Chapter IV

Histology

The hepatotoxicity caused by rifampicin is included under adverse effects of drug in the pharmaceutical codex (1979) and Martindale- the Extra Pharmacopoeia (1985). Survey of 63 patients taking rifampicin showed abnormalities in serum aspartate aminotransferase (SGOT) and bilirubin. This occurred during first 12 weeks of the therapy. Average duration of the abnormalities of serum was 14 1/2 days irrespective of whether treatment was interrupted or not (Lal *et al,* 1972). Skakun and Slivka (1992), Skakun, and Tabachuk (1992) showed cytolytic action of rifampicin that was confirmed in albino rats by Saraswathy *et al* (1998) when they tested effects of Liv 100 against rifampicin induced hepatotoxicity. The rifampicin-induced hepatotoxicity was also noted in the mouse (Ramana and Kohli, 1999) and in rat (Puri and Kohli, 1995).

Piriou *et al* (1987) with the high doses of rifampicin showed hepatotoxicity and damage to the hepatocytes. Sodhi *et al* (1998) with 50mg/kg-body weight dose for 2 weeks showed alterations in parenchymal hepatic cells of rats. Attari *et al* (2000) demonstrated with 50 mg/kg/day dose given for 21 days hepatic histological lesions.

In present project the histological features of the liver and kidney carried out the evaluation of the hepatoprotection.

For the experimental work, the male albino rats of age 25 - 30 days and of weights 110 - 120 g were grouped in four (I-IV) each containing 5 rats During experimental period they were maintained in animal house providing food and water *ad libitum.*

Group I - The rats were maintained as normal rats.

Group II - These rats were treated with daily dose of 50 mg rifampicin given orally in the morning (during 08 - 30 to 10 - 00 am) for three days.

Group III - To the rats of these groups rifampicin treatment was given as described under Group II but simultaneously mandur bhasma (mg/kg body wt/day for 30 days during 8-30 to 9-00 a. m.)

Group IV - The rats that were maintained under this group were treated with mandur bhasma alone (10 mg/kg body wt/day for 30 days during 8 - 30 to 9-30 a.m.).

On completion of the experimental schedule the rats were killed under deep anaesthesia and central lobe of liver was cut into pieces (transverse in case of kidneys) and were immersed in buffered paraformadehyde and Bouin's fixative. The wax sections were prepared using routine microtechnique. Eosin + Hematoxylin and triple staining

methods were used to prepare the stained preparations (given in detail in Chapter II). The histology of liver and kidney was observed and evaluated in the following observations.

RESULTS

The histological alterations occurred in liver:

The histological alterations occurred in liver of albino rats are given in Figs. 1 to 12.

Fig.1: Centrolobular region of normal liver -

It showed hepatic cords, with clear bile canaliculi and normally distributed Kupffer cells and sinusoidal cells.

Fig.2: Periarterial region of normal liver -

This part of liver showed well arranged hepatic cords; clear bile canaculi well distributed sinusoids and well distributed Kupffer and sinusoidal cells.

Fig. 3: Centrolobular region of liver of rifampicin treated rat -

This region of liver showed widened sinusoids and hepatic cords were poorly identified. Most of the hepatocytes showed centrally placed nuclei with dense perinuclear chromatin and inter chromatin granules. The cytoplasm of hepatocytes showed vacuolar empty appearance and to one of the sides of this part of cytoplasm nuclei were attached. In some cells

Captions To Figures

Liver of Normal Rat:

Fig. 1:- Centrolobular region shows well organized hepatic cords and normal distribution of Kupffer cells and sinusoidal cells X 250

Fig. 2:- Perarterial region shows well organized hepatic cords and normal distribution of Kupffer cells and sinusoidal cells X 250

