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ANATOMY AND HISTOPATHOLOGY OF ALIMENTARY CANAL OF *HALYS DENTATUS* (HEMIPTERA: PENTATOMIDAE) TREATED WITH DICHLOROVOS

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ABSTRACT *Halys dentatus* is well known sap sucker living under the bark and having the reputation of affecting nutritional supply of their host plants. Dichlorovos (2,2-dichlorovinyl dimethyl phosphate) is an organo-phosphorus compound previously used for controlling crawling and flying insect pests. In the present study dichlorovos has been tested as systemic poison for checking its lethality against plant bug *Halys dentatus*. The oral application has found to be effective, as it disintegrates components of gastro-intestinal tract affecting vitality of test individuals. The adult plant bug when treated with dichlorovos, alteration in histological structures of ventriculus has been observed. In most of the cases alteration in shape of ventricular components has been noticed; basement membrane has found to be collapse while longitudinal and circular muscles layers were also disrupted. The cell boundaries of epithelial cells found to be busted and secretory cells become denatured; nuclei were found to be stained intensely as compared with untreated histological sections.

KEYWORDS : *Halys dentatus, Dicholorvos*, Histology, Histopathology, Anatomy, Pest control

INTRODUCTION

Halys dentatus is distributed in North West Africa, China, Japan, India, Pakistan and Iran and commonly found on trees like *Samania saman, Millingtonia hortensis, Spathodea companulata, Acacia moniliformis* under loose bark of trees. *Halys dentatus* are the insect pest living under the loose bark and suck the sap affecting nutritional supply of their host plants. These insects are equipped with well-developed piercing and sucking mouth parts along with cibarial pump for sucking the sap through pierced epidermis of host plant.

Alimentary system is well developed and as usual divisible into foregut, midgut and hindgut and capable of digesting sap of plant. It is necessary to study anatomical and histological features to understand process of digestion and absorption. (Terra and Ferreira 1994). Harris (1938) studied anatomy and histology of alimentary system of *Murgantia histrionica* and reported nine distinct regions in alimentary canal viz. three stomodeal, three ventricular and three proctodeal of which stomodium comprises of functional mouth, cibarium and oesophagus while ventriculus divided into anterior, mesial and posterior ventriculus respectively; and proctodium consists of anterior intestine, posterior intestine and terminal anal canal. The histological structure of midgut of *B. tabidus* has been studied by Guedes et al. (2007) to observe structural alterations in the midgut when insects were starved and fed with their food at different time interval.

The hemipteran pests are often managed by application of various insecticides in agro-ecosystems but have deleterious effect on non-target species and host plant also. The potential of insecticide and their lethal activity on hemipteran pests has been studied by many workers but the impact of the insecticide on histomorphology of alimentary system is being neglected. In the present study the effect of locally popular insecticide Dichlorovos on histomorphology of alimentary canal of *Halys dentatus* has been investigated.

MATERIAL AND METHOD

The adult insects were collected from their host plants viz: *Millingtonia hortensis, Spathodea companulata, Acacia moniliformis, Acacia arabica, Pithocelebium dulce and Samania saman* etc. from K.T.H.M. College campus, Nashik by hand picking method and cultured at laboratory during the period of June 2011 to December, 2015. Insects were fed with pumpkin, potato, as food daily and acclimatized to temperature 28±1, relative humidity (RH) 65%, D/L-14:10 in laboratory. The healthy adults of same size and age were utilized for the experimental purpose.

For anatomical and histological preparations, adult *Halys dentatus* were anesthetized with chloroform and mounted in paraffin wax plate and dissected in insect saline (Lum, 1961) using stereoscopic binocular microscope. The exposed alimentary canal is isolated & biometry has been done using scale and photographed. An isolated alimentary canal is fixed in fixative followed by histological process to obtain sections of its various parts. Sections of 6-7 µm thickness were obtained using Leica microtome and permanent slides prepared using DPX. Screening and microphotography were done by using UV ultrascope 9.1 under trinacular research microscope.

