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HISTOPATHOLOGICAL ALTERATIONS IN THE KIDNEY OF *Cyprinus carpio* AFTER EXPOSURE TO DIMETHOATE (EC 30%)

RAM NAYAN SINGH[1]

Department of Zoology, Kamala Nehru Institute of Physical and Social Sciences, Sultanpur, U.P., India
E-mail: rnsingh_zool@rediffmail.com

ABSTRACT

This study was conducted to assess the histopathological damage of kidney in common carp, *Cyprinus carpio* after sub lethal exposure to dimethoate. In a short term (96 hr) study healthy juveniles of common carp were exposed to 0.96 mgl^{-1} of dimethoate (60% of 96 hr LC_{50}), and a parallel control was run simultaneously. Kidney of exposed individuals exhibited remarkable changes in their histology in comparison to control. Prominent changes include shrinkage of glomerulus, and dilation of tubular lumen. Vacuolization, desquamation, hydropic swelling and hyaline degeneration of tubular epithelium is also observed. Cyst formation and hemorrhage also appear in certain specimens. Duration of exposure appears to have profound effect on kidney as with increasing duration of exposure histopathological damages become more severe.

KEY WORDS: Histopathology, common carp, dimethoate, kidney, glomerulus, renal tubules

Heavy dependence of modern agriculture on agrochemicals such as fertilizers and pesticides is emerging as a threat to the ecological balance of aquatic ecosystems. Synthetic pesticides used for controlling pests in agriculture are one of the major causes of aquatic pollution. Sometimes pesticides are directly applied in water bodies for controlling pests and vectors but their residues mostly reach into aquatic ecosystems through surface run off and affect the health of non target organisms including fish. Among synthetic pesticides organophosphates are widely used in agriculture and in health and hygiene programs due to their high effectiveness as insecticide but less persistence in the environment. They are favoured over organochlorines which have long persistence and consequently easily bioaccumulate in food chain. The shift from organochlorines to organophosphates has resulted into increased occurrence of organophosphates into water bodies causing acute and chronic toxicity to fish fauna (Rao et al., 2005; Velmurugan et al., 2007; Singh et al., 2009).

Dimethoate, [IUPAC Name O, O dimethyl S - (N methylcarbamoylmethyl) phosphoro-dithioate], popular with common name - rogor, is a systemic organophosphate insecticide used widely for controlling insect pests of fruits, vegetables and crop plants. It is highly selective as insecticide because the relative rates of degradative enzymes viz, esterases and amidases are slower in insects than in mammals (Rose and Hodgson, 2004). Like other organophosphates, dimethoate is also an acetylcholinesterase inhibitor and primarily works as nerve poison. This is very toxic insecticide and has been classified as a possible carcinogen by USEPA based on occurrences of tumors in mice and is rated as moderately hazardous by W.H.O.

Specific lesions occurring in organs of fish exposed to toxic substances under laboratory conditions are helpful as biomarkers of exposure. As a result histopathological examination is increasingly being recognized as a valuable tool for assessing the impact of environmental pollutants on fishes (Teh et al., 1997; Handy et al., 2002). Kidney serves as a major route of excretion of metabolites of xenobiotics, and receives the largest proportion of postbranchial blood, and therefore, it is more likely to undergo histopathological alterations under pesticide stress (Ortiz et al. ,2003).

The present work is an effort to assess the toxic impact of dimethoate on the histology of kidney of common carp, a highly palatable and one of the most widely cultured fish in the Northern part of India. The common carp is a relatively hardy fish, can tolerate poor water quality and feeds on a wide variety of natural as well as artificial foods. This is a fast growing food fish of very high economic importance and easily breeds in confined waters.

