Original Research Article

ALTERATIONS IN STEROID SEX HORMONES (17β estradiol and testosterone) OF *Clarias gariepinus* EXPOSED TO DIFFERENT SUB-LETHAL CONCENTRATIONS OF CYPERMETHRIN

ABSTRACT

The study on the alterations in steroid hormone levels in *Clarias gariepinus* exposed to sub-lethal concentrations of cypermethrin was carried-out over a 28 days period. A total of one hundred (100) sub-adults of *Clarias gariepinus* with mean weight of 55.280 ± 6.281g were used through-out the study. Sub-adults of *Clarias gariepinus* were exposed to 0.00ppm, 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin. The Concentration of 17β-Estradiol and testosterone in *Clarias gariepinus* sub-adult exposed to 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of the toxicant for 7, 14, 21 and 28 days decreased significantly from the control at $p<0.05$ except for 17β-Estradiol and testosterone concentration in fish exposed to 0.0125ppm concentration of cypermethrin for 21 days and 7days respectively. The concentration of 17β-Estradiol and testosterone in *Clarias gariepinus* exposed to the sub-lethal concentrations of the toxicant during 7, 14, 21 and 28 days of exposure decreased with increase in the concentration of cypermethrin, except for very few groups. Despite the adverse effect of cypermethrin on fish reproduction (production of 17β-Estradiol and testosterone) and other physiological processes, it will be a setback to Agricultural productivity pesticides if the use of pesticides are totally banned. As a result, we recommend that the Government sensitizes the farmers properly on the proper quantity of pesticides to be applied at a time, enforce against excess application of pesticides in quick succession in order to ensure sustainability of our fishery resources. More of similar studies should be funded in order to continue to monitor the level of the effects of various pesticides on fishes and the aquatic eco-system at large.

KEYWORDS: Alterations, sex steroid hormones, *Clarias gariepinus*, sub-lethal, cypermethrin, concentration

INTRODUCTION

Pesticides are recognized world-wide as a veritable means of controlling pests, at the same time such chemicals are highly toxic to other species in the environment [1]. Presently, there is an increasing concern world over on the indiscriminate use of such chemicals that result in environmental pollution and toxicity risk to non-target organism [2]. Cypermethrin is a widely used pyrethroid pestiside, and is a broad based spectrum used against stored products pests, and control of ectoparasites in both land and aquatic animals [3, 4]. Responses to pyrethroid insecticides by fish are wide ranged, depending on the compound, exposure time, water quality, concentration and species [5]. Non-point source pollutants are mainly transported overland and through the soil by runoff [6]. These pollutants ultimately find their way into groundwater, wetlands, rivers and lakes and, finally, to oceans in the form of sediment and chemical loads carried by rivers [7]. The major insecticides that are usually applied in agriculture and public health sections include organophosphate, organocholorines, pyrethroids and carbamate. Contamination of water by insecticides is mainly due to intensive agriculture combined with surface runoff and subsurface drainage, usually within a few weeks after application [8]. Fishes are particularly sensitive to environmental contamination of water. Hence, pollutants such as insecticides may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes [8]. Endocrine disrupting chemicals (EDCs) are defined as chemical substances that alter the normal endocrine function [9], including either naturally occurring chemicals as phytoestrogen or synthetic chemicals such as Pesticides, plasticizers, polychlorinated biphenyls (PCBs) and alkylphenolic compounds. EDCs exert their effect either through mimicking (act like a natural hormone) such as methoxychlor pesticide, certain polychlorinated biphenyls (PCBs) and bisphenol (BPA) or antagonizing endogenous hormones such as tamoxifen or disrupt the synthesis and metabolism of hormones or interact with the hormone receptors [10]. In Ovary, estradiol must be synthesized in sufficient amount to stimulate liver to produce vitellogenin. In case of oocyte atresia; the estradiol hormone decrease lead to impaired vitellogenesis [11]. Nile tilapia also showed decrease in sex steroid hormones upon exposure to organochlorine pesticide hexachlorobenzene (HCB) [12]. [13], mentioned that *O. niloticus* fed on diet incorporated with malathion for 4 month exert endocrine disrupting effect on males than females through decreasing testosterone hormone; while dimethaote has pronounced effect on females through decrease 17 ß- estradiol hormone. [14] reported that male *Oncorhynchus mykiss* exposed to sublethal concentration of imethaote 0.735 mg/l for 30 day showed significant decrease in 17 ß- estradiol. The study

was aimed at evaluating the steroid sex hormones alterations in *Clarias gariepinus* sub-adult exposed to different concentrations of cypermethrin.

