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HISTOPATHOLOGICAL AND HISTOCHEMICAL CHANGES IN THE MIDGUT OF *CYBISTER CONFUSUS* SHARP (DYTISCIDAE:COLEOPTERA)

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ABSTRACT – In the present study the effects of sub-lethal doses of DDT and EDTA have been assessed on the histopathology and histochemistry of the midgut of *Cybister confusus* Sharp, after the exposure for 7 days. Exposure to DDT caused severe damages to the midgut epithelium and midgut crypts became highly pronounced. Where as in case of EDTA almost total disintegration of epithelium and the invasion of regenerative cells was observed. Crypts were found to be more pronounced. Muscular layer also showed atrophy. DDT and EDTA also adversely affected the histochemistry of the different layers of the midgut. Protein showed a sharp declination in different layers.

Keywords : Toxicants, histopathology, histochemistry, midgut, *cybister confusus*.

INTRODUCTION

Insecticides used to control pests and disease vectors ultimately find their way into aquatic habitats and have been found to be highly toxic not only to the insects but also other aquatic organisms. These pollutants alter the natural condition of aquatic medium that causes many changes in aquatic organisms. Histopathology and histochemistry has become a diagnostic tool in toxicological studies and has been proved a reliable and sensitive indicator of stress in various aquatic organisms. Further, these are the diagnostic methods widely used in human beings and vertebrates for the analysis of certain pathologies, using this as a parameter, these kind of studies started to be used in invertebrates with objective to identify cell damages caused by certain toxic substances (Triebskorn and Kaunast23 1990; Triebskorn *et al* 1999) These techniques are used to analyze the impact of environmental pollution in some animals (Triebskorn *et al* 1991). More over relationships between histology and metabolic process in aquatic organisms have widely been reported. (Sakr, and Jamal al Lail, 2005, Verma, *et al* 1983, Sancho *et al* 1998).

The insects have well developed and sophisticated gut with distinct anatomical regions. They feed upon a variety of food hence alimentary canal exhibits a corresponding diversity (Wiggles worth – 1939). One important modification found in the midgut of some Coleopteran, Mecoptera, (Grel – 1938), in which regenerative cells are contained in crypts. These reach a high degree of development in *Dytiscus* (Duspiva, 1939). In this species the regenerative cells occurs at the bottom of the crypts and from these the new cells are supplied to the inter-cryptal epithelium as old cells degenerate.

The alimentary is a vital organ and likely to be easily exposed to the pollutants which may adversely affect the histology and histochemistry. The epithelium of the foregut and hindgut generally exhibit very little change during the process of digestion but histology of midgut is of special interest because of the rapid degeneration and replacement of the epithelial cells. Further, more it is not lined by the Chitinous intima and epithelium is often in direct contact with the food content. That is why it is selected as target organ for the investigation of the effects of the sub-lethal concentration of the toxicants, DDT and EDTA.

Insect of alimentary canal has been studied by several workers, Days and Waterhouse (1953) studied the digestive system of insects in detail. Goodchild (1952) in cocoon bug and Waterhouse and Stay (1955) in blood fly larvae. Vanzyl *et al* (2000) studied anatomy of histology of alimentary canal in *Furgella intermedia* and *Papares annulatus,* with reference to their feeding physiology. General histochemistry of the gut and location of different enzymes have been studied by several workers in various insects.

Chawdhary and Hawaldar (1980) in Sarcophaga and Uma Nath Saha (1987) in certain dipterans. Pollutants or toxicants induced histopathological and histochemical changes in different organs of insects, diplopods and fishes, snails etc. have been performed by many workers like, Sakr and Jamal al Lail (2005) in the liver of cat fish.

Birgul Otludil *et al* (2004) on the great ramshorn snail. Juliana de Godoy and Fontanetti (2010) in the midgut of diplopods. Nogarol and Fontanetti (2010) in the midgut of diplopods.

Little is known about the damaging effects of DDT and EDTA on the midgut of *Cybister confusus* in particular about histopathological and histochemical aspects. Hence the work was taken to examine histopathological and histochemical alterations in the midgut.

