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DIMETHOATE INDUCED HISTOLOGICAL CHANGES IN THE INTESTINE OF FRESH WATER FISH *PUNTIUS TICTO* (HAM).

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ABSTRACT
Puntius ticto, a fresh water fish exposed to lethal [5.012ppm] and sublethal concentrations [2.506 and 1.253ppm] of Dimethoate for 96 hrs and 60 days respectively. After exposure period Histopathological changes in the intestine of *Puntius ticto* were assessed. Acute exposure resulted in to cloudy swelling and granular cytoplasm in mucosal cells, broken serosa, bulging and hypertrophic condition was noticed in columnar epithelial cells which secretes excess amount of mucus. Necrotic and bulging conditions were also observed at the tip of villi which lead to rupture of villi. Chronic exposure results in broken serosa, vacuolated submucosal layer, vacuolated longitudinal and circular layer, mucosal layer completely damaged, vacuolated villi and columnar cells were completely collapsed to higher concentration. The severity of damage in the intestine was found to be dose and time dependent.

KEY WORDS: Dimethoate, Intestine, Histopathology, *Puntius ticto.*

INTRODUCTION

With rapid industrialization and increase in human population, the pollution of water bodies has become a universal phenomenon in the present day world (Bela and Prasad 2008). The important sources of water pollution are industrial effluent, domestic sewage, drainage and pesticides, which pollute the river and major sources (Maruthanayagam and Sharmila, 2004). Pesticides and related chemicals destroy the delicate species that characterizes a functioning ecosystem (Khan and Francis,2005). Pesticides are not highly selective but are generally toxic to many macrophytes, non-target organisms such as fish (Ayoola,2008 Franklin et.al.,2010) Fish is good indicator of aquatic contamination because its biochemical stress responses are quite similar to those found in mammals (Mishra and Shukla, 2003). Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. For example, the mechanism of action of several xenobiotics could initiate the formation of a specific enzyme that causes changes in metabolism, further leading to cellular intoxication and death, at a cellular level, whereas this manifests as necrosis, i. e. histopathological biomarker on a tissue level. (Velkova-Jordanoska, 2002; Roganovic-Zafirova *et. al*., 2003).

Organophosphorus (OP) pesticides are finding increasing use in recent years since they are biodegradable and therefore persist in the environment only for a short time. Because of their low persistence, repeated applications of these pesticides are being practiced for the control of pests in agricultural fields and thereby large quantities find their way into water bodies (Jyothi and Narayan, 1999). These pesticides enter into the body of aquatic fauna by means of gills, oral membrane, and gastrointestinal mucosa & from general body surface. These are deposited in the tissues and produces toxic effects. Therefore it is necessary to study in detail on the histopathological alterations in different organs of fishes and thoroughly investigate them in order to assess the extent of damage.

The previous histopathological studies of fish exposed to pollutants revealed that fish organs are efficient indicators of water quality (Cardoso *et.al.,* 1996 & Cengiz *et.al.,* 2001). Many investigators have reported the histopathological changes in the intestine of different fish species exposed to pesticides (Kamble, 1983; Srivastava et al 2004; Bhatnagar et.al., 2007 and Banee et.al., 2013). However there has been little information on the histopathological impact of dimethoate on intestine of *Puntius* ticto. Therefore, the present investigation was undertaken with a view to study in detail about histopathological changes in the intestine of *Puntius ticto*, to dimethoate toxicity.

MATERIAL AND METHODS

The freshwater fish *P. ticto* were selected from the freshwater sources around Aurangabad city. They were acclimatized in aged, dechlorinated and well aerated water for two weeks in the laboratory. During acclimatization they were fed on alternate days with pieces of live earthworms. The LC_{50} values are determined by following the guidelines given by committee of toxicity tests with aquatic organism (Annon, 1975) and probit analysis method (Finney, 1971). The acclimated fish were exposed to lethal concentration (5.012 ppm) for 96 hrs and sublethal concentrations (2.506ppm &

1.253ppm) for 60 days. Simultaneously a control group of healthy fishes were maintained under identical conditions. The 20 healthy fishes showing normal activity were exposed for chronic study. After commencement of exposure period fishes were killed by decapitation and intestine is removed and fixed in Bouins fluid for 24hrs and processed according to standard procedure of routine microtechnique. For staining double stain method was followed by using Haematoxylin and Eosin and mounting was done in DPX.

