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Serum Hormone Profiles in freshwater Catfish *Clarias batrachus* Exposed to Lambda-Cyhalothrin

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ABSTRACT

Hormone control in aquatic creatures is disrupted by synthetic pesticides, particularly endocrine disruptors like pyrethroids. These substances interfere with cellular uptake by attaching to the receptors of hormones or plasma transport proteins, imitating or blocking hormones. Fish thyroid hormones, which are essential for metabolic rate and stress-induced glucose mobilization, are especially impacted. Organophosphates and organochlorides hinder reproduction by inhibiting pituitary gonadotropin release and gonadal development. Pyrethroids, like lambda-cyhalothrin, are known to change the structure of the gonads and hormone-related processes. The purpose of this study is to better understand how Lambda-cyhalothrin affects freshwater catfish *Clarias batrachus's* serum hormone levels and how it contributes to endocrine disruption and reproductive dysfunction. The results could help address more general ecological concerns about pesticide pollution in aquatic ecosystems by exposing hormonal abnormalities and gonad structural alterations. Serum hormone levels were assayed using standard biochemical methods. Using radioimmunoassay, plasma triiodothyronine (T3) and thyroxine (T4) were evaluated. Serum hormone levels in *Clarias batrachus* significantly decreased overall ($P < 0.01$) after exposure to sub-lethal amounts of lambda-cyhalothrin. Following exposure to the sub-lethal quantities of the pyrethroid, both groups exhibited an overall significant ($P < 0.01$) fluctuation in the blood cortisol levels, with T4 and estrogen (E2) levels dropping noticeably ($P < 0.05$). Progesterone levels were declined by 73.51% and 63.50% after 45 days in higher and lower exposure groups, respectively. These hormonal disruptions indicate endocrine interference by the pyrethroid.

INTRODUCTION

Endocrine system plays an important role in various physiological processes such as reproduction, metabolism, and growth of animals (Ankley et al., 1998). Many pesticides are now suspected of being endocrine disruptors. These endocrine disruptors are either by binding to the hormone's receptors and mimic the hormone or block the action of the hormone. Synthetic chemicals also interfere with hormone transport systems by binding to plasma proteins responsible for the distribution of endogenous hormones (Ankley et al., 1998). This interference thus ultimately affects the cellular uptake of hormones. Thyroid hormones change the metabolic rate by regulating oxidative metabolism at the mitochondrial level (Peter and Oomen, 1993; Sheridan and Kao, 1998). Thyroid hormones play an important role in stress related mobilization of glucose in fishes. Fish behavior has also been linked to thyroid hormones, according to Castonguay and Dutil (1990). The T3:T4 ratio has been shown to be considerably changed by stress. Dopamine, noradrenalin, and adrenaline are together referred to as catecholamines, and their amounts vary depending on the species (Mazeaud et al., 1977; Folmar 1993). The primary catecholamines implicated in fish reactions to stress, noradrenalin and adrenaline, have varying levels in the bloodstream depending on the species (Mazeaud et al., 1977).

The inter-renal tissue in the fish head kidney secretes cortisol, another important corticosteroid hormone (Chester-Jones et al., 1969). It has been documented that ambient chemical stressors like acid cause an increase in plasma cortisol (Brown et al., 1984; Goss and Wood 1988). Numerous investigations have documented the function of cortisol in lipid mobilization (Lidman et al., 1979). Enzymes like glucose-6-phosphate dehydrogenase of the glycolytic pathway and succinate dehydrogenase and malate dehydrogenase of the Kreb's cycle have been shown to be less active when cortisol is present (Barton et al., 1987; Andersen et al, 1991).

Certain environmental contaminants are also known to alter the reproductive physiology of animals by exerting their effects on the secretion of reproductive hormones by interfering with hypothalamic-pituitary-gonadal axis. Pesticides such as organophosphorous and organochloride compounds suppress reproduction, development of gonads and gonadotropin

secretion from the pituitary gland and decrease gonadal activity during different phases of reproductive cycle of the organisms exposed to them (Lal and Singh, 1987). Pyrethroids are also known to disrupt hormone-related function (Go et al., 1999).

