CHAPTER-IV

HISTOPATHOLOGICAL STUDIES
EFFECT OF HYBANTHUS ENNEASPERMUS ACTIVE PRINCIPLE ON HISTOLOGICAL CHANGES OF ACRYLAMIDE TREATED MICE KIDNEY AND TESTIS

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 compliment the knowledge gained from the anatomy, physiology and pathology. Induction of carcinogenesis 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 also 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. Animal susceptibility to chemical injury exhibits variation among the tissues and cells of the organ. 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 (Jayantha Rao, 1982) and concentration dependent (Thyagaraju et al., 2003)

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.

Hence 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 at various doses and with acrylamide, pathological changes were studied in testis of both treated and control (Devi, 1998). Similar studies were also conducted to observe the changes in structure of hepatocytes enzymes.
Therefore to observe the variations in the mice kidney and testis due to the influence of Hybanthus enneaspermus active principle extract on acrylamide treated tissues the following objectives were selected.

Objectives:

1. To study the histological changes of acrylamide treated mice kidney and testis tissues with dosage and time dependent manner.

2. To study the histological changes of mice kidney and testis by exposure with acrylamide and then with acrylamide and Hybanthus enneaspermus active principle extract with dosage and time dependent manner.
RESULTS

Histology experiments were performed for the above aims as mentioned in “Materials and Methods” chapter. Histological changes of kidney and testis were observed (Humason, 1972) under light microscope and the following results were observed.

Figure-32: Histological analysis of kidney sections (mice-control) under light microscope (50X40x) showing, medulla and cortex.

On 24 hours, 48 hours and 72 hours exposure of mice kidney and testis to 1mg, 2mg, 3mg and 4mg of AC treatment kidney sections showed pathological variations in cortex and medulla. On 24hours exposure to AC, the kidney showed mild degeneration of cortex at first and then mild degeneration of medulla was observed at 1mg, 2mg, 3mg and 4mg of AC, respectively (Fig. 33, 34, 35, 36). On 48hours exposure kidney section showed severe damage to medulla, (Fig. 37, 38, 39). The same results were observed on 72hours exposure to AC (Fig. 40, 41, 42, 43). However damage to cortex and medulla of mice kidney was reduced on exposure of mice to AC and HEAPE mixture. (Fig. 44, 45, 46, 47, 48, 49, 50, 51)
**Figure-33:** Histological analysis of kidney sections (mice-treated) under light microscope (50X40x) showing mild degeneration medulla and cortex 1mg acrylamide/24hr interval.

**Figure-34:** Histological analysis of kidney sections (mice-treated) under light microscope (50X40x) showing mild degeneration cortex 2mg acrylamide/24hr interval.
Figure-35: Histological analysis of kidney sections (mice-treated) under light microscope (50X40x) showing mild degeneration medulla 3mg acrylamide/24hr interval.

Figure-36: Histological analysis of kidney sections (mice-treated) under light microscope (50X40x) showing mild degeneration medulla 4mg acrylamide/24hr interval.
Figure-37: Histological analysis of kidney sections (mice-treated) under light microscope (50X40x) showing severe degeneration medulla 1mg acrylamide/48hr interval.

Figure-38: Histological analysis of kidney sections (mice-treated) under light microscope (50X40x) showing severe degeneration medulla 2mg acrylamide/48hr interval.
Figure-39: Histological analysis of kidney sections (mice-treated) under light microscope (50X40x) showing severe degeneration medulla 3mg acrylamide/48hr interval.

Figure-40: Histological analysis of kidney sections (mice-treated) under light microscope (50X40x) showing severe degeneration medulla 1mg acrylamide/72hr interval.
Figure-41: Histological analysis of kidney sections (mice-treated) under light microscope (50X40x) showing severe degeneration medulla 2mg acrylamide/72hr interval.

Figure-42: Histological analysis of kidney sections (mice-treated) under light microscope (50X40x) showing severe degeneration medulla 3mg acrylamide/72hr interval.
Figure-43: Histological analysis of kidney sections (mice-treated) under light microscope (50X40x) showing severe degeneration medulla 4mg acrylamide /72hr interval.

Figure-44: Histological analysis of kidney sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration in cortex 1mg /24hr interval.
Figure-45: Histological analysis of kidney sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration in cortex 2mg /24hr interval.

Figure-46: Histological analysis of kidney sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration in cortex and medulla 3mg /24hr interval.
Figure-47: Histological analysis of kidney sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration in cortex and medulla 4mg /24hr interval.

Figure-48: Histological analysis of kidney sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration in cortex and medulla 1mg /48hr interval.
Figure-49: Histological analysis of kidney sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration in cortex and medulla 2mg/48hr interval.

Figure-50: Histological analysis of kidney sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration in cortex and medulla 3mg/48hr interval.
Figure-51: Histological analysis of kidney sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration in cortex and medulla 4mg/48hr interval.
The control mice testis showed numerous seminiferous tubules (ST) with a connective tissue as a boundary line and developing spermatids and developed spermatozoa (Fig. 52). Histopathological analysis of testis tissues of mice in the present investigation revealed a pathological condition on acrylamide with different doses and different times.