MATERIALS AND METHODS

Fish were collected from ponds of local government hatchery with the help of fishermen. Fishes were caught by fishing net and carefully packaged into aerated polythene bags filled with tube well water. Fishes

[1]**Corresponding author**

were brought to laboratory and immediately given 0.05% potassium permagnate treatment for two minutes for disinfecting them. After disinfectant treatment they were transferred into plastic pools of 500 liter capacity for two weeks acclimatization to laboratory conditions. Fishes were starved for first 24 hr and then fed ad lib rice bran mixed with mustard oilcake in the ratio of 2:1, during acclimatization. Water of the pool was changed daily and dead fishes whenever located were removed immediately. A few mortalities occurred during first 48 hr of acclimatization, thereafter no mortality occurred and fishes appeared normal and healthy.

The experiment was conducted under natural photoperiod and temperature. Water quality was measured as per APHA (2005). 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 determined by Finney's probit method and found to be 1.60 mgl^{-1} (Singh et al., 2009). For the histological study, 0.96 mgl^{-1} (60% of 96 hr LC_{50}) of dimethoate (Rogor 30% EC, Rallis India Ltd, Mumbai) was selected as sub lethal concentration. Common carp individuals of size, 17-22 cm, and weight, 50-65 gm were sorted and starved for 24 hr before starting the experiment. Six specimens were exposed to the sub lethal dose for the 24, 48 and 96 hr and a control was run simultaneously.

Fish were sacrificed at 24, 48 and 96 hr of exposure. Fish were first immobilized in ice and then dissected out carefully; kidneys were removed and fixed in bouins fluid for 24 hr 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 and photographed for histopatholgical effects.

RESULTS AND DISCUSSION

Kidney of control fish is composed of numerous renal corpuscles with well developed glomeruli and a system of renal tubules (Fig. 1). At 24 hr exposure the changes in the kidney histology are not very conspicuous. After 48 hr exposure the shrinkage of glomerulus is easily recognizable and space between glomerulus and Bowman's capsule is increased. The degenerative changes in the epithelium of kidney tubules are mild and the nuclei and cells are largely intact. In some tubules, however, diameter of lumen is increased, and nuclei of some epithelial cells become pycnotic (Fig. 2). At 96 hr of exposure tubular lumen is remarkably widened. Degenerative changes in tubular epithelium are enormous and the epithelium is desquamated in some tubules. Hyaline degeneration, hydropic swelling and vacuolization are seen in most tubules. Nuclei show degeneration, and degenerating necrotic areas become visible and glomeruli exhibit conspicuous shrinkage (Fig 3 and 4).

Histological alterations in the kidney at the level of glomerulus and tubular epithelium in fish after exposure to toxic agents such as pesticides have been reported by many workers. Das and Mukherjee, (2000) reported dilation of renal tubules and necrotic changes characterized by karyorrhexix and karyolysis in *Labeo rohita* exposed to hexachloro-cyclohexane. Tilak et al., (2001) noticed severe necrosis, cloudy swelling in the renal tubules, cellular hypertrophy, granular cytoplasm and vacuolization in kidney tissues of *Ctenopharyngodon idella* after exposure to fenvalerate. Degeneration in the epithelial cells of renal tubules, pycnotic nuclei in the hematopietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intra cytoplasmatic vacuoles in the epithelial cells with hypertrophied cells and narrowing of the tubular lumen were observed in the kidney tissues of fish exposed to deltamethrin (Cengiz, 2006). Velmurugan et al., (2007) reported pycnotic nuclei in tubular epithelium, hypertrophied epithelial cells of renal tubules, contraction of the glomerulus and expansion of space inside the Bowman's capsule in the kidney of *Cirrhinus mrigala* exposed to monocrotophos. Gill et al., (1989) reported various histopathological changes such as degeneration of tubular epithelium, nuclear deterioration like karyorrhexis and karyolysis, and collapsing glomeruli in the kidney of *Puntius conchonius* following exposure to cadmium. They also found progressive increase in severity of degenerative changes with increasing duration of exposure.

The study thus shows that dimethoate is very toxic

to the fish even at sub lethal and short term exposure. It can seriously affect fish health by causing deleterious changes in the structure of kidneys.