Disruption of fish reproductive process with alteration in gonad structure due to pesticide exposure has been reported by various authors (Kelce and Gray, 1999). Hence, this study aims to study the effect on serum hormones of freshwater catfish, *Clarias batrachus* after exposure to synthetic pyrethroid Lambda-cyhalothrin.

MATERIALS AND METHODS

Acquisition of Lambda-cyhalothrin, Synthetic pyrethroid; collection, grouping, and maintaining the fresh water female catfish, *Clarias batrachus* for analysis was followed by the method Gulati et al., (2025).

Assay of Serum Hormones:

Radioimmunoassay of T3 and T4:

Plasma T3 and T4 were estimated using the RIA kits of Bhabha Atomic Research Centre, Bombay. 200 ml of 0.14 M Tris (pH 8.6) was taken in assay tubes along with 50 ml of plasma samples. To this 100 ml of + 25 1 labelled T3 and T4 were added. After gently mixing, the tubes were incubated for 45 minutes for T3 and 30 minutes for T4 at 37ºC. At the end of incubation 1.0 ml of polyethylene glycol was added to all tubes. The tubes were gently vortexed gently and centrifuged at 1000 rpm in a swing out rotor for 20 minutes. The supernatant was discarded and the precipitate was counted in Beckmann gamma counter (DP 5500) for one minute. Standard displacement curves were prepared using T3 and T4 standards in hormone free serum in a similar manner.

The plasma T3 and T4 was expressed as ng/ml.

Estimation of Plasma Cortisol:

The fluorimetric method of Mattingly (1962) was used to measure cortisol, and the plasma cortisol level was reported as ng/ml

Radioimmunoassay of 17ß- Estradiol and Progesterone:

17-ß Estradiol was estimated according to the procedure of Lamba et al. (1981). The serum 17-ß estradiol level was expressed as pg/ml.

The progesterone assay was performed using [1, 2, 6, 7-3H] progesterone (specific radioactivity 85G/mmol, ICN, CA, USA) and its antiserum. The assay procedure was similar to that of 17-ß estradiol. The cross reactivity of the antibody was 3% with 17ß hydroxyl progesterone and 1% with 17, 20ß-dihydroxy progesterone. The sensitivity limit of the assay was 10 pg/ml. The intra and inter assay coefficients of variations was 3.5 and 4.7% respectively. The serum progesterone level was expressed as ng/ml.

RESULTS

The variations in the serum hormone levels of both the groups of pyrethroid-exposed fishes are tabulated (Table 1a & 1b).

An overall significant decline ($P < 0.01$) was witnessed in the serum levels of the fishes exposed to the higher and lower sub-lethal concentrations of the pyrethroid. Though the fishes exposed to the higher sub-lethal concentration of lambda-cyhalothrin showed a significant decline ($P < 0.05$) in the serum T3 levels on the 30^{th} and 45^{th} days of exposure, the fishes exposed to the lower sub-lethal concentration of the pyrethroid showed significant variation ($P < 0.05$) at all durations of exposure.

The serum T4 levels also showed a significant decline ($P < 0.05$) in the pyrethroid-exposed fishes of both groups in comparison to the control group of fishes. A decline of 70.73% and 57.87% was witnessed in the serum T4 levels of the fishes exposed to the higher and lower sub-lethal concentrations of lambda-cyhalothrin respectively.

A significant increase ($P < 0.05$) was witnessed in the serum cortisol levels of both the groups of pyrethroid-exposed fishes in comparison to the control group. A similar trend of variation was witnessed in both groups of Pyrethroid-exposed fishes. An initial significant increase ($P < 0.05$) witnessed in the cortisol levels was followed by a significant decline ($P < 0.05$) as witnessed at the end of the exposure period of 45 days. However, both groups showed an overall significant ($P < 0.01$) variation in the serum cortisol levels after exposure to the sub-lethal concentrations of the pyrethroid.

The estrogen levels (E2) showed a significant drop ($P < 0.05$) in fishes after exposure to the sub-lethal concentrations of lambda-cyhalothrin in the present study. The fishes exposed to the higher sub-lethal concentration showed an overall decline of 76.28% in the estrogen levels after 45 days of exposure to the pyrethroid, while the fishes exposed to the lower sub-lethal concentration showed a decline of 59.59% in the serum estrogen levels.

