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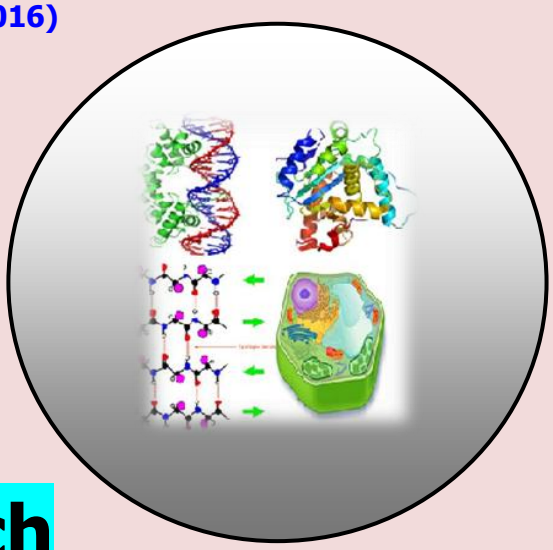

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RESEARCH PAPER

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Intestinal Histopathology in a Freshwater Cyprinid, *Garra mullya* (Sykes) Exposed to Sub-Lethal Levels of Carbosulfan and Bioneem

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ABSTRACT

Fresh water fish Garra mullya (Sykes) was exposed to sub lethal (LC $_{50}$/10 values of 96 hours) levels of carbamate pesticides i.e. Carbosulfan (0.6980 ppm) and biopesticide Bioneem (16.7455 ppm) for 21 days. After an interval of 7 days histopathological alterations in the intestine of G. mullya was investigated. Exposure to Carbosulfan resulted in progressive loss of mucosal epithelium, reduced lamina propria, atrophied mucosal epithelium followed by necrosis. Similar types of lesions were noticed in Bioneem treated fishes but they were less severe than carbosulfan treated fishes.
Keywords: Garra mullya, Carbosulfan, Bioneem, Sublethal conc., Intestine and Histopathology.

INTRODUCTION

Histopathology, the promising field of research provides the real pictures of toxicants effect in the vital functions of the living organisms. Structural changes at cellular level brought about by toxicants like pesticides alters the tissue structure and hence the whole organ. The extent of histopathological damage and the concentration of the pesticide can be correlated to access toxicity of pesticide. Carbosulfan is a carbamate pesticide and is extensively used in countries like Mexico, Brazil, Shi Lanka and India to control various pests on citrus, corn, potato and rice. Bioneem is a biopesticide containing azadirachtin, an active ingradient extracted from the seed kernels from Neem tree (*Azadirachta indica*). It disturbs vital physiological processes and affects activity of an insect (Anisuddin siddiqui, 2013). According to Johnson *et. al., (*1993), histopathological biomarkers provide a rapid and reliable method to detect effect of pesticides. Das and Gupta (2013 b) reported number of lesions in different organs of fish when exposed to chemical toxicants. Intestine being a multifunctional organ along with digestion and absorption of foodstuffs performs vital role in maintenance of water and electrolyte balance. It is having endocrine regulatory activity for digestion and metabolism. According to Mishra and Shukla (2003), responses given by the fishes to toxicant stress are quite similar to those shown by the mammals for which they can be considered as a good indicator of aquatic pollution. Being exclusively aquatic organism fishes comes in direct contact with toxicants. Gills and alimentary canal of fishes are found to be very much vulnerable to toxic substance. Several workers reported effect of pesticide and combination of pesticides in different organs of fishes [Banaee *et. al*., (2013); Ganeshwade *et. al*., (2013); Fayhaa and Saleh (2014) Suchismita Das and Abhik Gupta (2013)]. Histopathological alteration in various tissues of fish is a valuable tool to study toxic effect of different pollutants that will help to monitor

environmental pollution. Present work was also aimed with the study of effect of Carbosulfan and Bioneem on intestine of *G. mullya.*