Figure-52: Histological analysis of testis sections (mice-control) under light microscope (50X40x) showing seminiferous tubules.
Mice treated with (1mg/100 g body weight x 24 hours, 48 hours and 72 hours interval for four days) and the administration of acrylamide showed pathological symptoms according to dose and time. Mice testis of 24 hours (Fig. 53, 54, 55, 56), 48 hours (Fig. 57, 58, 59) and 72 hours (Fig. 60, 61, 62, 63) intervals showed more intensified changes these 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. 52) and tubules with degenerative changes and pyknotic nuclei and arrest of spermatogenesis and also degenerative changes in primary and secondary spermatogonia, observed, weakness and ataxia in hind limbs after one week, which progressed to paralysis with continued exposure. Other symptoms included testicular atrophy and degeneration of germinal epithelium. Arrest of spermatogenesis and also shows degenerative changes in primary and secondary spermatogonia, integration of seminiferous tubules deranged were observed. However damage to testis of mice to AC and HEAPE mixture. (Fig. 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74) were observed.
Figure-53: Histological analysis of testis sections (mice-treated) under light microscope (50X40x) showing mild degeneration seminiferous tubules 1mg acrylamide/24hr interval.

Figure-54: Histological analysis of testis sections (mice-treated) under light microscope (50X40x) showing enlarged seminiferous tubules 2g acrylamide/24hr interval.
Figure-55: Histological analysis of testis sections (mice-treated) under light microscope (50X40x) showing rings of enlarged seminiferous tubules 3mg acrylamide/24hr interval.

Figure-56: Histological analysis of testis sections (mice-treated) under light microscope (50X40x) showing degeneration of connective tissue seminiferous tubules 4mg acrylamide/24hr interval.
Figure-57: Histological analysis of testis sections (mice-treated) under light microscope (50X40x) showing severe degeneration of seminiferous tubules 1mg acrylamide/48hr interval.

Figure-58: Histological analysis of testis sections (mice-treated) under light microscope (50X40x) showing severe degeneration of seminiferous tubules 2mg acrylamide/48hr interval.
Figure-59: Histological analysis of testis sections (mice-treated) under light microscope (50X40x) showing severe degeneration of seminiferous tubules 3mg acrylamide/48hr interval.

Figure-60: Histological analysis of testis sections (mice-treated) under light microscope (50X40x) showing severe degeneration of seminiferous tubules 1mg acrylamide/72hr interval.
Figure-61: Histological analysis of testis sections (mice-treated) under light microscope (50X40x) showing severe degeneration of seminiferous tubules 2mg acrylamide/72hr interval.

Figure-62: Histological analysis of testis sections (mice-treated) under light microscope (50X40x) showing severe degeneration of seminiferous tubules 3mg acrylamide/72hr interval.
Figure-63: Histological analysis of testis sections (mice-treated) under light microscope (50X40x) showing severe degeneration of seminiferous tubules 4mg acrylamide/72hr interval.

Figure-64: Histological analysis of testis sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration seminiferous tubules 1mg /24hr interval.
Figure-65: Histological analysis of testis sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration seminiferous tubules 2mg /24hr interval.

Figure-66: Histological analysis of testis sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration seminiferous tubules 3mg /24hr interval.
Figure-67: Histological analysis of testis sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration seminiferous tubules 4mg/24hr interval.

Figure-68: Histological analysis of testis sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration seminiferous tubules 1mg/48hr interval.
Figure-69: Histological analysis of testis sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration seminiferous tubules 3mg/48hr interval.

Figure-70: Histological analysis of testis sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration seminiferous tubules 3mg/48hr interval.
Figure-71: Histological analysis of testis sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration seminiferous tubules 4mg/48hr interval.

Figure-72: Histological analysis of testis sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration seminiferous tubules 1mg/72hr interval.
Figure-73: Histological analysis of testis sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration seminiferous tubules 2mg /72hr interval.

Figure-74: Histological analysis of testis sections (mice-treated with AC and HE) under light microscope (50X40x) showing mild regeneration seminiferous tubules 3mg /72hr interval.
DISCUSSION

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, 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, 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 and spermatocyte chromosome aberrations.

Thyagaraju et al., 2003. reported oedema, congestion, 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.

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.

The most important organ, the brain, is responsible for thinking and feeling, the rational and emotional sides of our mental life and the clinical and experimental evidence would suggest its paired cerebral hemispheres are product of activity in neural networks.

Many detoxication enzymes exist as isozymes with multiple genes and exhibit differential expression with a wide variety of xenobiotics to prevent chemical lesions such as mutagenesis, carcinogenesis and tissue necrosis.

The mice Kidney size was presumably influenced both by genetic and environmental factors. The number of glomeruli at birth is presumably genetically
The size of kidneys is dependent on the number and size of nephrons. The total filtration surface area depends on the glomerular density and the glomerular surface area.

The kidney are highly vascularized organs that filter the blood and excrete waste materials. Each kidney is enclosed in a tough connective tissue capsule extending into the parenchyma and has two regions—the cortex and the medulla.

Mice treated testis of intervals showed more intensified changes these changes include: increase in the lumen of the seminiferous tubes, clear degenerative changes in seminiferous tubes, necrotic spermatids and atrophied seminiferous tubules, extensive interstitial oedema when compared to controls and tubules with degenerative changes and pyknotic nuclei and arrest of spermatogenesis and also degenerative changes in primary and secondary spermatogonia, observed, weakness and ataxia in hind limbs after one week, which progressed to paralysis with continued exposure. Other symptoms included testicular atrophy and degeneration of germinal epithelium. Arrest of spermatogenesis and also shows degenerative changes in primary and secondary spermatogonia, integration of seminiferous tubules deranged were observed. However damage to testis of mice to AC and HEAPE mixture.

The substrate specificity, purification, immunological and 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 previous reports.

My reports suggests that the plant product present in HEAPE may protect the cells and morphology of kidney and testis in mice.