Chapter - 3

Histopathological Studies
Histology in a precise sense is the study of the cytoarchitectural change of the body which envisage the anatomy and gives in sight into functioning of tissues and organs. The histology is a structural science and serves to complement the knowledge gained from the anatomy, physiology and pathology. Induction of tissues under the influence of chemicals modification of tissue results in the elevation of enzymatic activities coupled with histological studies provide a reliable study for monitoring the severity of chemical damage to the tissue. This study provide a reliable knowledge on tissue damage and further provide an information on the interlinks of enzymes and tissues.

It is obvious that any chemical insult could cause pathological or injury to cells in animal, if it is consumed beyond a required dose either slowly or fast. Animal susceptibility to chemical injury exhibits variation among the tissues and cells of an organ. The extent of severity of tissue damage is depends up on the function, concentration and potentiality of toxic compound (Jayantha Rao, 1982) (Thyagaraju et al., 2003). The earlier studies have reported these effects on various tissues of rats, rabbits, guinea pigs and mice.

The cytoarchitectural changes produced during chemical induced tissue modification can be identified by microscopic examination of the tissues and also explains the extent of tissue specificity to the chemical action. It can be suggested that both morphological and biochemical assays should be applied for more accurate evaluation of pathological concepts of organs and their tissues.

Hence histopathological studies would help in assessing the effect of carcinogens or chemicals at the initiation stage in various organs and organ systems of an organism. In rats the transformation process was induced by treating with acrylamide, β-methylcholangrene, phenobarbital etc at various doses in rat liver, brain and testis of both treated and control in our laboratory (Devi, 1998, Raveendra et al., 2008). Similar studies were also conducted to observe the changes in structure of hepatocytes of rat liver under the influence of acrylamide
at various doses upon prolonged treatment as described in the chapter materials and methods. The histopathological studies were related since acrylamide.

Therefore to observe the variations in the rat liver due to the influence of acrylamide treated tissues the following objective was selected.

**OBJECTIVE**

In this context was influencing at the level of enzymes activities and protein content.

To study the histopathological changes of rat liver tissues under the influence of acrylamide depending upon its concentration / variation intake (administration).

**Results**

The rats treated with acrylamide were sacrificed and the collected rat liver was used for histological analysis as described in “Materials and Methods chapter”.

The Control rat liver showed on microscopic observation normal hepatocytes with centrally placed nuclei (Fig 31). Upon serial dose administration ranging from of AC 16 mg to 96 mg showed hypertrophy of tissue (Fig 32) and nuclei was binucleated (Fig 33) pyknotic in hepatocytes, vascular congestion, haemorrhages, granularity in cytoplasm, (Fig 33) mild mononuclear round cell collections (Fig 34), mild vascular congestion, mild nuclear prominence, proliferation of sinusoidal bile ducts haemorrhages (Fig 35) and congestion with peliosis-hepatitis changes (Fig 36&37) (cystic spaces) were observed (Fig 32-37). The hypertrophy of nuclei was observed in this (Fig 35-37).
Fig-31: Control liver Shows normal hepatocytes with nuclei. (H&E 40X).

Fig-32: AC administered rat liver showing mild mononuclear round cell collections, mild vascular congestion, mild nuclear prominence with 16mg H&E 40X.
Fig-33: AC administered rat liver showing hypertrophy of nuclei and also the nuclei was binucleated and pyknotic in hepatocytes, vascular congestion, haemorrhages, granularity in cytoplasm observed with 32mg. H&E 40X

Fig-34: AC administered rat liver showing mild mononuclear round cell collections, mild vascular congestion, mild nuclear prominence with 48mg H&E 40X
Fig-35: Acrylamide administered rat liver showing hypertrophy of nuclei and also the nuclei was binucleated and pyknotic in hepatocytes, proliferation of sinusoidal bile ducts haemorrhages, congestion with peliosishepatitis changes (cystic spaces) with 64 mg H&E 40X

Fig-36: AC administered rat liver showing mild mononuclear round cell collections, mild vascular congestion, mild nuclear prominence with 80mg H&E 40X
Fig-37: Acrylamide administered rat liver showing hypertrophy of nuclei and also the nuclei was binucleated and pyknotic in hepatocytes, proliferation of sinusoidal bile ducts haemorrhages, congestion with peliosishepatis changes (cystic spaces) with 96 mg H&E 40X.
DISCUSSION

Liver is the major metabolizing organ which detoxifies a number of drugs and xenobiotics. Liver shall be affected with certain doses of phenobarbitol and β-methyl cholangthrene with hypertrophy of hepatocytes and also the nuclei shall be observed to contain binucleated pyknotic in hepatocytes (Devi et al., 2002; Raveendra et al., 2008). The chemicals also induce damage in brain and liver at the levels of centrlobular nerosis, hypertrophoid nuclei, adenomas, hepatocellular carcinoma. Kandarkar et al., 1983; Thygaraju et al., 2003, reported occlusion, congestion and degenerative changes with multiple doses of 3-MC in testis. Malathion induced histological alterations in the testis such as degeneration of germinal epithelial lining of seminiferous tubules shrinkage in sertoli cells, spermatocyte, spermatids, hypertrophy of sperms and cytoplasmic vacuolation in albino rats (Baroni et al., 1992).

Mild histological lesions observed in 16mg and 32mg, moderate histological lesions in 48mg and severe histological changes in 64mg, to 96mg, respectively were observed in liver of AC administered rats when compared to control livers (Figs 31-37), deleterious effects on liver upon the treatment of AC. My studies demonstrate that the acrylamide influences hepatocytes for degeneracy, hypertrophy with binucleated hemorrhages and cystic spaces. These studies were also in accordance with the earlier results on the enzyme activities of GST and GPx protein levels, and expression studies as evidenced by immunological cross reactivity analysis.

Acrylamide has been evaluated for reproductive toxicity in multigenerational studies in rats (Tyl et al., 2000a) In the studies with rat the increase of tumours was most evident in specific organs, e.g. mammary gland, uterus, adrenal gland and scrotal mesothelium. In mice there was an increase of lung and skin tumours. These cancer studies have been used for the assessment of the risk of cancer in humans due to acrylamide exposure upon intake of tobacco or fried foods. It should be noted that the genotoxic studies have indicated that there is no threshold value for the risk of cancer induced by acrylamide, i.e. there is no
dose of acrylamide so low that it does not increase the risk of cancer. In making these assessments it is assumed that man and rat have the same sensitivity for cancer induction by acrylamide. Acrylamide (10-20mg/kg body weight) caused testicular degeneration in mice (Shiraishi, 1978; Hashimoto and Tani, 1981) and spermatocyte chromosome aberrations. A marked degeneration of seminiferous tubules was observed by McCollister et al., 1964 in male rats during histological assessment. Our results with 60mg/kg body weight were also in agreement with these reports on liver. Following ip injection of 50 mg/kg body weight / day for 10 days Huang et al., 1982, reported atrophy of epididymal fat pad, accompanied by a severe triglyceride depletion.

The observed results showed some of the less deleterious effects in 16mg and 32mg when compared to other doses (48mg, 64mg, 80mg and 96mg). This indicates after five days of treatment and at the 6th dose treatment may act as a drug to overcome the previous deleterious effects. However it may need further work like immuno staining.