MATERIALS AND METHODS

The fresh water fish *Garra mullya* was collected from unpolluted Bhaware dam from Navapur Tehsil and acclimatised for several days under laboratory conditions. The acclimatized fishes *G. mullya* were exposed to sub lethal (LC50/10 values of 96 hours) concentrations of pesticides i. e. Bioneem (16.7455 ppm) and carbosulfan (0.6980 ppm). Test water was changed after every 24 hours. After an interval of 7 days fishes were sacrificed. Intestine was removed and fixed immediately in Bouin's fluid for24 hours followed by dehydration, clearing, infiltration and embedding. Sections were cut at 6µm thickness and stained with Harries Haematoxylene and Eosin. Pesticide induced changes in the intestine were photographed and analyzed by light microscope (400 X). Histopathological study was repeated after an interval of 7 days up to 21days for control and treated groups.

RESULTS

Present study was aimed with evaluation of Carbosulfan and Bioneem induced histopathological changes in the intestine of fresh water fish *Garra mullya*. During investigation no mortality was recorded for all treatment groups. Fishes are sensitive to the presence of xenobiotics in the water and their behavioral changes can be used as the most good indicators of toxic effect of pollutant. Fishes in control group showed normal behavior and swimming pattern but change in swimming behavior was recorded with exposure to carbosulfan than to bioneem. Increased opercular movements and excessive secretion of mucous was also reported with carbosulfan which was comparatively less in bioneem.

The intestine of *G. mullya* is divisible into four concentric layers viz mucosa, submucosa, muscularis and serosa (Fig.1). Mucosa, the innermost layer lies over lamina propria containing nerves and leucocytes (Stoskopf, 1993) and is supported by submucosa. Muscularis has inner circular and outer longitudinal layer of muscle fibers. Serosal layer is present in the coelomic cavity and consists of mesothelial cells and loose connective tissue with blood vessels (Genten *et. al*., 2009). The intestinal epithelium is made up of simple or pseudostratified columnar cells that can be of different types, namely columnar absorptive enterocytes, goblet type mucous cells, lymphocytes and enteroendocrine cells (Kapoor *et.al.,* 1975).

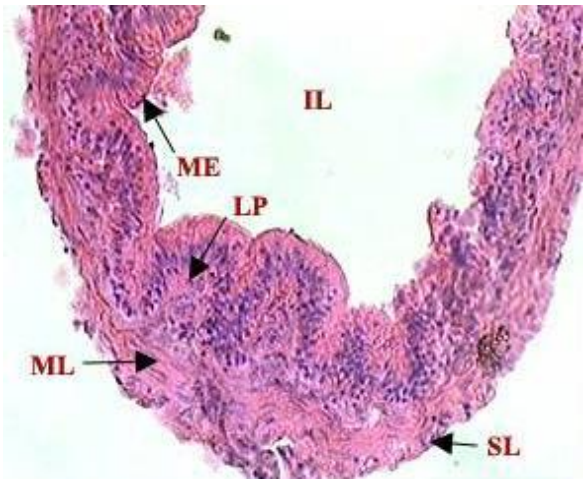

Figure 1. Microphotograph of Transverse Section of Intestine of *Garra mullya* (Sykes) showing Normal architecture (H and E; 400 X) SL: Serosal Layer; ML: Muscularis Layer; LP: Lamina Propria with Connective tissue; ME: Mucosal Epithelium with columnar cells and IL: Intestinal Lumen.

TREATMENT OF BIONEEM (Fig 2.1 to 2.3)

Deterioration of core, broken villi, thickening of muscle layer, degeneration in the serosa and muscularis layer and reduced lamina propria were marked degenerative changes. The histological structure of intestine was altered showing detached mucosal epithelium and vacuolated lamina propria. Intestinal lumen was decreased.

