Ultrastructural changes in testis of gobiid fish *Glossogobius giuris* (Ham) induced by fenthion

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Pesticides are known to affect the testis in fishes and cause cytomorphological changes or gross physiological alterations due to changes in its histology, retard gonadotrophic secretions thereby causing regression of the gonads. Light and ultrastructural studies were made on control and fenthion treated testis, interstitial gland and sperms of *G. giuris* during spawning phase after exposing them to different sub-lethal concentrations (0.05, 0.25 and 0.5 ppm) fenthion for a short-term period (24, 48, 72 and 96 hr). The results indicated extensive cytotoxic damages in the testis including atrophy of testis, reduction in lipoidal material in the interstitial cell and degeneration of sperms. The alteration in histology of testis, interstitial cell and sperms progressively increased with the increase in the sub-lethal concentration of pesticides.

Key words: Fish *Glossogobius giuris*, fenthion, testis.

Pesticide influx in the aquatic ecosystem is continuous due to its extensive and indiscriminate application for the agricultural purposes resulting in aquatic pollution. Fishes are one of the main victim of such pollution. In fishes, pesticides seem to retard gonadotrophic secretions, thereby causing inhibition of gonadal growth. Harilal and Sahai have reported that interstitial cells in the testis of *Mystus teengara* become necrotic after treatment with malathion and BHC. Similar observations have been reported in the gonads of *Symbranchus bengalensis*. Although, extensive light microscopic work has been done on control and pesticide exposed testis of fish, very little is known about the ultrastructural changes in the testis of different fishes. Hence, in the present investigation, light and ultrastructural studies including the interstitial gland and sperms in the testis of *Glossogobius giuris*, have been made during spawning phase, after exposing them to different sub-lethal concentration of fenthion, one of the widely used organophosphorus pesticides for the eradication of mosquito larvae.

Materials and Methods

The adult male fish, *G. giuris* (40-60g; length 90-120 mm) were collected from Sanathkumari tank, near Bangalore. They were maintained in the laboratory conditions in glass aquaria at 27°± 1° C and were fed with earthworms. Different concentrations of fenthion were prepared in alcohol medium (stock solution of fenthion - 0.5mg/l) and added separately to the test water to obtain the desired concentration by adopting serial dilution technique as outlined in APHA. The acclimated fishes were divided in 4 experimental groups of 10 each. Of these, three groups were placed separately in sub-lethal concentration of fenthion (0.05, 0.25 and 0.5ppm) while the fourth group served as control. They were starved for 24 hr prior to the start of the experiment and were not fed during the course of the experiment.

The fishes were vivisected at the interval of 24, 48, 72 and 96 hr from experimental as well as control groups.

**Light microscopic study:**

Pieces of testis from control and experimental groups were removed and fixed in Bouin’s fluid, dehydrated in grades of alcohol and embedded in paraffin for histological examination. Section (5μm thick) were stained in Ehrlich’s haematoxylin - eosin.

Since, cytotoxic effect was seen after 24 hr o treatment in higher concentration (0.5ppm) of fenthion, electron microscopic studies were conducted only for 24 hr period.

**Electron microscopic studies**

For these studies, small pieces of testis, from control and experimental fishes vivisected after 24 h (0.5ppm concentration) and fixed in 2.5% glutaraldehyde (0.1M phosphate buffer, pH 7.4) for 48 hr. After post fixation in 1% osmium tetroxide the specimen was dehydrated in grades of alcohol...
Fig. 1 — Cross section of the testis of *G. giuris* showing seminiferous lobules with masses of spermatozoa (SP) IG - Interstitial gland (H–E staining) × 600; Fig. 2 — Electronmicrograph of interstitial cell of control fish, *G. giuris*. (Note a clear perinuclear zonae (arrows), N - nucleus, M - mitochondria); Fig. 3 — Electronmicrograph of interstitial cell cytoplasm of the control fish showing mitochondria with tubular cristae (arrows), lipid globules (L) and smooth endoplasmic reticulum (ER); Fig. 4 — Electronmicrograph of spermatozoa of control fish showing prominent head (N-nucleus) neck and tail (T) consisting of axial filaments. Annulus mitochondria (M) and centriole (C) are present; Fig. 5 — Electronmicrograph of enlarged view of sperm nucleus (N) of the control fish showing dense homogenous chromatin in the nucleus and distinct vesicular cristae of rod shaped mitochondria (M).
4°C and embedded in epoxy resin. The specimen block was kept in incubator for hardening. Gross trimming and rough sections were made. Thick sections (1 μm) were stained with 1% toludine blue solution and examined under compound microscope. Ultrathin sections (700-800 μm) were placed on copper grid, stained with uranyl acetate and lead citrate and were then observed under electron microscope.