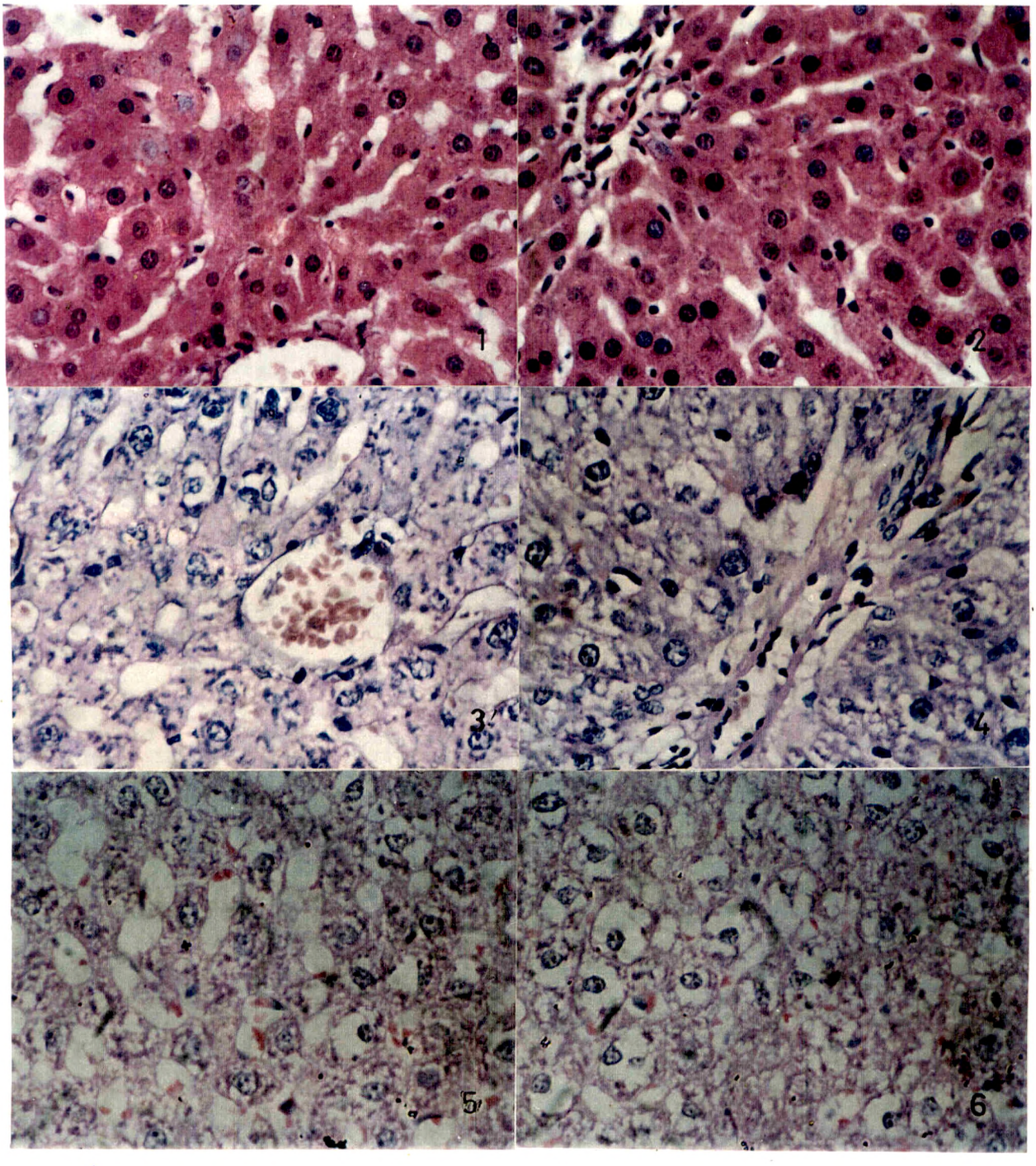
Liver of Rifampicin Treated Rat

Fig. 3:- Centrolobular region shows necrotic cells and sinusoidal cell distribution X 250

Fig. 4:- Periarterial region shows necrotic cells poor distribution of Kupffer and sinusoidal cells X 250

Fig. 5:- Transit zone of liver in the vicinity of centrolobular region exhibits large number of necrotic cells X 250

Figure 6. Transit zone in the vicinity of periarterial zone demonstrates large number of necrotic cells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Captions To Figures

Liver of rifampicin + Mandur Bhasma Treated Rats

Fig. 7:- Centrolobular region shows normal cells and some degenerating cells X 250

Fig. 8:- Transit zone between centrolobular and periarterial regions. Note most of the normal cells. Few shows vacuoles in the cytoplasm-the sign of degeneration X 250

