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TESTICULAR TOXICITY OF DIMETHOATE IN COMMON CARP *Cyprinus carpio* (Linn)

RAM NAYAN SINGH[1]

Department of Zoology, Kamla Nehru Institute of Physical and Social Sciences, Sultanpur, Uttar Pradesh, India

ABSTRACT

Dimethoate, a broad spectrum organophosphate insecticide was investigated for its sub lethal toxicity in common carp, *Cyprinus carpio*. The aim of present study was to assess the adverse effects of dimethoate on histology of male gonad (testis). In this study juvenile individuals of *Cyprinus carpio* were exposed to dimethoate at 0.96 mg/l and 0.48 mg/l respectively in the short term (96 hour) and long term study (36 days). For short term and long term study concentrations selected were respectively 60 % and 30% of LC_{50} value for 96 hours. Major histopathological changes observed in the testis were necrotic spermatogonia and spermatocytes and clumping of spermatids and spermatozoa. The study showed that dimethoate can adversely affect reproductive fitness of male common carps even at sub lethal concentration both in short term and long term exposure.

KEYWORDS: Dimethoate, Toxicity, LC_{50}, Gonad, Common Carp, Histopathology

During past few decades the use of synthetic chemicals has increased substantially in agriculture and in health and hygiene programmes. These synthetic chemicals in general persist in the environment for short to long term and adversely affect the health of non target organisms (Machado and Fanta, 2003). They are polluting air, water and soil but the health of aquatic ecosystem is at greater risk as it serves as ultimate sink for these pollutants. These chemicals reach into rivers, streams and other water bodies either as effluents from different industries or through runoff water from agricultural fields and other sites of application (Malik *et al*. 2009).The aquatic ecosystem as a result is facing the threat of shrinking genetic base and biodiversity.

In India, fish culture adjacent to agricultural crops, and paddy cum fish culture is a common practice. Therefore, a considerable proportion of applied pesticides easily find their way in to aquatic ecosystem in agricultural areas through overspray and surface runoff and alter physicochemical properties of water. Pesticides enter the fish ponds through run off mostly during southwest monsoon, just after sowing the rice crop and persist in ponds at a very low concentration. Although at low concentrations in ponds and other water bodies, due to their extensive range of biological activity, affinity and stability, pesticides create serious problems for non-target aquatic biota, especially the fishes.

Among aquatic fauna, fishes are one of the most susceptible animals to pesticide pollution because of their anatomy and physiology. Fishes live in intimate contact with surrounding water through their gills and branchial surface comprises over half the surface area of the body (Wood and Soivio, 1991). Only a few microns thick delicate gill epithelium separates the internal environment of fish from external aquatic environment (Wood and Soivio, 1991) which makes the fish very susceptible to aquatic pollutants. Therefore, contamination of water bodies by pesticides causes acute and chronic poisoning of fish and results in severe damage to vital organs (Velmurugan *et al*. 2007;Velisek *etal*. 2009).

Fishes are excellent model organisms for toxicological investigation as they are sensitive to very low concentration of toxic substances. Tissue changes and lesions in organs appear very early in the exposed fishes as manifestation of toxicity. Histopathology of fish can, therefore, be used as biomarker for assessing aquatic contamination in environment monitoring studies (Steinford et al. 2003). Many workers have studied histopathological changes in different organs of pesticide exposed fishes, but information on effect of pesticides on testis of exposed fishes is scarce. Present study was therefore carried out to assess the toxic effect of dimethoate, an organophosphate on the testis of common carp, a widely cultured and important food fish. .

Dimethoate [IUPAC Name- 0, 0 dimethyl S-(N methyl carbamoylmethyl) phosphoro-dithioate], CAS No.60-51-5, is an organophosphate available in the market by the trade name of Rogor. It is a systemic insecticide used for control of a wide variety of insect pests of fruits, vegetables and crop plants. Dimethoate is highly selective as insecticide because relative rate of degradative enzymes viz., esterases and amidases are very low in insects as compared with those of mammals

[1]Corresponding author

(Rose and Hodgson, 2004). Like other organophosphates, Rogor is also an acetylcholinesterase inhibitor (Begum and Vijayaraghavan, 1995), therefore, affects impulse conduction through synapses and neuromuscular junctions which is reflected in uncoordinated abnormal behavior of the fish soon after exposure to pesticide. Behavioral changes are physiological responses shown by the animal which are often used as the sensitive measure of stress in the organism experiencing it (Singh *et al*. 2009).