MATERIALS AND METHODS

Collection and transportation of test organisms

Clarias gariepinus sub-adults were collected from the University of Calabar fish farm (Hatchery) along with the culture water in a plastic bucket, and transported immediately to Zoology and Environmental Biology Laboratory, University of Calabar, where they were allowed to acclimate to the laboratory conditions.

Acclimation and maintenance of study organisms

Once the fish samples arrived the laboratory, they were allowed to acclimate to laboratory conditions at room temperature for a period of two (2) weeks during which they were fed with commercial fish feed (coppen) twice daily, at 4% of their body weight.

Range finding test

A range finding test was carried-out prior to the commencement of the research, during which the test fish were exposed to a wide range of different sub-lethal concentrations of cypermethrin, which revealed the most appropriate concentrations for the study.

Preparation of stock solution

A stock solution was prepared by adding nine hundred and ninety (990) ml of water to ten (10) ml of cypermethrin and shaken thoroughly to form 1000 ml of the cypermethrin stock solution through which further dilutions into 0.0125, 0.025, 0.100 and 0.400 ppm concentrations along with a control group.

Experimental design

Five (5), 25 x 15.5 x 15.5 cm^3 glass aquarium were used through-out for the study. A total of one hundred (100) sub-adults of *Clarias gariepinus* with mean weight of 55.280 ± 6.281g were used through-out the study. Twenty (20) sub-adults of the test fish was stocked in each aquarium containing twenty-five (25) litres of water in a 72 hourly renewable experiment. Four groups of the fish will be exposed to 0.0125, 0.025, 0.100 and 0.400 ppm of cypermethrin for 28 days, and there was also be a control group (0 ppm) where the toxicants was not introduced. Every 72 hours, the water and test solution was renewed and the fishes fed as well. After 7, 14, 21 and 28 days of exposure, five (5) fishes per group were punctured

around the cardiac area behind the anal fin using a 5ml syringe and needle into a serum bottle. The collected blood samples were immediately preserved in an ice chest before they are taken to University of Calabar teaching hospital for analysis of 17β estradiol and testosterone (sex steroid hormones) using enzyme-linked immunosorbent assay (ELISA) kits designed by Diametra S.R.I, Italy.

Determination of steroid sex hormones

Sex steroid hormones testosterone (T) and 17β estradiol (E2) were extracted from blood plasma using a method adapted from [15]. In short, 100 µl plasma was adjusted to 1 ml with milliQ water, after which 5 ml diethyl ether. After 45 seconds, samples were spun down for 3 min at 2500 rpm and snap-frozen for 7 seconds in liquid nitrogen. Subsequently the ether phasewas evaporated in a warm (40 °C) water bath under a gentle flow of nitrogen gas. The remaining pellets were reconstituted in 500 µl PBS+gelatin and stored at −80 °C until analyzed. Plasma T- and E2 concentrations were determined in triplicate by enzyme-linked immunosorbent assay (ELISA) kits designed by Diametra S.R.I, Italy.

Statistical analysis

The data obtained for 17β estradiol and testosterone in the exposed and control fish group were subjected to descriptive statistics (Mean and standard deviation). Analysis of variance (ANOVA) was used to test for the significance of the difference in cypermethrin induced alterations in steroid sex hormones in each group exposed to different concentrations of cypermethrin after 7, 14, 21 and 28 exposure days compared to the control. All analysis was be carried-out using prism graph pad at 0.05 level of significance and at their relevant degree of freedom.

RESULTS

Plasma concentration of estradiol (E2) in *Clarias gariepinus* sub-adult exposed to sub-lethal concentrations of cypermethrin

The summary of the trend of estradiol concentration in *Clarias gariepinus* exposed to 0.00ppm (control), 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin is shown in Figure 1 – 4. The Concentration of 17β-Estradiol in *Clarias gariepinus* sub-adult exposed to 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin for 7, 14, 21 and 28 days decreased significantly from the control at $p<0.05$ except for the group exposed to 0.0125ppm concentration of cypermethrin for 21 days (Fig 1 – 4).

The concentration of 17β-Estradiol in *Clarias gariepinus* sub-adult exposed to 0.00ppm (control), 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin for 7, 14, 21 and 28 days decreased with increase in the concentration of the test toxicant (Fig 1 – 4). For the 7 days exposure duration, the highest concentration of 17β-Estradiol in *Clarias gariepinus* sub-adult was observed in the control group (0.00ppm), having a mean and standard deviation of 212.4 ± 3.156 pg/ml, but reduced to 146.7 ± 9.393 pg/ml when exposed to 0.0125ppm of the toxicant. It further decreased to 140.2 ± 5.448 pg/ml for 0.025ppm group, 129.5 ± 10.13 pg/ml for 0.100ppm and 118.8 ± 9.682 pg/ml as the lowest, for the highest concentration group (0.400ppm) (Fig 1).

