CHAPTER 5
Histoarchitecture of liver and kidneys of 10 groups of rats were studied to evaluate the effect of methanolic root extract of *Achyranthes aspera* L. against aflatoxicosis in rats. Selected rats were weighed before being killed. A detailed necroscopy was then conducted. The liver and kidneys were removed and weighed. Tissue samples were collected in 10% neutral buffered formalin. After fixation with Zenker's fluid, samples were dehydrated in alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut out at 4μm and stained with haematoxylin and eosin (Thermoshandon, 15275, USA). Some sections were also stained by the methods of Van Geison and periodic acid schiff staining (Luna, 1968) and observed under microscope (magnification 10X, 40X, 100X, 200X and 400X).

Microscopically, hepatocellular degeneration in liver was graded following (M.Ortatatali, H.Oguz, 2005).

*Slight (degree: 1)*: Mild hepatocellular swelling due to hydropic degeneration and fatty changes only in centrilobular areas (fatty infiltration)

*Moderate (degree 2)*: Clear hepatocellular swelling in both centrilobular and midzonal areas.

*Severe (degree 3)*: Diffuse and hepatocellular swelling, cytoplasmic paleness and rupture (this grade was not seen in any treatment).

Liver and kidney are most important organs of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites are especially vulnerable to damage (Brzoska *et al.*, 2003). Histopathological examinations of liver and kidney
sections revealed typical lesions of aflatoxicosis. The aspect of aflatoxicity could be important also for risk assessment extrapolations, if the immune system, via the inflammatory process or other mechanisms, is involved in hepatotoxicity and/or carcinogenicity (Dennis et al., 2003). Thus, in the study presented herein, we investigated the effects of aflatoxin on the main cellular targets was investigated by histopathology.

5.1. The Liver Histopathology

It is well known that toxic effects of a xenobiotic can be modified by other substances (Skoczynska and Smolik, 1994; Brus et al., 1999; Institoris et al., 1999; Gupta and Gill, 2000). Ayd et al., 2003 reported the toxic effect of ochratoxin on liver and kidney curative effect of melatonin in albino rats. These results supported our findings against aflatoxicosis. The severity of the lesions was significantly reduced by the administration of Achyranthes aspera L. root extracts (100mg, 200mg/kg) and silymarin (25mg, 50mg/kg) (group III - X). Both prophylactic and anaphylactic measures of Achyranthes aspera L. root extract and silymarin were studied. Prophylactic measures show better curative effects (Group III and VI). These results revealed that aflatoxin induced significant histopathologic changes in liver and kidney tissue and administration of the plant root extract significantly reduced the toxic effect of aflatoxin on kidney and liver tissues of rats.

Histopathological examination demonstrated fatty degeneration of hepatocytes, mononuclear infiltration in peripheral region; hyperplastic bile ducts and hepatocellular degeneration in the liver of the group which received aflatoxin alone (group II). Although these lesions were also observed in livers of groups (III - X) which received Achyranthes aspera L. root extract + aflatoxin and silymarin +
aflatoxin (Prophylaxis and anaphylaxis), they were not as severe as those in group II. These results were coincided with the results of Gokhan et al., (2006). Livers of broiler chickens affected by aflatoxicosis characteristically showed biliary and nodular hyperplasia and are pale and enlarged as a result of aflatoxicosis (Kubena et al., 1990; Phillips, 1995). Chronic exposure to aflatoxins below the LD$_{50}$ can result in reduced weight gain, hepatocellular necrosis and bile duct cell proliferation (G.C.Llewellyn, 1986).

The liver of control rats showed a normal structure. It is composed of hexagonal or pentagonal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing kupffer cells. The Kupffer cells are fixed macrophages, which belong to the Mononuclear Phagocyte System. (Fig5.1) They are associated with the endothelial cells and possess cellular processes, which extend via the spaces between endothelial cells into the lumen of the sinusoid.
Hepatocytes are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution. Some cells have two nuclei each. Lipocytes or Ito cells are typically found in the space and have the ability to accumulate lipid droplets. They are the main source of vitamin A storage in the body and also play a role in wound healing (hepatic fibrogenesis). Following exposure to aflatoxin single dose (1mg), the trabecular structure of the lobules was blurred. Cytoplasm of hepatocytes contained empty vacuole like spaces and were enlarged. Some sinusoids were overfilled with erythrocytes and the walls of most sinusoids showed numerous kupffer cells. Locally mononuclear cell infiltrations were observed frequently in the hepatocytes of zone I. (Fig5.2).

