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Research article

STUDIES OF CYPERMETHRIN ACTIVITY ON STEROIDOGENESIS IN MALE *LABEO ROHITA*

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ABSTRACT: Cypermethrin is a widely used type II Pyrethroid. As such it has become a contaminant in fresh water aquatic ecosystems. To know its toxic effect on steroidogenesis in male fish *Labeo rohita,* we have exposed six male fish to 10 and 20 µg/L of Cypermethrin for six days. The plasma concentration of Vitellogenin (VTG), testosterone (T) and 11-ketotestosterone (11 KT) were evaluated. Vitellogenin levels increased with increasing concentration of Cypermethrin, where as Testosterone and 11-Ketotestosterone levels decreased with increasing concentration of Cypermethrin.

Key words: *Labeo Rohita*, Cypermethrin, Vitellogenin and Steroidogenic hormones.

INTRODUCTION

Cypermethrin is a synthetic Pyrethroid insecticide employed all over the world to control different pests of crops (cereals, cabbage, oil seed rape, soybean, etc.), flies and mosquitoes because of its high photo stability, rapid knock down effect, low mammalian toxicity, degradability and persistent nature (Elliot et al., 1978; Casida et al., 1983). A growing number of environmental toxicants like synthetic Pyrethroid are believed to have deleterious effects on the development of non-target organisms by disrupting hormone sensitive processes. Exposure to environmental estrogenic alkyl phenolic chemicals in rainbow trout resulted in a decreased fertility and egg production in females, reduction of gonadal size (Jobling et al., 2003; Ashfield et al., 1998), reduced sperm production, demasculinisation and presence of intersex gonads. (Nimrod and Benson, 1998; Gimeno et al., 1998) etc. Another frequently observed effect of exposure to environmental estrogens is the induction of the yolk precursor protein Vitellogenin (VTG) in male and sexually immature fish (Jobling et al., 2003; Folmar et al., 1996). The VTG is also known as a biomarker in toxicology, which represents intersex or testicular dysfunction such as alternations in steroidogenesis, sperm production and quality (Denslow and Sepulveda, 2008; Gregory et al., 2008). Impacts on VTG synthesis by androgens have been reported and exposure may result in either increases or reductions in the production of VTG (Hornung et al., 2004; Andersen et al., 2001; Orn et al., 2003; Zerulla et al., 2002). An increase in VTG levels has often been found in fish exposed to high levels of androgens and is probably due to an increased rate of conversion of the androgens to estrogens by the enzyme Aromatase (Ankley et al., 2001; Hornung et al., 2004; Zerulla et al., 2002). From the literature it is evident that there are no studies on the interference of Cypermethrin during the time the carps are in reproductive phase. Hence an attempt has been made to fill this gap by studying the effect on Cypermethrin an steroidogenesis in the edible fish, *Labeo rohita.*

MATERIALS AND METHODS

Procurement and maintenance of fish

Mature adult male fish, *Labeo rohita* were obtained from the local fisheries department and acclimatized to laboratory conditions for one week. During this time they are fed with commercial fish feed and continuous aeration was provided. All experiments were conducted during the time the fish were reproductively active.

Chemical

Technical grade Cypermethrin, RS-a-cyano-3-phenoxybenzyl, IRS, cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate was purchased from Sigma-Aldrich (99% pure; St. Louis, Mo, USA). The stock solution was prepared in ethanol (99%, Merck). 10mg of Cypermethrin was dissolved in 2ml of ethanol and stored at 4^0C. From this dilutions were made and used for daily renewal of the test solutions in the aquaria.

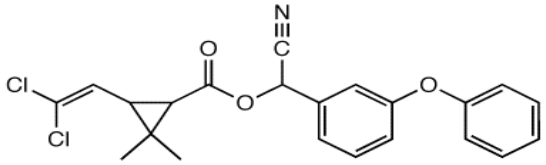

Figure 1: Structure of Cypermethrin

Experimental design

Six male fish were kept in 500L water tanks and fed with commercial fish feed and then exposed to 10 and 20 µg/L of Cypermethrin for six days. After the stipulated time blood was collected for analysis of different hormones involved in the steroidogenic pathway.

Blood collection from the caudal vein/artery

Immediately following anaesthesia, the caudal peduncle was severed transversely, and the blood was removed from the caudal artery/vein with a heparinized microhematocrit capillary tube.