For the 14 days exposure duration, the highest concentration of 17β-Estradiol in *Clarias gariepinus* sub-adult was observed in the control group (0.00ppm), having a mean and standard deviation of 210.5 ± 8.286 pg/ml, but reduced to 151.1 ± 13.510 pg/ml when exposed to 0.0125ppm cypermethrin. It further decreased to 121.0 ± 11.530 pg/ml for 0.025ppm group, 102.2 ± 11.090 pg/ml for 0.100ppm and 90.70 ± 7.553 pg/ml as the lowest for the highest concentration group (0.400ppm) (Fig 2).

For the 21 days exposure duration, the highest concentration of 17β-Estradiol in *Clarias gariepinus* sub-adult was observed in the control group (0.00ppm), having a mean and standard deviation of 131.70 ± 5.652 pg/ml, but reduced to 121.20 ± 5.524 pg/ml when exposed to 0.0125ppm of the toxicant. It further decreased to 113.40 ± 5.501 pg/ml for 0.025ppm group, 80.20 ± 6.582 pg/ml for 0.400ppm and 80.77 ± 6.882 pg/ml for the 0.100ppm group (Fig 3).

For the 28 days exposure duration, the highest concentration of 17β-Estradiol in *Clarias gariepinus* sub-adult was observed in the control group (0.00ppm), having a mean and standard deviation of 177.6 ± 12.250 pg/ml, but reduced to 106.5 ± 12.460 pg/ml when exposed to 0.0125ppm cypermethrin. It further decreased to 85.67 ± 11.910 pg/ml for 0.025ppm group, 75.20 ± 4.386 pg/ml for 0.100ppm and 52.77 ± 11.080 pg/ml as the lowest for the highest concentration group (0.400ppm) (Fig 4).

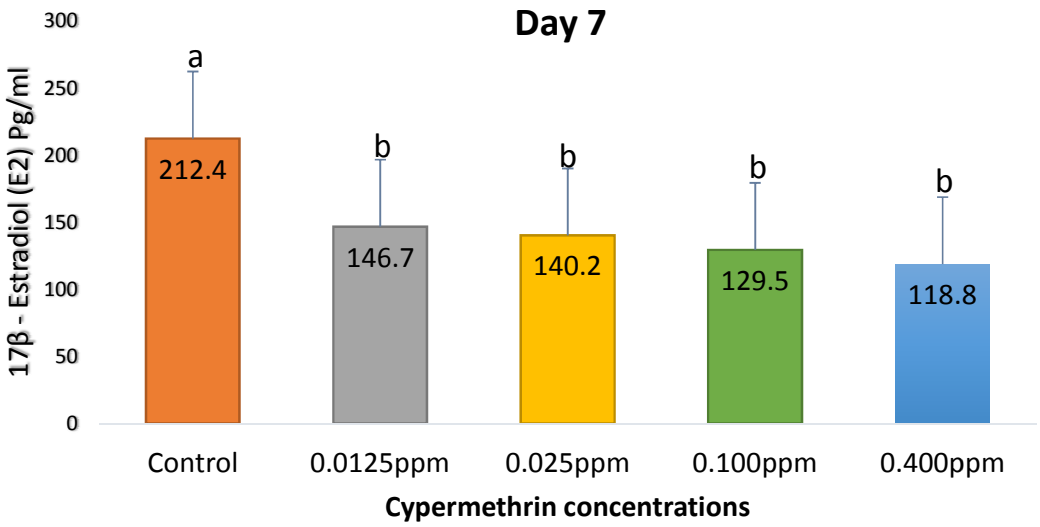

Fig 1: Graph showing the decrease in the concentration of 17β-Estradiol (pg/ml) in *Clarias gariepinus* sub-adult exposed to different concentrations of cypermethrin for 7 days. Concentration of 17β-Estradiol in *Clarias gariepinus* exposed to 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin decreased significantly from the control at $p<0.05$. Different alphabets for each group compared to control are significantly different from control at $p<0.05$.