The inflammatory cell infiltrates aforementioned were observed more frequently. However increased numbers of activated Kupffer cells characterized by sinusoidal walls with increased amounts of cytoplasm and vacuolated nuclei as well as small loci of extramedullary hematopoiesis (EMH) were also present. The walls of the sinusoids in both zones showed numerous kupffer cells. In zone I hepatocytic necrotic changes were evident, a small pycnotic cellular nucleus with condensed chromatin, lack of nucleolus and strongly acidophilic cytoplasm were observed. Mononuclear cell infiltration were also noted in zone I hepatocytes.

Further in some places infrequent small inflammatory cell infiltrates composed of lymphocytes, plasma cells, mononuclear cells, and few segmented neutrophils appeared to respond to degenerate vacuolated hepatocytes. There were early reports on inflammatory responses in rats due to AF-B₁-induced injury in the liver. Another report was made in aflatoxin treated chicken that showed relatively slight cellular degeneration and almost no necrosis, large lymphoid follicles appear in the areas of fatty change. (Kuester et al., 2002). The increase in the autophagosomes
has also been marked in hepatic cells of aflatoxin fed guinea pigs (Thurston et al., 1980). Rats treated with *Achyranthes aspera* L root extract (100mg) and single dose of aflatoxin (1mg) (group III) regained nearly normal structure of liver.

Fatty cells between hepatocytes disappeared, assumed their normal histoarchitecture (Fig 5.3). Kupffer cells number in the walls of sinusoids decreased significantly. In the rats pretreated with *A. aspera* L root extract (200mg/kg) and single dose of AF (1mg/kg) (group IV), nearly normal structure of liver was observed (Fig 5.4).
Granular or vacuolated degeneration and necrosis of the liver cells, sinusoidal and central vein dilatation, bile duct proliferation, enlargement of periportal areas with mononuclear cell inflammatory infiltration and mild degree fibrous tissue proliferation were completely get repaired. The similar progressive changes were observed in rats pretreated with silymarin (25mg and 50mg/kg) (Group V, VI) (Fig 5.5, 6). This investigation proved that Achyranthes aspera L. methanolic root extract shows antihepatotoxic effect like the hepatoprotective effect of the drug silymarin.

5.2. The Kidney Histopathology

There are an estimated 1.3 million nephrons in each kidney. Each nephron is about 50-55 mm long and it is estimated that the total length of all the nephrons of both kidneys is about 100km. Each nephron consists of: Renal corpuscle (Renal body, Malpighian body), proximal convoluted tubule, loop of Henle, distal convoluted...
Malpighian body), proximal convoluted tubule, loop of Henle, distal convoluted tubule and collecting duct. The renal corpuscles are round or ovoid structures (about 150-200mm diameter) located in the cortex. They have a double-walled epithelial capsule (Bowman's capsule). The outer or parietal layer of Bowman’s capsule is composed of much flattened (squamous) epithelial cells. The inner or visceral layer of Bowman's capsule is composed of specialized epithelial cells known as podocytes. An afferent arteriole enters the renal corpuscle at the vascular pole and forms a complex capillary bed (glomerular tuft), before leaving the vascular pole as an efferent arteriole.

The podocytes are associated with and surround the capillaries of the glomerular tuft and form part of the filtering apparatus. The proximal tubules originate at the urinary pole of the renal bodies. They consist of simple cuboidal epithelium, which is very acidophilic (owing to the activity of the numerous mitochondria). These epithelial cells are relatively larger in size. Their nuclei are regular and fairly central.