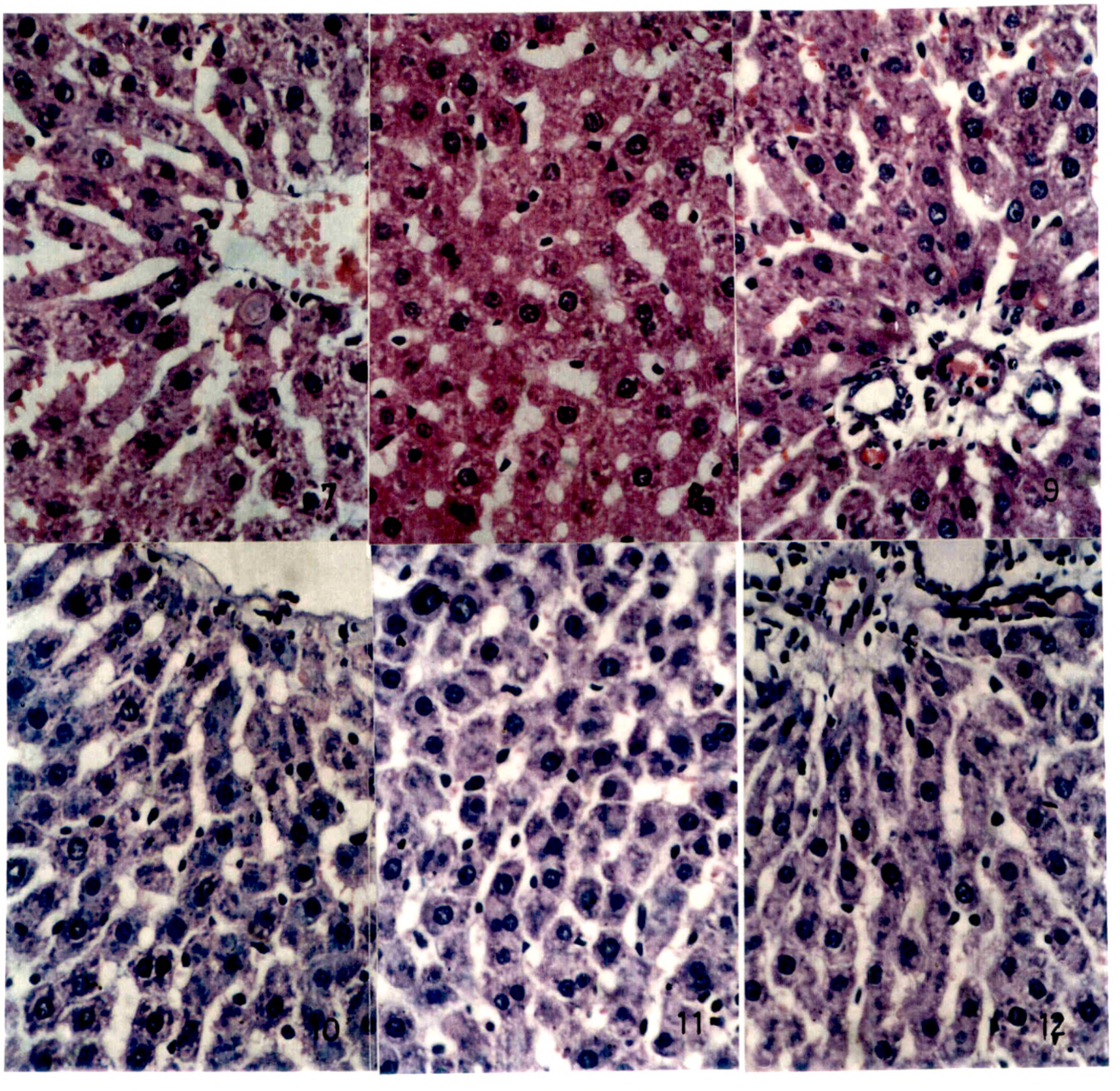
Fig. 9:- Periarterial region shows normal cells X 250

Liver of Rifampicin + Mandur bhasma Treated Rat

Fig.10:- Centrolobular regions shows distinct hepatic cords with normal hepatocytes and normal distribution of Kupffer cells and sinusoidal cells X 250.

Fig. 11:- Transit zone between centrolobular and periarterial regions exhibits healthy hepatocytes in clear hepatic cords. Distribution of Kupffer and sinusoidal cells is normal X 250

Fig. 12:- Periarterial region with normal hepatocytes and other histological architecture X 250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Captions To Figures

