlled by the shifting balance of the steroid hormones, it was not contradicting to observe that the phases of the estrous cycle were affected though the difference from the control was not significant. In agreement with our findings, Elbetieha et al. [20] reported that treatment of male rats with cypermethrin decreased the FSH, LH and testosterone levels.

From the study, β-cyp, a type II pyrethroid, may be responsible for the decrease in the estrogen level since pyrethroids have affinity for androgen or estrogen receptors. One of the pathways in the synthesis of estrogen in the ovaries is the conversion of androgens (testosterone and androstenedione) into estrogens by enzyme aromatase in the granulosa cells; activity stimulated by FSH [21]. This is in line with the findings of Trif et al. [22] who reported that the decrease in estrogen level could be the consequence of decrease in FSH concentration as a result of chromium exposure which led to decrease of the aromatase in the granulosa cells and androgen transformation into estrogen. According to Asuquo et al. [23], administration of *Spondias mombin* to non pregnant female rats caused estrogen inhibition. This reduction in the estrogen level in this study invariably affected other hormones.

At proestrus, under the influence of FSH from the anterior pituitary, maturation of the ovarian follicle and ovum occur. The mature follicle secretes increased amount of estrogen which in turn through a negative feedback mechanism acts on the pituitary to shut down FSH production and through positive feedback mechanism, cause a release of LH from the pituitary. This large surge of LH and the residual FSH causes ovulation at estrus [24]. The cause of this abrupt surge in LH is not known; however, it has been suggested that estrogen at this point in the cycle (estrus) has a peculiar positive feedback effect of stimulating pituitary secretion of LH and to a lesser extent FSH [21]. In view of these, it is therefore suggested that when the estrogen level is disrupted, the feedback mechanism may also be impaired as reflected in the serum level of the gonadotrophic hormones (FSH and LH).

These changes in the hormones also to a greater extent affected the phases of estrous observed in this study. The dose dependent increase in diestrus phases in the treated groups relative to the control as well as the persistent diestrus observed in the survivors in group 4 could be as a result of short supply of LH. According to [21], if the preovulatory surge of LH is not of sufficient magnitude, ovulation will not occur.

5. CONCLUSION

The study demonstrated that there was no significant difference in the phases of estrous cycle and the sex hormones of adult female albino rats treated with β-cyp. However, the altered levels of hormones recorded in this study suggests that β-cyp, could be a potential endocrine disrupting chemical to females exposed indiscriminately to this pesticide.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Table 1. Effect of beta cypermethrin on serum sex hormones

Serum sex hormones	Groups		
	Group 1	Group 2	Group 3
LH (mIU/ml)	1.96±0.32	1.70±0.22	1.72±0.21
FSH (mIU/ml)	3.52±0.42	2.94±0.56	3.48±0.42
Estrogen (pg/ml)	78.31±1.08	76.78±0.62	76.57±0.23
Progesterone (ng/ml)	18.21±1.27	18.60±2.22	23.75±3.38

Values are given as mean ± SEM for each group. indicate significant difference ($p<0.05$) compared to group 1 (control group). P: statistical level of significance as determined by one-way ANOVA*

Table 2. Effect of beta cypermethrin on estrous cycle

Phases of estrous cycle	Groups		
	Group 1	Group 2	Group 3
No. of Proestrus	3.00±0.00	2.20±0.58	2.60±0.51
No. of Estrus	3.80±0.49	3.20±0.58	2.00±0.71
No. of Metestrus	3.60±0.60	3.40±0.51	2.40±0.51
No. of Diestrus	3.60±0.60	5.20±1.07	7.00±1.41

Values are given as mean ± SEM for each group. indicate significant difference ($p<0.05$) compared to group 1 (control group). P: statistical level of significance as determined by one-way ANOVA*

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