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EFFECTS OF DIMETHOATE ON OVARY OF COMMON CARP, *Cyprinus carpio* (LINN)

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ABSTRACT

This study attempts to investigate the sub lethal effects of dimethoate, an organophosphate insecticide on ovarian tissue of common carp, *Cyprinus carpio*. Healthy adults of *Cyprinus carpio* were exposed to 0.96 mg/l (60% of LC_{50}) for 96 h in the short term and to 0.48 mg/l (25% of LC_{50}) for 36 days in the long term study respectively. At both exposure durations dimethoate caused considerable structural damage to ovaries, which included breaking of ovigerous lamellae, lifting and fragmentation of follicular lining and zona radiata of maturing oocytes. Degenerative changes in the form of nuclear and cytoplasmic retraction were also observed in certain oocytes. Extrusion of nuclei and whole cytoplasm resulting in empty follicles also occurred in some instances of long term exposure. However, most common symptom, observed in this study was increased atresia of both previtellogenic and vitellogenic oocytes in short as well as long term exposure. Extensive atresia of oocytes of stage II and III are observed at 96 h of short term and 36 day of long term exposure. Dimethoate induced damage to ovaries showed both concentration and time dependence.

KEYWORDS : Dimethoate, Common Carp, Ovary, Oocyte, Atresia

Deterioration in the quality of habitat translates into poor health and fitness of its inhabitants. Aquatic habitats are prone to contamination by more or less persistent chemicals as they serve as ultimate sink for pollutants. Pesticides whether applied in the field; or released by accidental spills ultimately reach into aquatic ecosystems either through surface runoff and /or as aerosols and pollute water. Heavy reliance of pest control practices on synthetic pesticides has made them an important group of aquatic pollutants which frequently causes acute and chronic toxicity among aquatic organisms including fish. (Heger et al., 1995; Saha and kaviraj, 2008; Singh, 2015).

Most pesticides used in agriculture and in hygiene programs are non selective, more or less persistent and bioaccumulate in the food chain and pose great danger to the health of non target organism in fresh water. Organophosphates have emerged as a popular class of synthetic pesticides due to their great efficacy and less persistence in the environment. Among organophosphates, Dimethoate (trade name Rogor) is very popular broad spectrum insecticide, extensively used in agriculture for controlling insects on fruits, vegetables, cotton, tobacco, sunflower, olives and ornamentals. It is non photodegradable, undergoes very slow hydrolysis and shows moderate persistence in water. It works as nerve poison by irreversibly blocking acetyl cholinesterase in synapses and neuromuscular junctions (Cope et al., 2004). It's very high toxicity for insects is accounted by less activity of degradative enzymes in insects than in mammals. Dimethoate is acutely toxic and classified as possible human carcinogen by USEPA based on tumor occurrence in mice. In the WHO acute hazard ranking dimethoate is rated as moderately hazardous.

Fishes are excellent model organisms for toxicological investigations because of their sensitivity to very low concentrations of toxic substances. Tissue changes and lesions in organs of toxicant exposed fish appear very early as manifestation of toxicity. Histopathology of fish can, therefore, be considered as a cost effective tool for determining health status of fish populations, which also reflects the health of water body. In the environment monitoring studies, histopathological changes in fish organs are, therefore, used as biomarkers for assessing aquatic contamination both in laboratory and field conditions (Stentiford et al., 2003).

Histopathological changes in different organs of pesticide exposed fish have been studied by many authors. But, very scarce information is available on histopathological changes in fish ovary due to organophosphate toxicity in general and dimethoate in particular. Hence, in the present study, an attempt has been made to document possible histopathological alterations in ovary of the freshwater fish *Cyprinus carpio* exposed to sublethal concentrations of dimethoate.

Cyprinus carpio, the common carp, is an important food fish of the region, available afresh round the year. This is also a popular culture fish due to hardy nature, omnivorous habit, fast growth rate and easy breeding in

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confined water. As a result, this exotic carp has now become common in natural and manmade water bodies and makes substantial proportion of the inland capture and culture fishery.

MATERIALS AND METHODS

Healthy adult specimens of common carp, *Cyprinus carpio* were collected from local ponds of Sultanpur district of Uttar Pradesh. They were caught by fishing nets and brought carefully to departmental laboratory in polythene bags filled with aerated water. The fish were stored in tap water filled plastic pools (500 liters) for two weeks for acclimatization to laboratory condition. Fish were fed ad lib rice bran and mustard oil cake, mixed in the ratio of 2:1 during acclimatization. Water of the pool was partially renewed every 24 hour and any dead fish if spotted were removed immediately to avoid fouling of pool water.