Kidney of Normal Rats

Fig. 13:- Cortex region shows Glomerulus Bowman's capsule X 250

Fig. 14:- Cortex region shows normal proximal and distal tubules X 250

Fig. 15:- Normal collecting ducts X 250

Fig. 16:- Inner medulla shows collecting duct in cross sections X 250

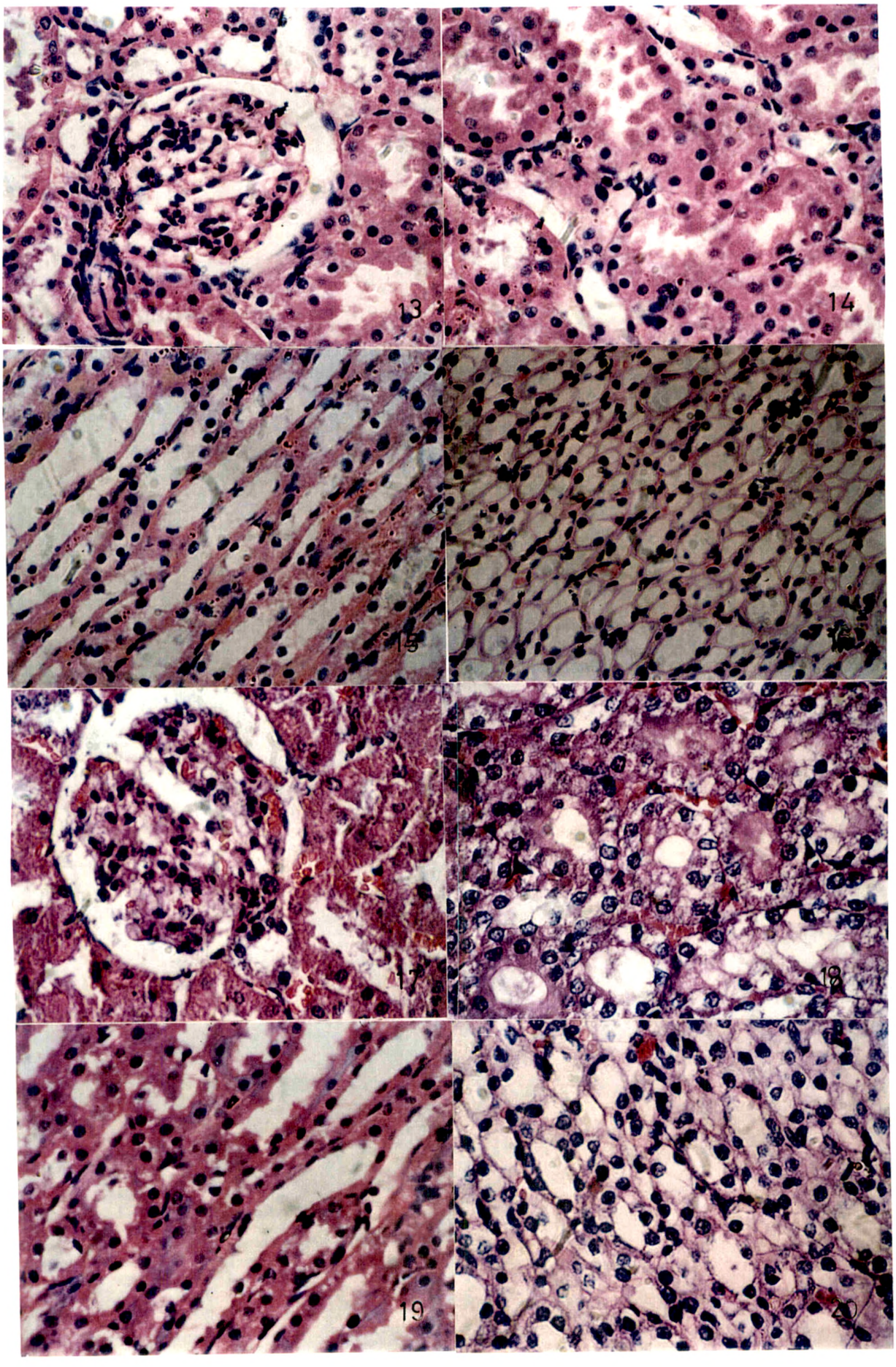
Kidney of Rifampicin Treated Rats

Fig, 17:- Cortex region note swollen glomerulus with blood filled capillaries dilated Bowman' capsules surrounded by blood filled capillaries X 250

Fig. 18:- Cortex region shows vacuolated cells in proximal and distal tubules. Note the blood in interspersed capillaries in tubules X 250

Fig. 19:- Outer medulla exhibits few vacuolated cells and few foggy cells in collecting tubules X 250

Fig. 20:- inner medulla shows vacuolated cells in proximal and distal tubules. Some tubules appear swollen. Capillaries show blood X 250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Captions To Figures

Kidney of Rifampicin + Mandur Bhasma Treated Rats

Fig. 21:- Cortex region with normal glomerulus and Bowman's capsule. Capillaries did not show stagnant blood X 250

Fig. 22:- Cortex region shows normal distribution proximal and distal tubules X 250

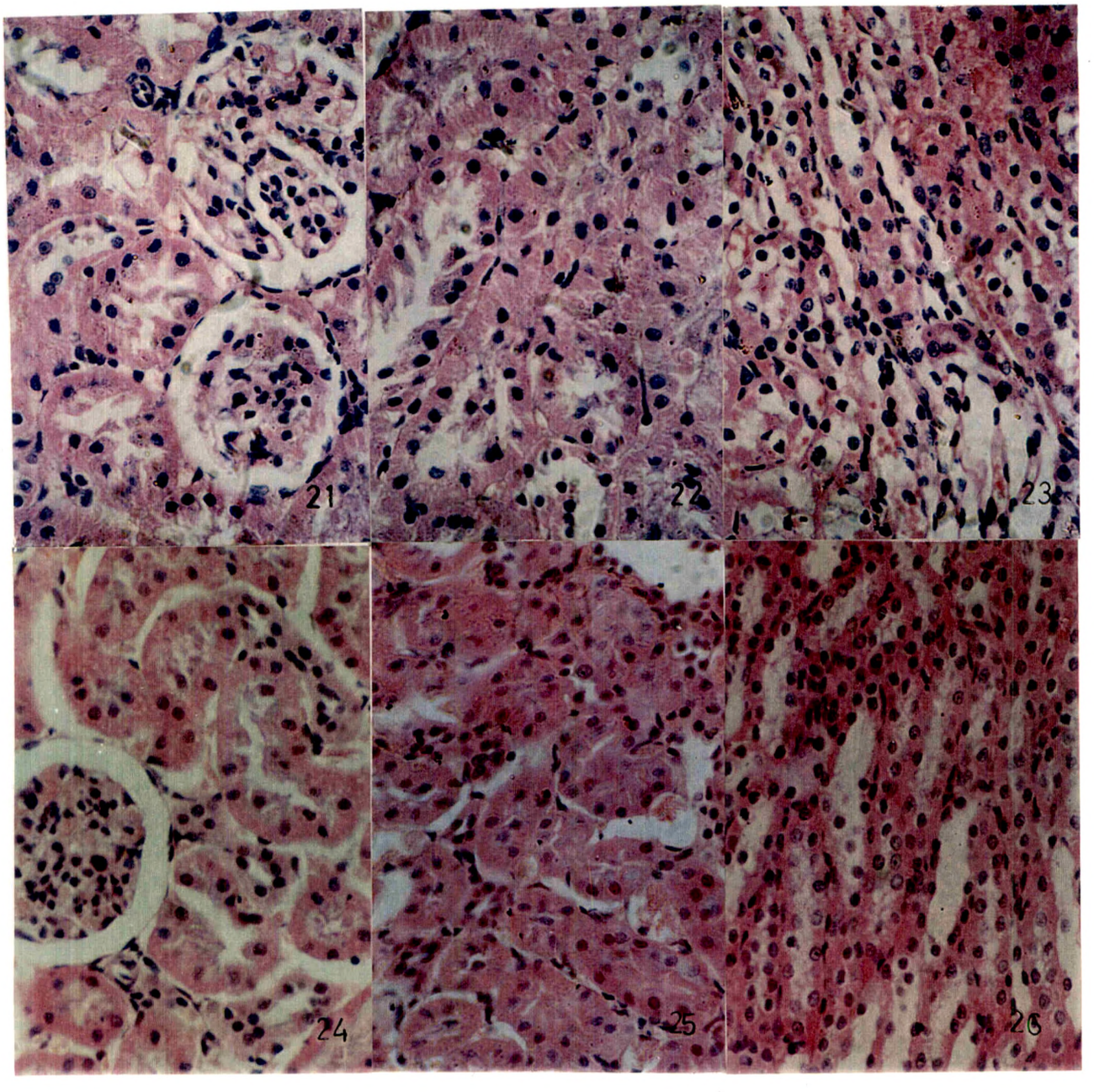
Fig. 23:- Medulla region demonstrated collecting tubules in cross sections as well as longitudinal sections X 250