Results

Histology of testis

The testis of *G. giuris* is an elongated organ, mainly composed of two tissues, the peripheral seminiferous tissue and central glandular tissue (Fig. 1). The seminiferous tissue is made up of single layer of seminiferous lobules whereas the glandular tissue consists of interstitial glandular cells.

Ultrastructure of interstitial cell and spermatozoa

Control:

In untreated fishes, the glandular cells are large and spherical, with eccentric nuclei and with a prominent nucleoli. The nucleus showed uniformly distributed chromatin material (Fig. 2). The cytoplasm contained a clear perinuclear zone and one/two lytic bodies and prominent smooth endoplasmic reticulum (Fig. 3) along with few lipid globules characteristic of steroid producing cells. Large number of mitochondria were present with dense matrix and tubular cristae.

The inner wall of each seminiferous lobule is covered with cysts in which germ cells develop. After metamorphosis from spermatids, spermatozoa are discharged from the cyst into the cavity of seminiferous lobule. Each spermatozoa consists of a prominent head, neck and tail consisting of axonemal complex. The nucleus of spermatozoa contains condensed chromatin material (Fig. 4) and in higher magnification the residual cytoplasm around the nucleus show few rod shaped mitochondria with distinct vesicular cristae (Fig. 5), while that surrounding the middle piece contains large number of mitochondria. The axonemal complex of the tail consists of single pair of centrally placed tubular filament and nine pairs of circumferentially arranged peripheral axial filaments. A few dilated smooth endoplasmic reticulum are seen surrounding the complex.

Fenthion treated

Extensive cytotoxic damage and other abnormalities were observed ultrastructurally in the testis including the interstitial gland cells as a result of fenthion treatment. Hence, after 24 hr of treatment with 0.5 ppm of fenthion shrinkage of interstitial cells and its nuclei led to the formation of condensed and clumped chromatin material. Nucleolus was in

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**Fig. 6**—Electronmicrograph of interstitial cells of fenthion treated fish showing clumped chromatin material in the nucleus (N), shrunk mitochondria (arrow) and a few vacuoles (V). Lip globules are absent; **Fig. 7**—Electronmicrograph of spermatozoe of fenthion treated fish showing less dense chromatin in nucleus (N), few empty mitochondria (M) and tail with discontinuous filaments (arrow), **Fig. 8**—Electronmicrograph of enlarged view of sperm nucleus (N) of the treated fish showing granulat chromatin material in the nucleus and hypoplastic cristae degenerated mitochondria (M).
degnerating condition. The cytoplasm was reduced to a granular material with fragmented endoplasmic reticulum and was almost devoid of mitochondria and lipid droplets. The clear perinuclear zone observed in control cell was absent in treated ones (Fig. 6).

The spermatozoa of the fenthion treated fish showed atrophy in the head and tail region. The shape of the head became irregular with disintegrating nuclear membrane (Fig. 7). The nucleoplasm consisted of granular material. The mitochondria showed degenerative changes mainly reduction in stainability and hypoplastic cristae (Fig. 8). The tail was reduced to thread-like structure.

Discussion

Ultrastructural study of the testis of the fenthion treated (for 24 hr in 0.5ppm) G. giuris, revealed that abnormality results in the atrophy of the nuclear material of the sperm, degenerative changes in the axonemal complex of the sperm tail and reduction in the lipoidal material in the interstitial cell, interstitial cell size and degenerative changes in its cytoplasm. Dutta and Kobayashi have shown various abnormalities and degenerative changes in the fine structures of rat testis as a result of cadmium treatment. Similar features were observed in G. giuris. In light of these findings it can be presumed that in G. giuris, the fenthion interferes with androgenic secretion in the testis, which in turn affect the reproductive potential of the fish.

References
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