Fig.1 :Microphotograph of part of control kidney (H/E × 400) showing Bowman's capsule (BC), Glomerulus (G), Capillaries in glomerulus (C), Blood vessel (BV), hematopoietic tissue (HPT) and Kidney tubules (KT)

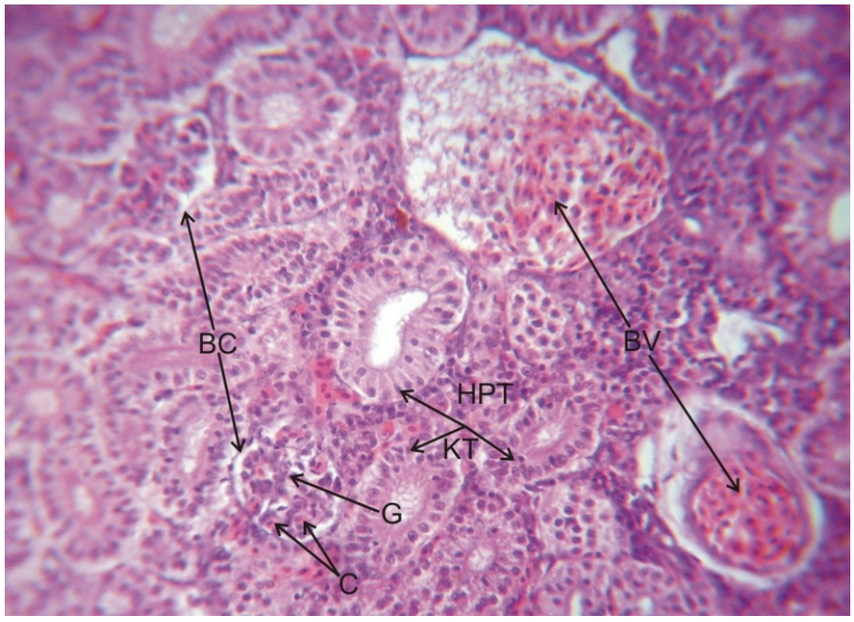


Fig. 2: Microphotograph of part of kidney after 48 hr exposure (H/E × 400) showing glomerular shrinkage (GS), increase in space between glomerulus and bowman's capsule (↔), increased tubular lumen (TL), Pycnotic nuclei (PN), and relatively intact tubules

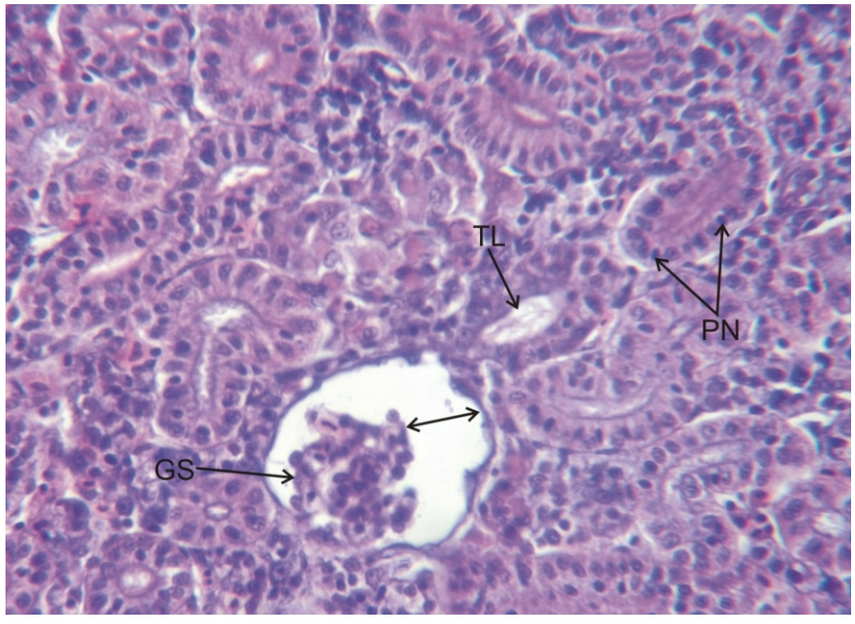


Fig. 3: Microphotograph of part of kidney after 96 hr exposure (H/E × 400) showing disorganized tubules (DT) Hydropic swelling (HS) Vacuolization (V), hyaline degeneration of tubular epithelium (HD), desquamation (D) and damaged blood vessel (DBV)