Kidney of Mandur Bhasma Treated Rats

Fig. 24:- Cortex region with glomerulus and Bowman's capsule showing normal appearance X 250

Fig. 25:- Cortex region having normal proximal and distal cells X 250

Fig. 26:- Medullary region with normal collecting tubules.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cytoplasmic strands were observed from nuclear periphery and in some in the intact region of cytoplasm basophilic granules were identified. Bile canaliculi were blocked. Sinusoidal and Kupffer cells were larger in appearance as compared to respective normal cells.

Fig. 4: Periarterial region of liver of rifampicin treated rat -

In periarterial region of liver of rifampicin treated rat the alterations were more prominent than that observed in centrolobular region except dilated sinusoids.

Fig. 5: *Transition region near to centrolobular zone of liver of rifampicin treated rat -*

The nuclei of the cells of intermediate to centrolobular zone and periarterial zone of liver of rifampicin treated rat were dilated and showed reduction in hematoxylin staining. While few of the cells had dense and small nuclei. In few of the cells the nuclei were supported peripherally by a thin rim of cytoplasm extending through the cytoplasmic strands into the peripheral area of cytoplasm of the cell. The sinusoids were dilated. Number of Kupffer cells was considerably reduced. Sinusoidal cells showed foggy appearance. The normal distribution of Kupffer and sinusoidal cells is disturbed and reduction in their number was conspicuous.

Fig.6: *Transition region near to peri centrolobular zone of liver of rifampicin treated rat -*

No hepatic cords were identified in the region away from immediate peri centrolobular zone. Many large hepatocytes exhibiting empty cytoplasmic area. Nuclei were attached to one of the sides of the cells through cytoplasmic strands. In nuclear staining internuclear granules were large. Some of the cells showed small intense nuclei attached to the sides of the cell. Cellular mass was so distributed that there was difficulty in identifying the sinusoids that were identified by the presence of weakly stained erythrocytes. Few of the sinusoidal cells that were identified were large and foggy in appearance. Kupffer cells were also foggy in appearance.

Fig.7: *Centrolobular region of Rifampicin + Mandur Bhasma treated rat -liver.*

The centrolobular region showed highly perforating sinusoids. The histological appearance of hepatocytes was normal and few of the cells showed few empty spaces in cytoplasm. The hepatic cords were identified. The distribution of sinusoidal cells and Kupffer cells appeared normal.

Fig.8: *The transition zone between centrolobular and periarterial region. of Rifampicin + Mandur Bhasma treated rat -liver.*

Most of the cells in the region were normal and sinusoids were also normal. Similarly distribution of sinusoidal and Kupffer cells. Few of the cells showed small emptied spaces distributed within cytoplasm.

Fig. 9: *Periarterial region of Rifampicin + Mandur Bhasma treated rat -liver.*

Well-traversed sinusoids were also evident in periarterial region of liver as in centrolobular region. Majority of the cells showed normal appearance.

Fig. 10: *centrolobular region of Mandur Bhasma treated rat-liver.*

Normal histological structure of liver was observed.

Fig.11: *Transition zone between centrolobular Region and periarterial Region of Mandur Bhasma treated rat-liver.*

The picture showed normal histological architecture but few of the hepatic cells showed small vacuoles distributed in the cytoplasm.

Fig.12: *Periarterial Region of Mandur Bhasma treated rat-liver.*

The histological architecture of this region was normal.

The histological alterations in rat kidney :

The changes in the histology of kidney of rats treated with rifampicin and rifampicin + mandur bhasma are given in Figures 13 to 26.