In addition to the variation in the estrogen levels of the pyrethroid-exposed fishes, a significant decline ($P < 0.05$) was also witnessed in the serum progesterone levels on the 15^{th}, 30^{th} and 45^{th} day of exposure in both the groups of pyrethroid-exposed fishes. A comparison of the progesterone levels of the fishes at different durations of exposure also showed significant variation ($P < 0.05$) in both the experimental groups. An overall decline of 73.51% and 63.50% was witnessed in the progesterone levels of the fishes after 45 days of exposure to the higher and lower sub-lethal concentrations of the pyrethroid respectively.

DISCUSSION

Cortisol is also suggested to play a vital role in reduction of ionic imbalance in stressed fish. The present study witnessed an initial significant experimental period. This increase in the cortisol levels indicates its role in increase in the cortisol levels followed by a decrease by the end of the gluconeogenesis whereby glucose production is enhanced via stimulation of protein catabolism (Lidman et al., 1979: Marshall Adams et al., 1985) which provides free amino acids that act as precursors for glucose synthesis and the process being regulated in the liver by cortisol (Lidman et al., 1979; Murat et al, 1981) by enhancing the activity of gluconeogenic enzymes such as PEPCK and pyruvate carboxylase (Mommsen et al., 1999) as also observed in the present study.

According to earlier reports based on in vitro research, the effect was exerted by stress-induced rises in cortisol levels in the bloodstream, which had a direct impact on gonadal steroidogenesis (Carragher and Sumpter, 1990). However, cortisol has a variable and weak inhibitory effect or sporadic stimulatory effect on ovarian synthesis of steroids making attempts to replicate this work mainly fruitless (Pankhurst, 1998). The impact of exogenous cortisol administered in vivo on reproductive endocrine systems and development was the subject of other investigations (Foo and Lam, 1993). However, the chronic nature of these experiments with wide range systemic effects of cortisol make it unclear whether this reflected a direct effect on reproduction or secondary effects resulting from the immunosuppressant and metabolic effects of chronically elevated plasma cortisol levels (Pankhurst and Van Der Kraak, 1997).

Chronic toxicity of cypermethrin is reported to have adverse effects on the reproductive parameters in mysid shrimps (USEPA, 2005). Lambda-cyhalothrin treatment has also been associated with overt signs of stress (Ratnasooriya et al., 2002). Earlier studies have shown that pyrethroids may act as hormone disruptors (Go V, 1999). In the present study, a significant decline was witnessed in the levels of serum 17-3 estradiol (E2) and progesterone in the pyrethroid-exposed fishes. Earlier studies have shown that the action of stress and cortisol is neither at the level of pituitary release of GtH nor the conversion of testosterone to E2 by ovarian follicles. Instead the inhibition may occur at some point between GtH-receptor interaction on the cell membranes and conversion of upstream steroid substrates to testosterone attributing the fall in the E2 levels to the reduction in the substrate pool of testosterone. Also the decline in the CYP19 aromatase activity could bring about a decline in the testosterone conversion to E2 (Vinggaard et al., 2000) leading to lowered plasma levels of E2. Similar reasons could be attributed to the lowered E2 levels in the fishes subjected to lambda-cyhalothrin exposure in the present study.

It is evident that different pesticides have varying effects on steroidogenesis. More molecular research is required to determine the precise point at which the pesticide used in this study, lambda-cyhalothrin, disrupts the synthesis of steroids.

Though it may target the pituitary-gonad axis and compete for their receptor binding sites, lambda-cyhalothrin can be regarded as an endocrine disruptor in fish, influencing gonadal activity. The current result confirms the earlier findings of Singh et al. (1993), who found that *Heteropnesutes fossilis* exposed to gamma-BHC had reduced ovarian steroidogenesis and steroid release into plasma. Chatterjee et al. (2001) similarly documented a similar decrease in 17-κ-Estradiol levels in the serum of *Heteropneustes fossilis* subjected to carbofuran. In an in vitro study Oduma et al. (2006) reported a significant inhibition of 17-ß Estradiol and Progesterone hormones in rat follicular and luteal cells when exposed pesticide heptachlor.

