CHAPTER - 3

Histo pathological studies
Induction of carcinogenesis results in the elevation of enzymatic activities coupled with histological studies provide a reliable study for monitoring the severity of chemical carcinogenesis. Histology in a precise sense is the study of the cytoarchitectural change of the body which envisage the anatomy and gives insight into functioning of tissues and organs. The histology is a structural science and serves to compliment the knowledge gained from the anatomy, physiology and pathology.

It is obvious that any chemical insult could cause pathological or injury to cells in animal if it is consumed beyond the safe doses. Susceptibility to chemical injury exhibits variation among the tissues and cells of the same animal. The extent of severity of tissue damage is a function of the concentration and potentiality of toxic compound effects in the tissues as it is time dependent (Jayantha Rao, 1982).

Alpha-acetylamino-fluorene, diethyl-nitrosamine, N-ethyl-N- hydroxyethyl nitrosamine, dihydroxy-n-propyl nitrosamine were used as initiators of hepatocarcinogenesis (Ito et al., 1989). The cytoarchitectural changes produced during chemical carcinogenesis can be identified by microscopic examinations 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.
Histopathological studies would help in assessing the effect of carcinogens at the initiation stage in various organs and organ systems of an organism. In rats the transformation process was induced by treating with β-methylcholanthrene and acrylamide, pathological changes were studied in testis of both treated and control rats. Liver also studied at the same doses for reference.

Aims:

1. To study the histological changes of both control and MC treated rat testis tissues with dosage and time dependent manner.
2. To study the histological changes of both control and acrylamide treated rat testis and liver tissues.

Treatment of rats with MC and Acrylamide:

The male albino rats weighing about 150 gm (3 Months old) were grouped into six rats for each set and they were treated with MC and acrylamide as discussed in materials and methods (pages 43-53). The tissues were prepared for histological analysis as mentioned in materials and methods.

Results:

The control rat testis showed numerous seminiferous tubules (ST) with a connective tissue (CT) as a boundary line and developing spermatids and developed spermatozoa (Fig.45). Histopathological analysis of testis tissues of rats in the present investigation revealed a pathological condition on exposure to β-MC with different doses and different times.
Fig. 45: Control rat testis showing seminiferous tubules (SFT) basement membrane (BM), spermatids (ST) and spermatozoa (SP) (H&E-20X)

LST : Lumen of seminiferous

CT : Connective tissue
Fig. 45: Control rat testis Showing seminiferous tubules (SFT) basement membrane (BM), spermatids (ST) and spermatozoa (SP) (H&E-20X)

LST : Lumen of seminiferous tubules
CT : Connective tissue
Rats treated with (4 mg/100 g body weight / 24 hours interval for three days) the single dose administration of 4 mg of MC did not show much pathological symptoms. From 8 mg onwards, rat testis (Fig-46) showed more intensified changes. With 12 mg, MC dosage the changes include, increase in the lumen of the seminiferous tubules, clear degenerative changes in seminiferous tubules, necrotic spermatids and atrophied seminiferous tubules, extensive interstitial oedema when compared to controls (Fig-47).

With long-term duration and with 1 mg /100 gm body weight / weekly interval for six weeks also the single dose administration and 2 mg, 3 mg did not showed much pathological change. Where as multiple dose i.e., 4th week to 6th week (4 mg, 5 mg, 6 mg) showed pathological changes. Whereas MC with 4 mg and 6 mg doses in long term treatment showed tubules with degenerative changes and pycnotic nuclei and arrest of spermatogenesis and also degenerative changes in primary and secondary spermatogonia. MC with 5 mg dose showed less degenerative changes when compared to 4 mg and 6 mg doses (Fig. 48-49).

