Chapter 8

Histology
INTRODUCTION

HISTOLOGY INVOLVES NOT ONLY ANALYSIS of three dimensional structure by the examination of two dimensional specimens, but also the application of whatever methods and techniques help the histology to understand structure, function and cellular interrelationships in a more dynamic way. Since the diseases, particularly tissue diseases are primarily because of the abnormal activity in cells and tissues, the close relations between cytology on the one hand and pathology on the other hand should be expected. In view of that the histopathological studies would help in evaluating the extent of the effects of toxic substance, an attempt was made to study the structural changes in midgut, fat body and malpighian tubules through Transmission Electron Microscopy (TEM). Compounds that enter the body via the intestinal system after oral feeding, bypass the fat body, accordingly they are not subjected initially either to the detoxifying reactions of the fat body are to excrete via the malpighian tubules. In effect, compounds transported by oral feeding can be distributed to all parts of the body in the un metabolized form.

Midgut

The alimentary canal in silkworm is more or less a straight tube from the mouth to the anus and can be divided into three main parts the foregut or stomodeum, midgut or mesenteron and hindgut or proctodeum. Foregut is formed from invagination of the ectoderm and divided into three regions: the oral cavity, the pharynx and esophagus. The larvae feed and swallow the diets by regulation of the action of pharyngeal muscles. The fore gut epithelial cells can secrete and produce the intima but cannot secrete digestive fluids and absorb the nutrients. Main functions of the fore gut are swallowing the diets and transporting into the mid gut as well as storing the diets for some time.
The midgut is a long wide cylindrical tube and it is narrow at the posterior end. The midgut is of endodermal origin, therefore its structure differs from those of foregut and hindgut, it comprises muscular layer basement membrane epithelium and peritrophic membrane from outer side to inner side. The cells of the midgut are actively involved in enzyme production and secretion, as well as in absorption of nutrients. In the muscular layer, inner longitudinal muscles and outer circular muscles are arranged in web like manner, in addition there are two stout closely ranged longitudinal muscles on the middle of dorsal surface, respectively. The basement membrane is a transparent structure less thin lining, and similar to that in foregut, it covers on the outside of the epithelium. The epithelial layer comprises cylindrical cells, goblet cells and regenerative cells. The cylindrical cells are main components of the epithelial layer, and more developed at the posterior midguts. Its nucleus is in the middle of the cell and look like a ball. The mitochondria and exasperates of endoplasmic reticula in the cell are more developed at feeding periods than at moulting periods. The golgi bodies form vacuoles, there are many black granules in the cytoplasm. The granules are secreted into the midgut cavity. The surface towards the cavity of epithelial cell forms brush border, there the ATPase has high activity, this suggests that there is a presence of initiative transportation. All the surface linked with basement membrane have many deep invaginations which are employed to increase the surface area for the exchange of the material with the body fluid. The epithelial cell can absorb the nutrient from the lumen as well as secrete the digestive fluid into the lumen. The goblet cells present mainly in epithelial layer at the middle midgut region, its small nucleus is at the base of the cell. The goblet cells are plenty of secretion. There are many processes in the cytoplasmic membrane which are full of mitochondria. The mitochondria furnish the goblet cells energy for transporting materials into the lumen.

The regenerative cells are scattered on the bases between cylindrical epithelial cells and goblet cells and are undifferentiated cells. They contain plenty of glycogen and small sized mitochondria. Keeping in view of the importance of the midgut the histological aspects were studied at control, sub lethal and sub-sub lethal doses treated silkworm in 2nd, 3rd and 4th day of V instar.
Fat body

The fat body is composed mainly of fat contained cells, its surface is covered by a thin membrane. Fat cells in the young larval stage are almost circular while they are multi angular in the older larval stage. Each fat cell normally has only one circular nucleus but some cells have two even more than three nuclei. During the feeding period the fat body cells increase gradually in size up to the middle of the stadium, they can divide, like the integument and in adult as discs. The fat body cells vary under the influence of hormones. On the 2nd to the 3rd day in the pupal stage, histolysis of the fat body occurs. Lipolysis is more conspicuous in female than in male. In male pupae newly formed body by the imaginal fat body discs could be observed on the 5th day. The newly formed fat body may be maintained and transformed into adult fat body.

