Electron microscopy is widely used as a basic research tool, and necessary for other components of a toxicity study such as biochemical, metabolism and kinetic studies. Transmission electron microscopy also has a well established role in the characterization of sub-cellular structural alterations in tissues which have been modified by the effects of xenobiotics. Despite the fact that electron microscopy only provides a static morphological organelles can provide valuable information about any functional deficits. Electron microscopy, in contrast to light microscopic examination, allows the characterization of changes and proliferation of the exclusion of sub cellular degeneration in vital organs such as the heart when unexplained macroscopic or weight changes are seen without a light microscopic correlation.

Despite the technological advances in transmission electron microscopy, it is highly selective and only small samples of tissues can be examined, so that appropriate and defined objectives are selected and examined within the context of a toxic study.

The use of larger resin embedded sections is a cost effective compromise between electron microscopy and conventional light microscopy. Sometimes termed ‘high resolution light microscopy’, light microscopic evaluation of semi-thin sections can provide a means of avoiding extensive use of the electron microscopy, because it can locate cytoplasmic organelles in a way sometimes not possible in paraffin embedded material.

The electron microscope (EM) permits a direct study of biological ultra structure. Its resolving power is much greater than that of the light microscope. In spite of the apparent similarities there are great differences between the light and the EM. In case of EM, molecules or supramolecular structures is now possible to obtain more detailed information (Bozzola and Russell, 1992; Aughey and Frye, 2001).

The toxicity of any chemical necessarily impairs the metabolic strategy of animal physiology. However, the physiological studies do not satisfy in complete understanding of the impact of any deleterious chemical. To have clear understanding, as to how these chemicals cause injury to the tissues, it is where in, one can envisage a better understanding of the pathological conditions of tissues under toxic stress of pestilent. Thus, histopathology helps in diagnosing the damages of the tissues of an
animal subjected to toxic stress of pestilence (Jayantha Rao et al., 1983). Several workers reported that the metals and nonmetals cause damage at cell organelles.

Fluoride is widely distributed in the natural environment and can lead to fluorosis due to excessive fluoride intake in many parts of the World. In addition to its well-known effects on the skeleton and teeth, fluorosis can also adversely affect soft tissues, such as the liver and testes.

Many studies have suggested that mitochondria are the major target of fluoride ion toxicity in the human kidney (Cittanova et al., 1996) and in rat liver and pancreas (Dabrowska et al., 2004). We also evaluated spermatozoa, which is widely used to characterize the functional status of mitochondria (Gravance et al., 2001), and found a significant decrease in fluoride-treated spermatozoa, indicating mitochondrial dysfunction. Mitochondrial dysfunction can promote high ROS production, and an inverse correlation between and ROS levels in spermatozoa in semen samples from patients with abnormal semen parameters has been observed.

In view of the histopathological changes observed in the present investigation (chapter V; plates 5.1 to 5.10.), an attempt has been made to study the transmission electron microscopy (TEM) to observe the possible changes at cell organelles under sodium fluoride and vitamin c intoxication in mice.

Results

Normal electron microscopic structure of mice testes

The testis of control mice under electron microscopy showed several seminiferous tubules. The seminiferous tubules are covered with connective epithelial layer, spermatids, mitochondria and spermatozoa, seratoli cells, lumen and leydig cells and cluster of leydig cells in between seminiferous tubules (Figs. A – C).

Ultra structural changes of mice testes under sodium fluoride intoxicification for 30 days

Electron Micrograph of experimental Albino mice testis under 5 ppm of sodium fluoride showed abnormal spermatozoa with seratoli cells, mitochondria in seminiferous tubules, leydig cells. Seratoli and leydig cells are showing altered sturucture with shrunken and swollen nucleous, disruption of chromatin material, shrunken mitochondria with vesciculation. Seminiferous tubules are showing
abnormal sperms (Figs. D – E). The combination of sodium fluoride and vit. C treated group showed recovery of altered structures of spermatids and spermatozoa (Fig. F).

**Normal electron microscopic structure of mice liver cells (hepatocytes)**

The cells of the liver are called hepatocytes. They are large sized and polygonal cells. Hepatocytes have prominent nuclei, uniformly distributed chromatin and centrally placed, uniform distribution of cell organelles, rough endoplasmic reticulum, smooth endoplasmic reticulum, Golgi apparatus, many mitochondria, lysosomes, rich in peroxysomes, rich in secretory vesicles and secondary lysosomes. They are rich in glycogen granules and fat droplets. Each cell has central nucleus with distinct nuclear membrane and one or more prominent nucleoli.