The experiment was conducted under natural photoperiod and temperature. The temperature of the experimental water was 23 ± 1.5^{0}C, pH was 7.2 ± 0.4, Dissolved oxygen was 7.2 ± 0.6 mgl^{-1}, free carbon dioxide was 6.2 ± 0.4 mgl^{-1} and total hardness as calcium carbonate was 112 ± 3.2 mgl^{-1}.

The 96 hr LC_{50} value of dimethoate for common carp fingerlings was found to be 1.60 mgl^{-1}(Singh et al., 2009). For the present study dimethoate as rogor (EC 30%) was procured from Rallis India Ltd. Mumbai. Stock solution of dimethoate was prepared in absolute alcohol. For short and long term test respectively, 0.96 mgl^{-1} and 0.48 mgl^{-1} dimethoate was selected as sub lethal concentration. Common carp individuals of size, 20 - 28 cm, and weight, 190 - 270 gm were sorted and starved for 24 hr before starting the experiment. Four specimens each were exposed to the sub lethal dose for the 24, 48, and 96 h in short term and for 12, 24 and 36 days in the long term along with a simultaneously running control.

Four individuals were sacrificed at different exposure periods in the short and long term test. Fish were first immobilized in ice and then dissected out carefully, and part of the gonads were removed and processed for study of histopathological effects of dimethoate. Removed tissue was fixed in bouins fluid for 24 h and then processed and embedded in paraffin for block preparation. The sections were cut at 5 6 micron and stained in haematoxylin and eosin. The slides were examined under light microscope for histopathological effects.

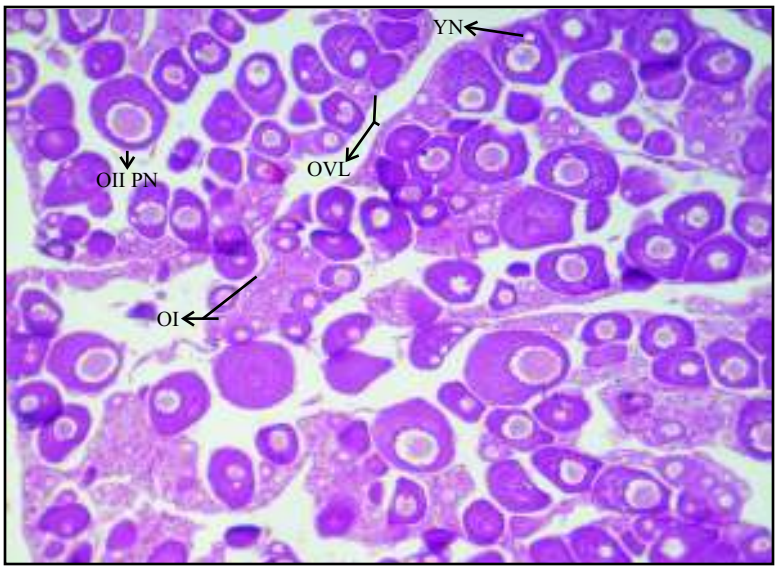

Figure 1 : Photo Micrograph of Part of Control Ovary of *Cyprinus carpio* Exhibiting Ovigerous Lamellae (OVL) With Closely Packed Developing Oocytes of Different Stages, Oogonials (OOG), Oocyte I (OI), Oocyte II of Perinucleolus (OII PN) Stage, Yolk Nucleus (YN). H/E -100 x

RESULTS AND DISCUSSION

The ovaries in the control common carp show typical teleostean structure. They are covered by a thin unicellular layer of peritoneum, overlying the tunica albuginea of connective tissue, muscle fibers and blood capillaries with ovarian epithelium as innermost layer. The ovarian tissue with connective tissue and blood vessel forms finger like projections called ovigerous lamellae into the lumen of the ovary. These folds enclose numerous ova at different stages of development and growth. Photo Micrograph of part of control ovary of *Cyprinus carpio* exhibits ovigerous lamellae (OVL) with closely packed developing oocytes of different stages, oogonials (OOG), oocyte I (OI), oocyte II of perinucleolus (OII PN) stage, yolk nucleus (YN) (Figure 1).

The oocytes which are unable to mature or those which fail to spawn undergo the process of resorption and are termed atretic follicles or corpora atretica. Atretic follicles may be seen in pre spawning, spawning and post spawning months. Number of atretic follicles, however, increases under pesticide stress, as at the end of both short and long term exposure of this test, extensive atresia is seen the ovary. Generally, atresia occurs in the vitellogenic and fully mature eggs and atresia of previtellogenic oocytes is rare under normal conditions but under exposure to toxicant both previtellogenic and vitellogenic atresia is common (Figure 4 & 5). At 24 h in the short term and 12 day of long term exposure changes in the ovarian histology are not very conspicuous. But by 48 h of short term and 24 day of long term exposure pathological alterations in the ovary are obvious (Figure 2 &3). Nature of damage includes lifting and rupture of ovigerous lamellae, follicular epithelium, zona radiata; nuclear retraction, cytoplasmic retraction, cytoplasmic clumping, empty follicles, extrusion of ooplasm, and increased atresia of moderate degree. At 96 h of short term and 36 day of long term exposure extensive atresia and necrosis of previtellogenic and vitellogenic oocytes is seen (Figure 4 & 5).