Fig. 13: *Normal kidney - Glomerulus and Bowman's capsule-*

The normal appearance of glomerular region and Bowman's capsule is also evident

Fig. 14: *Proximal and distal tubules of Normal kidney-*

Proximal and distal tubules exhibited normal nuclear staining.

Fig. 15: *Collecting ducts of normal rat kidney-*

Collecting ducts of normal rat kidney were normal in apearance.

Fig. 16: *Central Medullar area of normal kidney-*

Medullar area of normal kidney showed normal nuclear staining and clear lumina.

Fig. 17: *The kidney of rifampicin treated rat -*

Oral treatment of rifamicin to male albino rats caused the alterations in the appearance of glomerulus and Bowman's capsule, proximal and distal tubules around Bowmasn's capsule. Nuclear staining of cells was distinctly reduced. In some cells nuclei were small and dense. The glomeluar cells showed the empty spaces. The nuclei of glomerular cells were stained densely and dilated. The glomerulus and the surrounding tubular region showed dilated capillaries filled with RBCs. Phagocytotic cells could be identified in inetrtubular zones

Fig. 18: *Proximal and distal tubules in inner cortex of kidney of rifampicin treated rat -*

Proximal and distal tubules in inner cortex region away from the glomerular region exhibited turgid appearance of tubules with distinct lumina. The cells of tubules showed many empty spaces. Most of the nuclei were dilated and showed intensely stained perinuclear and intranuclear granules. Numerous scavenger cells were evident with their darkly stained appearance with amoeboid nature.

Fig. 19: *Collecting ducts in inner cortex of kidney of rifampicin treated rat -*

Collecting ducts also did not exhibit normal architecture. Many cells showed empty cytolpasmic areas while distinct number of cell were foggy in appearance, however nuclear staining was not affected. Nuclei were intensely stained.

Fig. 20 : *the tubules of inner medullar region of kidney of rifampicin treated rat -*

The cells of the tubules of medullar region exhibited enlarged nuclei. Few of the nuclei were empty centrally and the staining was predominant in perinuclear chromatin. Intranuclear granules became distinct and were stained intensely. Scavenger cells were identified by their intense stain and amoeboid nature.

Fig. 21 *Glomerulus and Bowman's capsule region of kidney of rifampicin mandur bhasma treated rats -*

Kidney of rifampicin + mandur bhasma treated rats demonstrated protection of histological architecture of kidney. Glomerulus, Bowman's capsule in the outer cortex, proximal tubules and distal tubules in outer cortex and inner cortex and medullar ducts retained their normal structure.

Fig. 22 *Proximal and distal tubules of kidney of rifampicin + mandur bhasma treated rats -*

Normal appearance of proximal and distal tubules is evident in the histological architecture.

Fig. 23 *Medullar region of kidney of rifampicin + mandur bhasma treated rats-*

The microphotograph is taken in transit zone of internal medulla and the exterior of the medullar zone. The tubules in exterior zone are nearly normal but few of them show some cells with empty spaces surrounding the nuclei. There are hardly any foggy basophilic contents in cells. The internal medullar region on the left side of the picture is also nearly normal except few cells that show empty area surrounding nuclei.

Fig. 24 *Glomerus and Bowman's capsule region in kidney of mandur bhasma treated rats-*

Kidney structure was not altered in outer and inner coretx and Glomerus and Bowman's capsules were normal in histological appearance.

Fig. 25 *Proximal and distal tubules of kidney of mandur bhasma treated rats -*

Proximal and distal tubules of kidney were normal in many tubules lumen was not distinct as observed in inner cortex but appearance of cells was distinctly normal in medulla by the treatment of only mandur bhadsma.

Fig. 26 *Medullar region of kidney of mandur bhasma treated rats-*

Outer medullar ducts collecting ducts showed normal histological picture, as it is evident from the picture. Internal tubules were also normal and are not included here. They had also shown normal histological picture in our earlier studies also (kanase *et al*, 1998).

DISCUSSION

Rifampicin treatment showed distinct necrosis in centrolobular region. The cells showed large empty areas around nuclei. Nuclei were supported by the extended thin extensions of peripheral cytoplasm (Type I Hepatocytes) indicating necrotic condition of the cells. The cells without such areas exhibited dilated nuclei with distinctly stained perinuclear chromatin with clear nucleoplasm (Type II hepatocytes). Cytoplasm showed

Table 1 - Differnital distribution of hepatocytes in rat liver in various experimental groups

No.	Group	Normal cells	Necrotic cells Type-I	Stressed cells Type-II	Less stressed/ recovery	Cells with shrunken nuclei
	Normal Rat					
	I) Centrolobular region	93.51	-	-	-	6.49
1	II) Centrolobular transit zone	95.23	-	-	-	4.73
	III) Periarterial region	91.00	-	-	-	9.00
	IV) Transit zone	93.00	-	-	-	7.00
	Rifampicin treated Rat					
	I) Centrolobular region	-	76.00	14.50	-	9.50
2	II) Centrolobular transit zone	-	78.41	9.09	-	-
	II) Periarterial region	-	90.00	5.00	-	5.00
	IV) Transit zone	-	94.00	4.00	-	2.00
	Rifampicin + Mandur Basma treated Rat					
	I) Centrolobular region	83.84	6.60	-	7.00	2.50
3	II) Centrolobular transit zone	80.70	-	-	17.00	2.30
	III) Periarterial region	91.45	6.10	-	-	3.45
	IV) Transit zone	9.45	7.00	-	-	2.55
	Mandur Bhasma treated Rat					
	I) Centrolobular region	98.56	-	-	-	1.44
4	II) Centrolobular transit zone	98.89	-	-	-	1.11
	III) Periarterial region	97.50	-	-	-	2.50
	IV) Transit zone	98.12	-	-	-	1.88