MATERIALS AND METHODS

Fish Collection and Acclimatization

Fish were brought from ponds of Uttar Pradesh state government's local hatchery (Bhojpur, Sultanpur) and treated with 0.05% potassium permanganate for two minutes. They were acclimatized to laboratory conditions for two weeks into plastic pools of 500 liter capacity. Fishes were fed ad lib rice bran mixed with mustard oilcake in the ratio of 2:1, and three-fourth of the pool water was renewed daily during acclimatization.

Water Quality Parameters

The experiment was conducted under natural photoperiod and temperature. The temperature of the experimental water was 23 ± 1.5^0C, pH was 7.2 ± 0.4, Dissolved oxygen was 7.2 ± 0.6 mg/l, free carbon dioxide was 6.2 ± 0.4 mg/l and total hardness as calcium carbonate was 112 ± 3.2 mg/l.

Preparation of Stock solution and Exposure of fish

The 96 hr LC_{50} value of dimethoate for common carp fingerlings was determined as 1.60 mg/l by Finney's probit method (Singh *et al.* 2009). Based on 96 hr LC_{50} value, a sub lethal concentration of 0.96 mg/l (60% of LC_{50}) and 0.48 mg/l(30% 0f LC_{50})dimethoate was selected for the experiment. Technical grade dimethoate (ROGOR 30% EC, Rallis India Ltd, Mumbai) was procured and stock solution (1mg/ml) was prepared in absolute alcohol. Glass troughs of 30 liter capacity were filled with 25 liter of water in which 24 ml and 12 ml of stock solution in short and long term test respectively was mixed thoroughly before releasing the fishes. In control trough the commensurate absolute alcohol quantity was mixed in 25 liter of water. Thirty individuals of common carp (age, six months, size, 22-27 cm, and weight, 190-250 gm) were sorted and separated in groups of six fish each irrespective of sex from the acclimatized stock. Feeding was stopped 24 h before the experiment and was not resumed during the course of the experiment. Six fish were released in each test and control trough. No mortality occurred in test and control groups during the experiment. At the 24 and 96 h exposure in short term test and at 9 days and 36 days in long term test fishes were sacrificed for collecting gonads for observing histopathological effects.

Histopathology

Fish were first immobilized in ice and then dissected out carefully so as to expose their gonads. Samples from male gonads (testis) were removed and fixed in bouins fluid for 24 h and processed for histopathological study. Paraffin embedded tissues were molded into blocks and sections were cut at 5 – 6 micron and stained in haematoxylin and eosin. The slides were examined under light microscope (Olympus CH 20i) and photographed for histopathological effects.

RESULTS AND DISCUSSION

The control testis is seen to consist of a large number of closely packed seminiferous lobules separated by interlobular septa of connective tissue fibers, blood capillaries and interstitial cells. The testicular lobule comprises numerous germ cells in different stages of maturity. It includes resting germ cells, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. In the maturing stage all maturation stages of spermatozoa are present in lobules but in the mature and ripe testis lobules are seen studded with spermatids and spermatozoa. On the basis of their size, nuclei and the nature of cytoplasm, different maturation stages can be distinguished in *Cyprinus carpio* (Fig. 1 and 2). Resting germ cells occur singly along interlobular wall and have large spherical outline (17.45 µm). Spermatogonia are smaller (12.69 µm) with indistinct cell boundaries buried in the connective tissue of lobular wall. Primary spermatocytes are formed from spermatogonia by mitotic division and measure 4.76 µm. They do not have distinct nuclear membrane as they have entered in meiotic prophase but their chromatin is deeply stained. No nucleolus occurs in this stage.The secondary spermatocytes are still smaller (3.17 µm) than primary spermatocytes. The deeply stained chromosomal material is seen in the form of dark thick clumps.

Spermatids measure around 2.76 µm which in due course give rise to spermatozoa.

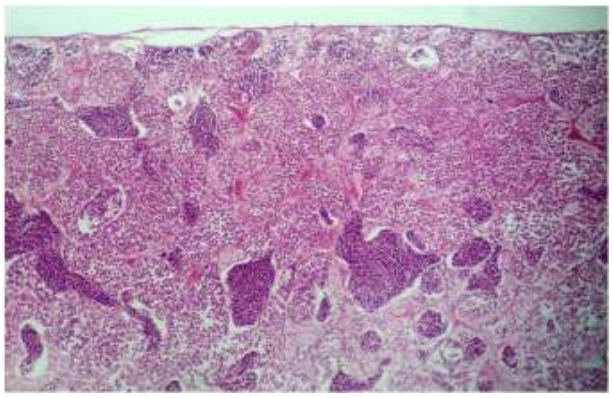

Figure 1: Photo Micrograph of part of control testis of *Cyprinus carpio* (H/E – 100X) of ripening stage showing different stages of spermatogenesis, with thin lobular wall (TLW) and increased vascularisation (VAS).