The fat body is the principle site of intermediary metabolism as well as a site for storage of metabolic reserves. The fat body serves as the main site of fat synthesis and storage as an energy reserves, fat is transferred from the saccharide. The fat body also serves as the main site of glycogen synthesis and storage. In larval stage, the glycogen amounts are more for fore moulting than after moulting. In larval stage the glycogen amounts in the middle IV instar and in the V instar are among the highest. Further the fat body also takes part in the metabolism of proteins and amino acids. Parts of the synthesized protein stay in the fat body as storage protein. The fat body may synthesize the vitamin-C and contain a lot of xanthane oxidase and degenerate the amino acids into the uric acids. Which are transferred into the body fluid and then expelled.

Malpighian tubules

Malpighian tubules are yellowish curved slender tubes and distributed on the lateral sides of the posterior alimentary canal, their basal apertures open inward the digestive tract at ventro lateral sides between the small intestine and the colon, its basal portion of the tube bulges to form a capsule and the chamber is called urinary bladder, from which two branches arise, one run towards the dorsal side and the other runs towards the ventral side.
The malpighian tube except the portion of urinary bladder is composed of in the order internally the connective tissue layer, basement membrane and the tubular cellular cell layer. The cells are large, two large hugging tubular cells from the cavity in a cross section. The cell counts has been fixed since the embryonic development stage, in the post embryonic development stage. The cell counts are that added, but the cell volume is continuously increased with the growth of the larvae. The nuclear form varies depending upon the various developmental stages, the form for the first instar larva is ball shaped while the form for the late instar larvae is branching. Many granular or short thread like mitochondria occurs in the plasma, they are cell organelles for the energy transformation. The major component of the urine is uric acid, in addition there are some inorganic materials such as phosphoric acid and sulphuric acid. The excretory amount of malpighian tubules varies depending upon various developmental changes.

RESULTS
Midgut

Transmission Electron Microscopic (TEM) studies on silkworm, *Bombyx mori* (PM x CSR₂) exhibited the histological structure in the midgut (Plates I-a, II-a and III-a). The gut lumen, plasma membrane and microvilli were prominent and clear in control and contain no cellular material. The microvilli surface is dense and even and no numerous vacuoles are visible in the cell cytoplasm.

In the control silkworm sacrificed at the onset of experiment (0 h) and after 2\textsuperscript{nd} day, 3\textsuperscript{rd} day and 4\textsuperscript{th} day the structure of the midgut was not significantly altered. The midgut epithelium is thin, slightly folded in the anterior region while uniformly folded in the posterior region. It consists of tall columnar cells and is interspersed apically with the goblet and basally with regenerative cells. The columnar cells contain large spherical nuclei in the middle of apical region and the cytoplasm is granular. The columnar cell possesses a fine brush border facing towards lumen and large number of vesicles discharging into extra peritrophic region of the lumen. The goblet cells are flask shaped with oval nuclei and a bulk of cytoplasm in the basal region. The regenerative cells are small spherical or elongated, and are basally located. They contain large spherical nuclei at the center and a granular cytoplasm. The peritrophic membrane is well evident in the lumen of the midgut.
After exposure to sub lethal dose at 2nd day, the nucleus was condensed at the basal region of the goblet cells while it was granule in the columnar cells. The peritrophic membrane was not closely lying to the epithelial cells and the space in between the epithelium and the peritrophic membrane was build with cytoplasmic vesicles (Plate I-b). On the 3rd day clusters of small round regenerative cells were prominent adjacent to the basal membrane. The goblet cells were empty and formation of vacuoles is observed (Plate II-b). On the 4th day of exposure the nuclei were significantly condensed in some columnar cells, while remaining cells were filled with inclusions, further the columnar cells reproduce membranous vesicles filled with secretory material indicating resumption of secretory activity by the midgut epithelium. The destruction of the gut epithelium was completed and only disorganized layer of shrunken columnar cells remain along with condensed nuclei. Vacuolated goblet cells are observed extending to the basal membrane itself (Plate III-b).