Histopathological analysis of testis tissues of rats in the present investigation revealed a pathological condition on exposure to acrylamide with 6mg/100gm body weight dosage effects were more pronounced than other doses. Studies have shown that animals (rats,) receiving 60 mg/kg of body weight per day/24 hours interval 1 (12 doses, total 72 mg) exhibited weakness and ataxia in hind limbs after one week, which progressed to paralysis with continued exposure. Other symptoms included testicular
Fig. 46: Experimental rat testis showing mild degenerative changes in seminiferous tubules (DGST) in 8 mg/48hr injected $\beta$-MC). (H&E-20X)

Fig. 47: Experimental rat testis showing necrosis in connective tissue (NCT), degenerative changes in seminiferous tubules (DGST) and increase in lumen of the seminiferous tubules (ILST) of rats received 12 mg/72hr $\beta$-MC (H&E-20X).
Fig. 46: Experimental rat testis showing mild degenerative changes in seminiferous tubules (DGST) in 8 mg/48 hr injected β-MC. (H&E-20X)

Fig. 47: Experimental rat testis showing necrosis in connective tissue (NCT), degenerative changes in seminiferous tubules (DGST) and increase in lumen of the seminiferous tubules (ILST) of rats received 12 mg/72 hr β-MC (H&E-20X).
Fig. 48: Experimental rat testis showing degenerative changes in seminiferous tubules (DGST), necrosis in connective tissue (NCT) with 4 mg (1 mg/week) injected β-MC (H&E-20X).

Fig. 49: Experimental rat testis showing mild degenerative changes in seminiferous tubules (DGST) with 5 mg (1mg/week) injected β-MC (H & E-20X).
Fig. 48: Experimental rat testis showing degenerative changes in seminiferous tubules (DGST), necrosis in connective tissue (NCT) with 4 mg (1 mg/week) injected 3-MC (H&E-20X).

Fig. 49: Experimental rat testis showing mild degenerative changes in seminiferous tubules (DGST) with 5 mg (1mg/week) injected β-MC (H & E-20X).
Fig. 50: Experimental rat testis showing necrosis in connective tissue (NCT), degenerative changes in seminiferous tubules (DGST). Showing damage in tubules with piknotic nuclei and also shows arrest of spermatogenesis received 6mg (1mg/week) β-MC (H&E-20X).
Fig. 50: Experimental rat testis showing necrosis in connective tissue (NCT), degenerative changes in seminiferous tubules (DGST). Showing damage in tubules with piknotic nuclei and also shows arrest of spermatogenesis received 6mg (1mg/week) β-MC (H&E-20X).
atrophy and degeneration of germinal epithelium. Arrest of spermatogenesis and also degenerative changes in primary and secondary spermatogonia and integration of seminiferous tubules deranged were observed (Fig-51). Control liver Shows normal hepatocytes with nucle (Fig. 52). Multiple dose of MC administration (12 mg MC/72hrs) of rat liver showing hypertrophy of nuclei and also the nuclei was binucleated and pycnotic in hepatocytes, vascular congestion, haemorrhages and granularity in cytoplasm (Fig.53), whereas 6 mg MC (1mg/week) administered rat liver showing mild mononuclear round cell collections, mild vascular congestion and mild nuclear prominence (Fig.54). Multiple dose of acrylamide administered rat liver showing hypertrophy of nuclei and also the nuclei was binucleated and pycnotic in hepatocytes, proliferation of sinusoidal bile ducts haemorrhages, congestion with peliosis-hepatis changes (cystic spaces) 60mg/kg body wt for 12 i.p injections (Fig 55).
Fig. 51: Experimental rat testis showing severe necrosis in connective tissue (SNS), showing atrophied seminiferous tubules (AST), degenerative changes in seminiferous tubules (DGST) and increase in lumen of the seminiferous tubules (ILST) of rats received acrylamide 60mg/kg body wt for 12 i.p injections (H&E-20X).
Fig. 51: Experimental rat testis showing severe necrosis in connective tissue (SNS), showing atrophied seminiferous tubules (AST), degenerative changes in seminiferous tubules (DGST) and increase in lumen of the seminiferous tubules. (ILST) of rats received acrylamide 60mg/kg body wt for 12 i.p injections. (H&E-20X)
Fig. 52:  Control liver Shows normal hepatocytes with nuclei.(H&E-40X).

