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Effect of Alphacypermethrin (Pyrethroid) on the Thyroid Gland of Fresh Water Air-Breathing Fish *Clarias Batrachus* (Linn)

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Abstract: ***Modern agricultural practices results in discriminate use of various agro- chemicals in the field has the potential to change the aquatic medium affecting the tolerance limit of aquatic fauna and flora as well as creating a danger to the ecosystem. The present study reports the chronic sub-lethal toxicity of alpha cyper-methrin to fish Clarias batrachus (Linn) at histopathological level of the thyroid gland. It is in this perspective, the present investigation was undertaken to find out histopathological alteration in experimental fish Clarias batrachus (Linn) exposed to sub lethal dose of alphacypermethrin (0.95 mg/L) for a period of 21 days to explore histological changes which may affect physiology of the gland and health of fish. Thus the thyroid gland of Clarias' batrachus after alpha-cypermethrin treatment showed hyper trophy hyperplasia and mild hyperemia.***

Keywords: Alphacypermethrin, Fish, $LC_{50,}$ Toxicity, Concentration, Sub-Lethal, Thyroid, Metamorphic

1. Introduction

Thyroid gland is a dumb-bell shaped bilobed gland. This gland is endodermal in origin arising from the primitive forgut. It is the largest endocrine gland. It controls basal metabolic rate. It increases the activity of ($^{Na+ - K+)}$ ATP. Histopathological changes have been widely used as bio-markers in the evaluation of the fish health exposed to contaminants. The alterations found in the organ can easily be identified than functional one and serve as warning sign of damage to animal health (Fanta et.al. 2003, Hinton and Laveen-1990). The function of thyroid gland is to elaborate, store and discharge secretions. Its basic function is to concentrate iodine and synthesize the thyroid hormone. The thyroid has greater capacity to store its secretions called colloid.

Thyroid is the only gland which has greater capacity to store its product outside the cells (=Lumen of follicle). When the gland is active there is tendency for colloid to accumulate and the epithelium to become low colloidal to squamous or flattened. When the gland is over active the colloidal storage are depleted and the epithelium becomes columnar.

The alterations of the hormonal level may be used as a potential bio marker and also can be established as the ability to endocrine tissue to respond to their appropriate releasing factors.

In fish hormones are critical towards maintaining proper physiological function and amongst the many hormones thyroxine (T_4) and tri-iodo thyroxine (T_3) are known to play important role in fish growth (Higgs et.al. 1982, Miwa and Inui-1985) and early development (Brown.1997).

When fishes are exposed to stressors, the levels of thyroids hormones have been demonstrated to be decreased (Pickering . 1993 Deane et.al. 2001)and chemical pollutants have been reported to detrimentally affect thyroid hormones status in number of fish species (Xu-et.al. 2002, Brown-et al 2004, Scolt and solmon-2004 Vander van et.al. 2006).

Studies have found, fish in contaminated water with reproductive defects as well as alarmingly high ratios of female to male fish in some locations (Kct-2009). One potentially devastating threat to wild fish populations comes from male fish exposed to estrogen becomes feminized, retards normal sexual maturation including egg production (Hogan, 2008).

It is reported that population living around the active agricultural regions are highly prone to cancer. Thyroid and bone cancers are prevalent in agricultural regions where fungicides are extensively used (Schreinemachers et.al. 1999).

Recent studies have shown that the incidence of hormone related organ cancers of hormonal cancers have increased among farmers exposure to endocrine disrupting (ECDs) pesticides particularly DDT and phoenix herbicides is the suspected cause in some of these hormonal cancers (Buranana trevedh and Roy 2001).

2. Materials and Methods

The air breathing *Cat-fish*, *Clarias batrachus* (*Linn*) commonly called '*MAGUR*' in local language were selected for the present studies as the test animals. These fishes belong to the family *Claridae* of the order *Siluriformes* (Lacking scales on the body). Live fishes of average length 18-22 cm and weight- 30~40 gm were procured from local non polluted ponds and were brought to the laboratory in large earthen pots half filled with water and covered with mosquito net. They were thoroughly washed and rinsed in 0.1% KMno4 solution to remove dermal infections if any, before transferring them 40 litre rectangular glass aquaria for acclimation to various laboratory conditions viz: Light, temperature and physico-chemical characteristics of test water (running tap water). Acclimation period lasted for a fort-night and no acriation

was done during the entire period of experimentation. The fish were allowed to adjust to natural photo-period and ambient temperature. No food was given to fish for the initial four days of acclimation with a view to prepare them to accept artificial food. Small pieces of egg white were supplied to them as food, every day adlibitum at 10.30 AM. The feeding schedule was strictly followed and was stopped 24 hours before the start of the after the end of exposure period (Day-21) to study histological changes in glands of fishes from treated and control group were sacrificed by decapitation.