The midgut of the silkworm on exposure to sub-sub lethal dose exhibited little regression in the columnar epithelial cells. The nuclei were well evident in the apical region of columnar and goblet cells. Cytoplasmic granules and globules from the columnar cells into the space lying in between peritrophic membrane and epithelium was observed in 2nd day after exposure of fluoride (Plate I-c). On the day 3rd the columnar cells of the anterior midgut were swollen apically and began to extrude large cytoplasmic vesicles in to the gut lumen. The goblet cells were vacuolated, the membranous vesicles were absent, the regenerative cells remain unaffected (Plate II-c). However on the 4th day exposure of sub-sub lethal dose of fluoride there are no significant conspicuous changes. The goblet and columnar cells discharged membranous vesicles indicating normal functioning of the midgut after the due recovery from the histopathological injury (Plate III-c).

**Fat body**

The principle cells like trophocytes or adipocytes were observed in the control silkworm (Plate IV-a, V-a and VI-a). These are held together to form sheets of tissues by desmosomes and gap junctions and the whole tissue is clothed in a basal lamina attached to the cell by heavy desmsomes. In addition to the trophocytes there many urate cells, tracheal cells, mycetocytes, hemoglobin and other oenocytes. The
structure of a trophocyte is almost multi angular in shape and it has one circular nucleus and some times more than two nuclei.

Silkworms on exposure to sub lethal dose of fluoride at 2nd day exhibited alterations when compared to controls (Plate IV-b). The changes like hypertrophied condition and vacuoles appeared in the cytoplasm were some what less and membranous sheath surrounding the fat cells were slightly destructed on the 3rd day exposure at sub lethal dose and also showed hyperplasia and fatty degeneration (Plate V-b). On the 4th day of exposure there were severe structural alterations like cell necrosis of reticuolo endothelial cells, cell outline disappearance, degeneration of the nuclei, block of unity distributed all over nucleoplasm, fat bodies are plenty, varied in size (Plate VI-b).

On exposure to sub-sub lethal dose on 2nd day (Plate IV-c) also appeared cell outline disappearance, degeneration of nuclei cell necrosis and hypoplasia of reticulo endothelial cells was also observed. Silkworms on exposure to sub-sub lethal dose at 3rd day (Plate V-c) exhibited appreciable changes pushing up the nucleus to the peripheral regions of the fat body cells, degeneration of nucleus, clubbing of nuclei due to cytoplasmic disappearance. However the silkworm on exposure to sub-sub lethal dose at 4th day (Plate VI-c) showed only mild structural changes with insignificant necrosis and no conspicuous changes were observed in fat body cells.

Malpighian tubules

Electron micrographic examination (TEM) of the malpighian tubules in control silkworms showed a normal ultra structure. The malpighian tube contained in the order internally the connective tissue layer, basement membrane and tubular cellular cell layer. The cells are large to large hugging tubular cells from the cavity in a cross section. The nuclear form varies many granular or short thread like mitochondria were observed in the plasma (Plates VII-a, VIII-a and IX-a).

On exposure to sub lethal doses of fluoride at 2nd day (Plate VII-b) the malpighian tubules exhibited certain alterations in their fine structure. The microvilli decreased in density and contain fewer mitochondria interspersed within the microvilli. The basal portion of the cell remains unaffected. In malpighian tubules
observed on 3rd day exposure of fluoride treated silkworms (Plate VIII-b). On the day 4th at sub lethal dose of fluoride treatment exhibited cellular disruption. Apical microvilli are much thinner and fewer in number and mitochondria have disappeared. The cytoplasmic granule substance is disrupted and only a greatly infolded plasma membrane, few mitochondria, and occasional strands of granular endoplasmic reticulum remain. The lumen of the malpighian tubule is no longer empty and is filled with material. Infoldings of the basal plasma membrane are no longer evident and only a thin basement membrane remain intact. Further, the integrity of the cytoplasmic ground substance is destroyed, and ruminates of mitochondria, some vacuoles and disrupted membranes. Microvilli on the apical surface of the primary cell disappear (Plate IX-b).