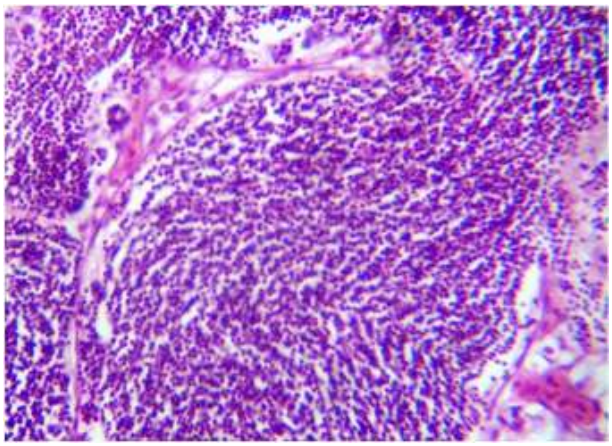

Figure 2: Photo Micrograph of part of control testis of *Cyprinus carpio* (H/E – 100X) of ripe stage showing , lobules filled with spermatids and spermatozoa (SPM), blood vessel (BV), and interstitial cell (IC).

In the test group dimethoate causes mild to severe damage to the testis in both short and long term exposure. The damage in gonad became visible at 24 h of short term test and 9 day of long term test. Damage gradually increased with passage of time in both short as well as long term test. Damage to testis includes breaking of tunica albuginea, clumping of spermatozoa and spermatids, necrotic changes in spermatogonia and spermatocytes and vacuolization and rupturing of lobular wall. At 24 h exposure damage in the form of clumping (CLP), of spermatids and spermatozoa (SPM) around wall of seminiferous lobules is visible. Vascularization (VAS) is, however, normal (Fig. 3). At day 9 in the long term test, testis of common carp shows damage in the form of thickening and breaking of tunica albuginea (BTA), degeneration in primary and secondary spermatogonia (PSG, SSG), and necrotic spermatocytes and spermatids (SC). Spermatogonia in some lobules leave the peripheral positions and spread out in the entire available space within lobule (Fig.4).

Terminal sacrifice in short term was done at 96 h and at 36 day exposure in long term study. Damage in testes showed positive correlation with concentration and duration of exposure. At96 h exposure testis of common carp shows damage in the form of clumping of spermatozoa (CL SPM) on one side, leaving lobular lumen empty (EL), and ruptured lobular wall (RLW). Necrosis still is not very conspicuous even at 96 h exposure in short term (Fig. 5). At 36 day exposure in the long term experiment testis shows damage in the form of necrotic spermatogonia (NSG), indistinct primary and secondary spermatogonia (PSG, SSG), and vacuolization. Necrosis is extensive and every stage of spermatogenesis including primary and secondary spermatocytes and spermatids degenerate and die out under influence of dimethoate leaving the seminferous lobules almost empty. Interstitial cells also become necrotic. Vascularization is reduced and walls of lobules occasionally break. The interstitial cells may vary in shape from oval to elongated and possess centrally placed nuclei with one or two nucleoli. The nuclear membrane is ill defined and cells are strongly basophilic in control testis which at 36 day exposure exhibit only dark clump of cytoplasm.

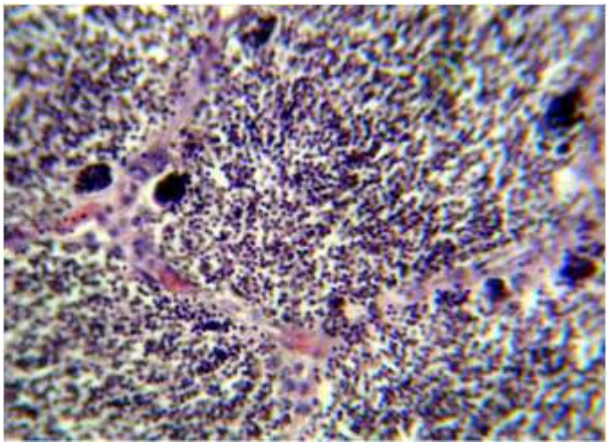

Fig. 3 Photo Micrograph of part of testis of common carp at 24 hexposure to 0.96 mg/l dimethoate (H/E – 400X) showing clumping of spermatids

