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Effects of Beta Cypermethrin Exposure on Male F1 Generation of Albino Rats during Perinatal Development

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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, wrote the manuscript, managed the analyses of the study and literature searches. Author GOA supervised the study. Both authors read and approved the final manuscript.

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ABSTRACT

Cypermethrin, a type II pyrethroid, widely used for pest management causes environmental pollution and health hazards. Previous studies have shown that cypermethrin has teratogenic effect on rat foeti born to exposed dam or buck with no information on its effect on their reproductive parameters when assessed at maturity. The present study was therefore carried out to evaluate the reproductive effect of perinatal beta cypermethrin (β-cyp) exposure on male F1 generation albino rat. Fifteen pregnant animals (Day 0 = day of mating, average body-weight = 190 g) were randomly divided into 3 groups. Group I (Control) received 0.5ml olive oil, Group II(15 mg/kg β-cyp) and Group III (30 mg/kg β-cyp) by oral gavage from gestational day (GD) 1 – post natal day (PND) 20. On PND 21, the pups were weaned and bred to 12 weeks of age (maturity). At maturity, 5 males were randomly taken from each group. The animals were anaesthetized, testes were collected for histopathological study and caudal epididymides used for determination of sperm characteristics. Blood was collected for hormonal assay (testosterone) using Enzyme Immunoassay.

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Histopathological study of the testes was conducted. β-cyp had a dose-dependent non-significant decrease ($p>0.05$) on mean testicular and body weights, testosterone level and sperm count relative to the control. β-cyp had no significant effect ($p>0.05$) on sperm cell characteristics. No abnormality was observed in the testicular sections of exposed F1 generation rats. It is therefore concluded that reproductive effect of perinatal β-cyp exposure of male F1 generation rats assessed at maturity is not significant. However, the dose-dependent decrease in the male reproductive parameters recorded in this study suggests that β-cyp, can impair spermatogenesis in male offspring exposed during the perinatal period.

Keywords: Beta cypermethrin; pyrethroid; testosterone; perinatal; male; sperm.

1. INTRODUCTION

Pesticides are used in agriculture and public health to control pests and vectors of disease. However, they cause hazardous effects at different levels to non target species. Pesticide toxicity has become a major reason for morbidity and mortality in developing countries. Extrapolations of data from developing countries suggest that pesticide poisoning causes more deaths than infectious diseases [1-2]. Pesticide poisoning has been implicated in reproductive toxicity in both males and females.

Animal studies on pyrethroid pesticides such as cypermethrin, deltamethrin and fenvalerate have demonstrated insecticide-related reproductive adverse effects both in male, female and foetal organisms [3-8]. Other studies also showed growth retardation and/or foetal loss due to exposure of pregnant animals to pyrethroids [9-10]. Pyrethroids have also been reported to cause developmental neurotoxicity [11].

Previous studies on the teratogenic effect of pyrethroids in rodents are limited to the maternal exposure during the period of gestation [10,12-13]. However, the present work, using beta cypermethrin as an example, extended the maternal exposure to the period of lactation after which the F1 generation was raised to maturity before collection of samples for analyses.

The aim of the present study, therefore, is to evaluate the reproductive effect of beta-cypermethrin exposure on male F1 generation albino rat during the perinatal development.

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

Fifteen mature female albino rats weighing an average of 190g, procured from the Animal House of the Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria were used for the study. The rats were acclimatized for two (2) weeks before commencing the study. They were fed *ad libitum* with commercially sourced feed (Top Feeds Nigeria Limited) and supplied with clean drinking water all through the study. The rats were handled humanely throughout the study in line with the ethics for animal handling and research as stipulated by NHMRC [14].

Following acclimatization, one female was paired with a male in a cage overnight. On the following morning, vaginal washings (lavage) were taken for each female using a dropping pipette containing few drops of normal saline. The wash solution was spread on a clean microscope slide, stained with Eosin blue dye and viewed under the light microscope. The presence of sperm cells in the smear indicated insemination and designated day zero (0) of gestation (Assayed et al., [12]. Thereafter, they were then grouped into 3, each group housed in a cage. Group I (Control) received 0.5 ml olive oil, Group II (15 mg/kg β-cyp) and Group III (30 mg/kg β-cyp,) by oral gavage from gestational day (GD) 1 to postnatal day (PND) 20. Animal's weight was taken daily and the dose adjusted accordingly.