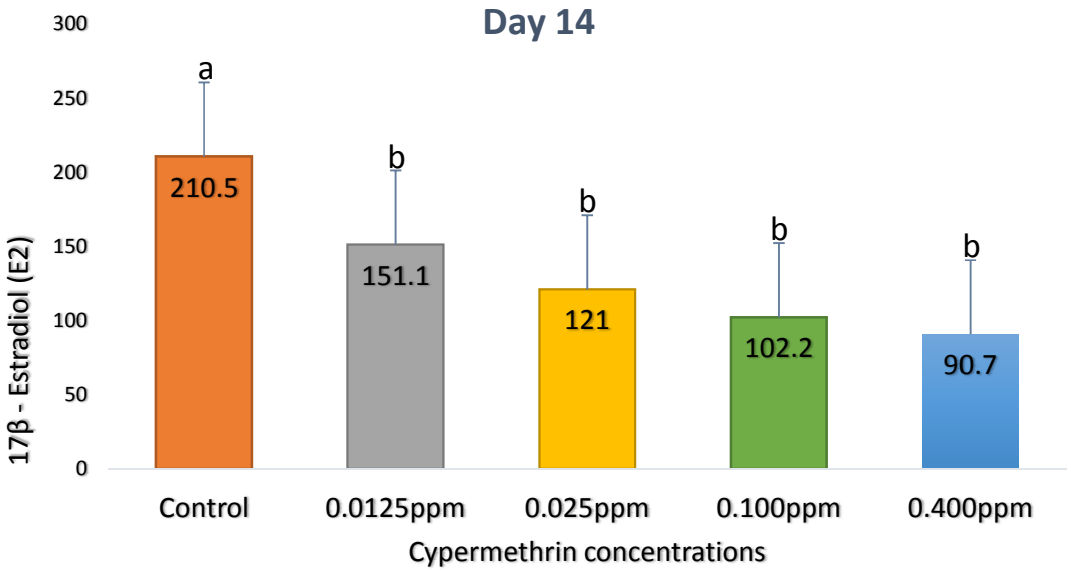

Fig 2: Graph showing the decrease in the concentration of 17β-Estradiol (pg/ml) in *Clarias gariepinus* sub-adult exposed to different concentrations of cypermethrin for 14 days. Concentration of 17β-Estradiol in *Clarias gariepinus* exposed to 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin decreased significantly from the control at $p<0.05$. Different alphabets for each group compared to control are significantly different from control at $p<0.05$.

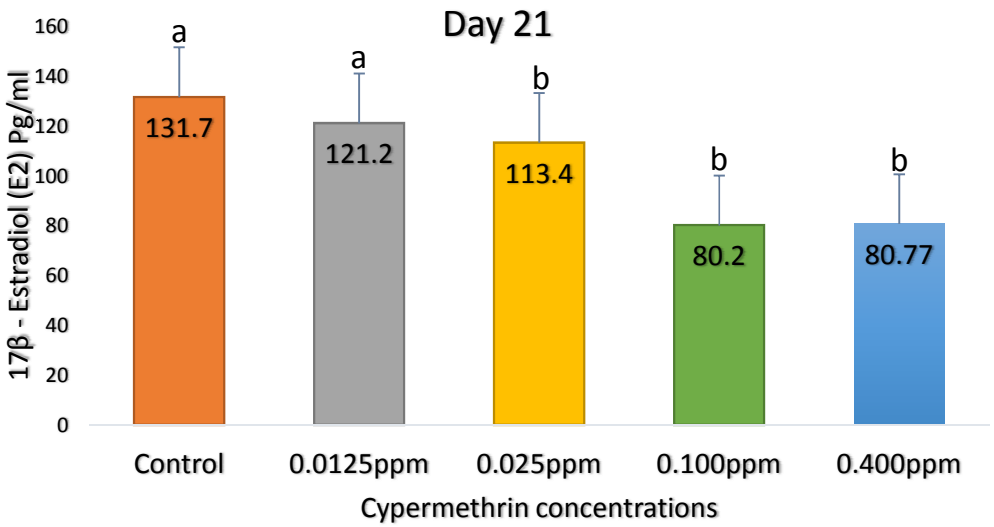

Fig 3: Graph showing the decrease in the concentration of 17β-Estradiol (pg/ml) in *Clarias gariepinus* sub-adult exposed to different concentrations of cypermethrin for 21 days. Concentration of 17β-Estradiol in *Clarias gariepinus* exposed to 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin decreased significantly from the control at $p<0.05$. Different alphabets for each group compared to control are significantly different from control at $p<0.05$.

