INTRODUCTION AND REVIEW OF LITERATURE

The liver is the largest gland in the body. It is both an endocrine and exocrine gland, releasing several substances directly into the blood stream and secreting bile into a duct system *(Williams and Harry, 1994).*

Normally the liver has a minimal content of connective tissue in its interior. It presents a remakrably uniform appearance and structurl subunits which are not easily identifiable. It is possble, however, to detect a repeating pattern of roughly polygonal areas, in which fenestrated plates of parenchymal cells (hepatocytes) are arranged radially arround a central vein. At some corners of these polygonal areas, there are small triangular areas of connective tissue (the portal areas) enclosing a branch of the portal vein, a branch of the hepatic artery, a small branch of the bile duct and a lymph vessel. The branch of the portal vein is wide and thin walled. That of the hepatic artery is narrower, thicker-walled delivering blood to the sinusoids between the hepatocytes. The small branch of bile duct, recognizable by its lining of simple cuboidal epithelium carrying bile towards the porta hepatis. The thin walled lymphatics drain lymph in the same direction *(David, 1993).* These polygonal units were called the hepatic lobules and for a century were considered to be the structural and functional units of the liver. They are now commonly referred to as the classical lobules *(Ham and Cormack, 1987).*

The portal lobule is another type of hepatic subunits. It consists of the mass of parenchyma around each portal area, including all cells

The transaminases, glutamyl transpeptidase and alcohol dehydrogenase enzymes are predominant in zone 1 hepatocytes, whereas NADH and NADPH reductases and esterases are more abundant in zone2. Functional changes accompany zonal differences, such as bile acid uptake by zone 1 hepatocytes and drug detoxification by zone 3 hepatocytes ***(Popper and Schafner, 1963).***

About 80% of liver volume and 60% of its cells are hepatocytes. They are polyhedral, with five to twelve, sides and are from 20-30 μm across. Their nuclei are spheroidal and euchromatic and often polyploid or multiple 2 or more in each cells) ***(Doljansky, 1960).*** Their cytoplasm typically displays much granular and agranular endoplasmic reticulum many mitochondria, many lysosomes and well developed Golgi bodies, features indicating a high metabolic activity. Glycogen granules and lipid vacuoles are usually prominent ***(Loud, 1968).*** The plasma membrane has a triple-layered structure (unit membrane) about 10 nm in width and consisting of two dense outer layers and a light intermediary one. Functionally it differentiates into : sinusoidal, bile canalicular and lateral surfaces ***(Tanikawa, Egnchi and Ikejiri, 1979).*** Both sinusoidal and bile canalicular surfaces have numerous microvilli, about 0.5 - 1.0μm in length and 0.1 μm in diameter which effectively increase the surface for uptake or secretion ***(MacSween and Scothorne, 1979).*** Many coated and non-coated vesicles, referred to as pinocytotic vesicles, are usually observed in the cytoplasm along the sinusoidal surfaces. The lateral surface is usually straight and smooth, but occasionally protrudes into the opposed surface of the adjacent hepatocyte. The hepatocyte adheres to adjacent cells by means of tight and gap junctions and desmosomes ***(Goodenough, 1972).*** The previous three types form the junctional complex (terminal bar), limit

the bile canalicular lumen and form a seal between the bile canaliculus and space of Disse *(Tanikawa and Ikejiri, 1977).*