2.3 Sample Collection

On PND 21, the rat pups were weaned and bred for another 9 weeks in order to attain maturity.

At maturity, 5 males were randomly taken from each group. Thereafter, the animals were anaesthetized under chloroform. Blood samples were collected from the retro orbital plexuses using the microhaematocrit capillary tube. 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℃ until analysis. The serum was later subjected to the hormonal assay for assessment of Testosterone levels using Accu-bind ELISA kits (Testosterone Test System Product Code: 3725-300) from Monobind Inc. Lake Forest, CA 92630, USA.

The testes were carefully removed, cleared of adhering tissues and weighed. The caudal epididymides were homogenized and used for determination of sperm characteristics. The testes were fixed in Bouin's Solution, and then processed as described by Lillie [15], embedded in paraffin, sectioned at 4-5 µm and stained by Haematoxylin and Eosin blue.

2.4 Statistical Analysis

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

3. RESULTS

3.1 Mean Body Weights and Testicular Weights

There was non-significant decrease ($p>0.05$) in the mean body weights of beta-cypermethrin exposed F1 generation rats relative to that of the control (Fig. 1). Beta cypermethrin caused a non-significant decrease ($p>0.05$) in the left and right testicular weights of exposed F1 generation rats when compared with the control (Fig. 2 and 3)

3.2 Hormonal Level

Non-significant reduction ($p>0.05$) in the testosterone levels of beta-cypermethrin exposed F1 generation rats relative to that of the control (Fig. 4) was observed.

3.3 Sperm Count and Sperm Cell Characteristics

Perinatal beta cypermethrin exposure caused a non-significant ($p>0.05$) decrease in sperm cell count of F1 generation rats relative to the control (Fig. 5). There was no significant ($p>0.05$) effect on the sperm characteristics of beta-cypermethrin exposed F1 rats relative to that of the control. However, it had a dose-dependent increase ($p>0.05$) in abnormal sperm morphology when compared with the control (Table 1).

3.4 Histological Findings

Histoarchitecture of Testes of beta-cypermethrin exposed male F1 generation rats at maturity showed no obvious abnormality in comparison with the control (Plates 1-3).

4. DISCUSSION

Pyrethroids are known to act as endocrine disruptors. Endocrine disruptors are involved in the induction of developmental abnormalities. However, these effects may be reversible or irreversible, acute or latent and not expressed for

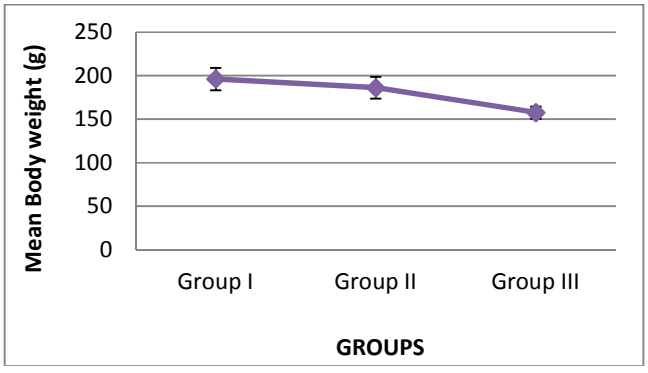


Fig. 1. Effect of beta cypermethrin on mean body weight of male F1 generation rats at sexual maturity