On exposure to sub-sub lethal dose of fluoride the plasma membrane is still highly infolded, but mitochondria and granular endoplasmic reticulum, are no longer evident in the cytoplasm on 2nd day of exposure (Plate VII-c). On the day 3rd also observed a highly infolded plasma membrane and less number of mitochondria and granular endoplasmic reticulum. Further vesicles possible indicating of pinocytosis occur along the inner surface of the basement membrane. Extensive cellular debris is evident (Plate VIII-c). On the exposure of 4th day exhibited a mild changes in the gross structure of malpighian tubules (Plate IX-c). This indicates the recovery of the silkworm on prolonged exposure of fluoride.

DISCUSSION

Midgut

Since the midgut is the first organ of the silkworm to be attacked by excessive ingestion of fluoride, it caused a structural change to the midgut epithelium of Bombyx mori L. The results of our studies were lead to cell structure damage caused by the high fluoride concentration as evidence by the studies of Chen et al., (2005), who reported that fluoride resulted in the massive release of ACPase from midgut cells due to cell structure damage caused by the high fluoride concentration. The vacuolization of midgut epithelium was also reported after the treatment of orcinides (Rizvi and Khan, 1973) and organophosphates (Deshmukh and Tembhare, 1998) in various insects. Some authors exhibited elongation and regression of the epithelial cells and deposition of numerous vesicles in the extra peritrophic space mostly due to hypersecretary activity after the treatment of insecticides (Deshmukh and Tembhare,
Blackburn et al., (1998) reported that apical swelling and blebbing of large cytoplasmic vesicles by the columnar cells led to the eventual extrusion of cell nuclei in vesicles into the gut lumen and lysis of the gut epithelium in *Manduca sexta* on exposure to photorhabdys linenscens toxin complex. Toppozadi et al., (1968) exhibited condensation of the nuclei of midgut epithelium of the Egyptian cotton leaf worm *spodoptera littoralis* on exposure to insecticides. In the present study the changes induced by the fluoride are greater on the 4th day at sub lethal dose, when compared to the 2nd and 3rd days. The studies of bioaccumulation of fluoride coincides with the duration and dose of exposure, the accumulation and rate of accumulation was more on 4th day than 2nd and 3rd days. The higher level of accumulation might have resulted in extensive degeneration of the structure of midgut which may be due to the failure of detoxification mechanisms. Most histological alterations described in mammals after fluoride poisoning are nuclear degeneration, hyperplasia and necrosis (Kaur and Singh al., 1980). Our results are also in concomitant with the findings of Chui et al., (1995), who reported that fluoride ion induced a direct injury to the midgut cells accompanying the degeneration of organellies.

A large number of vacuoles appeared followed by the formation of large auto phagosomes in the cytoplasm finally degenerated cells were emitted into the midgut cavity. More importantly goblet cells diminished than columnar cells. The effects of fluoride on midgut cells of silkworm were related to dose and time after exposure. The midgut cells showed a slow necrosis, rounding up of the cells and swelling of organellies, the connective tissue beneath the epidermal layer became enlarged with many invading cells/nuclei. However, on the 4th day the histopathological effects were not severe and a mild necrosis in cells, and swollen appearance of goblet cell lumens were observed. Swelling of goblet cell lumens suggest that pre exchange of fluid between the goblet lumen and the exterior does not occur. The partial loss of microvilli and vacuolated cytoplasm was also observed. The anomalous dose response of structural changes in midgut on 4th day of Sub-sub lethal dose can be explained by the recovery of silkworm on prolonged exposure. Further the less rate of accumulation also supports our studies, that for the chronic exposure, the animal could resist the fluoride toxicity by detoxification.
Fat body