In the present study, a significant decline was seen in the plasma Ts and Te levels throughout the period of exposure to the sub-lethal concentrations of the pesticide. A significant and progressive reduction in the serum Te levels was reported by Kirubagaran and Joy (1994) and Bhattacharya et al. (1989) in *Clarias batrachus* and *Channa punctatus* respectively. The decline in the serum Ta levels could be attributed to the impairment of hormone synthesis and its release into the circulation under conditions of toxicant stress (Kirubagaran and Joy, 1994). Bhattacharya et al (1989) attributed the decline in the T4 levels to the inhibition in the activity of lysosomal protease (cleaves T4 from thyroglobulin for release into the blood) and cytosolic iodide peroxidase (oxidizes inorganic iodide to active elemental iodine). Similarly reasons could also be attributed to the decline in the plasma Ta and T_4 levels in the present study. The site of interference of lambda-cyhalothrin with the thyroid hormone levels in the serum requires further investigation.

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TABLES:

Table 1a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on serum hormone levels of *Clarias batrachus*

Hormone	F Value	P value	Control	Experimental Days			
				15	30	45	Recovery
T3 (ng/ml)	134.92	0.000**	2.877^{d}± 0.205	2.628^{cd}± 0.171 (-8.65)	1.438^{b}± 0.118 (-50.01)	0.397^{a} ± 0.161 (-86.20)	2.053^{c} ± 0.207
T4 (ng/ml)	504.30	0.000**	6.708^{e}± 0.107	5.648^{d}± 0.164 (-15.80)	3.678^{b}± 0.182 (-45.16)	1.963^{a}±0.188 (-70.73)	5.108^{c}± 0.167
Cortisol (ng/ml)	301.94	0.000**	3.333^{a}± 0.147	4.922^{c}± 0.171 (+47.67)	6.690^{d}±0.228 (+100.72)	4.073^{b}±0.113 (+22.20)	3.294^{a}± 0.077
E2 (pg/ml)	8241.17	0.000**	4350.5^{e}± 21.016	4144.3^{d}± 38.082 (-4.73)	2323.8^{b}± 30.869 (-46.58)	1031.8^{a}±32.510 (-76.28)	3448.0^{c}±27.166
Prg. (ng/ml)	248.73	0.000**	4.550^{e}± 0.132	3.138^{c}± 0.163 (-31.03)	2.495^{b}± 0.214 (-45.16)	1.205^{a}± 0.162 (-73.51)	3.662^{c}± 0.108

Table 1b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on serum hormone levels of *Clarias batrachus*

Hormone	F Value	P value	Control	Experimental Days			
				15	30	45	Recovery
T3 (ng/ml)	275.60	0.000**	2.915^{d}± 0.047	2.370^{c}± 0.153 (-18.69)	1.343^{b}± 0.216 (-53.92)	1.148^{a}± 0.043 (-60.62)	2.188^{c} ± 0.103
T4 (ng/ml)	343.23	0.000**	6.588^{e}± 0.144	6.065^{d}± 0.119 (-7.93)	4.275^{b}± 0.216 (-35.10)	2.775^{a}± 0.158 (-57.87)	2.598^{c}± 0.169
Cortisol (ng/ml)	474.27	0.000**	3.628^{a}± 0.092	5.137^{c}± 0.083 (+41.59)	6.193^{d}± 0.153 (+70.70)	4.168^{b}± 0.090 (+14.88)	3.603^{a}± 0.073
E2 (pg/ml)	6303.04	0.000**	4334.5^{e} ± 25.955	4231.5^{d}± 28.525 (-2.37)	3043.3^{b}± 27.035 (-29.78)	1751.5^{a} ± 25.013 (-59.59)	3646.0^{c}± 26.495
Prg. (ng/ml)	336.49	0.000**	4.658^{e}± 0.127	3.608^{c}± 0.125 (-22.54)	2.775^{b}± 0.136 (-40.42)	1.700^{a}± 0.104 (-63.50)	3.973^{d}± 0.128