Many workers investigating effect of pesticide exposure on reproductive physiology of fish have reported similar changes in different fish. They have noted pesticides as important inhibitors of reproductive activity which retard gonadal development of fish to a great extent. Decrease in gonosomatic index and increased follicular atresia are the most common effect of pesticide exposure in female fish. Damage to ovaries of fish after toxicant exposure has been characterized by reduction in ovarian diameter, atresia of oocytes and fall in Oocyte II: Oocyte I ratio. Dutta and Dalal

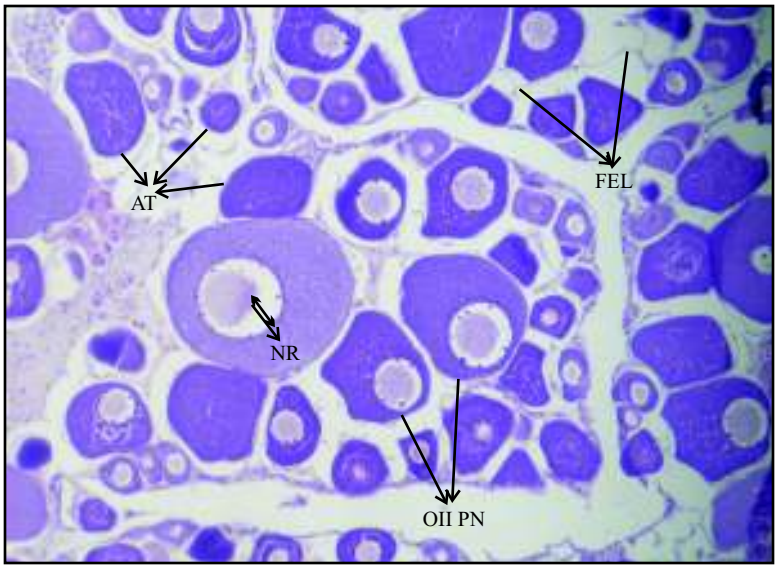

Figure 2 : Photo Micrograph of Part of Ovary of Common Carp at 48 h Exposure to 0.96 mg/l Dimethoate Showing Lifting of Follicular Epithelium (FEL), Nuclear Retraction (NR), Atresia (AT); Oocyte of Stage II in Perinucleolus Stage (OIIPN). H/E - 100 x

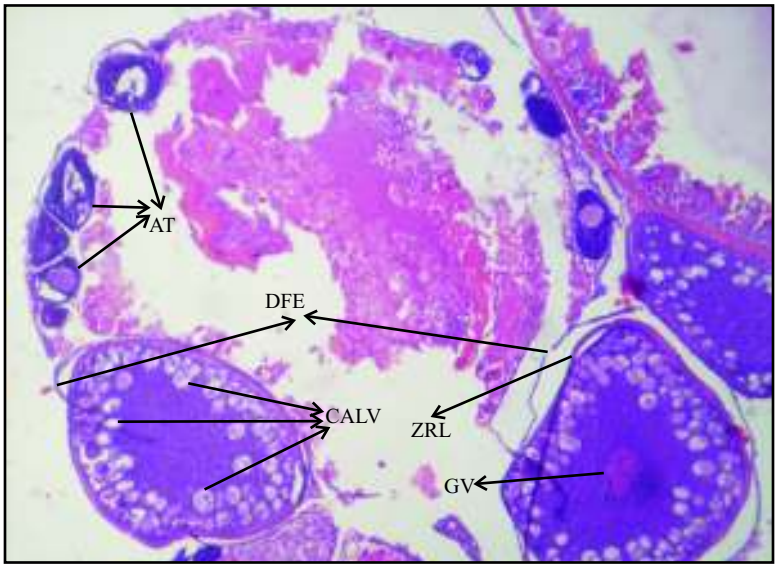

Figure 3 : Photo Micrograph of Part of Ovary of Common Carp at 24 Day Exposure to 0.48 mg/l Dimethoate Showing Damaged Follicular Epithelium (DFE), Lifting of Zona Radiata (ZRL), Increased Atresia (AT); Cortical Alveoli (CALV), and Germinal Vesicle (GV) in Ripe Egg. H/E -100 x