Insecticides on entry into the tissues are excreted often after being metabolized. The changes resulted in the compound become less toxic, but are converse sometimes true and toxicity is enhanced. Insecticides extensively damaged the histology of fat body in insects (George, 1996) and caused lysis of intercellular connecting material resulting into gaps between epithelial cells causing frilled appearance. Cellular degeneration, vacuolization, appearance of fat globules, vacuolization caused by exfoliation of cells and inter lumen migration of broken cells, degeneration of nucleus. Some compounds totally that are water soluble are metabolized to components and they are subsequently incorporated into the insects primary metabolic pathways. Most of the lipophilic substances are first converted into water soluble components and are excreted. Many different enzyme systems are known to be involved in these reactions and some systems are almost certainly ubiquitous. This process may occur in a variety of tissues as there is no organ comparable with the fat body i.e., the focus for comparable reactions. Activities of the appropriate enzymes often occur in the midgut, fat body and malpighian tubules. Different species differ widely in their ability to metabolize toxic substances. For example plant feeding insects exhibits host plant specificity. The caterpillar of Manduca sexta, for example habitually feeds on alkaloid containing plants and it is able to do this because it detoxifies the alkaloids (Snyder et al., 1994). Eventually the end products of these metabolic processes are commonly excreted, there some times is sequestered. In some species the compound has been stored in the cuticle. This is perhaps minimizing the risk to the insect. However, some insect species store the defensive substances in glands which are in the haemolymph. Similarly silkworm fat bodies accumulates most of the toxic substances for the detoxification. In our study at sub lethal doses on 2nd, 3rd and 4th days exhibited greater accumulation of fluorides and might have caused an extensive structural degeneration of fat body and it may be due to the failure of detoxification mechanisms. The most significant changes observed are the appearance of vacuoles in the cytoplasm, hypertrophoid condition, degeneration of fat cells, cell necrosis, cell out line disappearance, degeneration of nuclei, most histological changes exhibited in fat body of silkworms are also appeared on exposure to different pesticides such as BHC, Carboryl, γ-BHC, phosphomidon, malathion. Further the silkworms exposed to pathogens like Bm NPV and CPV also exhibited alterations such as vacuolization, fatty degeneration,
hypoplasia and cell necrosis (Smitha, 2006; Ingram, 1955, Lockau and Ludicke, 1952; McMullen, 1965 and Misra, 1981). The appearance of destructed membranous sheath surrounding the fat cells and hypertrophoid condition could be correlated with the excessive fluoride accumulation in fat body and haemolymph. Consequently extra vasitions of fluoride ions diffusing into surrounding areas might be responsible for excessive damage. According to Misra (1981) the fat cells are either destroyed or dissolute and scattered nuclei and hypertrophoid on exposure to insecticides. A typical virogenic structure was observed in fat body nuclei on exposure to CPV in the fat body nuclei. Tukra fed silkworm larvae exhibited mild destructivity and normal vacuolization in the cell cytoplasm of the fat body (Jagadish, 2005). Since soft tissues retain only a small fraction of dietary fluoride (Shupe et al., 1962) it is likely that the structural alterations observed in the present study are consequent of the fluoride extra vesation and tissue retention. Hence the present study showed that fluoride ions exert toxic effects on fat body. Shupe (1980) also reported similar findings in liver tissues of cattle. Further the alterations observed in the fat body of silkworm are dose and time dependent. The findings have been further substantiated by biochemical disorders which were observed in the fat body of silkworm treated with different doses of fluoride on sub lethal doses. The vaculation in fat body cells, shrinkage of trophocytes and dissolution of laminar structure suggests that the depletion in its glycogen reserves. Corresponding to the cellular damage of the fat body tissue the decrease in proteins and increase in ammonia, impaired the oxidative metabolism which reflect the potent toxicity of the fluoride to fat body.