Table 2 - Distribution of Kupffer and sinusoidal cells per 100 hepatocytes in microscopic field

No.	Group	Kupffer cells	Sinusoidal cells
	Normal Rat		
	IV) Centrolobular region	11.00	20.00
1	V) Centrolobular transit zone	12.00	18.00
	VI) Periarterial region	11.00	18.00
	IV) Transit zone	12.00	17.00
	Rifampicin treated Rat		
	I) Centrolobular region	12.50	14.20
2	II) Centrolobular transit zone	8.35	8.35
	II) Periarterial region	2.00	5.00
	IV) Transit zone	8.00	8.00
	Rifampicin + Mandur Basma treated Rat		
	I) Centrolobular region	6.00	7.50
3	II) Centrolobular transit zone	14.95	16.10
	III) Periarterial region	8.05	10.35
	IV) Transit zone	2.50	8.80
	Mandur Bhasma treated Rat		
	I) Centrolobular region	7.77	8.88
4	II) Centrolobular transit zone	1.11	11.10
	III) Periarterial region	2.50	8.88
	IV) Transit zone	1.25	8.00

lumpy foggy basophilic granules interrupted with unstained areas. Sinusoidal and Kupffer cells were large and intensely stained. In periarterial region type I cells were large in number as compared to centrolobular zone. Trafficking scavenger cells were more crowded as compared to centrolobular zone, but they were more frequent in the vicinity of artery. The transit zones between centrolobular and periarteial zone exhibited two distinct areas i) rich in type I cells and ii) Type II cells. Zone rich in type II cells was perforated by numerous distinct sinusoids, while in zone rich type I cells sinusoids were not distinct, although their intermingled appearance could be identified. In both these zones sinuusoidal and Kupffer cells wherever identified exhibited foggy appearance. All these observations are clear indications of cellular necrosis. Type I cells may be in advanced necrotic condition (or totally necrotic cells). On the contrary type II cells may be late initiating stages of necrosis (or stressed cells). The cytoplasmic empty spaces are the remanant area of accumulated fat from the hepatocytes as it is known that refampicin causes the fatty liver (Khedun *et al*, 1992). The sinusoids and their appearance indicate stagnancy of blood. This may be the consequences of rifampicin toxicity and/or cause of rifampicin toxicity.

Simultaneous treatment of mandur bhasma showed normal appearance of sinusoidal spaces in centrolobular region. Many normal hepatocytes and type II hepatocytes could be identified in hepatic cords. The picture was more normal in periarterial zone. Only single type of intermediate zone between centrolobular periarterial zone was identified. It

showed normal histological picture, but little number of cells showed vacuoles in the cytoplasm. Distribution of Kupffer cells and sinusoidal cells was distinct.

Treatment of only mandur bhasma demonstrated healthy appearance in all three zones of the liver. Hepatocytes were normal small number of basophilic granules were observed in healthy cytoplasm.

For the quantitative analysis of the histological elements of the liver; the liver histology was considered in the four histologically identifiable zones 1) Centrolobular region 2) Centrolobular region associated transition zone 3) Periarterial region 4) Periarterial region associated zone. The cells identified with their morphological feature as normal hepatocytes, hepatocytes under stress (Type II), necrotic hepatocytes (Type I), partial necrosis showing cells (Cells described under Fig.11 or less stressed cells) with few vacuoles and cells with shrunken nuclei. This differential count of hepatocytes from the livers of the experimental animals is given in Table No 1.The analysis of the count indicates that; in normal liver the normal hepatocytes varied between 91 - 95 % in zones studied, lowest being in periarterial region and highest being in centrolobular region. Shrunken nuclei showing cells occurred in the range of 5 - 9 % highest being in periarterial region and lowest being in centrolobular region.

In rifampicin treated rats normal liver cells were absent after one month of the treatment. The necrotic cells in advanced condition ranged

between 76 - 90 % and highest were in periarterial region associated transitory zone and lowest in the centrolobular region; while stressed cells were in the range of 4 - 14.50 %. Highest number was noted in the centrolobular region and lowest number was noted in the periarterial region. Cells with shrunken nuclei were hardly distributed in centrolobular associated transitory zone but were 9.5 % *i.e.* highest in number in centrolobular region while they were lowest in number in periarterial region associated transition zone. In the rifampicin + mandur bhasma treated rats necrotic cells were hardly noted in centrolobular region but 17 % less stressed cells, 2.3 % Cells with shrunken nuclei and remaining normal cells were observed. The normal hepatocytes in this region were in the range of 80.70 - 91.45 % highest being in periarterial region and lowest being in the centrolobular associated transit zone. Only mandur bhasma treated rats showed 97.5 - 98.89 % normal hepatocytes with equal distribution in all the zones. Similarly the cells having shrunken nuclei were ranged between 1.11 - 2.5 % showing large number of cells in periarterial region.