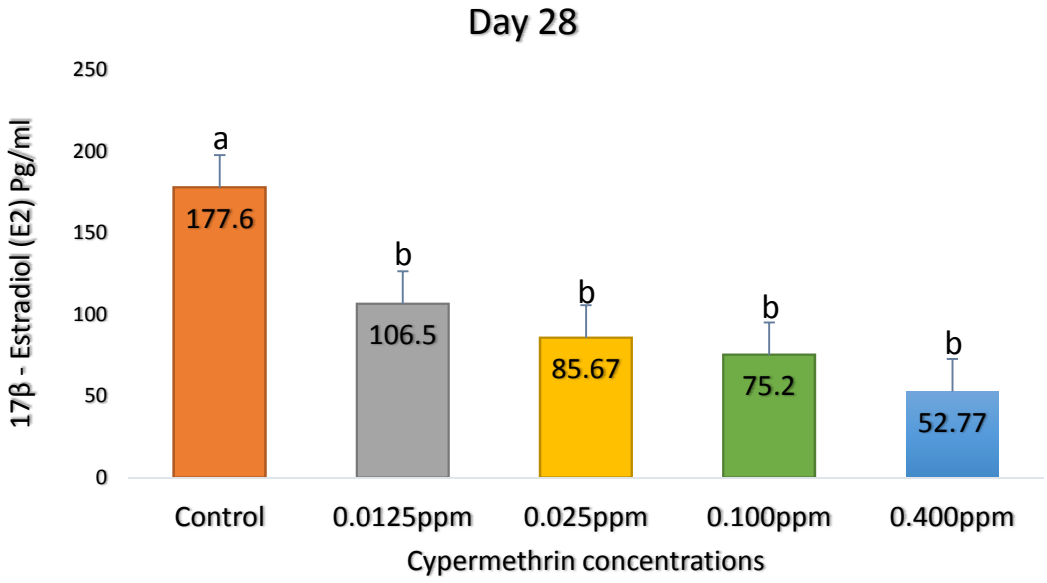

Fig 4: Graph showing the decrease in the concentration of 17β-Estradiol (pg/ml) in *Clarias gariepinus* sub-adult exposed to different concentrations of cypermethrin for 28 days. Concentration of 17β-Estradiol in *Clarias gariepinus* exposed to 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin decreased significantly from the control at $p<0.05$. Different alphabets for each group compared to control are significantly different from control at $p<0.05$.

Plasma concentration of Testosterone (T) in *Clarias gariepinus* sub-adult exposed to sub-lethal concentrations of cypermethrin

The summary of the testosterone concentration in *Clarias gariepinus* exposed to 0.00ppm (control), 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin is shown in Figure 5 – 8. The Concentration of testosterone in *Clarias gariepinus* sub-adult exposed to 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin for 7, 14, 21 and 28 days decreased significantly from the control at $p<0.05$ except for the group exposed to 0.0125ppm concentration of cypermethrin for 7 days days (Fig 5 – 8).

The concentration of testosterone in *Clarias gariepinus* sub-adult exposed to 0.00ppm (control), 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin for 7, 14, 21 and 28 days decreased with increase in the concentration of cypermethrin, except between the 0.025ppm and 0.100ppm group after 7 days exposure, 0.0125ppm and 0.025ppm after 14 days exposure and 0.100ppm and 0.400ppm group after 28 days of exposure (Fig 5 – 8). For the 7 days exposure duration, the highest concentration of testosterone in *Clarias gariepinus* sub-adult was observed in the control group (0.00ppm), having a mean and standard deviation of 2.367 ± 0.208 pg/ml, but reduced to 1.733 ± 0.351 pg/ml when exposed to 0.0125ppm cypermethrin. It further decreased to 1.167 ± 0.251 pg/ml and 1.167 ± 0.351 pg/ml for 0.025ppm and 0.100ppm group respectively and then 0.823 ± 0.276 pg/ml as the lowest for the highest concentration group (0.400ppm) (Fig 5).

For the 14 days exposure duration, the highest concentration of testosterone in *Clarias gariepinus* sub-adult was observed in the control group (0.00ppm), having a mean and standard deviation of 2.700 ± 0.200 pg/ml, but reduced to 1.300 ± 0.200 pg/ml when exposed to 0.0125ppm cypermethrin. It then slightly increased to 1.367 ± 0.208 pg/ml for 0.025ppm group, but decreased to 0.933 ± 0.251 pg/ml for 0.100ppm and 0.466 ± 0.152 pg/ml as the lowest for the highest concentration group (0.400ppm) (Fig 6).

For the 21 days exposure duration, the highest concentration of testosterone in *Clarias gariepinus* sub-adult was observed in the control group (0.00ppm), having a mean and standard deviation of 3.200 ± 0.300 pg/ml, but reduced to 2.167 ± 0.251 pg/ml when exposed to 0.0125ppm of the toxicant. It further decreased to 1.733 ± 0.351 pg/ml for 0.025ppm group, 1.067 ± 0.251 pg/ml for 0.100ppm and 0.300 ± 0.100 pg/ml for the highest concentration group (0.400ppm) (Fig 7).