Sub-sub lethal treated silkworm also showed some histopathological changes as seen in sub lethal fluoride treated silkworms. The histopathological changes were more at 3rd day than 2nd and 4th days. This could be attributed to the rate of fluoride accumulation from 2nd day to 4th day, on the day 4th. The rate of accumulation was less when compared 3rd and 2nd days. Hence, the lower amounts of fluoride ions resulted in very mild histopathological changes in the silkworms at 4th day on sub-sub lethal dose. Accumulation and excretion studies have confirmed the marked decrease in the absorption of fluoride in silkworm and less accumulation in fat body as well as marked increase of faecal fluoride excretion.
Malpighian tubules

Since malpighian tubules are concerned with the elimination of undetoxified and unwanted substances from the haemolymph of the insect it is more prone to the toxicity of various pollutants. Fluoride treated silkworms in all the groups exhibited significant changes in the architecture of the malpighian tubules. The decreased microvilli in density and few mitochondria within the microvilli, cellular disruption, cytoplasmic granules substance, thin basement membrane, vacuolation, remnants of mitochondria could be resulted due to increased fluoride ions accumulation.

Administration of calcium fluoride to the V instar larvae of Bombyx mori., at high concentration exhibited fluoride accumulation in malpighian tubules whereas low concentration of fluoride has a positive effect on the fecundity and distribution in the highly fluoride resistant variety, Zhenong-1 (Chen et al., 1996). High concentration of F⁻ (142.5 mM) is strong enough to cause binding, thereby blocking the passage of cl⁻ in the malpighian tubules, hence, fluoride is highly reactive halide (Ming-Juin and Klaus E. 2001) to cause histopathological changes in the malpighian tubules. According to Kawahara (1956), rabbits treated with 30-32 mgF/kg body weight per day orally for 14 to 150 days, developed inflammatory kidney changes in the glomeruli with the increased cellularity, capillary hyperemia, hypertrophy or atrophy, tubular degeneration, cloudy swellings and protein casts were in blood in the lumen. Similar reports were pointed by Shashi et al., (2002), that no significant clinical signs of toxicity were found in animals exposed to the lowest dose. At the higher doses, however the cytoarchitecture of the kidneys exhibited increasing amounts of cloudy swellings, degeneration of tubular epithelia, tissue necrosis, extensive vacuolization in renal tubules, hypertrophy and atrophy of glomeruli, exudation, interstitial edema and interstitial nephritis. These changes in the kidneys result in impaired renal function in chronic fluoride intoxication. During the present investigation the malpighian tubules of silkworm traeated with sub lethal (5.5 mg/ kg b.wt.) and sub-sub lethal dose of fluoride (2.75 mg/kg b.wt.) showed cellular disruption, thinner and fewer microvilli and disappearance of mitochondria and disruption of greatly infolded plasma membrane, lumen with filled material, thin basement membrane vacuolization and extensive cellular debris were observed. Cloudy swellings, the intensity of such swellings has been observed with others to increase with increasing dose of fluoride (Machle et al., 1992 and Geiger, 1936).
Further chronic fluoride intoxication at a level 14 mgF/kg b.wt. per day and higher has been claimed to result in renal lesions (Rohlem, 1937; Bond and Murre, 1952). The findings of the present investigation are showing extensive destruction to the malpighian tubules of fluorotic silkworm, substantiate the observations of the above studies. Higher levels of fluoride in the haemolymph and malpighian tubules also support the cytotoxicity to the cells of the malpighian tubules. On the other hand, sub-sub lethal treated silkworm also produced certain histopathological changes on 2nd day and 3rd day. The accumulation of fluoride in haemolymph and malpighian tubules was also greater in these groups of silkworm than in group of 4th day. The physiological and biochemical alterations were very less and more or less similar to controls in the 4th day group of silkworm. Correspondingly the structural damage to the malpighian tubules in these groups are very less. This could be attributed to the recovery of silkworm to the lower dose at prolonged period due to development of detoxification mechanism to the fluoride toxicity.
a, b & c: TEM photographs of the Midgut of V instar silkworm, *Bombyx mori* L. (PM x CSR₂) exposed to sub lethal and sub-sub lethal doses of fluoride at 2\textsuperscript{nd} day. X 7280.