MATERIALS AND METHODS

*Cybister confusus.*Sharp is a common, carnivorous fresh water beetle found in ponds. The insects were collected from the local ponds and kept in glass aquaria along with few snails and small fishes for few days to acclimatize to the laboratory conditions. The rearing insects were divided into three group, one reared in normal laboratory conditions without application of toxicants and were treated as control. Remaining two groups were exposed to the sub-lethal concentration of DDT and EDTA. The sub-lethal concentration of DDT was found to be 0.005% (100mg of 50% DDT dissolved in 1ml acetone then dissolved in 2l tap water) and of EDTA it was 0.01% (100mg EDTA dissolved in 1l of tap water).

The control insect was dissected out in normal saline and similarly the treated insects after 7 days (168 hours) of toxicants exposure. The alimentary canal was rinsed with distilled water and fixed in 10% formaline and Bouins fluid.

The fixed tissues were processed for histological and histochemical studies. 8µm thick section were cut and following conventional histological and histochemical techniques were applied.

Harris's Haematoxylin and acetic Eosin for general histological slides, PAS-reaction after de-Tomasi(1956) for polysaccharides, Alcian Blue after Steadman(1950), modified for acid mucopolysaccharides and Bromophenol Blue after Mazia, (1953) for protein in general.

RESULT AND DISCUSSION

The alimentary canal of *C. confusus* is highly coiled tube measuring about 4 inches and divisible into three regions viz, foregut, midgut and hindgut. The midgut was about one inch long of nearly uniform diameter, at the junction of gizzard and midgut were found eight blind hepatic caeca.

Histology:

The midgut epithelial cells of control insects were elongated, peritonium and muscularis were thin, cuticular intima and peritrophic membrane were absent (Fig.-01) The epithelium forms crypts visible as small papillae on outer surface of the midgut.

Histology of the gut of insects has been extensively investigated and well understood before the beginning of the twentith century. In the present investigation the midgut epithelium was highly raised to form crypts and regenerative cells were contained in these crypts. Chitinous intima as well as the peritrophic membrane were absent. These findings are in conformity of the findings of Rungius (1911); Duspiva (1939) and Wiggles worth (1939).

Effect of DDT:

The midgut epithelium showed disintegration after DDT treatment. The crypts became more pronounced. The muscular layer and peritonium remained unaffected (Fig.-02)

Effect of EDTA:

The midgut epithelium exhibited a high degree of disintegration. Midgut crypt became more pronounced. The invasion of regenerative cells were observed. Muscular layers also showed degeneration. Peritonium seems to be delaminated from muscular layer at several places (Fig.-03).

Histopathological effects of pollutants and toxicants in different animals has been studied by various workers. The midgut of the nymph of *Locusta* was destroyed following the ingestion of sodium arsenite and sodium fluorosilicate Pilat (1935).

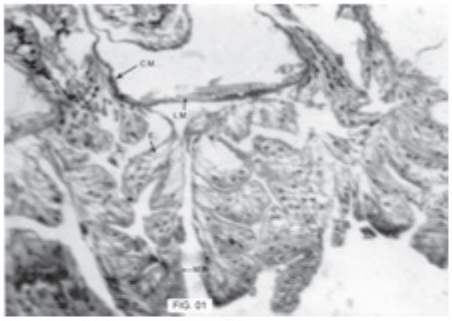
Break down of digestive epithelium from exposure to toxicants have been extensively described in different animals by several workers like Dhawale *et al* (1978) in *Fundulus heteroclitus,* Pipe and Moore (1985) in the gills of blood calm, *Anandra granosa*, Saber *et al* (1994) in the liver and small intestine of chicken and Vijaya Raghwan (2000) in *Spodoptera litura*(Fab).

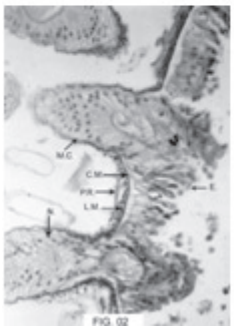
M. Rosety *et al* (2001) observed distruction of the structure of villi and increased thickness of other layers in the intestine of young giltheads (fish nursery) *Sparus aurata* by acute action of alkyle benzene sulphonate.

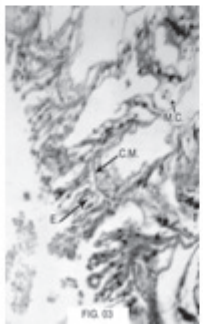
Effect of endosulfan on the ramshorn snail, *Planobarius corneus* was studied by Birgul Otludil, *et al* (2004). They observed the degeneration of cells and atrophy in the connective tissue, desquamation of epithelial cells and atrophy of muscle fibre in foot and mantel tissue.