After 14 days lesions were continued with atrophy of mucosal epithelium that increased lumen. Intestine exposed to bioneem for 21 days showed degenerated serosa and muscular layer necrosis of epithelium was observed.

TREATMENT OF CARBOSULFAN (Fig3.1 to 3.3)

After treatment of Carbosulfan, notable effect in the structure of an intestine was observed which was severe than Bioneem treated fishes. Detachment of muscular epithelium and degeneration of muscular layer was the prominent lesion seen in the intestine after sublethal exposure. The villi were ruptured at some places. The intestine showed progressive loss of mucosal epithelium, reduction in lamina propria, atrophy of mucosal epithelial cells and necrosis as the fishes were exposed to toxicant for prolonged duration. Severe damage in the intestine was observed after 21 days exposure.

Comparison of Severity of lesions after exposure to bioneem and carbosulfan in the intestine of *G. mullya* is given in Table 1.

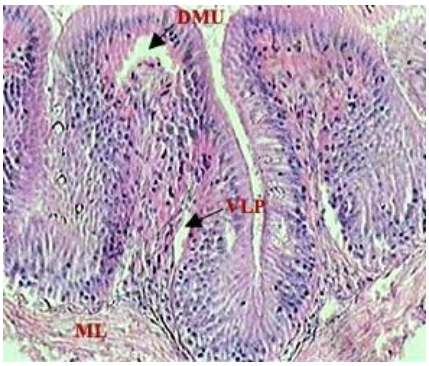

Figure 2.1. Bioneem 7 Days exposure

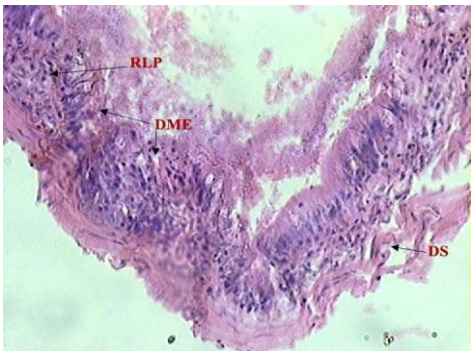

Figure 2.2. Bioneem 14 Days exposure

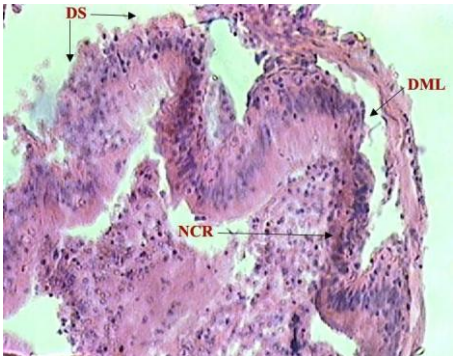

Figure 2.3. Bioneem 21 Days exposure

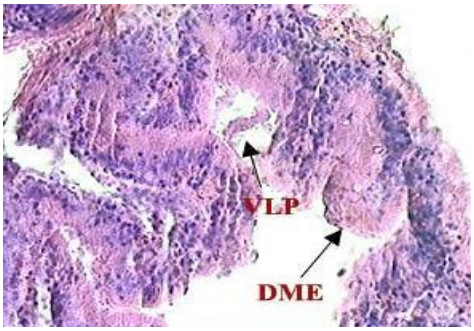

Figure 3.1. Carbosulfan 7 Days exposure

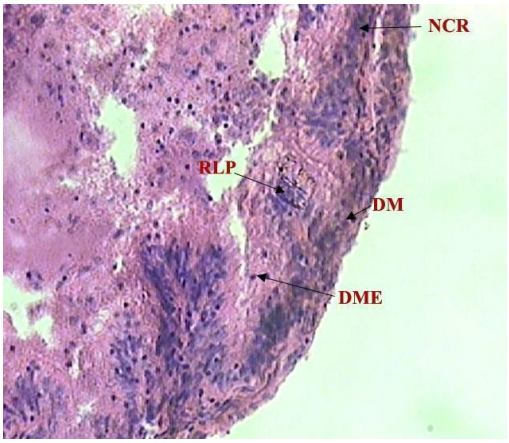

Figure 3.3. Carbosulfan 21 Days exposure.