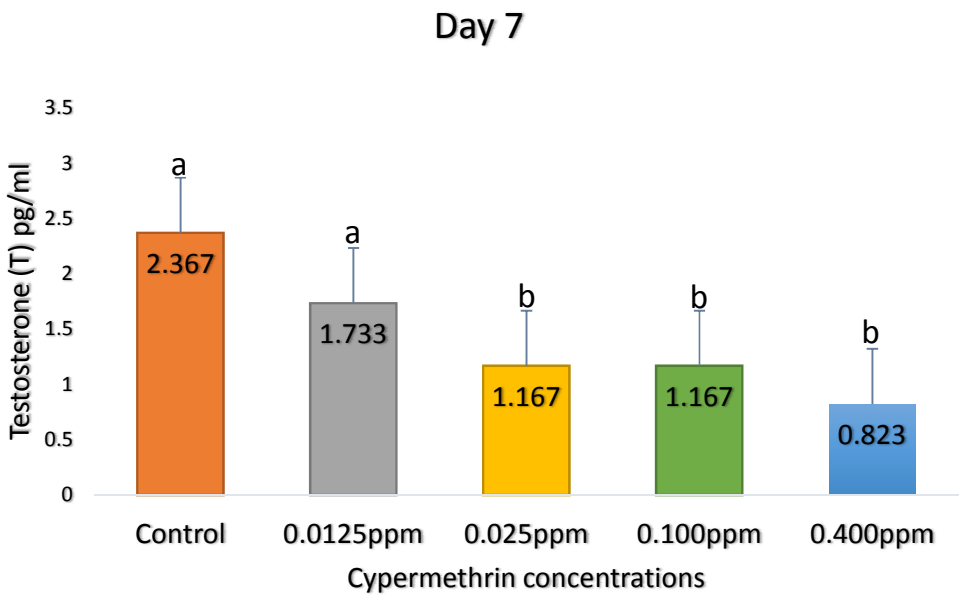

Fig 5: Graph showing the decrease in the concentration of Testosterone (T) (pg/ml) in *Clarias gariepinus* sub-adult exposed to different concentrations of cypermethrin for 7 days. Concentration of Testosterone (T) in *Clarias gariepinus* exposed to 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin decreased significantly from the control at $p<0.05$. Different alphabets for each group compared to control are significantly different from control at $p<0.05$.

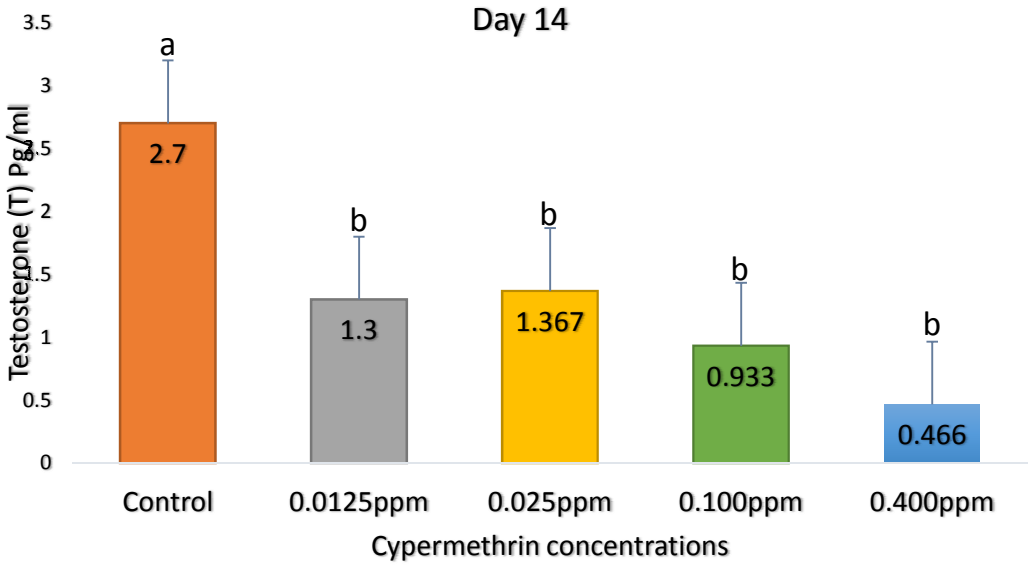

Fig 6: Graph showing the decrease in the concentration of Testosterone (T) (pg/ml) in *Clarias gariepinus* sub-adult exposed to different concentrations of cypermethrin for 14 days. Concentration of Testosterone (T) in *Clarias gariepinus* exposed to 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin decreased significantly from the control at $p<0.05$. Different alphabets for each group compared to control are significantly different from control at $p<0.05$.