Liver cell were found to be degenerated with necrosis. Marked cytoplasmic vacuolization were also observed in fenvalerate induced effects in catfish by Sakr and Jamal Al Lail (2005).

After exposure to industrial effluents the distegration







Abbreviations Used

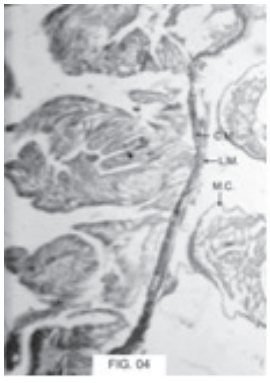
C.M. : Circular Muscle

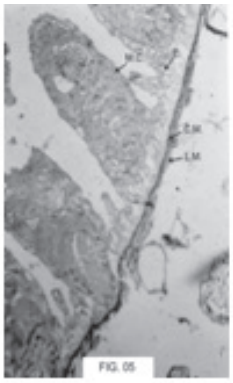
E. : Epithelium

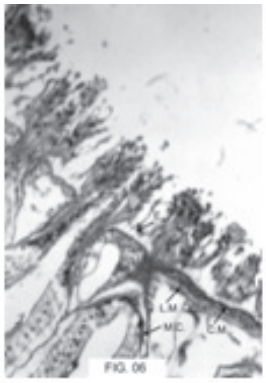
L.M. : Longitudinal Muscles

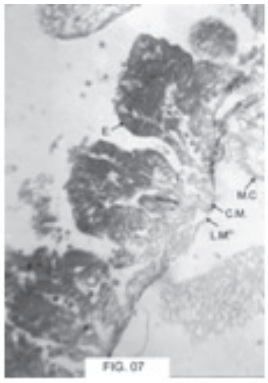
M.C. : Midgut Crypts

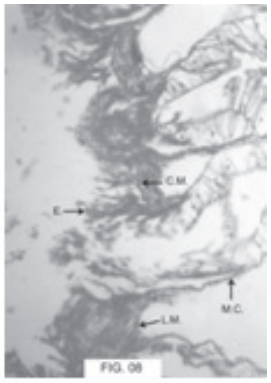
N. : Nucleus

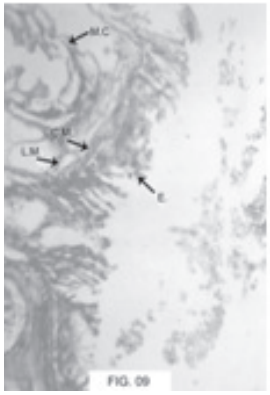


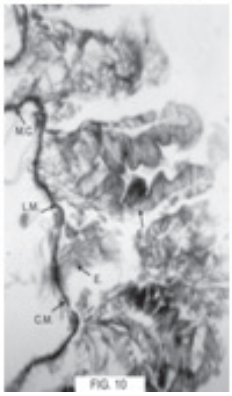












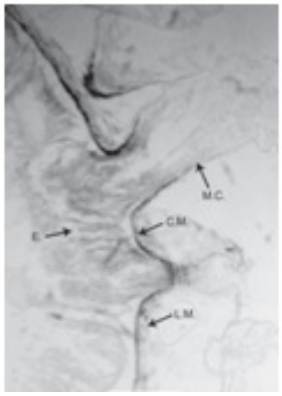


Fig. 01 : T.S. Midgut control, *C. confusus* Haematoxyling + Eosin. X 100
Fig. 02 : T.S. Midgut of DDT treated *C. confusus,* after 7 days Haematoxylin + Eosin X 100
Fig. 03 : T.S. Midgut of EDTA treated *C. confusus,*after 7 days Haematoxylin + Eosin X 100
Fig. 04 : T.S. Midgut control, *C. confusus*, PAS reaction. X 100
Fig. 05 : T.S. Midgut of DDT treated *C. confusus* after 7 days, PAS reaction. X 100
Fig. 06 : T.S. Midgut of EDTA treated *C. confusus* after 7 days, PAS reaction. X 100
Fig. 07 : T.S. Midgut control, *C. confusus,* Alcian blue. X 100
Fig. 08 : T.S. Midgut of DDT treated *C. confusus,* after 7 days, Alcian blue. X 100
Fig. 09 : T.S. Midgut of EDTA treated *C. confusus,* after 7 days, Alcian blue. X 100
Fig. 10 : T.S. Midgut control, *C. confusus,* Bromophenol blue. X 100
Fig. 11 : T.S. Midgut of DDT treated *C. confusus,* after 7 days, Bromophenol blue. X 100
Fig. 12 : T.S. Midgut of EDTA treated *C. confusus,* after 7 days, Bromophenol blue. X 100

of epithelial cells and disquamated epithelium in gill lamellae of esturine fish, *Lates calcarifer* was observed by Chezhian *et al* (2010).