At sacrifice the lower jaws with thyroid were decalcified in 6% solution of formic acid in 5% formaldehyde for about a week.

The tissues were dehydrated by passing through various grades of alcohol cleared in xylem embedded in paraffin wax (56- 58^0 c).

Blocks were prepared and sections of 5-6 µm were cut from wises rotator microtone and were strained with eosin and ehrlich's haemotoxyline.

Assessment of thyroid activity was measured by microscopic examination of transverse section (5-6 µm) of lower jaw in both control and experimental groups. This was supplemental with measurements in the following parameters:

1 Average number of follicles in a section (5-6 section/fish).
2 Average follicle cell height.

It was calculated by measuring 5 to 6 cells per follicle at random in each of the 4 to 5 follicle per sections. These measurements were carried out in 5 sections per animals. Thus the average epithelial cell height of the animals was calculated.

3 Average size of the follicle:

Average size of the follicle was estimated by measuring diameter of 5-6 follicles per section and 5 sections per animal to find out the mean diameter of follicles of thyroid gland in both control and treated fish.

4 Average numbers of cells per follicles:

The number of cells per follicles was counted randomly in follicles per sections. The procedure was followed for 5-6 follicles per section and 5-6 slides per animal tissue which yielded the average number of cells per follicles.

The data obtained after various measurements were tested for statistical significance. The experimental values were evaluated statistically by student-t-test.

3. Observation

- The thyroid of Clarias batrachus in normal histology showed: Presence of follicles of various sizes filled up with colloid.
- The thyroid gland was found not covered by definite wall encapsulated and somewhat elongated.
- Each follicle contained a central cavity surrounded by a wall composed of single layer of epithelial cells.
- The epithelial cells were of two types:

(i) Chief cells which were columnar or cubical in shape having oval nucleus and clear cytoplasm.
(ii) Colloid cells or Benstay cells.

They contained droplets of secretary materials. The follicles were supported in position by connective tissue fibers which surrounded them. The central lumen of follicle was found filled with colloid containing chromophobic and chromophilic vacuoles.

Marked histological changes were observed in alpha-cypermethrin treated thyroid of fish *Clarias batrachus* showing:

- Increased number of follicles which appeared irregular in shape.
- The diameter of the follicle was found decreased as compared to control but the height of the follicle was found increased
- The number of cells per follicles showed a significant reduction in number.
- In some follicle, cells lining the follicle were found multiplied and projecting into the follicular cavity.
- In some follicles, loss of colloid material observed. These follicles were found closely packed and widely distributed in the lower jaw region.
- The follicles were seen with irregular shape and the follicles gradual increase in number in some section.
- In some follicles, cells lining the follicle were found multiplied and projecting into the follicular cavity.
- In some follicles loss of colloid material was observed. There follicles were found closely packed and widely distributed in the lower jam region.
- Some follicles in some section were seen with irregular shape and the follicles showed gradual increase in number.
- The follicular diameter was reduced and the secretary cell showed hyper-trophy and loss of colloid in some follicles.
- Vascularity of the thyroid gland in general was found increased. Blood cells were also observed with follicles.

Table

Morphometric analysis of thyroid gland in control and cypermethrin treated fish *Claries batrachus* for a period of 21days values are ± SE of 6 readings (0.9mg of ∝ -cypermethrin)

Parameters	Experimental period (21 days)	
	Control	Treated
1.Follicles per section	**-28.4 ± 2.7**	**103.9 ± 6.2 NS (265.9 ↑)**
2.Cell height(µm)	**-3.97 ± 0.25**	**5.95 ± 0.23 (48.76 ↑)**
3.Follicular diameter	**-59.6 ± 3.16**	**21.40 ± 2.2** (64.10 ↓)**
4.Cells per follicle	**-34.8 1± 3.5**	**19.39 ± 2.1*** (44.3 ↓)**

Values in parenthesis indicates percent change
P-values=*<0.1 **<0.01 ***<0.001

4. Results and Discussion

Thyroid gland regulates the organ, organ system and the metabolic process. The thyroid gland of Ambystoma maximum releases optimal amount of hormone necessary for metamorphosis following proper TRH stimulation (Darra and Kuhn - 1982).

In *Clarias batrachus* the thyroid gland are concentrated around the ventral aorata, middle ends of two pairs of afferent areteries and the paired interior jugular veins and is not covered by definite walls.

The thyroid in fish is not a discreet organ but fused in kidney also hence it is known as heterotrophic-thyroid. The thyroid follicles are scattered in the hemopoietic tissue of the head kidney (Mesonephric lobe) in air breathing fishes.