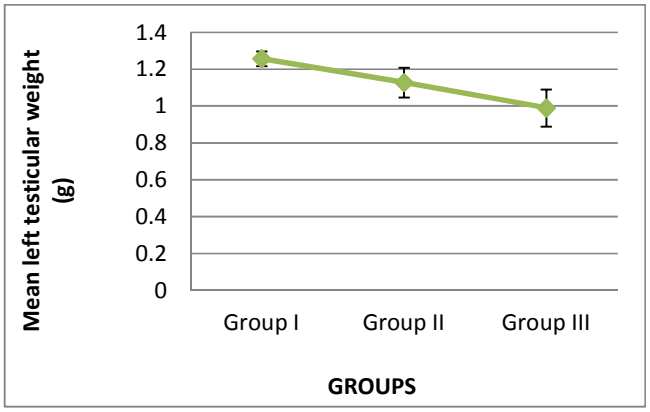


Fig. 2. Effect of beta cypermethrin on left testicular weight of male F1 generation rats at sexual maturity

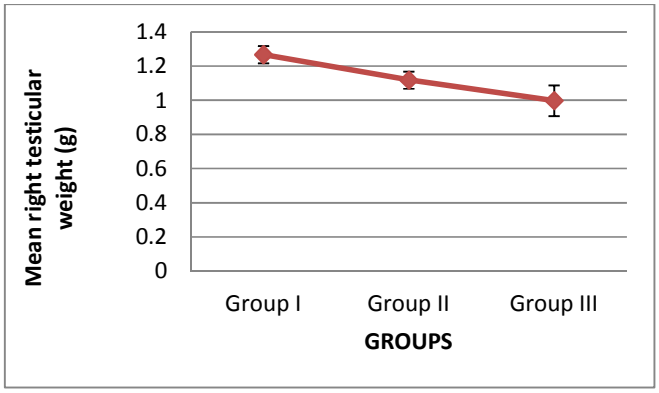


Fig. 3. Effect of beta cypermethrin on right testicular weight of male F1 generation rats at sexual maturity

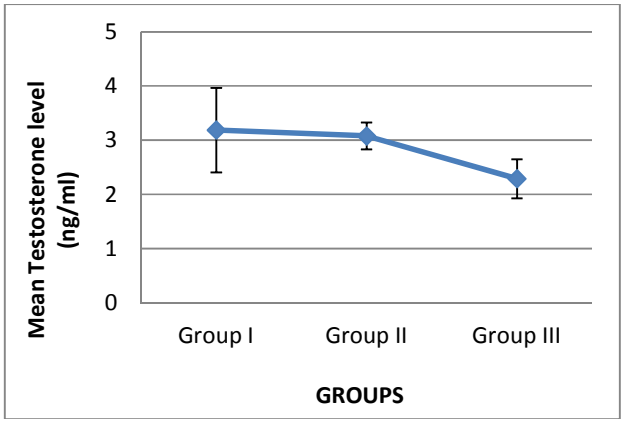


Fig. 4. Effect of beta cypermethrin on mean testosterone level of male F1 generation rats at sexual maturity

a period of time [16]. Higher damage caused by these endocrine disrupting pesticides usually occurs during gametogenesis and the early development of the foetus; and the effects may be apparent in adulthood damaging the reproductive system as well as the fertility [17]. These chemicals can be transferred prenatally to the developing foetus or postnatally

from breast milk to the nursing infant. Exposures through breast milk can be substantial, especially when the mother has significant ongoing exposures or has accumulated an unusually high body burden of persistent chemicals [18].

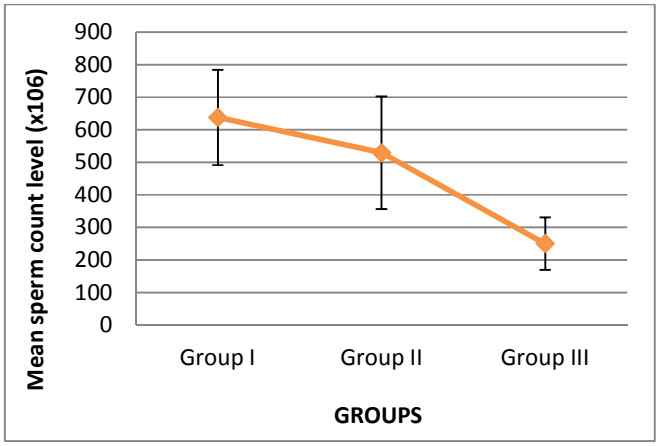
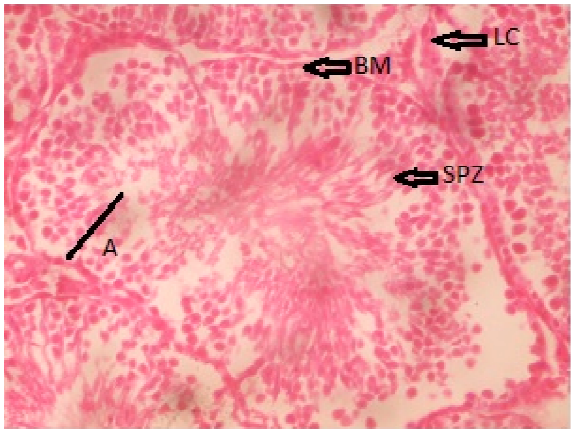