Collected blood volumes varied from 4-10µl depending on fish size. An equal volume of Aprotinin buffer (6µg/ml in Phosphate Buffered Saline) was added to the Microcapillary tube, and plasma was separated from the blood by centrifuging at 1500rpm for 5 min. Following that capillary tubes were freeze dried in liquid nitrogen and stored at -80°C until used for analysis of different hormones

Hormonal assay

The plasma Vitellogenin (VTG), testosterone (T) and 11-ketotestosterone (11 KT) were carried out using kits from Biosense laboratories (Cayman chemicals company, USA) by Enzyme linked Immunosorbent assay (ELISA) using µ quant plate reader from Bio-Tek USA. Protocol supplied by the manufacture was followed separately for each hormone.

RESULTS AND DISCUSSION

The present study demonstrates varied effect of Cypermethrin in male *Labeo rohita* on levels of plasma Vitellogenin (Fig.2 and Table1). In male fish, Vitellogenin level increased with increasing concentration of Cypermethrin from 10µg to 20µg/L after 6th day.

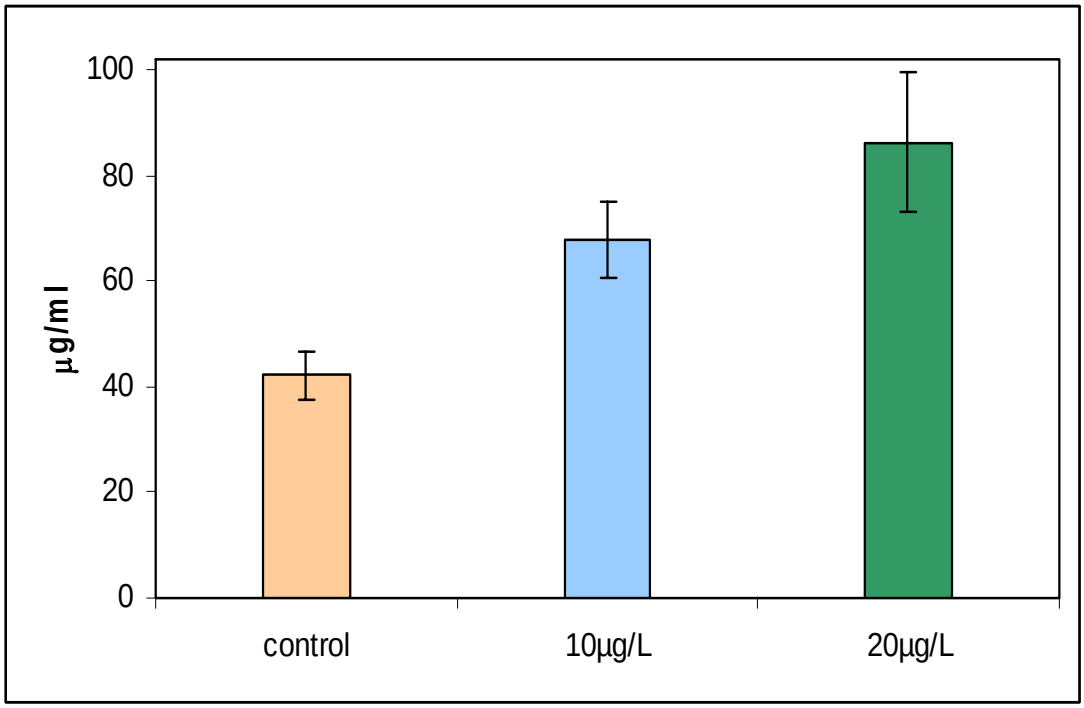

Figure 2: Levels of Vitellogenin in plasma of male *Labeo rohita* exposed to Cypermethrin.

Table 1: Levels of Vitellogenin in plasma of male *Labeo rohita* exposed to Cypermethrin.

S.No	Sample	Mean ± SD (µg/ml)	DMR-Test
1	Control	42.3 ± 4.6	-
2	10µg/L	67.9 ± 7.1	Significant over control
3	20µg/L	86.3 ± 13.1	Significant over control and 10µg/L

Note: Values are mean ± SD (n=6)

In this investigation, we also examined the effect of two different concentrations i.e. 10µg/L and 20µg/L of Cypermethrin on plasma testosterone in male *Labeo rohita* for 6 days. The results presented in Figure 3 and Table 2 which showed that testosterone level in male has decreased.