The nucleus is round or oval in shape and about 10 μm in diameter, but its size may vary from cell to cell. It is central in position and vesicular with a few scattered chromatin clumps and one or more nucleoli. Most cells have single nuclei, but about 25% are binucleated or with more nuclei. The electron microscopic picture shows the nuclear envelop with its outer and inner membranes [about 7 nm in thickness] separated by a space of 10-15 nm (perinuclear space). The outer nuclear membrane is studded with ribosomes, but the inner one is smooth. In many places, the outer and inner membranes are fused with each other, forming nuclear pores about 70 nm in diameter. The chromatin in the hepatocyte nucleus is represented by ill defined masses of fine filaments or granules of moderate density. Somewhat larger granules 300 nm in diameter, called perichromatin granules are located near the masses of chromatin. They are usually surrounded by a clear zone of about 25 nm *(Watson, 1962).* Interchromatin granules from another type of nulcear granules, 20-30 nm in diameter and distributed in the interchromatin spaces *(Ma and Biempica, 1971).* The nucleolus is the site of ribosomal RNA synthesis and it is about one μm in diameter. Multiple prominent nucleoli are usually present. They disperse and disappear during cell division but reapear in the telophase of mitosis. The nucleolus is usually basophilic when stained with hematoxylin and eosin. As seen with the electron microscope, the nucleolus consists of 3 distinct components. The first component is one to several pale-staining regions, composed of nucleolar-organizer DNA-sequences of bases that code for rRNA. Closely associated with the nucleolar organizers are densely packed 5 to 10-nm ribonucleoprotein

fibers, the pars fibrosa. The pars fibrosa is composed of primary transcripts of rRNA genes. The third componenet of the nucleolus is the pars granulosa, consisting of 5- to 20- nm granules, which are maturing ribosomes. The pars fibrosa and pars granulosa form a thread like structure called the nucleolonema by light microscopists, the pale staining nucleolar-organizing region has been termed the pars amorpha. Heterochromatin (nucleolus- associated chromatin) is often seen attached to the nucleolus, but the functional significance of this association is not known ***(Bruni and Porter, 1965).*** Nucleoplasm is an amorphous matrix that fills the space between the chromatin and the nucleoli in the nucleus. It is composed mainly of proteins, metabolites, and ions.

Extraction of nuclear nucleic acids shows that nucleoplasm contains continuous fibrillar structure called nuclear matrix. It binds to hormone receptors and to recently synthesized DNA ***(Blom and Fawcett, 1994).***

Mitochondria are usually round, oval or oblong in shape, 0.5 - 1μm wide and can attain a length of up to 16 μm. Each is composed of a homogenous matrix of moderate electron density surrounded by a double membrane, outer and inner. Both membranes are about 0.5 nm in thickness and separated from each other by a narrow intermembranous space of about 7-10 nm in width. The inner membrane is characterized by a number of folds known as a cristae that project into the interior of the organelle. A small number of electron dense granules can be observed in the matrix and considered as accumulations of ions such as calcium and magnesium ions required for the operation of the mitochordrial enzyme system ***(Peachy, 1964 and Sutfin, Holtrof and Ogilive, 1971).*** Crystalline inclusions in the mitochondrial matrix, often observed in human diseased liver, are rarely

seen in normal human liver *(Willis, 1965).* DNA filaments about 30-50 nm in width are noticed *(Nass and Nass, 1963).* RNA containing granules (mitochondrial ribosomes) were observed in the mitochondria, suggesting that they may serve as an extra nuclear genetic system or may have a self replication system. There is a multi-enzyme system for the manufacture of ATP in the mitochondria. The mitochondria in hepatocytes are usually located very close to rough endoplasmic reticulum, near the nucleus and along the sinusoidal surface, suggesting a functional relationship among those organelles *(Lesson, et al., 1990).*

Within the hepatocytes is a complex network of cisterns, saccules, tubules and vesicles that constitute the endoplasmic reticulum. It is markedly convoluted but its membrane is thought to form a continuous sheet *(Rohr, Luthy, Gudat, Oberholzer, Gysin and Bianchi, 1976).*