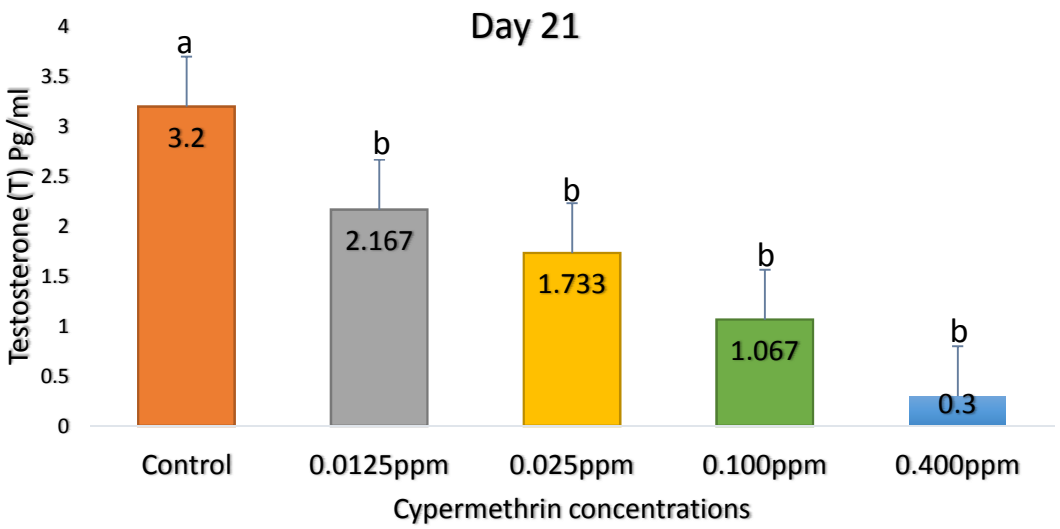

Fig 7: Graph showing the decrease in the concentration of Testosterone (T) (pg/ml) in *Clarias gariepinus* sub-adult exposed to different concentrations of cypermethrin for 21 days. Concentration of Testosterone (T) in *Clarias gariepinus* exposed to 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin decreased significantly from the control at $p<0.05$. Different alphabets for each group compared to control are significantly different from control at $p<0.05$.

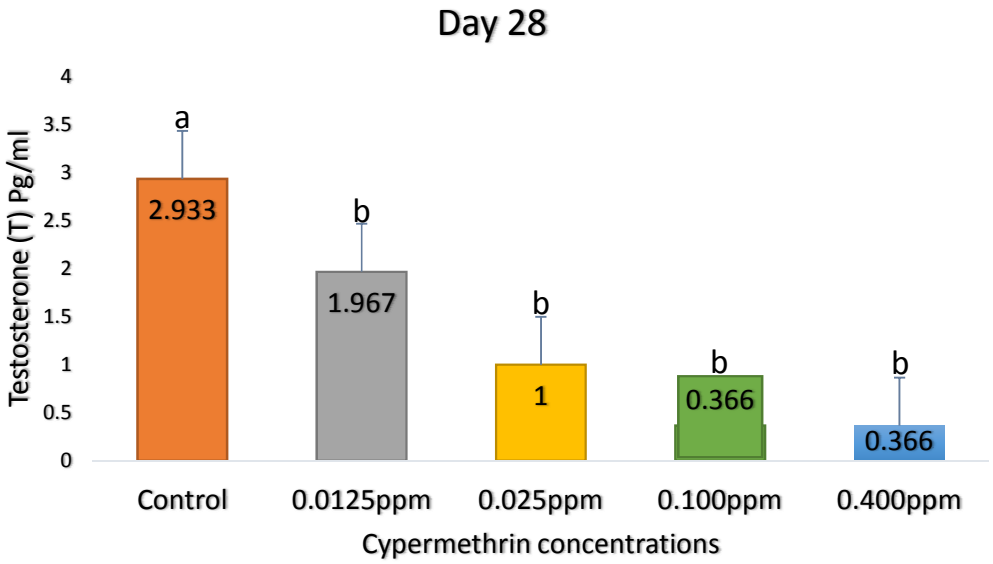

Fig 8: Graph showing the decrease in the concentration of Testosterone (T) (pg/ml) in *Clarias gariepinus* sub-adult exposed to different concentrations of cypermethrin for 21 days. Concentration of Testosterone (T) in *Clarias gariepinus* exposed to 0.0125ppm, 0.025ppm, 0.100ppm and 0.400ppm of cypermethrin decreased significantly from the control at $p<0.05$. Different alphabets for each group compared to control are significantly different from control at $p<0.05$.