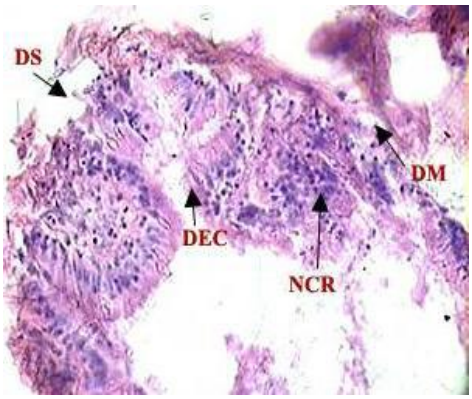

Figure 3.2. Carbosulfan 14 Days exposure

Fig 2.1 to 2.3: T.S of Intestine of *G. mullya* Exposed to Sub lethal Conc. (16.7455 ppm) of Bioneem. And Fig 3.1 to 3.3: T. S. of Intestine of *G. mullya* Exposed to Sub lethal Conc. (0.6980 ppm) of Carbosulfan (H and E; 400 X).

Table 1. Histopathological lesions in the intestine of *Garra mullya* (Sykes) exposed to sublethal concentration of Bioneem and carbosulfan.

Exposure	VLP			RLP			DME			DEC			DM			DS			NCR		
	7d	14d	21d	7d	14d	21d	7d	14d	21d	7d	14d	21d	7d	14d	21d	7d	14d	21d	7d	14d	21d
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bioneem	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-	+
Carbosulfan	+	-	-	+	-	-	+	+	+	-	-	+	-	+	+	-	-	+	-	+	+

(-) No Lession; (+) Lession

VLP: Vacuolated Lamina Propria; **RLP**: Reduced Lamina Propria; **DME:** Degenerated Mucosal Epithelium, **DEC:** Degenerated Epithelial Cells; **DM:** Degenerated Muscularis **ML**: Muscular Layer **DS:** Degenerated Serosa, **DML:** Detached Muscular layer and **NCR**: Necrosis.

DISCUSSION

Agriculture, pesticides and economic benefit of human are the inseparable aspects in this 21st century. There is significant contribution of green revolution in the development and application of pesticides in agriculture and stored products that diminishes food quality and quantity (Ecobichon, 2001). According to Mathur (1979) death of the fishes exposed to pesticide are due to pathological changes in the vital organs like lever, kidney and intestine of the fish. Abnormality in the vital organs alters the normal functioning that leads to death. Several investigators reported significant consequences of pesticides on aquatic organisms (Richards and Kendall, 2002; Banaee *et. al*., 2008). Krishna Gopal and Ram (1994) reported degeneration and necrosis of submucosa in the intestine of *Channa punctatus* when exposed to Carbofuran. Shawka et. al., studied the effect of metal stress in the intestine of *Clarius batrachus* and revealed damages in mucosal epithelium, vacuolation, hypertrophy and degeneration of mucosal epithelium. Similar results were observed by Ganeshwade *et. al*., (2013) in the intestine of *Puntius ticto* exposed to dimethoate. Das and Gupta (2013 b) exposed *Esomus danricus* to Malathion to find out histopathological changes in the intestine. It was reported from their study that Malathion exerted toxic effect on different layers of intestine. The degenerative changes reported in the intestine of *Cyprinus carpio* exposed to the pesticide atrazine were hyperemia, loss of structural integrity of mucosal folds, hypertrophy, vacuolation and necrosis (Walsh and Ribelin, 1975). Similar types of lesions are also observed in the present investigation. Histopathological changes in various tissues of fish are a valuable tool to find out toxic effect of xenobiotics which helps to monitor environmental pollution.

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