There are two components, the rough endoplasmic reticulum (RER) and the smooth endoplasmic reticulum (SER). The RER is distributed mainly around the nucleus in close association with mitochondria and toward the vascular and biliary poles. It tends to be arranged in parallel stacks. Its outer surface is studded with ribosomes. It is the site of protein synthesis especially that for secretion *(Palade, 1975).* Ribosomes are small electron dense particles about 15-30 nm in diameter. Two forms of ribosomes, one being attached to RER and the other free in the cytoplasm, are observed and consist of rRNA and ribosomal protein. They are the site where amino acids are incorporated into proteins *(Novikoff and Essner, 1960).* Each ribosome consists of a large and a small subsunits *(Florendo, 1969).* Several ribosomes aggregated and attached to strands of messenger RNA to form polysomes, which are usually in the spiral form. The free

ribosomes synthesize the structural proteins of the cell itself, a process particularly active during development and regeneration *(Redman and Sabatini, 1966).* In case of attached ribosomes, the large subunits are the site of attachment. The attached ribosomes have been demonstrated to synthesize the secretory proteins *(Redman, 1969 and Schwartz, 1969),* which are transmitted to cisternal lumen of RER through channels in the larg subunits then via the smooth endoplasmic reticulum to the Golgi apparatus to be discharged at the sinusoidal surface by exocytosis. This traditional view, full established for other protein secreting glands, has been disputed for the liver by *Lin and Change (1975),* The RER cooperate with the smooth one in the synthesis of enzymes associated with lysosomes and peroxisomes which remain within the cell *(Orlandi and Koch, 1975).*

Smooth endoplasmic reticulum (SER) cisternae are more tubular and more likely to appear as a profusion of inter connected channels of variable shape and size. SER membranes arise from rough endoplasmic reticulum *(Feldman, Swarm Becker, 1980).* It is abundant in liver cells, where it is responsible for the oxidation, conjugation, and methylation processes employed by the liver to neutralize or detoxify certain hromones and noxious substances, such as alcohol and insecticides. SER is also involved in the breakdown of glycogen in the liver cells *(Macsween and Scothorne, 1979).* SER is usually observed in the peripheral region of the cytoplasm, and occasionally adjacent to the glycogen area *(Karrer, 1961; Porter, 1961 and Cardel, 1971).*

Peroxisomes (microbodies) are ovoid membrane bounded granules, and were renamed to reflect their role in the metabolism of hydrogen

peroxide *(DeDuve and Baudhuin, 1966).* Peroxisomes, occupy 2% of total hepatocellular volume. They are limited by a single membrane and their matrix is finally granular. Peroxisomes may occur randomly within the cell but their membranes are usually intimately associated with the membranes of the adjacent SER *(Sternlieb and Quintana, 1977).* Peroxisomes conain a variety of oxidases and a large quantity of the catalaze. The oxidases are capable of oxidizing a number of substrates to form hydrogen peroxide. The catalazes mediate decomposition of peroxidase to water. The enzymes of those peroxisomes which remain attached to the endoplasmic reticulum circulate within the endoplasmic reticulum to detoxify accumulating H_2O_2 *(Rods, Rassat and Themann, 1981 and Hinkle, 1982).* Peroxisomes protect the cell from the effects of hydrogen peroxide, which could cause irreversible damage to numerous important cellular constituents *(William and Harry, 1994).*

The term microperoxisomes was coined fro peroxisomes which are small in size, devoid of a nucleoid, and are attached to the endoplasmic retciulum *(Novikoff, Novikoff, Quintana and Daves, 1973 and Novikoff, 1982).*

The Golgi apparatus consists of three to five parallel flat saccules or cisternae, each having one convex and another concave surfaces, with numerous vesicles and vacuoles beside it. The convex surface represents the forming face, where materials synthesized by ER are transferred into the Golgi apparatus by vesicles. The concave surface represents the maturing face from which materials are released into cytoplasm in a packed form. The Goligi apparatus in hepatocytes concentrates and transfers the secretory products, such as the low density lipoprotein *(Stein*

and Stein, 1967) and albumin *(Glumann and Ericsson, 1970)* toward the cell surface facing the space of Disse. Glycoprotein formation by the addition of carbohydrate to protein seems to occur in such apparatus. It is usually observed adjcent to the bile canaliculi and occasionally near te spaces of Disse and the nucleus. So, participation of the Golgi apparatus in bile excretion has been suggested *(Beams and Kessel, 1968 and Schachter, Jabbal, Hudgin and Pinteric 1970).*

