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CASE REPORT

HISTOPATHOLOGICAL IMPACT OF DIMETHOATE ON THE INTESTINE OF FRESHWATER FISH, *GARRA MULLYA* (SYKES)

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ABSTRACT

The present investigation was carried out to the effect of dimethoate on histopathological changes in intestine of freshwater fish, *Garra mullya*. Fishes was exposed to sub lethal concentration of dimethoate (0.0238ppm of 96hrs.) for 7, 14, 21 days. Apart from its role in preparing and absorbing nutrients, the intestine is the first line of defense against chemical insult through oral route. Fishes exposed to dimethoate were characterized by findings revealed that the intraperitoneal administration of pesticides produced various histopathological changes which include mitotic inhibition, dead mitotic figures, loss of villi, vacuolated and pyknotic nuclei in crypts and presence of notches in villi. Vacuolation are also observed in the mucosal part of the tissue. Several breaking of villi and mucosal cell membrane. Cell necrosis is observed. In chronic treatment of dimethoate exposure may pose serious threat to fish health and affect their population.

INTRODUCTION

Histopathology was used as a tool to detect the abnormalities caused by toxic stress of heavy metals, pesticides and industrial effluents because they are known to cause several histopathological changes in the tissue of organism. Uncontrolled discharge of untreated or primary treated industrial effluents in to inland water cause serious deterioration in water quality, which may lead to mortality of aquatic organisms. A number of pathological changes have been reported in fishes on exposure to different organochlorine, organophosphate, carbamate and synthetic pyrethroid pesticides, Tilak and Yacobu, **(**2002). The synthetic pesticides due to their long-term persistence in water and fish body adversely affect the quality of fish and their status, Waliszewski et.al, (1999). The aquatic ecosystem as a great part of the natural environment is also faced with the threat of a shrinkage genetic base and biodiversity due to indiscriminate use of pesticides, Omitoyin et al., (2006).

Fishes are largely being used for the assessment of the quality of aquatic environment and as such can serve as bioindicators of environmental pollution Dautremepuits et. al., (2004). Pollution of aquatic environment by pesticides are used indiscriminately in large amounts causing environmental pollution and potential health hazards. Dimethoate is systemic insecticides produced by reacting salts of Dimethyldithio-phosphoric acid with N-methylchloroaecetamide, in aqueous medium in the presence of some organic solvents is widely used against a broad range of insects pest and mites and is also used for indoor control of houseflies. The extensive use of DM poses a health hazard to animals and humans because of its persistence in soil and crops, WHO/IPCS, (1996). On the other hand, they cause much damage to the non-target organisms both in terrestrial and aquatic environment. Fishes are accumulating pollutants directly from contaminated water and indirectly via food chain by Sasaki, et al., (1997). Histopathology deals with the study of pathological changes induced in the microscopically structure of body tissue. Any alteration in normal structure of tissue indicates presence of disease or the effect of toxic substances like heavy metal and pesticides. Sprague, (1973) described histopathology as important tool for evaluating the action of any toxicant at tissue level. The present study was under taken to analyze the impact of chronic concentration of dimethoate in intestine of fish, *Garra mullya.*

Thus these histological studies have a way for understanding the pathological conditions of the animal by helping in diagnosing the abnormalities or damages of the tissues of an animal subjected to toxic stress of pesticide.

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MATERIALS AND METHODS

Healthy adult fish, *Garra mullya* were collected from local river Shivan Dist. Nandurbar, India in the month of December, 2015. Fishes were washed with 0.1% of potassium per magnate ($KMnO_4$) solution to avoid dermal infection. They were then rinsed in water and acclimatized to the laboratory conditions in the department of Zoology for two weeks in 200 liter. capacity glass aquaria. Dead fish were removed immediately that such mortality may deplete dissolved oxygen with resultant effect on other fishes. During acclimatization fishes were fed with pieces of live earthworm on alternate days. Water also changed once in every day. The experiment was conducted natural and photoperiod of temperature 25·1 ± 3·20C. Water quality was measure as per by APHA (2005), Conductivity- 0.64 ± 0.3, Dissolved O2- 6.3±1.1 (ml/L), pH- 8.60 ± 0.3, Acidity- 2.5± 0.1, Alkalinity- 44.1 ± 0.5, Total hardness- 67.5 ± 0.3. LC50 of dimethoate for 96 hours was determined by probit analysis method (Finney, 1971). The animals were dissected and intestine tissues carefully removed. Tissues were immediately washed in 1% saline solution to remove the adhering mucus and blood and soaked between the blotting papers. The tissue from the control and exposed batches were taken out and preserved in aqueous Bouin's fluid for 24-48 hrs. Tissues were cleared in xylene and embedded in paraffin wax (at 58˚- 60˚ C.). The tissue was then processed routinely and prepared into paraffin block cut at 6µm thickness using microtome and stained with Haematoxyline and Eosin by Luna (1968). Standard histopathological procedures were followed for histopathological investigations by Roberts, (1989). Observations were taken under light microscope.