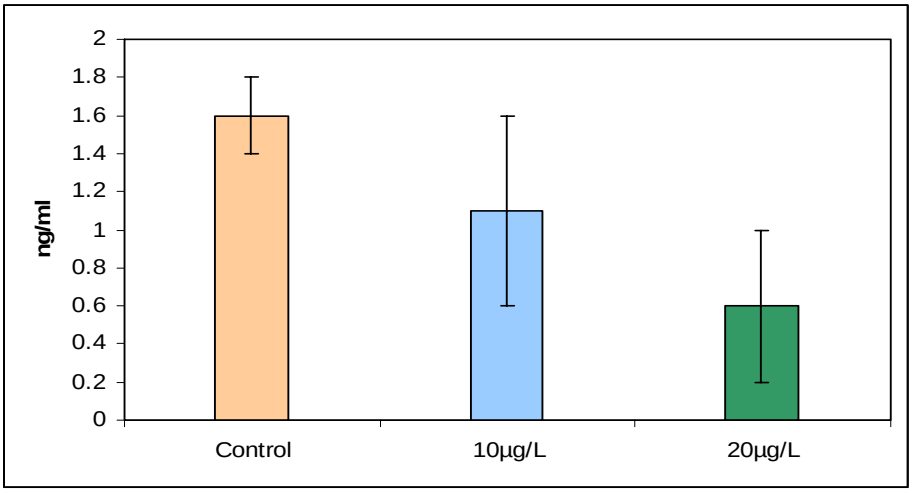

Figure 3: Levels of Testosterone in plasma of male *Labeo rohita* exposed to Cypermethrin

Table 2: Levels of Testosterone in plasma of male *Labeo rohita* exposed to Cypermethrin.

S. No	Sample	Mean ± SD (ng/ml)	DMR-Test
1	Control	1.6 ± 0.2	-
2	10µg/L	1.1± 0.5	Significant over control
3	20µg/L	0.6 ± 0.4	Significant over control and 10µg/L

Note: Values are mean ± SD (n=6)

With regard to 11-Ketotestosterone levels in male *Labeo rohita* decreased with increasing concentration of Cypermethrin from 10µg to 20µg/L after 6th day (Fig.4 and Table 3).

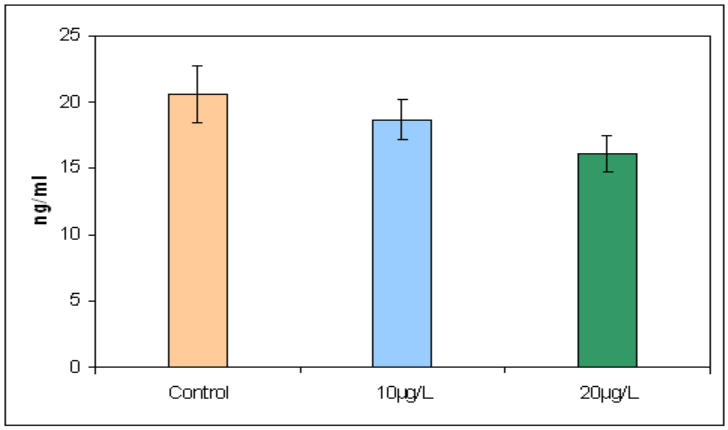

Figure 4: Levels of 11-Ketotestosterone in plasma of male *Labeo rohita* exposed to Cypermethrin.

Table 3: Levels of 11-Ketotestosterone in plasma of male *Labeo rohita* exposed to Cypermethrin.

S. No	Sample	Mean ± SD (ng/ml)	DMR-Test
1	Control	20.6 ± 2.1	-
2	10µg/L	18.7 ± 1.5	Significant over control
3	20µg/L	16.1 ± 1.4	Significant over control and 10µg/L

Note: Values are mean ± SD (n=6)

The total findings in the male *Labeo rohita* steroidogenesis were identified and shown in the Figure 5.