Fig. 5. Effect of beta cypermethrin on sperm cell count of male F1 generation rats at sexual maturity

Table 1. Effect of Beta cypermethrin on sperm cell characteristics of male F1 generation rats at sexual maturity

Groups	Spermatozoa morphology (%)		Sperm cell parameters (%)			
	Normal	Abnormal	Active	Sluggish	Motile	Dead
1	81.25±4.27	18.75±4.27	67.50±6.29	11.25±1.25	78.75±6.57	21.25±6.57
2	74.00±6.96	26.00±6.96	71.00±6.96	8.00±1.22	79.00±6.00	21.00±6.00
3	69.00±4.85	31.00±4.85	60.00±5.24	13.00±2.55	73.00±3.00	27.00±3.00

No significant variation exists across the table at 95% confidence interval (P>0.05)



Group I - Control

Plate 1. Photomicrograph of testicular section from control F1 generation rats stained with H & E (× 400) showing seminiferous tubule from the basement membrane (BM) to the lumen. It contains spermatogonia, primary and secondary spermatocytes, and spermatids occupying the region labelled 'A'. Mature spermatozoa (SPZ) occupying the luminal border. Interstitial spaces containing Leydig cells (LC)

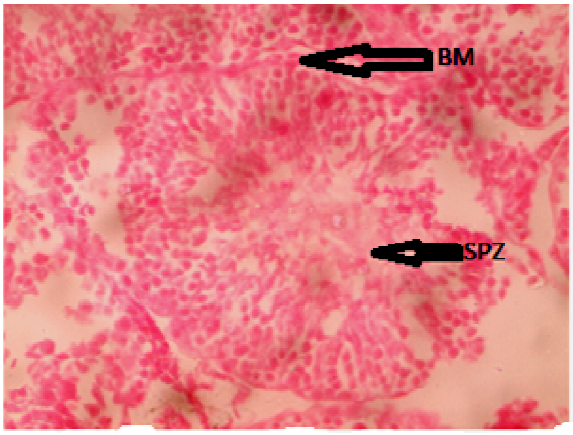


Plate 2. Photomicrograph of testicular section from 15 mg/kg β-cyp exposed F1 generation rats stained with H & E (× 400). It shows the seminiferous tubule from the basement membrane (BM) to the lumen. Mature spermatozoa (SPZ) occupying the luminal border. No abnormality in the testicular histoarchitecture is seen

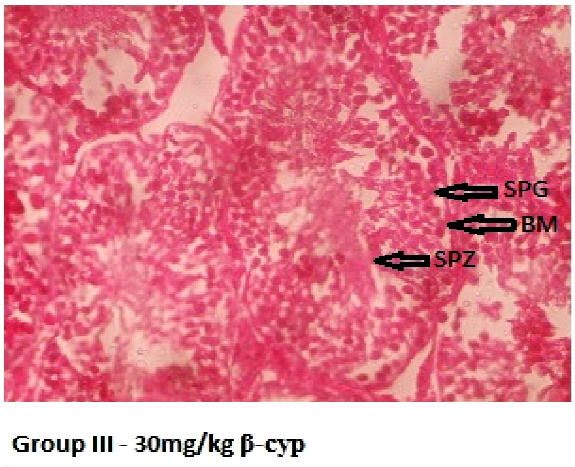


Plate 3. Photomicrograph of testicular section from 30 mg/kg β-cyp exposed F1 generation rats stained with H & E (× 400). It shows seminiferous tubule from the basement membrane (BM) to the lumen. It contains spermatogonia (SPG) with the mature spermatozoa (SPZ) occupying the luminal border