OBSERVATION AND RESULT

Anatomy:

The alimentary canal of *Halys dentatus* is distinguish into stomodaeum (foregut), ventriculus (midgut) and proctodeum (hindgut) and provided with pair of salivary glands **(Fig.1).** The alimentary canal at certain places shows the vulvular processes to allow or prevent the entry of fluid material known as valves. Such valve is present in between the stomodeum and former ventriculus is the stomodeal or cardiac valve and the valve in between the later of ventriculus and proctodeaum is the proctodeal or pyloric valve.

The stomodaeum forms preoral cavity forms sucking pump which is cuticulus at beginning but becomes thin and transparent as it meets into short oesophagus. The cibarium enlarged to the posterior region of head capsule which surpass posterior region of the brain and attached to the esophagus and extend into the thorax. It has a valvular process that prevent entry of liquid material and allow it when requires, the valve is present in between stomodaeum and ventriculus I.

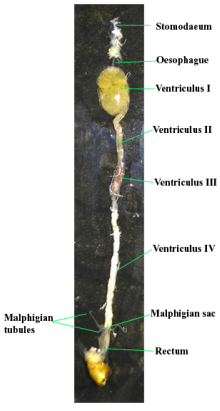

Figure 1: Alimentary Canal of *Halys dentatus*

The short, thin, semitransparent esophagus opens into ventriculus which is the middle portion of alimentary canal known as mid gut. It is longer and convoluted as compared to stomodeum and proctodeum and further differentiates into ventriculus I, II, III and IV (Fig.1).Ventriculus I is bulbus, elongated, folded, sac like structure, its former region continuous with stomodial valve, measures 7-8 mm, which further leads into ventriculus II. The ventriculus II is narrow, tubular brownish canal measures 5-6 mm. The ventriculus III is swollen bulb like, dark brown to blackish in colour measures 3-5 mm

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long and stores the food temporarily. The ventriculus IV is longest of all three ventriculus, it varies from creamy white to dark pinkand measures 13-15 mm. Ventriculus IV forms convoluted structure with four rows of caeca twisted around each other.

The proctodeum is short, consisting of anterior intestine, rectal sac and anal canal collectively measures 3-5 mm long. A pair of long malpighian tubules arises on ventral wall of intestine and hangs freely at lower abdomen. The rectal sac is thin membranous structure which terminates into rectum. The proctodeal valve is present in between the posterior of ventriculus IV and former of proctodeum.

HISTOLOGY:

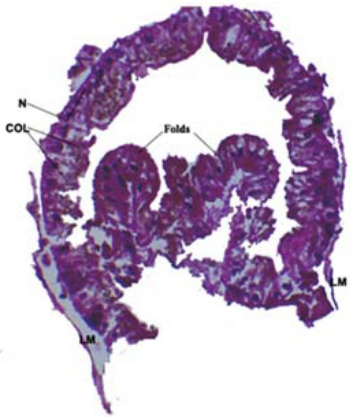


Figure 2: T.S. of oesophagous of H. dentatus (10X)

COL: Columnar epithelium, LM: Longitudinal muscle, N: Nucleus

STOMODEUM

Histologically foregut typically composed of six layers viz: innermost intima, epithelial layer, basement membrane, longitudinal muscle layer, circular muscle layer and outermost peritoneum. Oesophagus has a circular muscle layer which is not attach directly to longitudinal muscle layer. Columnar epithelial cells are with the folds inside to form loops and having basal nuclei. Inside the lumen there observe a folds of epithelial layer (Figure 2). The stomodeal valve shows cuboidal epithelium at exterior end and towards the lumen cells are of columnar type (Figure 3).

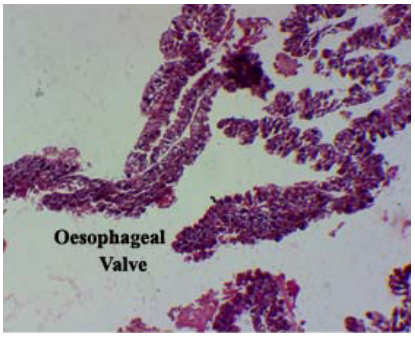


Figure 3: L.S. of Oesophageal valve of H. dentatus (10X)