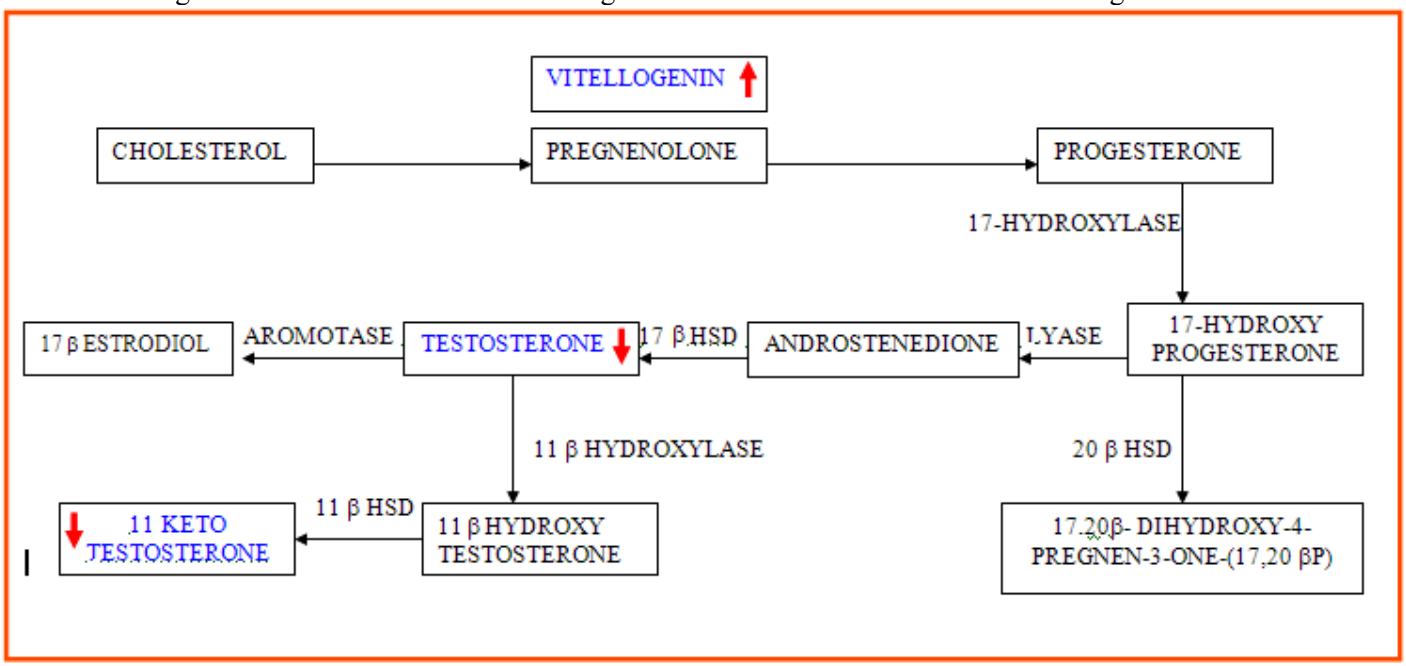

Figure 5: Summary of Findings in Male *Labeo Rohita* Exposed to Cypermethrin

DISCUSSION

Fish are often used as sentinel organisms for ecotoxicological studies because they play number of roles in the trophic web, accumulating toxic substances, responding to low concentration of mutagens (Cavas and Ergene-Gozukara, 2005) and also serve as bio-indicators of environmental pollution playing a significant role in assessing potential risk associated with contamination in aquatic environment since they are directly exposed to chemicals resulting from agricultural production via surface run-off or indirectly through food chain of ecosystem (Lakra, 2009). In our work we have selected male Labeo rohita as experimental animal to study the effect of Cypermethrin on different enzyme levels.

Sexual reproduction in vertebrates is mediated by steroid hormones and numerous endocrine disrupting chemicals (EDCs) discharged into the environment have been shown to interfere with hormone signaling via several mechanisms. In vertebrates including teleosts, reproduction is regulated by the brain-pituitary-gonadal (BPG) axis. Gonadotropins (GTHs), follicle stimulating hormone (FSH) and luteniging hormone (LH), are synthesized in the pituitary and directly act on gonads by circulation. Thus, these hormones are crucial modulators of steroidogenesis and gonadal development. Extensive *in vivo* and *in vitro* studies of salmonids have provided evidence that synthesis and release of FSH and LH are regulated by gonadal steroids (Tyler and Sumpter, 1996; Yaron et al., 2003). Especially, it showed that E_2, testosterone (T) and 11-ketotestosterone (11KT) have positive and negative feedback actions that take place in the pituitary, which has an important role in controlling gonadotropin synthesis and release(Yaron et al., 2003). Thus, it is anticipated that environmental estrogenic and androgenic compounds affect gonadotropin synthesis and release. Effects of environmental chemicals on fish reproductive activites have been investigated in freshwater teleosts such as zebrafish, goldfish, carp, mosquito fish and salmonids (Thomas, 2000). In this study we have studied the effect of Cypermethrin on Vitellogenin, testosterone and 11-ketotestoterone of male labeo rohita.