The apical membrane of each cell is covered with large numbers of microvilli to form a distinct "brush border", which is coated with a PAS-positive glycocalyx. The lateral membranes of the cells are modified to form numerous lateral processes, which interdigitate with similar processes from adjacent cells. The basal region of the cells has many invaginations of the basal plasmalemma. Numerous large mitochondria are situated between the basal invaginations. These mitochondria and invaginations result in basal striations, which are very well seen in histological preparations stained with iron hematoxylin.
Lysosomes and peroxisomes are common in the apical regions of the cells. Each proximal convoluted tubule continues as the descending thick segment of the Loop of Henle, which continues as the thin segment. The thin segment of the loop is characterized by its extremely flattened epithelial cells, which possess relatively few organelles. The site of the U-shaped loop is an area of extremely concentrated urine production. The loop of Henle continues as an ascending thick segment. The epithelial cells of the distal convoluted tubule are smaller and less acidophilic (fewer mitochondria) than the proximal convoluted tubule cells. They lack basal striations and also lack brush borders on their apical surfaces. Collecting tubules are the main component of the medullary rays of the cortex; however the collecting tubules dominate in the medulla (in the pyramids). They consist of straight tubules of cuboidal epithelium with central nuclei. The cells are stained weakly and the borders between adjacent cells are distinct. Near the apex of the pyramid the cuboidal epithelium becomes a columnar epithelium and the ducts are much larger and typically ovoid. These are known as the papillary ducts of Bellini, and open into the minor calyces.

Normal structure of the cortex and medulla was observed in the kidney of control rats. Renal glomeruli show normal structure. The first row renal tubules are lined with typical thick cubic epithelium. Second row renal tubules showed a considerably lower cubic epithelium. The tubules have a relatively regular distinct lumen. Lobular organization
of the glomeruli and a flat epithelium lining of the glomerular capsule can be also seen. (Fig 5.7)

Hypertrophy of epithelial cells and degeneration of epithelia of renal tubules with infiltration of mononuclear cells, dilation of glomeruli as well as hyperaemia of medullary and cortical parts with mononuclear cell infiltrates were evident in all animals treated with aflatoxin (1mg). Similar findings were reported in the rats exposed cadmium (Cd) and ethanol (EtOH) (M.M. Brzoska et al., 2003).

Degeneration of renal tubules with sincitial cells, degeneration of basement membrane of the Bowman’s capsule, diffused glomeruli and vacuolation of glomeruli was reported in TCA and t-butyl alcohol administered rats (Acharya et al., 1997).

The most advanced change after exposure was the dilation of capillaries filled with erythrocytes both in cortical and medullary parts of the kidney. Enlargement of renal glomeruli and epithelial cells of the I row tubules in the cortical part of the kidney were found. A few renal tubules showed single epithelial cells dissquamated to their lumen (Fig 5.8).
Mononuclear cell infiltrates were observed in some places of the medullary parts of the kidney and at these sites the inflowing cells blurred the tubular structure. Some typical changes were observed at ultra structural levels in aflatoxin treated rats.

The rat treated with aflatoxin exhibited alterations in ultra structure of proximal tubules. Changes in the constitution of mitochondria in the epithelial cells of proximal tubules along with presence of intracytoplasmic desmosomes in basement membrane epithelial cells indicate damage due to aflatoxin.

Effects of toxic substances or carcinogenic chemicals on kidney proximal tubules were reported by Ghadially, 1982a, b. Whereas rats intoxicated with the pretreatment using *A. aspera* L root extract and silymarin showed moderate affected renal tubules, glomeruli. Pretreatment with 100mg of *A. aspera* L (groupIII), 200mg of *A. aspera* improved partial retension of normal glomerlus, renal tubule structure. (Fig 5.9, 10). In kidney, recovery was slow as indicated by the presence of vauolation and
necrosis of the cells of many renal convoluted tubules three weeks after withdrawal of the experimental ration (Dafalla et al., 1987). The standard hepatoprotective drug, silymarin pretreated (25mg, 50mg/kg)

animals showed normal glomeruli and Bowmann’s capsule with space (Fig 5.11 & 12).

Curative effect of plant extract and silymarin was observed better in 100mg/kg A.aspera root extract treated rats. Improved changes in liver and kidneys pretreated rats evidenced that Achyranthes aspera L. is as effective as silymarin in curing hepatic damage.

Fig5.12: Kidney of rat exposed to Silymarin (50mg/kg) and aflatoxin (1mg/kg).slightly bulged renal tubule and glomeruli with desquamated cells were observed.