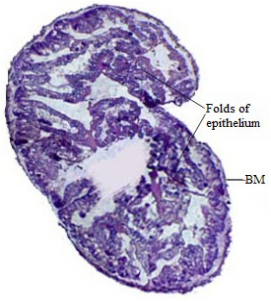
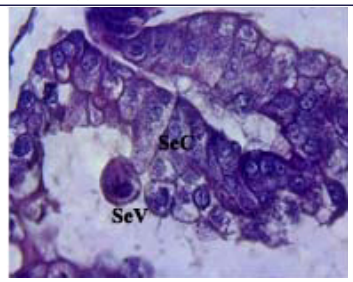


Figure 4: L.S. of ventriculus I of H. dentatus (10X) BM: Basement Membrane



VENTRICULUS: The wall of ventriculus typically composed of five layers viz. innermost columnar epithelium, based on basement membrane, circular muscle layer, longitudinal muscle layer and outermost peritoneal membrane. The epithelial cells of ventriculus I are more compact as compared to other regions of ventriculus. These cells are resting on thin basement membrane. The longitudinal and circular muscles are fewer as compared to rest of the alimentary canal. This region shows many folds of columnar epithelial cells (Figure 4 & 5) which are in active secretory phase.

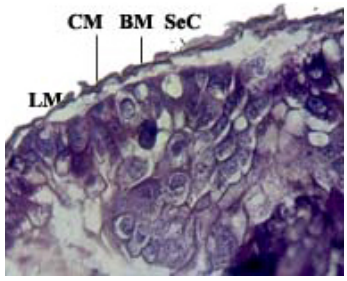


Figure 5: Magnified portion of T.S. of ventriculus1 of *H. dentatus* (40X) showing secretory phase of various cells

SeC: Secretory cell, SeV: Secretory vesicle,
BM: Basement Membrane, CM: Circular muscle, LM: Longitudinal muscle

The columnar epithelium composed of chiefly four kinds of cells viz. columnar secretory cells, regenerative cells, goblet cells and peptic cells. The secretion is of apocrine type because the secretion accumulated at the distal end of cell and pinched off at the time and released directly into the lumen of ventriculus (Figure 5).

The columnar cells characterized by their shape and apical microvillar border. Their lateral walls are straight while basal wall is folded along with invaginations. Of the basement membrane, secretory granules are present in variable density in the cytoplasm. Goblet cells are scattered among the columnar cells and characterized by invaginated luminal plasma membrane forming flask shaped large cavity. Endocrine peptic cells are intercalated among columnar and regenerative cells. They are somewhat bowl shaped or pyramidal basal lamina and basement membrane as well as apical membrane are unfolded.

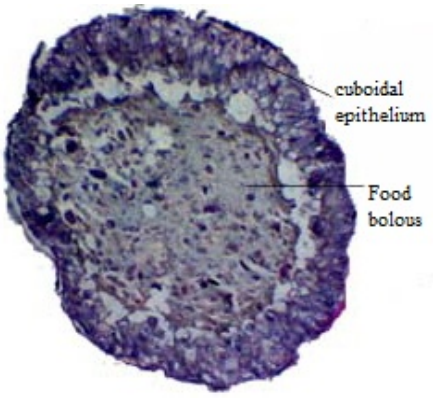


Figure 6: T.S. of ventriculus II of *H. dentatus* (10X)

The ventriculus II appears round in shape in transverse section. The musculature is well developed. The epithelium represents the cuboidal cells (Figure 6). The cells are resting on thin basement membrane, the longitudinal muscles, circular muscles and peritoneal sheath are also present at the exterior (Figure 7).

The food bolus is separated from lumen by peritropic membrane. It shows large nucleus with granular cytoplasm. The cells at the base are cuboidal whereas towards the lumen at its distal end have elongated in form. The secretory cells inflate and pinched off the secretory fluid in the lumen of ventriculus (Figure 7).

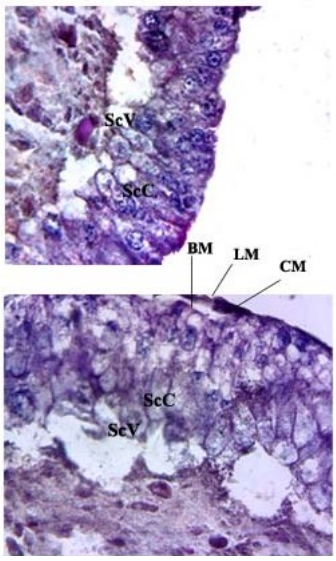


Figure 7: Magnified portion of T.S. of ventriculus II (40X)