RESULTS AND DISCUSSION

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory, Thophon, et al., (2003) and field studies by The, et al., (1997). Histological studies revealed that the intestine sections from control fish. Fig.-1 shows normal structure of intestine showing well marked. Mucosa the innermost layers shows finger like projection called villi. Intestine showing serosa, muscularis mucosa, submucosa and mucosa. Apart from its role in preparing and absorbing nutrients, the intestine is the first line of defense against chemical insult through oral route and intraperitoneal administration in abdomen. A major factor favoring absorption in the intestine is the presence of microvilli which increase the surface area for absorption. The cells in the active mitosis are sensitive to stress. Intestine of fishes exposed to 0.0238ppm dimethoate for 7,14 and 21 days resulted showed the changes such as inflammation at the base of the villi, vacuolation of cells of villi epithelium and compaction of the villi, hyperplasia of the epithelial cell. Fig. 2: Intestine of *G.mullya* exposed to dimethoate for 7 days showing damaged inflammation at the base of the villi, vacuolation of cells of villi epithelium and compaction of the villi. Fig. 3: After 14 days Intestine showing such as lesions in the villi, rupture of villi epithelium, massive infiltration of inflammatory cells throughout the villi and degeneration of villi epithelium. Structure of intestine showing the atrophy of the epithelial cells in mucosal layer was the significant change along with the vacuolation in the submucosal fold. Fig. 4: Intestine of fishes exposed to dimethoate for 21 days resulted structure of intestine shows vacuolation in villi and the wall in the necrosis in the mucosal membranes are observed. Massive infiltration of inflammatory cells throughout the villi. Several breaking of villi and mucosal cell membrane. Cell necrosis is observed.

Fig 1 shows normal structure of intestine,

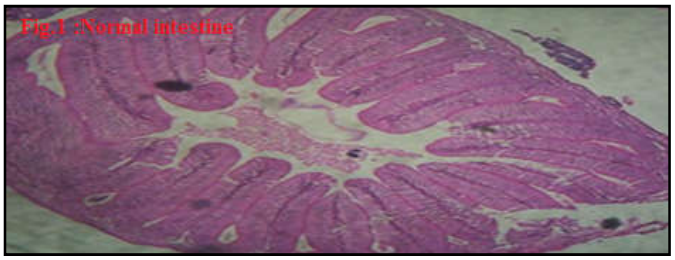

Fig 1 Normal structure of intestine showing well marked serosa, muscularis mucosa, sub-mucosa and mucosa. (H&E, 450X).

Fig.-2 shows histological structure of intestine on exposure of Dimethoate

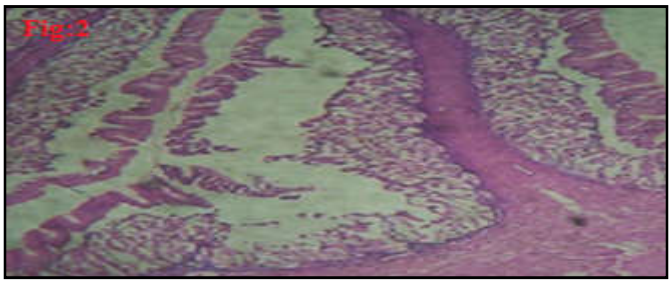

Fig 2 intestine shows the vacuolation in the sub-mucosal layer, hyperplasia of the epithelial cell. (H&E, 450X).

Fig 3 shows histological structure of intestine on exposure of Dimethoate

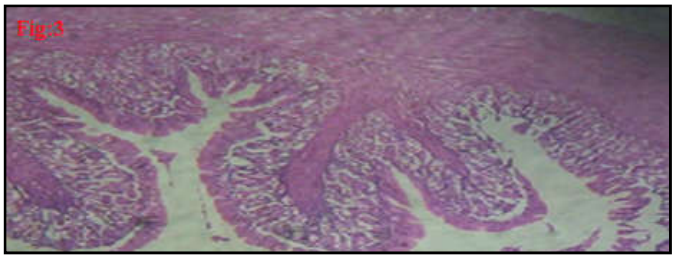

Fig 3 intestine showing the atrophy of the epithelial cells in mucosal layer was the significant change along with the vacuolation in the submucosal fold. (H&E, 450X).

Fig 4 shows histological structure of intestine on exposure of Dimethoate

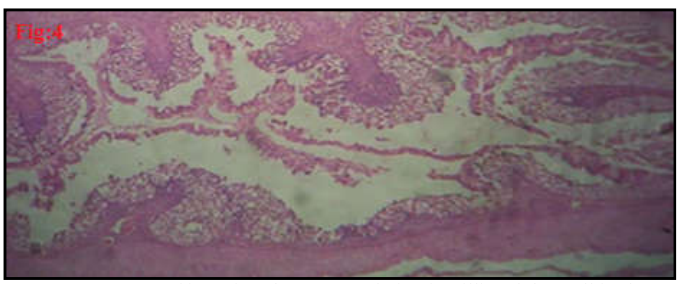

Fig 4 structure of intestine shows vacuolation in villi and the wall in the necrosis in the mucosal membranes are observed. (H&E, 450X).

CONCLUSION

The present study shows that dimethoate is toxic to fish, *Garra mullaya* and affects the structure and functions of intestine at sub-lethal concentrations causing considerable deterioration of

fish health and affect their population. The lesions in the vital organ might have resulted in physiologic and metabolic deregulations, which further led to behavioral alterations and growth impairment. Dimethoate used to protect many fruits, vegetables and field crops against disease, hence farmer come direct contact it and may affect their health.

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