RESULTS

The normal histological structure of intestine wall of *Puntius ticto* was composed of four layers viz, mucosa, submucosa, muscularis and serosa (Fif.1). The mucosa was thrown into prominent finger like projections forming villi. The mucosa was composed of columnar epithelium consisting of absorptive and mucous secreting cells or goblet cells. The submucosa, made up of loose connective tissue, was vascular and extended into the villi as lamina propria which formed the core of the villi. The muscularis was formed of the inner circular and the outer longitudinal layer of smooth muscle fibers. Serosa formed the outermost thin layer of flattened epithelial cells. Marked degenerative changes were observed in intestine of *Puntius ticto* under dimethoate toxicity stress. Acute exposure resulted in to cloudy swelling and granular cytoplasm in mucous cells, broken serosa, bulging and hypertrophic condition was noticed in columnar epithelial cells which secrete excess amount of mucous. Necrotic and bulging conditions were also observed at the tip of villi which lead to rupture of villi. The necrotic condition was found by dark staining and some spaces were formed in submucosal layers. (Fig.2)

Chronic or long term exposure shows broken serosa, vacuolated submucosal layer, vacuolated longitudinal and circular layer, mucosal layer completely damaged; vacuolated villi and columnar cells were completely collapsed to higher concentration (2.506 ppm) (Fig.3). The lower concentration (1.253 ppm) of dimethoate results in to broken serosa, less damage of longitudinal and circular muscles, vacuolated and less damaged submucosal layer, less damage in the epithelial cells of mucosal layer and villi was observed. So the above changes showed that the severity of damage is dependent upon the dose of concentration. (Fig.4).

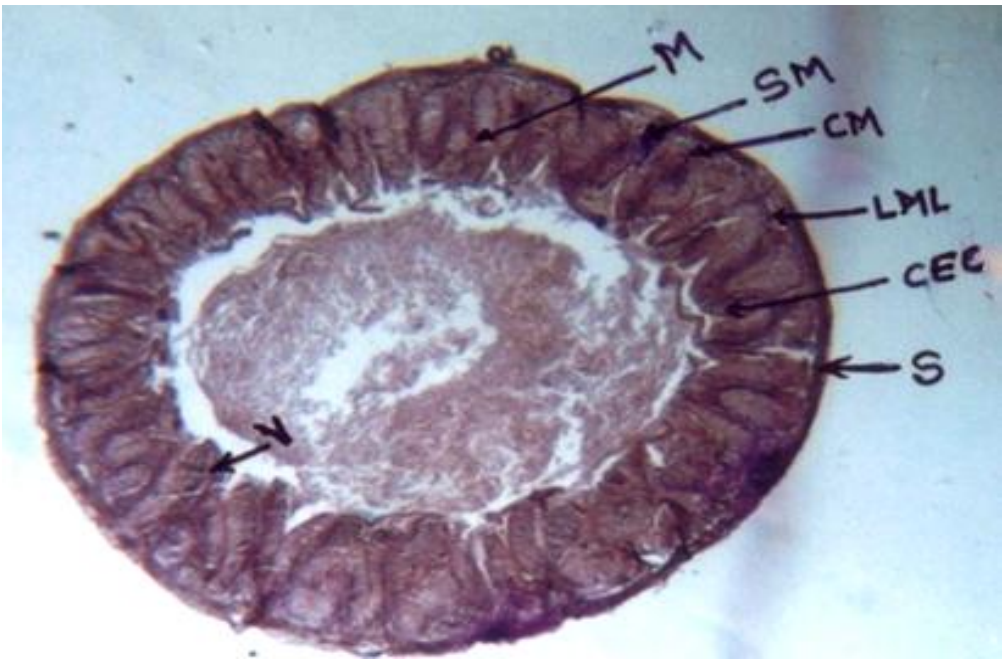

Fig.1: T.S. of intestine of *Puntius ticto* (Control): Haematoxylin/Eosin 40X

S	Serosa	LML	Longitudinal muscular layer
CEC	Columnar epithelial cells	CM	Circular muscle layer
SM	Sub mucosa	M	Mucosa
V	Villi		

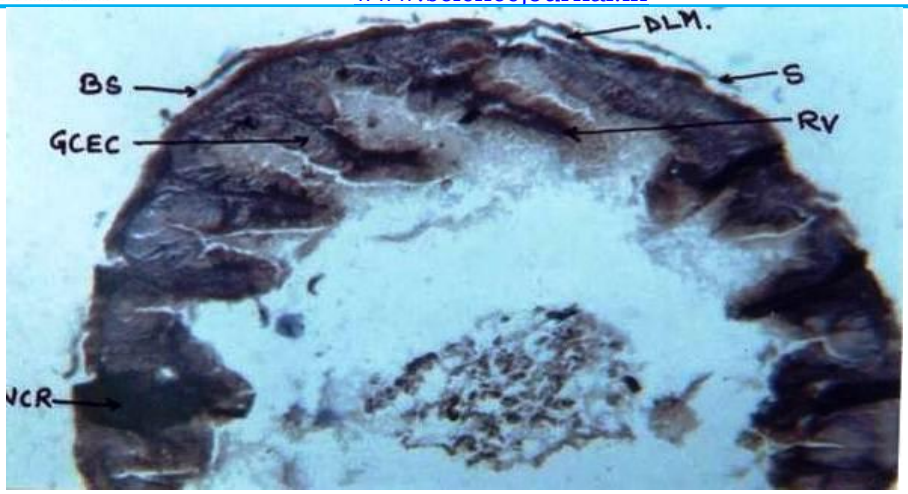

Fig.2: T.S. of intestine of *Puntius ticto* after 5.012 ppm exposure to dimethoate: Haematoxylin/Eosin 100X