Fig. 53:  Multiple dose of MC 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 12mg MC/72hrs.H&E 40X
Fig. 52: Control liver shows normal hepatocytes with nuclei. (H&E-40X).

Fig. 53: Multiple dose of MC 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 12mg MC/72hrs. H&E 40X
Fig. 54: Multiple dose of MC administered rat liver showing mild mononuclear round cell collections, mild vascular congestion, mild nuclear prominence with 6mg MC(1mg/week) H&E 40X

Fig. 55: Multiple dose of 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 60 mg / kg body wt for 12 i.p injections. H&E 40X
Fig. 54: Multiple dose of MC administered rat liver showing mild mononuclear round cell collections, mild vascular congestion, mild nuclear prominence with 6mg MC(1mg/week) H&E 40X

6mg/6th week 3-MC LIVER

Fig. 55: Multiple dose of 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 60 mg / kg body wt for 12 i.p injections. H&E 40X
DISCUSSION

In the present investigation the MC administration to rats induced pronounced pathological changes in testis and liver when exposed to multiple doses. The liver here is used for comparative purpose.

The phenobarbital (Thyagaraju et al., 2003), butylated hydroxy toluene and poly chlorinated biphenyls for liver (Pitot and Sirica, 1980), prolactin for mammary glands (Welsch and Nagasawa, 1977) and acids for colon (Narisawa et al., 1974; Reddy, 1977) and diet has been found to influence carcinogenesis in several systems.

Liver is the major metabolizing organ which detoxifies a number of drugs and xenobiotics. Liver was affected with PB and MC with certain doses with hypertrophy of hepatocytes and also the nuclei was binucleated and pyknotic in hepatocytes (Devi et al., 2002) centrilobular nerosis, hypertrophoid nuclei, adenomas, hepato cellular carcinoma after time periods in Benzene hexa chloride (Kandarkar et al., 1983). Thyagaraju et al., 2003 reported oedema, congestion, degenerative changes with multiple doses of β-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).

On short term treatment, with doubling dosage (4-12 mg for 24 hrs-72 hrs), male rat reproductive system affected with more degenerative changes. But on long-term treatment with low dosage of MC with prolonged time (1-6mg for 1-6 weeks), there was mild degenerative changes in testis by 4mg
MC/4th week treatment and there was some regenerative changes were observed with 5mg/5th week dosage of MC compared to control rat testis. Severity of histological lesions had been observed in multiple doses of short-term (12 mg/72 hours) and also in 6 mg/42 days (6th week) MC administered rats when compared to control, they caused deleterious effects and making them less fit for better survival.

In testis as reported earlier the degenerated spermatozoa, reduced seminiferous tubules and reduced basement membrane were observed by β-MC with respect to control. Hence the results in testis by the influence of β-MC are in agreement with the above reports.

We observed some of the less deleterious effects in 5th batch (5 mg/35 days or 5th week) when compared to other doses (4th and 6th batch). This indicates after 4 weeks of treatment the 5th dose treatment may be act as a drug to overcome the previous deleterious effects. This may need further work.

The tissue degeneracy of testis and hypertrophy with binucleated hepatocytes in liver was compared with the enzyme activities of GST and GPx protein levels and were further confirmed by immunological studies using their subunit specific and affinity protein antisera.

Acrylamide has been evaluated for reproductive toxicity in multigenerational studies in rats (Tyl et al., 2000a). In studies with rat and 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. It should be noted that the genotoxic studies have indicated that there is no threshold value for the risk of cancer induced by acrylamide. 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 (Shiraishi, 1978; Hashimoto and Tanii, 1981) and spermatocyte chromosome aberrations in mice. 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. Following ip, injection of 50 mg/kg body weight / day for 10 days Huang et al., 1982 has reported atrophy of epididymal fat pad, accompanied by a severe triglyceride depletion.

The substrate specificity studies, purification studies, immunological studies, sperm morphological studies correlate with the degenerated changes occurred in testicular tissue after treatment with acrylamide. These results are in agreement with the results of Dearfield et al., 1988 and Tyl et al., 2000b.