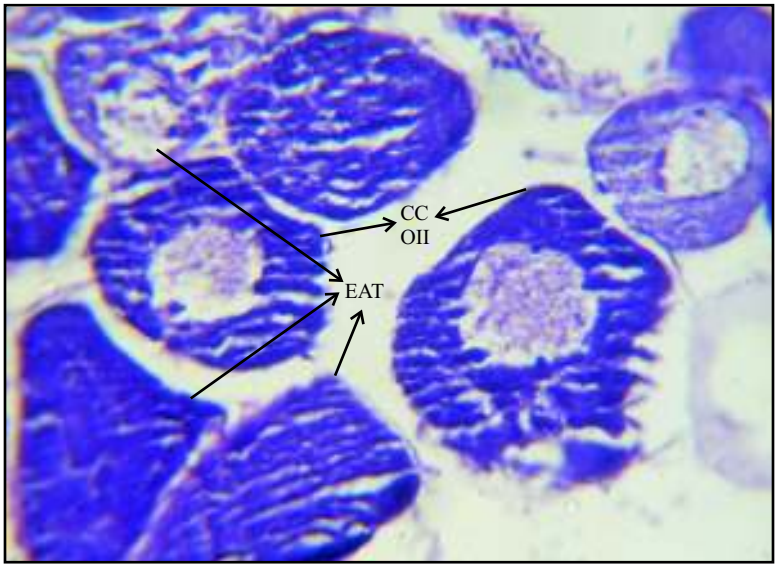

Figure 4 : Photo Micrograph of Part of Ovary of Common Carp at 96 h Exposure to 0.96 mg/l Dimethoate Showing Extensive Damage in the Form of Highly Increased Atresia (EAT) and Cytoplasmic Clumping (CC) of Oocytes of Stage II (OII). H/E -100x

(2008) observed damaged stroma, cytoplasmic and nuclear retraction in oocytes of stage II and III, adhesion between oocytes, empty follicles and cytoplasmic clumping in oocytes of stage III and IV, necrotic nuclei and increase in number of atretic follicles in bluegill sunfish after endosulfan exposure. However, Inbaraj and Haider (1988) have reported disappearance of oocytes of stage II and III in *Channa punctatus* exposed to malathion and endosulfan. Thickening of ovarian wall and increase in stromal tissue were also observed in addition to degeneration of oocytes of stage II and increase in atretic follicles by Saksena and Saxena (1999) in *Channa orientalis* exposed to

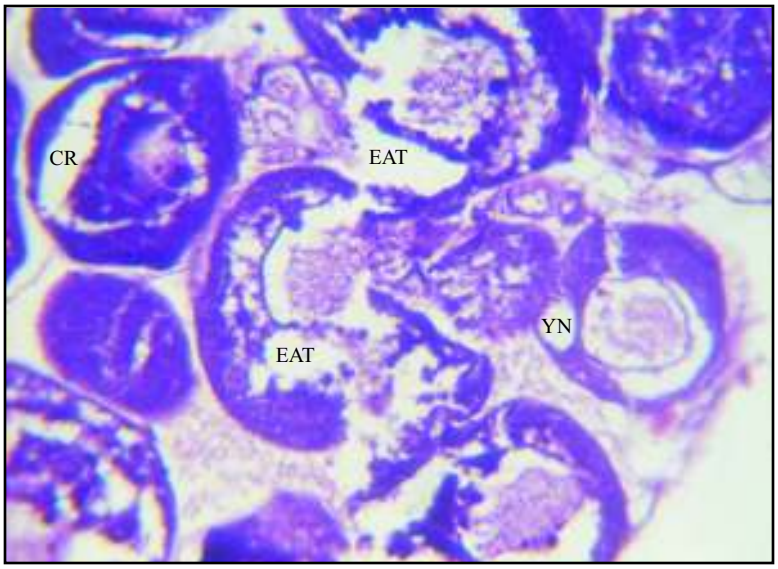

Figure 5 : Photo Micrograph of Part of Ovary of Common Carp at 36 Day Exposure to 0.48 mg/l Dimethoate Showing Extensive Damage in the Form of Extensive Atresia (EAT), Cytoplasmic Retraction (CR) of Oocytes of Stage III (OIII); Yolk Nucleus (YN). H/E 100 x

organophosphorus pesticides. Dutta et al. (1992) also reported clumping of cytoplasm, degeneration of stage I oocytes and increase in atretic follicles in Heteropneustes fossilis exposed to malathion. Degenerative changes in oogonials and immature oocytes, mainly ooplasmic and nuclear dissolution resulting in presence of debris in fishes with normal behavior and absence of early vitellogenic oocytes and variable degree of degenerative changes of oogonials and immature oocytes resulting in huge mass of debris in ovary of fishes with abnormal behavior at the same concentration of $HgCl_2$ were reported by Masud et al. (2009). A change in gonadotrophic hormone level or the imbalance of hormones caused by pesticide might be the reason of ovarian degeneration. This study shows that dimethoate in short as well as long term sub lethal exposure causes considerable harm to reproductive health of female fish.

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