a : Group 1 (Control)
b : Group 2 (Sub lethal)
c : Group 3 (Sub-sub lethal)
Plate - 1: Midgut - 2nd day

a

b

c
Plate - II

a, b & c: TEM photographs of the Midgut of V instar silkworm, *Bombyx mori* L. (PM x CSR₂) exposed to sub lethal and sub-sub lethal doses of fluoride at 3rd day. X 7280.

a : Group 3 (Control)
b : Group 4 (Sub lethal)
c : Group 5 (Sub-sub lethal)
Plate II: Midgut - 3rd day
Plate - III

a, b & c: TEM photographs of the Midgut of V instar silkworm, *Bombyx mori* L. (PM x CSR₂) exposed to sub lethal and sub-sub lethal doses of fluoride at 4^{th} day. X 7280.

a : Group 7 (Control)
b : Group 8 (Sub lethal)
c : Group 9 (Sub-sub lethal)
Plate III: Midgut - 4th day

a

b

c
Plate - IV

a, b & c: TEM photographs of the Fat body of V instar silkworm, *Bombyx mori* L. (PM x CSR\textsubscript{2}) exposed to sub lethal and sub-sub lethal doses of fluoride at 2\textsuperscript{nd} day. X 7280.

a : Group 1 (Control)
b : Group 2 (Sub lethal)
c : Group 3 (Sub-sub lethal)
Plate IV: Fat body - 2nd day

a

b

c
Plate - V

a, b & c: TEM photographs of the Fat body of V instar silkworm, *Bombyx mori* L. (PM x CSR₂) exposed to sub lethal and sub-sub lethal doses of fluoride at 3rd day. X 7280.

a : Group 3 (Control)
b : Group 4 (Sub lethal)
c : Group 5 (Sub-sub lethal)
Plate - V: Fat body - 3rd day
Plate - VI

a, b & c: TEM photographs of the Fat body of V instar silkworm, *Bombyx mori* L. (PM x CSR₂) exposed to sub lethal and sub-sub lethal doses of fluoride at 4\textsuperscript{th} day. X 7280.

a : Group 7 (Control)
b : Group 8 (Sub lethal)
c : Group 9 (Sub-sub lethal)
Plate VI: Fat body - 4th day

a

b

c
Plate - VII

a, b & c: TEM photographs of the Malpighian tubules of V instar silkworm, *Bombyx mori.*, L. (PM X CSR₂) exposed to sub lethal and sub-sub lethal doses of fluoride at 2nd day. X 7280.

a : Group 1 (Control)
b : Group 2 (Sub lethal)
c : Group 3 (Sub-sub lethal)
Plate VII: Malpighian tubules - 2nd day

(a) Image a
(b) Image b
(c) Image c
Plate - VIII

a, b & c: TEM photographs of the Malpighian tubules of V instar silkworm, *Bombyx mori.*, L. (PM X CSR₂) exposed to sub lethal and sub-sub lethal doses of fluoride at 3\textsuperscript{rd} day. X 7280.

a : Group 3 (Control)

b : Group 4 (Sub lethal)

c : Group 5 (Sub-sub lethal)
Plate - IX

a, b & c: TEM photographs of the Malpighian tubules of V instar silkworm, *Bombyx mori.*, L. (PM X CSR₂) exposed to sub lethal and sub-sub lethal doses of fluoride at 4\textsuperscript{th} day. X 7280.

a : Group 7 (Control)

b : Group 8 (Sub lethal)

c : Group 9 (Sub-sub lethal)
Plate IX: Malpighian tubules - 4th day