SeC: Secretory cell, SeV: Secretory vesicle,
BM: Basement Membrane, CM: Circular muscle, LM: Longitudinal muscle

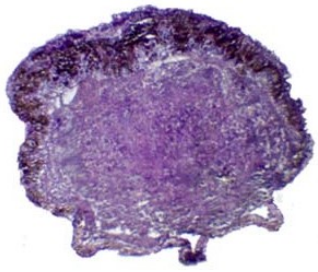


Figure 8: T.S. of ventriculus III of *H. dentatus* (10X)

The ventriculus III is sac of stored food material. It appears elliptical; at its one side the cells show folds at exterior in cross section (Figure 8). The longitudinal and circular muscle layers are presenting at outer to the basement membrane. The columnar epithelium shows a striated boarder at its outer (Figure 9).

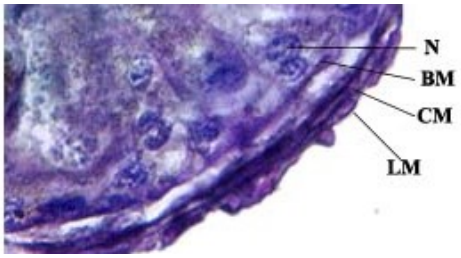


Figure 9: Magnified portion of T.S. of ventriculus III of *H. dentatus* BM: Basement Membrane, CM: Circular muscle, LM: Longitudinal muscle, N: Nucleus

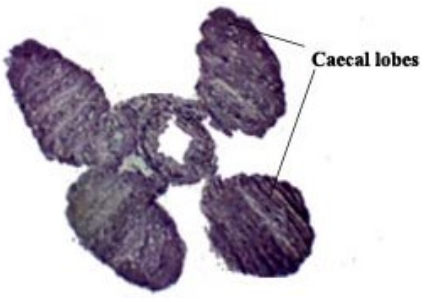


Figure 10: T.S. of ventriculus IV of *H. dentatus* (10X)

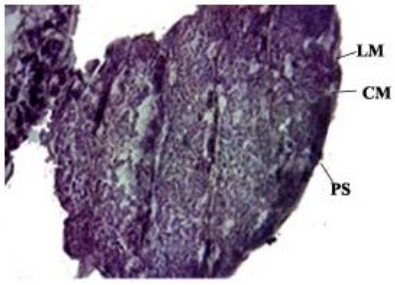


Figure 11: Magnified portion of the caeca in T.S. of ventriculus IV of *H. dentatus* (40X)

CM: Circular muscle, LM: Longitudinal muscle, PS:

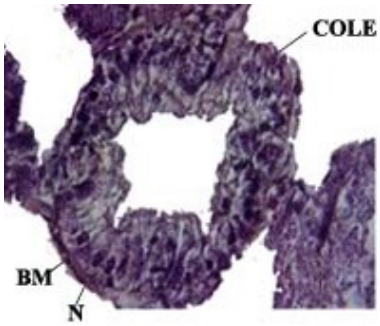


Figure 12: Magnified central portion of T.S. of ventriculus IV of *H. dentatus* (40X)

BM: Basement Membrane, N: Nucleus, COLE:

The ventriculus IV shows twisted four lobed caeca, surrounding the thin layer of epithelium with the presence of nuclei (Figure 10). The ventriculus IV shows columnar epithelium with small lumen. The longitudinal and circular muscles, peritoneal sheaths are exterior to the basement membrane (Figure 11 and 12).

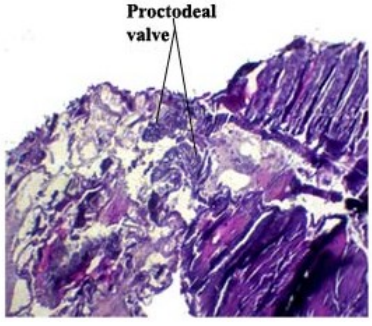


Figure 13: L.S. of hindgut showing proctodeal valve (10X)

Proctodeum:
The proctodeum is posterior region of alimentary canal, like the stomodeal valve the proctodeal valve is with less musculature and present in between the ventriculus IV and proctodeaum. It shows the

columnar cells of ventricular epithelium resemble with the anterior intestine. The wall of anterior intestine folded at the region of proctodeal valve. The expanded wall of ventricular epithelium exerts pressure on the content of the proctodeum by valvular action to prevent regurgitation (Figure 13).

