Chapter III

HISTOPATHOLOGY
Under physiological conditions the diversified structural and biophysicochemical components of the cells act and maintain a dynamic synergistic equilibrium (anatomophysiologic synergism) determined and regulated in an orderly manner by intrinsic mechanisms in conjunction with the environmental conditions of the cell. In abnormal conditions of a structural, metabolic or functional character, reversible changes (histometabolic dysergia) take place. However, if such endogenous or exogenous phenomena are repeated or perpetuated without control and exceed the physiologic endurance, then eventually disorganisation or dissolution of the integrated anatomophysiologic synergistic equilibrium follows with consequent irreversible change of structural (degeneration), metabolic (histometabolic pathergia) and functional (dysfunction or paralysis) character (Minckler, 1971). In the cell, one perturbation may trigger another in a cascading series of reactions that may intensify the potential of harm and spatially and temporally, obscure the initial triggering insult. At some point in the series of reactions, the system is irreversibly altered, even doomed (Brabec and Bernstein, 1981).
Although much can be learned from the careful pathological examination of gross specimens, the finer cytoarchitectural changes produced during chemical intoxication can be traced by microscopic examination of the tissues. Such studies may explain to certain extent, the tissue-specificity of drug or chemical agent action. The accessibility of a particular cellular structural component, as well as the component's influence on the integrity of one or other cell organalle, determine the specificity of attack by a toxic compound, more accurately. However, an alteration of cell morphology, even when localized and confined does not unequivocally, indicate where or how a molecular derangement occurs, because they are several steps away from the initial disturbance. More recently the biochemical studies are given much importance in the toxicological assessment of harmful chemicals (Brabec and Bernstein, 1981; Richardson, 1981; Aldridge, 1983; Chen, 1984). It can be suggested that both morphological and biochemical assays should be applied for more accurate evaluation of pathological concepts.

The incorporation of the parent compound or their metabolites in lower organisms in the tissues of fishes, birds, wild animals and humans was found to cause serious
morphological alterations in vital tissues of the organisms even at very low levels (Vijay Joseph and Jayantha Rao, 1990).

In general, any chemical substance can cause damage/injury to an animal, if taken beyond the safe level. The liver is the main organ for its accumulation to a greater extent (Edwards, 1973). Johnson (1973) and Verma et al., (1974) pointed out that a prolonged period of exposure to chemical compounds with very low concentrations results in the accumulation of it in the organs.

The investigation of histopathological effects have not been pursued with the same vigour compared to biochemical aspects. A large number of reports in the toxicity of organo chlorine insecticides to various animal species have been published (Pawar and Katdare, 1984; Zaidu et al., 1985; Anand et al., 1986; Fox et al., 1986). Histopathological changes in different organs of rat, fish and mouse were reported after acute and chronic sublethal exposure to endosulfan (Satyaprasad, 1983; Kulshrestha, 1984). Some more workers reported on organo chlorine insecticides and pointed out architectural damage to the gill, kidney, liver and intestine of various animals (Madhu, 1983; Philip, 1984;
Radhaiah, 1985; Girija Moses, 1987). Somasundara et al., (1978) reported histopathological changes in the skin and increased liver protein in response to dieldrine treatment. Reports on tumorogenic potential of endosulfan in mouse are unequivocal (Ionnes et al., 1969; Thrope and Walker, 1973). Several investigations were carried out on histopathological changes under insecticidal and herbicidal treatment in different animals (Kim et al., 1985; Ehan et al., 1986; Korolev et al., 1986; Anthony et al., 1987; Ozata and Atalay, 1987; Ramalingam, 1988; Sonneschein et al., 1989; Varshneya et al., 1989).

Dietary induced hyperlipidiemia in uremic rat showed proteinuria and renal histologic abnormalities consisting of xanthoma like glomerular lesions, infiltrates and fibrosis (Reichenberg et al., 1991). In uremic serum high molecular substances (greater than 50 KDa) may show cytotoxic effect (Rada and Otting, 1990). Grinshle and Linev (1989) reported morphofunctional damage to the biomembranes in the lipid peroxidation in patients with chronic kidney failure. De Deyn et al., (1991) demonstrated epileptogenicity of some of the guanidine compounds which might contribute to the pathogenesis of seizures in hyperargininemia. Asaka et al., (1988) investigated natural Killer (NK) cell activity of
peripheral blood mononuclear cells (PBMC) in uremia. It has been suggested that defective NK cell activity in uremic patients explains in part their susceptibility to malignancy and infection.

To have a clear understanding, as to how these chemicals cause injury to the tissue, it is essential to have an insight into the histopathological analysis of the tissues. In view of this an attempt has been made to study certain histopathological consequences of guanidine hydrochloride administration to experimental animals.

NORMAL HISTOLOGY OF THE LIVER:

The liver of rat comprises a continuous mass of hepatic cells (hepatic parenchyma) arranged in cords. There is no clear division of the hepatic cells into lobules as in the case of other mammals. The hepatocytes are large in size and possessing centrally placed nucleus (Fig. 9a). The pancreatic tissue is distinct with a well organized cellularity and a large number of blood sinusoids in the hepatic mass.

NORMAL HISTOLOGY OF THE KIDNEY:

Histologically the cortical part of the kidney is made up of a large number of tiny filters called nephrons. Each
nephron consists of two major parts viz., the glomerulus and the tubules. The intertubular spaces are filled with evenly distributed hemopoietic tissue (Fig. 10a). The cells are parenchymatous in nature, round to polygonal in shape with distinct nucleus in the centre.