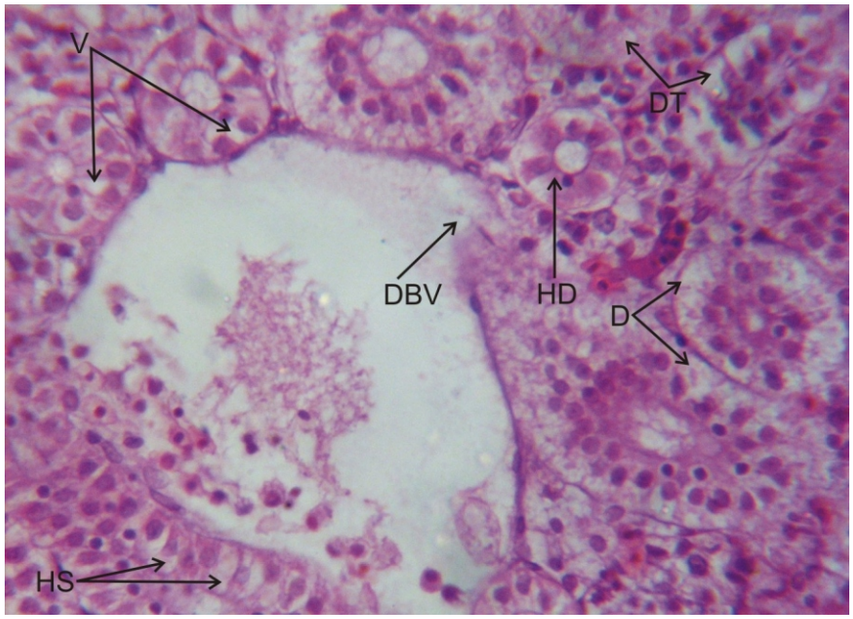
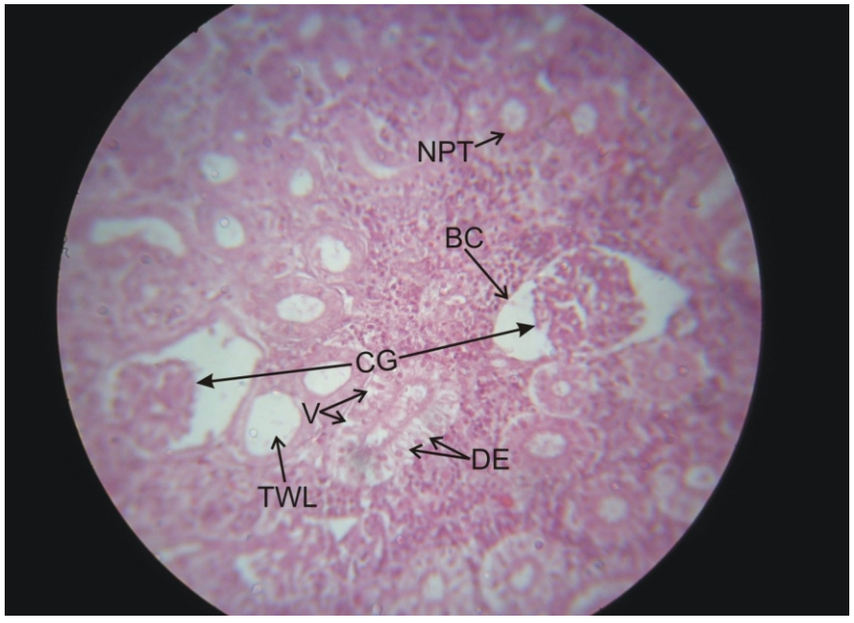


Fig. 4 :Microphotograph of part of kidney after 96 hr exposure (H/E × 400) showing collapsing glomeruli (CG), necrotic proximal tubules (NPT), tubules with widened lumen (TWL), degenerating epithelium (DE), vacuolized (V) and indistinct necrotic cells and nuclei of tubular epithelium



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