The anterior intestine is thicker and constitutes columnar epithelium cells, the longitudinal muscles are very few and the circular muscles are absent. The rectal sac represents variation in thickness of epithelium either having large nuclei and some cells are compacted.

HISTOPATHOPATHOLOGY:

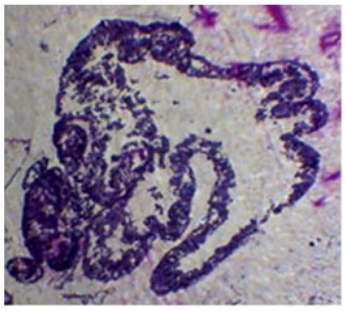


Figure 14: T.S. of ventriculus I of *H. dentatus* treated with dichlorovos (10X)

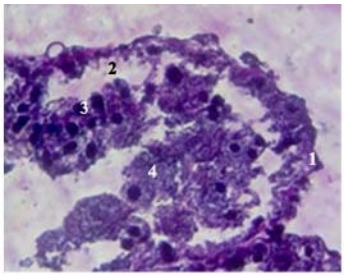


Figure 15: Magnified portion of T.S. of ventriculus I of *H. dentatus* treated with dichlorovos showing histo-pathological changes (10X)

1: detachment of basement membrane from disrupted epithelium, 2: disruption of longitudinal and circular muscles, 3&4: Busted cell boundaries of epithelial cells with darkly stained nucleus lies outside.

After exposure of pesticide dichlorovos 76% EC the ventriculus I of treated insect shows alteration in histology. The shape of ventriculus is altered with highly irregular structure; basement membrane found to be disturbed; the longitudinal muscles and circular muscles remain uninterrupted. The bursting of cell boundaries of epithelial cells, nucleus is darkly stained shifted to periphery of cells as compared to control. The damage to secretory cells cause to lose ability of secretion (Figure 14 and 15).

Figure 16: T.S. of ventriculus II of *H. dentatus* treated with dichlorovos showing histo-pathological changes (10X)

1: Detachment of circular and longitudinal muscle layers and peritoneal sheath from basement membrane, 2: epithelial lining folded inside, 3: secretory cells with obliterated with darkly stained nucleus, 4: distortion of peritoneal membrane, 5: denatured granular cytoplasm

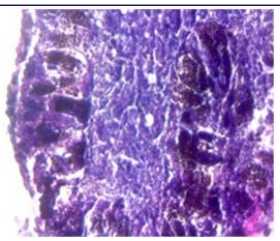


Figure 17: Magnified portion of T.S. of ventriculus II of *H. dentatus* treated with dichlorovos (40X).

The ventriculus II also shows alteration in histological pattern. The round shape of control changed to oval. The detachment of muscle layers like longitudinal, circular and peritoneal sheath from the basement membrane. The epithelium lining shows much folds, the secretory cells obliterated with darkly stained nucleus. Distortion of peritrophic membrane and food bolus fused with epithelium. The cytoplasm is vacuolated or compacted (Figure 16 and 17).

Figure 18: T.S. of ventriculus III treated with dichlorovos showing histo-pathological changes (10X)

The ventriculus III in treated insects shows drastic alteration in histology. The normal shape of ventriculus changes to oval. Musculature distorted from basement membrane. The epithelium shows irregular folds with columnar cells in some areas, double layer of epithelium also observed. The epithelial cells show stretching with the darkly stained nuclei (Figure 18).

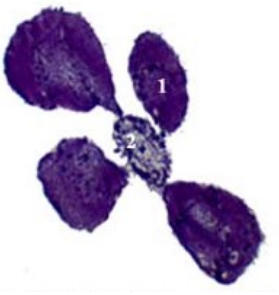


Figure 19: T.S. of ventriculus IV of *H. dentatus* treated with dichlorovos (10X).

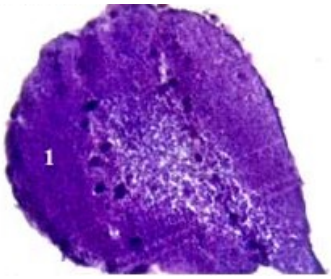


Figure 20: Magnified portion of the caeca in T.S. of ventriculus IV of *H. dentatus* treated with dichlorovos showing disappeared peritoneal sheath and secretory cells (40X).