HISTOPATHOLOGICAL LESIONS IN LIVER UNDER GUANIDINE HYDROCHLORIDE TREATMENT:

The microscopic observation of the liver sections of the experimental rats showed more pathological changes. These are cytoplasmic degeneration, necrosis in hepatocytes, exudation of nucleus, binucleate condition, degeneration of hepatocytes, nuclear degeneration and pycnotic nuclei in hepatocytes (Fig. 9b, c, d, e and f).

HISTOPATHOLOGICAL LESIONS IN KIDNEY UNDER GUANIDINE HYDROCHLORIDE TOXICITY:

The microscopic observation of the kidney sections of experimental rats showed necrosis in interstitial cells, luced lumen of proximal tubule, damaged glomeruli with ached stalk and atrophy of glomeruli is prominent (Fig. and c).
From the above observation it is evident that guanidine o:ochloride (GuHCl) induced marked histopathological changes in both tissues namely liver and kidney. The extent liver damage observed in the present investigation indicate that GuHCl treatment to animal causes impairment to the architecture of the tissue. Since liver is the major metabolis centre in the detoxification of foreign compound, it is susceptible to greater degree of disruption in its structural organization due to toxic stress. Degeneration of cytoplasm, necrosis in hepatocytes, exudation of nucleus, nuclear degeneration and pyknotic nuclei were observed in the liver due to guanidinc dosing.

A few reports pertaining to pathogenesis of liver with different drugs were also documented earlier. Philip et al., (1989) reported congestion of portal vessels and central vein swollen periportal hepatic cells showing parenchymatous degeneration, severe fatty changes in periportal cells in field mice treated with Benzene hexachloride (BHC). Prasad (1986) also reported similar changes in the liver of albino mice during hexachlorophene (HCP) treatment. Bhatnagar and Jain (1986) reported cloudy swelling and vacuolization of
hepatocytes, enlargement of nuclei, mild cytoplasmic degeneration apart from congestion of sinusoids and midzona; and periportal necrosis of hepatocytes. Various histopathological changes were reported in hepatic tissue of different animals exposed to various pesticides (Datta and Dikshith, 1973; Anthony et al., 1987; Ramalingam, 1988 Sonnenschein et al., 1989). Hence the changes observed in the present study might be attributed to the action of guanidine on liver cell membrane, microsomes and mitochondria (Lotina et al., 1973a; Lotina et al., 1973b).

 Pronounced changes were also noticed in the kidney of guanidine treated rats. As kidney forms the main organ of excretion the changes noticed are quite prominent. During excretion the undetoxified molecules pass through kidney and appears to have caused considerable damage to tissue. Glomerular damage, atrophied glomeruli, reduced lumen of proximal tubule, necrosis in the interstitial cells and detached glomerular stalk were conspicuous due to guanidine dosing.

 Earlier, Diane courteny and Moore (1971) report kidney malformations and reduced foetal weight in th
2,4,5-T (Trichlorophenoxy acetic acid) exposed mice. In ma
paraguat toxicity caused kidney and liver damage followed by respiratory distress (Galloway and Petrie, 1972). Chronic BHC administration induced congestion of blood vessels and glomerular tufts, cystic dilation of tubules and interstitial hemorrhages in the medulla were noticed in the kidney of mice (Philip et al., 1989).

The histological studies clearly demonstrate that the tissue structural integrity is disrupted to a large extent indicating that guanidine causes deleterious effects on architectural characteristics of cells.

The pathological changes observed in liver and kidney may be related to increased free radical production due to elevated lipid peroxidation, xanthine oxidase and depleted glutathione (GSH) levels. Decrease in GSH levels led to the enhanced formation of reactive free radical intermediates by increased peroxidation of membrane lipids and consequent damage to tissue organisation which in turn leads to functional derangement.

The histopathological alterations observed in the present study are in agreement with previous reports on tissue damage with other toxic chemicals (Anundi et al., 1979; Dwivedi et al., 1984; Vani, 1991; Peter et al., 1992 and Aslam et al., 1992).
Legend for Fig. 9

a. Control liver (H & E) x 50

N = nucleus
S = sinusoids

b. GuHCl treated liver (H & E) x 100

CDA = cellular disarray
NDG = nuclear degeneration
NH = necrosis in hepatocytes
BiN = binucleate condition

C. GuHCl treated liver (H & E) x 100

BiN = binucleate condition
CEDGH = cellular degeneration of hepatocytes
d. GuHCl treated liver (H & E) x 100

CDG = cytoplasmic degeneration

e. GuHCl treated liver (H & E) x 100

PYN = pycnotic nuclei in hepatocytes
CDG = cytoplasmic degeneration

f. GuHCl treated liver (H & E) x 100

NC = Necrosis in hepatocytes
EXN = exudation of nucleus from hepatocytes
CDG = cytoplasmic degeneration
Legend for Fig. 10

a. Control kidney (H & E) x 50

G = glomeruli
GS = glomeruli with stalk

b. GuHCl treated kidney (H & E) x 50

AG = atrophy of glomeruli
NIC = necrosis in interstitial cells
DGS = detached glomerular stalk

C. GuHCl treated kidney (H & E) x 50

DG = damaged glomeruli
DGS = detached glomerular stalk
RLP = reduced lumen of proximal tubule