The distribution may be the effect of the zonal distribution of metabolites since such zones are reviewed in the liver (Oinonen and Lindras, 1998) where the organelle distribution within the cell is varied so also the distribution of the metabolic content. The zonation is extended in an ascending /descending gradients from the portal to the central vein within the acinus the microcirculatory unit (Gumuco and Chinchale, 1988 and Gebhardt, 1992). The normal liver did not showed stressed or necrotic cells

but cells with shrunken nuclei were identified other cytoplasmic observations. It ranged within difference of 5% and may be associated with the metabolic stress. Cell renewal is the specific feature of the liver (Roullier, 1963; Guyton, 1992). Rifampicin toxicity resulted in the necrotic cells that were in high number in periarterial associated transitory zone and periarterial region. This is highly affected area may be due to decreased oxygen content which may have further decreased due to the stagnancy observed in sinusoidal flow of the blood in periarterial region associated zone. Shrunken nuclei containing cells were increased in centrolobular region than the normal liver. Stressed cells are also in high number in this region. The differential distribution of hepatocytes shows the clear indication of protection of hepatocytes by mandur bhasma in rifampicin + mandur bhasma treated rats. Only mandur bhasma treatment had raised healthy normal hepatocyte count to 98.89 % and varied in the range of 1.5 %. Even cells with shrunken nuclei were also decreased to 1/4th in higher range 1/5th in lower range. The distribution of sinusoidal and Kupffer cells showed remarkable decrease in their distribution in the all the regions of the liver. On rifampicin + Mandur bhasma treatment the distribution was further reduced in centrolobular region, their number being nearly half. But it doubled in transitory zone near centrolobular region. In periarterial region only sinusoidal cell- distribution was marginally high but not that of Kupffer cells. Kupffer cells were also decreased in the region of transition nearer to periarterial zone. The roles of liver sinusoidal cells are involved in pinocytosis and their lysosomal system of these cells is also well - developed

(Hinton and Grasso, 1993). Kupffer cells are the fixed macrophages, which are found within the sinusoids. They attach both to the endothelial cells and hepatocytes through the fenestration of endothelial cells. They are active phagocytes and contain an elaborated system of lysosomal enzymes and play active role in bile pigment formation as well as in removal of degeneration products and degradation of other waste materials and they are also known to secrete hepatocyte growth factor (Matsumoto and Nakamura, 1992). In present observation the roles of both the sinusoidal cells and Kupffer cells seems to be affected by their decreased distribution inperiarterial region, transit region near centrolobular region and also in the transition region near periarterial region on treatment of rifampicin; while, simultaneous treatment of mandur bhasma protected the liver to maintain the histological architecture of liver; during which the distribution of both the trafficking cells was nearly normalised in the transition zone that was near to the centrolobular zone and was increased but was not fully normalised in periarterial region. The role of the protected metabolisms in hepatocytes may have helped them by reduction in degenerative processes. The distribution of these drugs also indicated what chung *et al,* (2000) found. They reported that irrespective of the cause / s of pathological cell deaths - the necrotic-like deaths required the same set of engulfment genes' expressions that is used in programmed cell death. (This idea would fit neatly with the known ability of mammalian scavenger receptors such as SR-RB and CD36 to bind anionic phospholipids such as PS though may be the signals of two deaths differ altogether (Zhou *et al,* 2001). Mandur

bhasma is derived basically from iron ore and may be the final form of the processed iron in mandur bhasma that seems to be involved in protective metabolisms. The different treatments used in the preparation may have selectively converted iron into biologically active form that can take part in metabolic protection of liver. Similarly the liver originated wastes as well as other waste products affected the kidney adversely as it is reflected in histological alterations similarly in mandur bhasma mediated protective changes it is totally protected indicating its waste clearance may have shared by biliary system or reduced in the protective processes.

The histolgical alterations in kidney on administration of rifampicin orally indicated the degenerative changes in glomerular epithelium and the dilated capillaries are indicators of the stagnancy of blood and slow filtration. The bulged proximal and distal tubules are also indications of stagnancy and impairment fluid clearance, as a result some of the cells are showing degenerative changes in early and advanced stages of degeneration as light microscopic observations showed in proximal and distal tubules and also in medullar tubules in inner and outer medulla. The alterations may be the results of metabolism of rifampicin, accumulation of rifampicin leading to altered functioning of kidney resulting into the partial glomerular and tubular necrosis. These alterations were counter acted by the simultaneous treatment of mandur bhasma. The stagnancy of blood was eased so also the dilation of tubular regions and associated lowering in the necrotic cells. As shown mandur bhasma had not altered the normal histological architecture

of the kidney. Since the liver histology is protected so also the functioning (Chapter I) that may have given the relief to the kidney filtration and hence total histological protection was noted. The most of the antibiotic load may have driven through the bile pathway and in rat continuously which may have also helped to clear the kidneys.