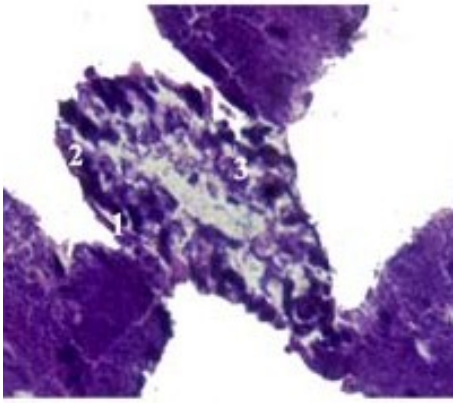


Figure 21: Magnified central portion of T.S. of ventriculus IV of *H. dentatus* treated with dichlorovos (40X)

1: Destructed peritoneal sheath, 2: destructed longitudinal and circular muscle layer, 3: denatured columnar cells appeared as compact mass.

The ventriculus VI after treatment shows the effect on caeca by changing its shape (Figure 19). The ventriculus shows destruction in the musculature like peritoneal sheath, longitudinal and circular muscles. The epithelial cells denatured to form compact mass (Figure 20 and 21).

DISCUSSION

The alimentary canal in *Halys dentatus* shows generalized plan as typical hemipterans i.e. oesophagus is short tube opens into ventriculus, which is the longest tubular part, posteriorly opens into proctodaeum. According to Dan et al. (1980) pentatomidae species represents a similar anatomical pattern for the alimentary canal being ventriculus is the longest part; in the present anatomical studysame observation has been noted. As per Harris et al. (1938) ventriculus III was not distinguishing histologicaly from ventriculus II reasonably they considered as part of ventriculus II. However, Kurup (1963) reported these two regions were fairly distinct. In *Halys dentatus* the anatomical distinction between the two regions viz. ventriculus II and III were confirmed from histological studies (Figure 6 and 8).

Presence or absence of gastric caecae in hemiptera has phylogenic significance and also important from nutritional point of view (Elson, 1937). Glasgow (1914) studied gastric caecae of hemipteran bugs where he reported caecae contained micro-organisms that inhibit the growth of foreign bacteria in the mid intestine. Primarily sap feeder species such as *L. trivittalus* occasionally feeds on fluids of animals and are lacks gastric caecae Glasgow (1914). Presence of gastric caecae in two rows*A. tristis* while four rows in *B. quadripushtalata* phytosuccivorous hemipteran were confirmed by Breaky (1936) and Dan (1980) respectively. In the present study Ventriculus IV in *Halys dentatus* longest of all the ventriculus; and bears four rows of gastric caecae twisted around themselves.

The histology of alimentary canal of hemiptera form more or less consistent pattern. Woolley (1949) and Breaky (1936) reported observation on histological pattern of oesophagus of *Leptocoris triritatus* and Anasa tristis showed the presence of cuboidal cells in the oesophagus. Whereas, the oesophagus of Murganntia histrionica contained the columnar epithelial cells was mentioned by Harris (1938). Latif et al., (1960) studied *Drosicha stebbingi* and mentioned the epithelium of oesophagus showed the intermediate pattern, the cells in this region are flattened but typical columnar shape with basal nuclei. Histologically oesophagus of *Halys dentatus* shows outer circular muscle layer which is not attaches directly to longitudinal muscle layer. The columnar epithelial cells are present with having folds inside to form loops and having basal nuclei.