For the 28 days exposure duration, the highest concentration of testosterone in *Clarias gariepinus* sub-adult was observed in the control group (0.00ppm), having a mean and standard deviation of 2.933 ± 0.251 pg/ml, but reduced to 1.967 ± 0.251 when exposed to 0.0125ppm of the toxicant. It further decreased to 1.000 ± 0.200 pg/ml for 0.025ppm group and then to 0.366 ± 0.115 pg/ml and 0.366 ± 0.208 pg/ml concentration group for 0.100ppm and 0.400ppm concentration group respectively (Fig 8).

DISCUSSION

Fishes are often used to assess the health of aquatic environment and physiological changes occurring as a result of pollution in an aquatic environment [16]. The physiological alterations caused by some of chemicals like; cypermethrin have been found to affect even the endocrine system. Endocrine disrupting chemicals (EDCs) like; cypermethrin act mainly by interfering with natural hormones due to their strong potential to bind to estrogen or androgen receptors, causing impairment of fish fecundity, semen quality, hatchability and survivability of fishes [17]. Sex steroid hormones, vitellogenin, organosomatic index and histopathology are considered as biomarker tools used for assessing the endocrine disrupting effect of pesticides on fish. Steroid hormones are one of the several hormones that influence fish reproduction, and as such the major androgens produced by testicular tissue differ from one fish species to another.

This study revealed a decrease in the concentration of 17β-Estradiol and testosterone in *Clarias gariepinus* sub-adult with increase in the concentration of the cypermethrin for most fish group after 7, 14, 21 and 28 days exposure period. Also, the concentration of 17β-Estradiol and testosterone in *Clarias gariepinus* sub-adult exposed to different sub-lethal concentration of cypermethrin decreased significantly from the control for the 7, 14, 21 and 28 days exposure period, except for estradiol and testosterone concentrations for the 0.0125ppm groups after 21 days and 7days of exposure respectively, causing vitellogenesis. This result corroborated with the findings of [18]; who reported decrease in testosterone and estradiol levels in *Oreochromis niloticus* compared to the control when exposed to Chlorpyrofos; [12], who reported that Nile tilapia also showed decrease in sex steroid hormones upon exposure to organochlorine pesticide hexachlorobenzene (HCB); [13] who reported endocrine disrupting effect on males than females through decreasing testosterone hormone for *O. niloticus* exposed to malathion and [11]; who reported that exposure to pesticide causes decrease in estradiol level compared to the control, leading to impaired

vitellogenesis since estradiol must be synthesized in sufficient amount to stimulate liver to produce vitellogenin. The decrease in the concentration of estradiol and testosterone in the sub-adult of *Clarias gariepinus* when exposed to sub-lethal concentrations of cypermethrin compared to the control group could be due to the impairment of the secretion of the 11-ketotestosterone from the sertoli [19], 17ß- estradiol from the follicular cells [20], molecular mimickery of the hormones which stimulates a negative feedback in gonadotrophin secretion, resulting in suppression of the synthesis of endogenous estrogen [21] and increase in the steroid metabolizing enzymes activities [22]. The reduction in the concentration of testosterone and estradiol in *Clarias gariepinus* exposed to the pesticide could also be due to interference with the production of free cholesterol, the sex hormone precursor which would have been converted into testosterone (T) and estradiol (E2) by the enzyme aromatase, or to 11- Ketotestosterone by the enzyme cytochrome P450 11ß- hydroxylase (CYP 11ß), thereby reducing steroid production [23].

The decrease in the testosterone and estradiol concentration in *Clarias gariepinus* exposed to the toxicant, was contrary to the findings of [24] who reported an increase in both sex hormones, and this could be due to the difference in duration of exposure, frequency of exposure, toxicity of the chemical, age of fish, season, sex and species. Abnormal behavioural responses were also observed on exposure to cypermethrin which resulted in in-active swimming, loss of equilibrium, hanging vertically in water, less feeding, erratic swimming and air gulping at water surface. Appearance of mucous covering on the gills along with the change in the colour of the lamellae were also observed as part of the adverse physiological effects of cypermethrin on the test fish.

Consequently, the result reveals alterations in the rate of spermatogenesis and oogenesis due to apoptosis, leading to the disturbance in normal hormone physiology and unexpected pressures on endocrine homeostasis.

CONCLUSION

In conclusion, the study revealed the sub-lethal toxic effect of cypermethrin on the level of steroid hormone production by decreasing the levels of production of 17β-Estradiol and testosterone by the fish, and subsequently leading to impaired vitellogenesis and disruption of endocrine glands in general. The study also revealed that the decrease in the concentration of 17β-Estradiol and testosterone in the fish was cypermethrin concentration

dependent, except for few groups. Also, the important roles of sex steroids (androgens and estrogens) in the regulation of reproduction and other physiological processes was revealed.

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