The mitochondria of the hepatic cells are spherical, rod shaped or filamentous, depending on the location of the cell within the lobule and on the functional state. The Golgi apparatus lies either near the edge of the cell or close to the nucleus. The rough endoplasmic reticulum shows continuity with smooth endoplasmic reticulum. Well-defined cell junction and the cell shows glycogen in the form of retsettes of dense granules. Clear granular mitochondria were seen (Agarwal, 2001; Aughey and Frye, 2001; Lodish et al., 2004) (Figs. G&H).

**Ultra structural changes of mice liver hepatocytes under sodium fluoride intoxication for 30 days**

Ultrastructural changes in hepatocytes during sodium fluoride showed shrunken nucleus vacuoles in cytoplasm with numerous mitochondria, disrupted endoplasmic reticulum, extensive vesicular cytoplasm, margination of chromatin material, severe vesiculation, loose cell junction, ruptured nuclear membrane irregular shape and size of peroxysomes (Figs. I&J). The combination of sodium fluoride and vitamin C-treated group showed recovery of liver in 30 days (Figs. K&L).
Discussion

The electron microscopic observation of testes and liver under sodium fluoride exposure showed pronounced pathological changes in cell organelles. These changes were intensified in 30 days of sodium fluoride.

In the present investigation mice testis exposed to 30 days of 5 ppm fluoride, several pathological changes and these architectural changes were recovered in mice exposed to fluoride along with vit. C (Figs. A-F).

Several authors reported ultrastructural changes in testes in different animal models under sodium fluoride toxicity. Spermatozoa motility has been proposed to be associated with the functional status of spermatozoa mitochondria (Gravance et al., 2001). Indeed mitochondrial alterations can result in decreased spermatozoa motility since motility is ATP-dependent (Ford and Rees, 1990).

Jianhong Zhou et al. (2014) reported after arecoline exposure, morphological abnormal spermatozoa and vacuoles in the exposed testis and the supplied vitamins (C, E) in the arecoline-expose seemed to have no abilities to alleviate these pathological changes in testes of mice.

Susheela and Arbind Kumar (1997) reported tests of rabbit treated with fluoride for 23 months showing either isolated or small clusters of leydig cells in the vicinity of blood capillaries and between the seminiferous tubules, degenerated and shrunken cells of interstitial tissue.

Sumedha and Miltonprabu (2014) reported displaying irregular membrane of spermatogonia nucleus with vacuolated cytoplasm where endoplasmic reticulum found to dilate and mitochondria are seem to swollen and vacuolated in the testes of rats exposed to Arsenic.

Parul Kaushal et al. (2014) observed the electron dense cytoplasm with increased number of lysosomes in the seratoli cells swollen and disorganized network of SER aggregated near acrosome formation in spermatids, the electron dense cytoplasm with increased number of lysosomes in the Sertoli cells in the testes of rats exposed to sodium arsenate. Fluoride exposure caused a decrease in spermatozoa has been reported Izquierdo-Vega et al. (2008).
Ultrastructural changes in hepatocytes during sodium fluoride exposure showed shrunken nucleus, vacuoles in cytoplasm with numerous mitochondria, disrupted endoplasmic reticulum, extensive vasicular cytoplasm, margination of chromatin material, severe vesiculation, loss of cell junction, ruptured nuclear membrane irregular shape and size of paroxysomes (Figs. G – L).

Fluoride can cross cell membranes by simple diffusion and enter soft tissues. The liver is one of the target organs attacked by fluoride. Numerous studies have revealed that excessive amounts of fluoride disturb the metabolic processes and detoxication capabilities of the liver (Shashi and Bhardwaj, 2011).

The pathological changes observed in the present investigation clearly indicate that sodium fluoride not only caused damage at cellular level of these organs but also cause damage at sub cellular level. Degeneration in the endoplasmic reticulum and mitochondira observed in the present investigation might have resulted in drastic reduction in ATPase activity noticed in the present investigation. The elevated levels of alkaline and acid phosphatases in the present investigation might be due to the cellular and sub-cellular damage which was also reported by Srinivasan et al. (1991). Several authors reported ultrastructural changes in liver hepatocytes of different animal models under sodium fluoride pesticide and metal toxicity.

Madhaveelatha (2006) reported reduction in nucleus size, scattered chromatin, and necrotic changes in the mitochondrial membrane and degeneration in endoplasmic reticulum in mice liver hepatocytes exposed to monocrotophos. In the liver of frog exposed to azadirachtin (Madhava Rao, 2007) and liver of mice exposed to azadirachtin and monocrotophos. Sivaiah (2006) several pathological lesions were observed.