The ultrastructural analysis of midgut of a diplopod, *Rhinocricus padbergi* after 2 weeks exposure to substrate containing sewage sludge showed a complete distructuring of epithelium with a total disintegration of cellular structure.

The present study is in confirmity of the earlier workers. The highly pronounced midgut crypts in the toxicants treated insects might be an attempt to renovate the epithelium with the invasion of regenerative cells as observed by J.A.P. de Godoy and Carmen S. Fontanetti10 (2010) in the midgut of diplopod, *R. padbergi.*

Table 1 : Histochemical observation of carbohydrates (PAS reaction) in the mid-gut of *Cybister confusus.*

Layers	Control	DDT after 7 Days	EDTA after 7 Days
Peritonium	++	++	++
Muscle	++	++	+
Epithelium	+++	++	++

Table 2 : Histochemical observation of acid Mucopolysaccharides (Alein Blue) in the mid-gut of *Cybister confusus.*

Layers	Control	DDT after 7 Days	EDTA after 7 Days
Peritonium	+	+	+
Muscle	+	+	++
Epithelium	++	+	++

Table 3 : Histochemical observation of Protein (Bromophenol Blue) in the mid-gut of *Cybister confusus.*

Layers	Control	DDT after 7 Days	EDTA after 7 Days
Peritonium	++	–	–
Muscle	+++	+	+
Epithelium	+++	++	+

– = No Activity; + = Little Activity; ++ = Medium Activity; +++ = Intense Activity

Histochemical Tests

The histochemical analysis for polysaccharides, acid mucopolysaccharides and total protein presented the pattern described as follows.

Polysaccharides :

The peritonium and muscles showed a medium PAS positive activity where as epithelium was strongly PAS posite, in control group (Fig.-04). In DDT treated insect a marked reduction of polysaccharides in all layers was noticed (Fig.-05). Where as in EDTA treated insects both muscular layers and epithelium exhibited reduction in carbohydrates as shown in (Fig.-06 and Table-1).

Acid Mucopolysaccharides :

A little activity of acid mucopolysaccharides was noticed in the peritonium and muscles but medium activity was observed in the epithelium of control group (Fig.-07). DDT treated insects exhibited a little activity in all three layers (Fig.-08). EDTA treated insect showed little activity in peritonium and medium activity in muscles and epithelium (Fig.-09 & Table-2).

Protein (in general) :

Peritonium showed medium activity where as muscles and epithelium exhibited intense activity in control group (Fig.-10). There was no activity in peritonium and little activity was observed in epithelium of DDT treated insect (Fig.-11). Where as EDTA treated insects shows further declination of protein (Fig.-12 and Table 3).

DDT and EDTA adversely affected the histochemistry of the gut. These findings may be correlated with biochemical analysis by several workers. Carbohydrate decreased as a result of exposure to a sub-lethal concentration of aldrin and formothion in liver and muscles of fish, *Heteropneustes fossilis* (Singh and Srivastava 1981). Sastry and A. Siddique (1984) reported reduction of glycogen in liver of *Channa punctatus* after exposure to sub-lethal concentration of quinalphos – pesticide.

According to Dhavale and Masurekar (1986) decreased level of carbohydrate constituents in the tissue of toxicant exposed animal may be due to prevalence of hypoxic condition in the tissue as a result of pollutant stress.

Similarly the protein content showed noticeable decrease after DDT exposure and almost no activity after EDTA exposure for seven days. This finding is in accordance with the findings of Chezhian1 (2010) who also noticed the declination of carbohydrate and protein in endosulfan effluent exposed fish, *Lates calcarifer.* They suggested that chemical stress causes rapid depletion

of stored carbohydrate primarily in hepatocytes and other tissues. The histopathological changes can provoke the functional disorders in affected organs (i.e. damage to the gut epithelium) could lead to the inhibition of both amino acid and sugar absorption and transport(Rosety; *et al* 2001)

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