S	Serosa	BS	Brokened serosa
DLM	Detached longitudinal muscular layer	RV	Ruptured villi
GCEC	Granular cytoplasmic epithelial cells	NCR	Necrosis

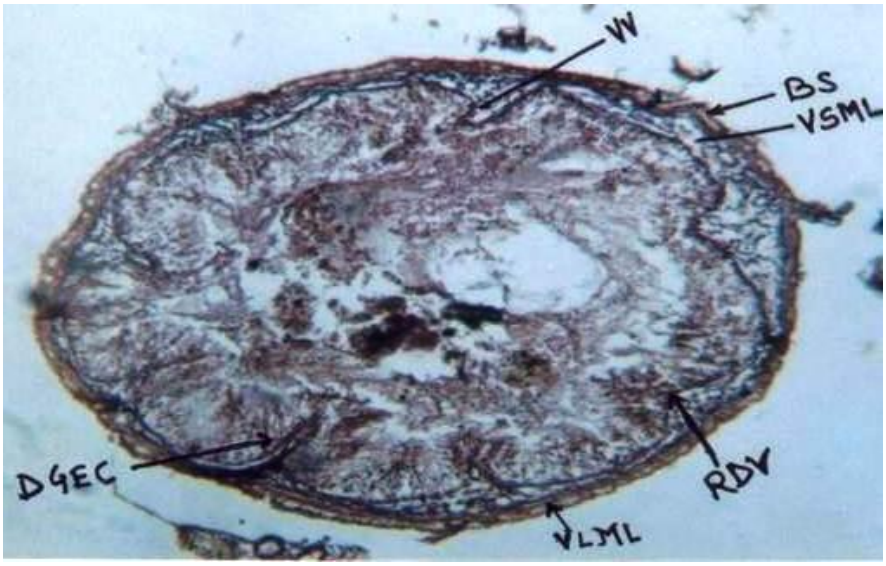

Fig.3: T.S. of intestine of *Puntius ticto* after 2.506 ppm exposure to dimethoate: Haematoxylin/Eosin 100X

BS	Brokened serosa	DGEC	Degenerated epithelial cells
RDV	Reduced villi	VLML	Vacuolated longitudinal muscular layer
VSML	Vacuolated sub mucosal layer	VV	Vacuolated villi

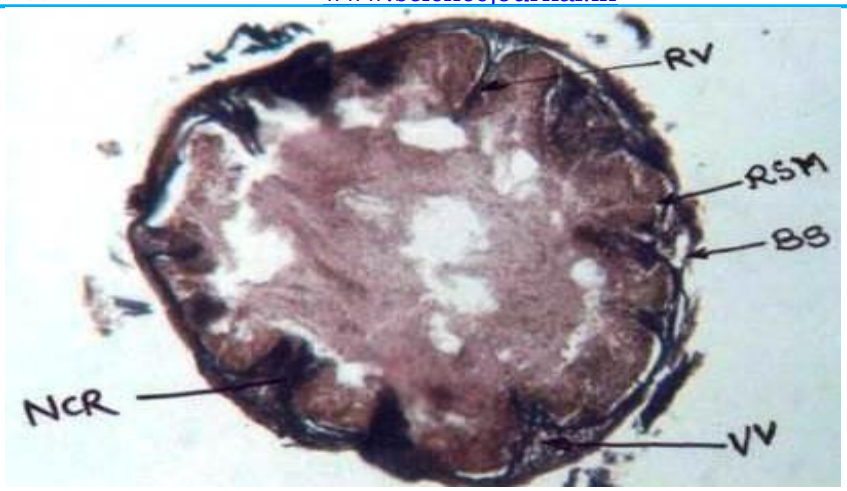

Fig.4: T.S. of intestine of *Puntius ticto* after 1.253 ppm exposure to dimethoate: Haematoxylin/Eosin 100X

BS	Brokened serosa	NCR	Necrosis
RSM	Reduced sub mucosal layer	RV	Ruptured villi
VV	Vacuolated villi		

DISCUSSION

Mathur (1979) reported that fishes which died due to pesticide toxicity showed pathological changes; these changes were present in the liver, kidney and intestine of Guppies and Brown trout exposed to sublethal concentration of DDT. Mathur *et al* (1981) studied the pathological alterations in the liver and intestine of *Rana cyanoflictis* in aldrin toxicity and found pyknotic nuclei, cytoplasmic vacuolations and cell necrosis. Takashi (1982) reported that pesticides treatment induced several abnormalities in the tissues; ulceration of the gastric mucosa, lack of appetite, reduction in growth and reproduction. Crespo *et al*. (1986) studied intoxication due to dietary cadmium and lead and observed increased goblet cells and enlarged apical pits and suggested that heavy metal exposure triggers mucous cell activity and production of mucous as a protective measure from the irritating agents.