Jayakumar (2008) observed disappearing of cell boundaries are disappeared, nucleus at the periphery of the cell, ruptured nuclear membrane, fragmentation of rough endoplasmic reticulum, swollen endoplasmic reticulum, enlarged and ruptured mitochondria showing clearance of matrix and destruction of cristae, decrease in the number of mitochondria, absence of secretory vesicles are of cytoplasmic organelles, irregular shape and size of peroxysomes and lucent areas of cytoplasm consist of residues of cell organelles in liver hepatocytes of frog exposed to cypermethrin.
Zhanxue Liang et al. (2012) observed abundant mitochondria polymorphic, with their cristae oriented transversely. Matrix granules were numerous, revealing that high electron density, rough endoplasmic reticulum, and glycogen granules were all discernible in the liver of rabbit exposed to high fluoride.

Kotarina et al. (2013) reported damaged hepatocytes having markedly electron dense cytoplasm, irregular nuclei dilated mitochondria, dilated inter cellular spaces liver of turkey’s exposed to cadmium. Cytomorphosis was observed in 100 mg/L fluoride in the liver of rat (Zhuo Zhang et al., 2014).

There is growing evidence that endocrine disruptors in the environment might be playing a substantial role in adversely influencing the reproductive system (semen quality is deteriorating by as much as 3% per year). Potency of fluoride as an endocrine disruptor has been reported with studies establishing alterations in leydig cell structure and function following exposure to NaF.

Vitamins C is known to be antioxidant. Vit. C is a water-soluble chain-breaking antioxidant and can scavenge superoxide and hydrogen peroxide. Moreover, vit. C is found to have the ability to restore the antioxidant abilities of vit. E. However, to the best of my knowledge and several workers reported that the effects of vit. C reduces the toxicity induced by sodium fluoride especially on the hepatotoxicity and testicular toxicity (Uzun et al., 2009; Adikwu and Deo, 2013; Mongi et al., 2011). Hepatotoxicity and testicular toxicity of sodium fluoride also reduced. In the present investigation there is a clear indication that vit. C has a protective role with NaF.
Fig. A: Electron Micrograph of mouse control testis – showing Seminiferous tubules (SFT), Mitochondria (M) and Leydig Cells (LC). 2.8 KX
Fig. B: Electron Micrograph of mouse control testis – showing Spermatids (SPD), Spermatozoa (SP), Seratoli Cells (SeC), Lumen (L) and Leydig Cells (LC). 3.8 KX
Fig. C: Electron Micrograph of mouse control testis – showing cluster of Leydig cells (LC) in between Seminiferous tubules and Sertoli Cells (SeC). 4.8KX
Fig. D: Electron Micrograph of experimental mouse testis under 5ppm of sodium fluoride showing abnormal Spermatozoa (SP) with Seratoli Cells (SeC) and Mitochondria (M) in Seminireous tubules. 5.7KX
Fig. E: Electron Micrograph of experimental mouse testis under 5ppm of sodium fluoride showing abnormal Spermatozoa (SP) and Seratoli Cell (SeC). 5.7KX
Fig. F: Electron Micrograph of experimental mouse testis under 5ppm of sodium fluoride and Vit. C showing normal Spermatozoa (SP) among a few abnormal ones. 5.7KX
Fig. G: Electron Micrograph of control liver of mouse – showing Vacuolization (V), Mitochondria (M) and Nucleus (N) with Chromatin material (CM) in Cytoplasm. 4.8KX
Fig. H: Electron Micrograph of control liver of mouse – showing Vacuolization (V), Mitochondria (M) and Nucleus (N) with Chromatin material (CM) in Cytoplasm. 7.7 KX
Fig. 1: Electron Micrograph of experimental Mouse liver under 5ppm of sodium fluoride showing Shrunken nucleus (SN), Vacuoles (V) in cytoplasm with numerous Mitochondria (M). 3.8KX
Plate: 5.20

Fig. J: Electron Micrograph of experimental Mouse liver under 5ppm of sodium fluoride showing Severe degeneration of cell organells (SDGCO) in hepatocytes with Shrunken nucleus (SN). 4.5KX
Plate: 5.21

Fig. K: Electron Micrograph of experimental mouse liver under 5ppm of sodium fluoride and vitamin C showing regeneration of hepatocytes and reduced Vacuolation (V). 3.8KX
Plate: 5.22

Fig. L: Electron Micrograph of experimental mouse liver under 5ppm of sodium fluoride and vit. C showing regeneration of hepatocytes and reduced Vacuolation (V). 5.8KX