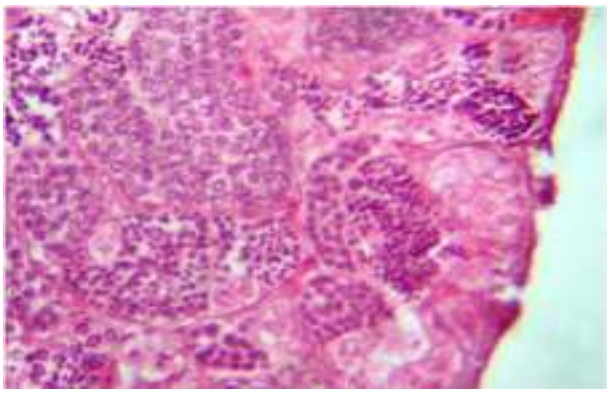

Figure 4: Photo Micrograph of part of testis of common carp at 9 day exposure to 0.48 mg/l dimethoate (H/E – 400X) showing breaking of tunica albuginea and necrosis primary and secondary spermatocytes

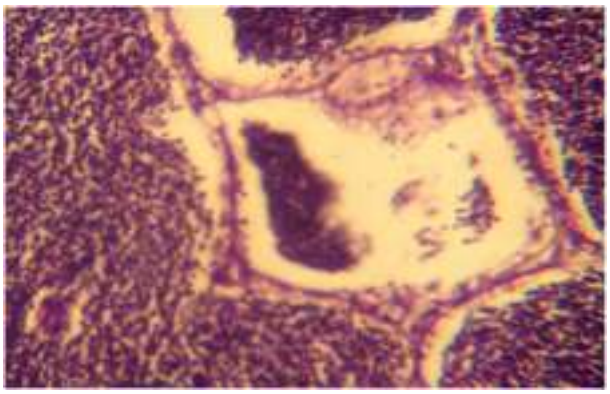

Figure 5: Photo Micrograph of part of testis of common carp at 96 hexposure to 0.96 mg/l dimethoate (H/E – 400X) with clumping of spermatozoa on one side of lobule and rupturing lobular wall

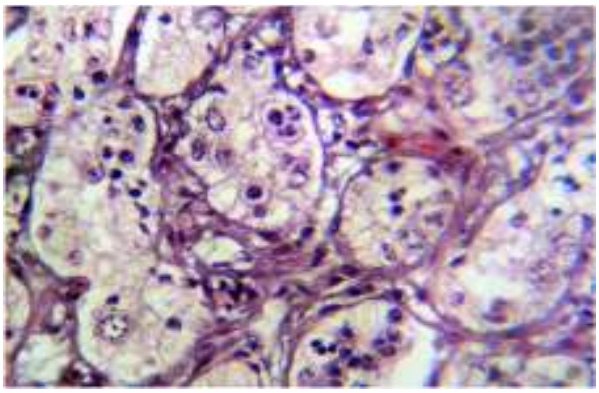

Figure 6: Photo Micrograph of part of immature testis of common carp at 36 day exposure to 0.48 mg/ldimethoate (H/E – 400X) showing extensive necrosis

In the present study testis in male shows clumping of spermatozoa, necrotic changes in spermatogonia and spermatocytes, vacuolization and breaking of tunica albuginea (Fig. 3-6). Necrotic changes are however conspicuous in the long term experiment. Since necrosis is not visible at 24 h and hardly detectable at 96 h but is rampant at 36 day exposure, it appears that necrosis sets in only when exposure is prolonged. Similar clumping of spermatogenic cells, intra and intertubular vacuolization have been observed in *Heteropneustes fossilis* exposed to linear alkyl benzene sulphonate by Kumar *et al*. (2007). Zutshi (2005) also observed reduction in size of testis, degeneration of spermatids and spermatozoa, reduction in gonosomatic index and necrosis of interstitial cells in *Glossogobius guiris* after fenthion exposure.

Since spermatogenic stages undergo degeneration after exposure to dimethoate, formation of mature and functional spermatozoa may be reduced considerably. Impairment of hypothalamo-pituitary-gonadal complex in fishes exposed to dimethoate may be responsible for deterioration of reproductive capacity. Ghosh *et al.* (1990), have shown decreased potency of hypothalamic extract in stimulating release of pituitary gonadotrophic hormones in murrel, *Channa punctatus* after carbaryl exposure.

Effect of pesticide exposure on reproductive physiology of fish has been studied by many workers (Dutta et al., 1992, Masud et al., 2009, Singh, 2015). They are found to retard gonadal development to a great extent and considered as important inhibitors of reproductive activity (Kime, 1995). And testicular inflammation has is considered as one of the commonest responses in both aquatic and terrestrial animals exposed to environmental toxicants (Sokal *et al*. 1985). This study shows that dimethoate at sub lethal concentration can cause severe structural and functional damage to testis of common carp and reduce reproductive fitness of the male fish resulting in reduced production of mature and functional spermatozoa.

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