Degenerative changes and rupture in tip of villi, loss of structural integrity of mucosal folds and degeneration & necrosis of submucosa in the intestine of *Channa punctatus* after the exposure to carbofuran are reported by Krishna Gopal and Ram (1994). Necrosis, degeneration, and accumulation of lymphocyte in lamina propia were observed in the intestine of mosquito fish, *Gambusia affinis*, exposed to thiodan and deltamethrin (Cengiz *et al*., 2001; Cengiz and Unlu, 2006). Srivastava *et. al.*, (2004) studied toxicological effects of malachite green on fishes and found histopathological changes in intestine. These changes includes necrosis, desquamation and degeneration of epithelial cell lining, cytolysis and increase in goblet cell population, rupture of tip of intestinal villi, breakage of mucosal folds, necrosis and disorganization of muscularis and serosa . Destruction of columnar epithelium, submucosa fused with muscles and serosa was found in broken condition after 10 days exposure to malathion (Kasotiya, 2004). Sabae and Ali (2004) reported epithelial degeneration, inflammatory cells infiltration in the submucosa as well as submucosal edema in the intestine of tilapia fish exposed to carbofuran. Al-Mansoori (2006) reported shortening villi, enlarged goblet cell , degeneration in mucosa, submucosa and muscularis, damage in mucosa, inflammation response with mucosa and submucosa, shortening villi with degeneration at tip of villi in the intestine of *Carassius carassius* (L) under cadmium (0.5 ppm) toxicity stress. Bhatnagar et.al., (2007) studied fluoride induced histopathological changes in the intestine of freshwater fish *Labeo rohita* and observed degenerative changes in the mucosal lining and villi of the intestine. The villi tend to become flattened and sloughing off of the mucosal lining. Hypertrophy of epithelial cells, swelling or oedema of lamina propria, and fusion of villi due to excessive hypertrophies, ultimately leading to rupture of villi at tip, are also evident.

Fatma (2009) studied histopathological changes in Tilapia zilli and Solea vulgaris and reported degenerative and necrotic changes in the intestinal mucosa and submucosa with necrotized cells aggregated in the intestinal lumen, hemorrhage in the submucosa and aggregations of inflammatory cells in the mucosa and submucosa with edema between them. Dilation in blood vessels of serosa and atrophy in the muscularis and Submucosa are noticed. Shawkat

et. al., (2010) reported damage of the mucosal epithelium, detachment of epithelium from the basal layer, especially at the tips of the villi, vacuolation in the epithelium, hypertrophy and degeneration of the mucosal epithelial cells in the intestine of *Clarius batrachus* under metal stress. Similar results were observed during present study and same findings are observed by Virk *et. al.,* (1987), Pandey *et.al,* (1994), Begum *et. al.,* (2001).

Mohanta et. al., (2010) reported, degeneration of columnar epithelium, necrosis at the tips of the villi and distortion of basement membrane and goblet cells in the intestine of Channa punctatus treated with tannery effluents. Ghanbahadur and Ghanbahadur (2012) reported destruction of mucosa and particularly of columnar epithelial cells has been prominent besides the granular degeneration, vacuolization and necrosis in intestine of *R. daniconius* exposed acutely and chronically to endosulfan. Shete and Patwari (2012) studied acute toxicity of $CuSO_4$ in the freshwater fish *Macrones cavasius* and reported histopathological changes in the intestine. These changes are epithelial cells and intestinal glands were enlarged; broken villi, Vacuolation at absorptive edges, cloudy swelling and damage of mucosal folds at some places. Satyanarayan *et.al.,* (2012) studied histopathological changes in the intestine of *Cyprinus carpio* under aldrin, dieldrin, BHC and DOT toxicity stress. They reported that DOT exposed intestine showed more flattening of intestinal folds followed by dieldrin and aldrin. In 30 days exposure of aldrin dieldrin resulted in completely flattened intestinal folds, reducing the surface area and necrosis. In case of BHC vacuolation and acute necrosis was observed. Banaee *et. al.,* (2013) observed atrophy and necrosis of mucosal cell, exfoliate of mucosal epithelium, lymphocyte infiltration to lamina propria, reduction in the elastic properties and capillary bleeding in intestine tissue of fish exposed to diazinon .

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