The follicular epithelium is visible and contains colloid which is strongly acidophilic, dense, homogenous and non-vacuolated. It appears that the thyroid follicles are migrating from the pharyngeal region to the kidney (Baker 1958 a and b).

The increase in quantity and activity of follicle is due only to continuous stimulation of thyroid from TSH .This results in colloidal content and cells become columnar in shape and increase in size.

In absence of TSH, synthesis of thyroid hormone is minimum, as a result the follicle cells become flattened and lumen remains enlarged and full of colloid.

Thyroid follicles have a remarkable ability to trap inorganic iodine (I_2) which can be stored and incorporated into hormones which are stored in follicle cavity.

The tri (T_3) and tetra (T_4) iodothyroxine (=thyroxine) derived from mono and di (T_1 and T_2) iodo–thyrosine undergoes further condensation process. The T_1 & T_2 are de-iodinated within the follicular lumen while the T_3 & T_4 are released into blood for circulation.

According to Sinha and Singh (1990), extra thyroidal conversion of T_4 to T_3 increases during spawning phase as compared to pre-spawning in fresh water *Catfish Clarias batrachus.*

In the present study histomorphometric study clearly reveals that pyrethroid alpha-cypermethrin induced changes in number, diameter and cell number of follicles & alteration in mean cell height of thyroid follicle. This clearly indicates departure from normal function of thyroid gland under pyrethroid stress.

Therefore, pyrethroid alpha-cypermethrin acted as an endocrine disruptor (EDC), in present study. The EDC are compounds that alter the normal function of endocrine system of wild life and humans. A large number of chemicals have been identified as EDC among them are several pesticides.

In particular EDCS can bind to and activate various hormone receptors (R) Such as AR, ER, AhR, PX-R, CA-R, ER-R. And then mimic the natural hormone action (agonist action). EDCs may also bind to these receptors without activating them. This antagonist action blocks the receptors and in hibits their action blocks the receptors too and inhibits their action.

EDCs may also interfere with the synthesis, transport, metabolism and elimination of hormone, thereby decreasing the concentration of natural hormone. For example, thyroid hormone production can be inhibited by some ten endocrine disruptor pesticides (EDCs). These include Amitrole, Cyhalothrin, Fipronil, Ioxyynil, Maneb, Mancozeb, Penta chloro benzene, Pro-diamine, Pyremethanil, Thiazopyr, Ziram, Zinecb etc. (Cocco-2002, Akhtar et.al. 1996, Leghait.et.al. 2009, Sugiyama et.al. 2005).

Endocrine Disrupting Effects on thyroid are well documented which includes some insecticides like Cyper methrin (Chen et.al, 2002, MC Carthy et.al. 2006). Dimethoate (Rawlings et.al. 1998, Mahjoubisamet et.al. 2005), Trichloroform (Nicolau et.al. 1983), Herbicideslike Acetachlor (Rollrrova et.al. 2000, Crunp et.al. 2002), Metribuzin (Porter et.al. 1993) and fungicides like HCB (Ralph et.al. 2003, Verreault et.al. 2004).

Acetachlor affects thyroid hormone gene expression; Dimethoate causes disruption of thyroid hormone action where as HCB severely disrupts thyroid hormone production Metribuzin causes hyper thyroidism and Trichloroform causes alteration of thyroid function.

This result indicates "limit the use of synthetic pesticides" and limitations and/ or bans should be sought along with alternative solution that are safer and non toxic to environments. One such alternative is so called "Natural Pesticides" (that are not synthetic) botanical pesticides (Pyrethrum, Limonene and many others) microbial/ biological agents (microbes and parasites) and inorganic minerals (boric acid, Lime stone and diatomaceous earth).

Available experimental results and morphometic analysis of thyroid gland of *Clarias batrachus* point towards endocrine disruptor capability of alpha-cypermethrin. Unfortunately concerned literatures were not available suggesting that it is first time reported experiment based information.

Chronic exposure of 50 days, the server I_3 (Tri-iodothyroxine) level decreased in the three major carps to organophosphate insecticide Chloropyrifos (Tilak et.al. 2005). Anterior pituitary in response to negative feedback mechanism was found involved in concentrating of free plasma T_3.

Such pesticides induced changes in circulatory thyroid hormone has been observed by earlier authors, Sinha et.al (2005) in *Clarias batrachus.*

In fish blood there are several thyroxine (TH) binding properties including TTR (Trans. thyretin) which has been recently identified in fish (Power et.al. 2000). It is known that some proteins bind to human TTR inhibiting TH binding and transport (LANs et.al. 1994).

5. Conclusion

In conclusion, the thyroid gland of *Clarias' batrachus* after alpha-cypermethrin treatment showed hyper trophy hyperplasia and mild hyperemia. The morphometric analysis of the thyroid gland with statistically analyzed data is presented in table to substantiate the statement.

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