The production of vitellogenin (VTG) is an estrogen-dependent yolk protein precursor, which serve as a valuable biomarker for exposure to estrogen in oviparous vertebrates. This biomarker can be used in both *in vitro* (e.g., hepatocyte cultures) and *in vivo* studies. VTG is normally present only in the plasma of female fish, but males do have the VTG gene, and exposure of male fish to environmental estrogens can trigger the gene's expression, leading to VTG accumulation in the blood (Purdom et al., 1994; Copeland el al., 1986). Vitellogenins (Vg) are the major precursor of the egg-yolk proteins, vitellins (Vn), which provide energy reserves for embryonic development in oviparous organisms. They are high-density (300–700 kDa, according to species) glycolipophosphoproteins having Ca and Zn ligands, Vg synthesis in mature females occurs in response to endogenous estrogens, which are released into the bloodstream and stored in developing oocytes (Wahli et al., 1981). Liver is generally recognised as the organ responsible for vitellogenesis in fish. Both field and laboratory studies have shown the value of VTG as a rapidly inducible biomarker for estrogens and antiestrogens in both adult and juvenile fish (Jobling et al., 1998; Nilsen et al., 2004; Panter et al., 2002; Sumpter and Jobling 1995; Thorpe et al., 2000). VTG is a large glycol-lipid protein and, in some species, is particularly labile. The susceptibility of VTG to cleavage can lead to products with distinct antigenic profiles, which potentially can cause problems with accurate VTG quantification using immunology-based detection methods (Folmar et al., 1996; Hiramatsu et al., 2005; Larsson et al., 1999; Tatarazako et al., 2004). Useful features of VTG induction as a biomarker are the specificity for estrogens, the sensitivity, and the magnitude of the response possible. [Plasma VTG may increase by up to a millionfold, from nanograms per milliter to milligrams per milliliter concentrations (Tyler et al., 1990)].

Very high levels of VTG synthesis in adult fish can induce kidney failure (Herman and Kincaid 1988) and cause disruption in blood dynamics and function (Scholz and Gutzeit 2000). Field studies on wild roach in U.K. rivers have also shown an increased content of plasma VTG in intersex fish (Jobling et al., 1998) and that there exists a correlation (not necessarily a causation) between elevated VTG levels and the presence of intersex gonads.VTG levels increased significantly in fish exposed to methoxychlor (MXC) and 4-tertoctylphenol (OP), with respect to controls. (Ortiz-Zarragoitia and Cajaraville, 2005). In our results also levels of VTG were decreased when exposed to Cypermethrin dosage.

Versonnen et al., (2004) conducted an experiment to determine the estrogenic capacity of methoxychlor (MXC) in adult zebrafish. They exposed fish to 0, 0.5, 5, and 50µg MXC/L for 14d. Induction of Vitellogenin (VTG) in males was detected at 5 and 50µg MXC/L. Plasma VTG was significantly higher in male fish exposed to atrazine (Bringolf et al., 2004). Continuous exposure of adult zebrafish male and female to 2µg and 5µg/L of deltamethrin for 6 days caused changes in the four different hormones by effecting components of the reproductive endocrine system. Pronounced differences in plasma 11-ketotestosterone like changes in the dynamics were observed in male goldfish exposed to atrazine (Moore and Waring, 1998). From this they suggested that sensitive axis to the disruption by atrazine was effects on sex streroidogenesis.

Decrease in levels of plasma 11-ketotestosterone with testicular degeneration and alteration in the testis gene expression were reported in zebra fish exposed to benzafibrate (Velasco-Santamaria et al., 2011). Lowered 11-ketotestostrerone levels were shown in fathed minnow exposed to 1mg/L (Corton et al., 2000) which agreegated with our data in Table 3.

CONCLUSION

In conclusion we demonstrate that male *Labeo rohita* when exposed to Cypermethrin at a concentration of 10 and 20 µg/L for six days, interferes with the reproductive activity by altering the three hormones namely Vitellogenin, Testosterone and 11-Ketotestosterone.

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