The midgut of Hemipteran was usually differentiated into three to four ventriculi. According to Richard and Davis (1957) certain families of Hemiptera which contained gastric caecae, the midgut divided into four ventriculus. After comparing the midgut of various hemipterans the Glasgow (1914) believed that it divided into four ventriculus. According to Breaky (1936), Harris (1938) and Hamilton (1931) certain Hemipteran species showed three ventriculus. The observation of Snodgrass (1935), Richard and Davies (1957) and Latif et al., (1960) on the histology of *Drosicha stebbingi* showed the midgut was differentiated into four ventriculus and the regenerative cells were more in fourth ventriculus therefore its lumen become narrow. The general histological structure of ventriculus was same in many Hemiptera except the arrangement of musculature. It includes the columnar epithelial cells based on thin basement membrane followed by longitudinal, circular muscle and peritoneal sheath. The circular muscles were absent in *Anasa tristis* reported by Breaky (1936). The findings on *Drosicha stebbingi* by Latif et. al. (1960) the histological structure of first and second ventriculus was same. The observation of Harris (1938) on hemipteran species showed the anterior region of second ventriculus contains a cuboidal epithelium, the cellular arrangement of third and fourth ventriculus was near about similar all over the hemiptera. Each ventriculus was showed basic histological pattern such as the epithelium rest on basement membrane followed by the longitudinal and circular muscle layer. In the present study of *Halys dentatus* midgut also distinguished into four ventricles showing typical histological pattern. The epithelial cells of ventriculus I are more compact as compared to other regions of ventriculus; these cells are resting on thin basement membrane; the longitudinal and circular muscles are fewer as compared to rest of the other. The musculature is well developed in ventriculus II; cuboidal epithelial cells resting on thin basement membrane, the longitudinal muscles, circular muscles and peritoneal sheath are also present at the exterior while ventriculus III represents columnar epithelial cells with striated outer border.

The cellular arrangement of proctodeaum or hindgut in hemiptera showed the presence of flattened cells. At the anterior the proctodeaum was less distinguished and contained columnar cells as compared to posterior end. Woolley (1949) showed in case of *Leptocoris triviitatus* the cells of lumen were columnar in shape but it may changed to cuboidal at the joining of ileum and malphigian tubules. The proctodaeum of *H. dentatus* containing columnar epithelium cells, the longitudinal muscles are very few and the circular muscles are absent. The rectal sac contains both large and short size of epithelium either having large nuclei and some cells are compacted.

The observations of George (1996) on histology of alimentary canal of three reduviid bug, the insecticide caused the lysis of intercellular connecting material which caused the gaps between the epithelial cells therefore it appeared frilled, cells degenerated, intestinal mucosa get damaged, pycnotic nucleus, vacuolization, obliteration of peritrophic membrane, appearance of fat globules, evagination of inner cellular tips and subsequent projection into the lumen, vacuolization caused exfoliation of cells and inter luminal migration of broken cells, degenerated nucleus, condensed chromatin and darkly and evenly stained perinuclear cytoplasm. The effect of two plant extract *Eupatorium odoratum* (Compositae) and *Vitex negundo* (Verbenaceae) were applied on alimentary canal of *Dysdercus cingulatus* where the ventriculus first showed pushing towards the lumen from the basement membrane mentioned by Pramila et al. (1995). The reduction of secretory material, the accumulation of secretory granules at tip of the cells which were not discharged into the cell lumen, while in control the columnar cells arranged normally and discharge their content into the lumen. As compared to control the ventriculus II was disrupted and between the cells gaps appeared. The ventriculus IV showed the condensed cell as compared to control while the midgut showed dearangement of epithelial cells. Grover and Hiradhar (1992) studied the effect of Ipomea leaf extract on midgut cells of *Periplaneta americana*, showed the disturbance in cell continuation. Nasiruddin and Mordue (1993) studied on *Schistocerca gregaria* and *Locusta migratoria* to check the effect of azadiractin on midgut, it showed the disturption of epithelium.

In present study of *Halys dentatus*, exposure to pesticide dichlorovos shows alteration in histology of ventriculus. The shape may changes to original, the disturbance of basement membrane, longitudinal muscles and circular muscles. The bursting of cell boundaries of epithelial

cells, outside nucleus, darkly stained the denaturation of secretory cells. The ventriculus II shows alteration such as the detachment of musculature like longitudinal, circular and peritoneal sheath from the basement membrane. The epithelium lining shows folds inside the cytoplasm, the secretory cells obliterated with darkly stained nucleus. The distortion of peritrophic membrane and food bolus fused with epithelium. The granular cytoplasm is denatured and compacted as compared to control. The caeca of ventriculus IV are disturbed and show change in shape, the ventriculus shows destruction in the musculature like peritoneal sheath, longitudinal and circular muscles, the epithelial cells denatured to form compact mass.

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