In previous studies, Beta cypermethrin was found to alter the Biochemical [19] and Haematological [20] profiles of rat offspring exposed during the perinatal period. In the present study, perinatal beta cypermethrin exposure of male f1 generation rats, when assessed at maturity, caused a dose-dependent non-significant decrease ($p>0.05$) in the testosterone level, sperm count and mean testicular and body weights. The reduction in serum testosterone level can have the adverse effect on spermatogenesis as evidenced in the reduced sperm count observed in the study. Spermatogenesis depends on testosterone produced in the Leydig cells of the testis. In the absence of testosterone or the androgen receptor, spermatogenesis does not proceed beyond the meiosis stage [21].

Sperm characteristics such as sperm motility and morphology as well as sperm cell count are key indices of male fertility, since they are the prime markers in testicular spermatogenesis and epididymal maturation [22]. Reduced sperm motility and viability may be an indication of alterations in spermatogenesis [23].

In other studies on beta cypermethrin and male reproductive parameters, Wang et al., [24] demonstrated that beta cypermethrin when administered to male mice for 35 days at the doses of 10 and 20 mg/kg not only decreased body weight and the weight of the testes, epididymides, seminal vesicles, and prostate but also reduced the serum testosterone concentration and the expression of steroidogenic acute regulatory protein in addition to damaging the seminiferous tubules and sperm development. Beta cypermethrin administered to adult male rats at the doses of 15 and 30mg/kg daily for 15 days was reported to cause substantial decreases of both sperm head counts and daily sperm production as well as decrease in androgen Receptors [25].

The result of this study contrasted with the findings of Huang and Li [26] who reported that intragastric exposure of pregnant mice to doses of 0.12, 1.2, and 12.0 mg/kg/day cypermethrin from embryonic day 0.5 (day of plug) to weaning (postnatal day 21.5) affected the body and organ weight of the offspring. The serum testosterone level was decreased and histopathological analysis of the testes revealed a thinner seminiferous epithelium layer at PD21.5, interstitial hyperplasia at PD45.5, and germ cell vacuolization at PD90.5 in the 12 mg/kg/day CYP group.

The reason for the disparity in the results may be attributed to the isomeric form of the insecticide administered in the studies. Whereas Beta cypermethrin was used in the present study, the isomeric form of cypermethrin used in the other study was not specified. According to Crane et al., [27], cypermethrin molecule contains three chiral centres giving rise to 8 stereoisomers. Cypermethrin is made up of a mixture of 4 *cis*- and 4 *trans*- isomers. There are four main isomeric mixtures of cypermethrin namely alpha-cypermethrin, beta-cypermethrin, theta-cypermethrin and zeta-cypermethrin [28]. The isomers of cypermethrin are not likely to be found in isolation in the environment, but are most likely to occur as different mixtures of isomers present at ratios that relate to their parent formulations [27].

Beta-cypermethrin consists of 4 (2 *cis*-isomers and 2 *trans*-isomers) of the 8 stereo-isomers that comprise cypermethrin. The *cis*- isomers have been reported to be more toxic than the *trans*-isomers [29] since the *trans*- isomers degrade more rapidly than the *cis*- isomers [30]. The environmental data package indicated that the *cis* and *trans*-isomers may degrade at different rates under some circumstances [30].

5. CONCLUSION

The result of this study shows that perinatal beta cypermethrin exposure of male f1 generation rats had no significant effect on the reproductive parameters when assessed at maturity. However, the dose-dependent decrease in the male reproductive parameters recorded in this study suggests that β-cyp, can impair spermatogenesis in male offspring exposed during the perinatal period.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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