Lysosomes are vesicles bounded by a single membrane, that contain a large variety of hydrolytic enzymes whose main function is intracytoplasmic digestion *(Essner, 1961).* Lysosomes are usually spherical, range in diamter from 0.05 to 0.5 μm. Lysosomal enzymes are synthesized and segregated in the rough endoplasmic reticulum and subsequently transferred to the Golgi complex, where the enzymes are modified and packed as lysosomes *(Hopsu-Havu, Arstila, Helminen and Kalime, 1967).* Lysosomes that have not entered into a digestive event are identified as primary lysosomes. They can be very small (0.05 μm in diamter) membrane limited vesicles with a clathrin coat. Secondary lysosomes are those in which digestion is occuring. They are generally 0.2 - 0.5 μm in diameter and, in electron micrographs, present a heterogenous appearance owing to the wide variety of materials they may be digesting. Following digestion of the contents of the secondary lysosomes, nutrients diffuse through the lysosomal limiting membrane and enter the cytoplasm. The remaining undigestable compounds are retained within the vacuoles, which are now called residual bodies. In hepatocytes, large quantities of residual bodies accumulate and are referred to as lipofuscin or age pigment *(Hayashi, Nakajima and Fishman, 1964 and Miller and Palade, 1964).* Lysosomes can digest materials taken into the cell from its environment, a

Bile canaliculi are minute canals passing between hepatocytes. Each canaliculus runs between an adjacent pair of cells. They are limited by only the plasma membranes of hepatocytes which have a small number of microvilli in their interior ***(Yamamato and Phillips, 1984).*** They form a complex anastomosing network progressing along the plates of the liver lobule and terminating in the region of the portal canals. The bile flow progresses in a direction opposite to that of the blood, i.e. from the center of the classic lobule to its periphery ***(Phillips, Oshio, Miyairi, Katz and Smith, 1982).*** Along the margins of the canaliculus the membranes of the opposing cells are fused to form an occulding junction to prevent the canalicular contents from escaping into the narrow intercellular clefts on either side ***(Oda, et al., 1974 and Yamamato, Fisher and Phillips, 1985).***

The hepatic sinusoids are larger than blood capillaries and have more irregular shape. The electron microscopy has greatly enhanced the knowledge of the structure and function of sinusoids. The hepatic sinusoidal wall consists of endothelial cells, Kupffer cells, Ito cells and possibly Pit cells ***(James, Siria, Jacqueline and Pedro, 1987).***

Endothelial cells represent 44% of the sinusoidal cell volume. There is no basement membrane. They are flattened and elongated cells provided with numerous slender, cytoplasmic projections. Gaps or fenestrae between these projections give the endothelium a very characteristic appearance, of sieve plates ***(Wisse and Deams, 1970; Wisse, DeZanger, Jacobs and McCuskey, 1983 and Wisse, DeZanger, Charels, VanDer smissen and McCuskey, (1985).*** Fenestrae permit the endothelium to function as a filtration barrier and provide communication between the sinusoids and the space of Disse ***(Wisse and Knook, 1979).*** Each

endothelial cell is loosely attached to neighbouring endothelial cells. The nucleus is usually elongated, the cytoplasm contains few mitochondria, smooth and rough endoplasmic reticul, Golgi appratus and lysosomes *(Yee and Revel, 1975).* A cardinal function of sinusoidal endothelium is the protection of the hepatocytes from the trauma of hepatic blood flow. It permits contact between hepatocytes and the plasma but excludes the cellular components of the blood, and is responsible for endocytosis of many compounds such as lipoproteins, glycoproteins, lipopolysaccharides and mucopolysaccharides *(James et al., 1987).* A characteristic of sinusoidal endothelium is the presence of pinocytotic vesicles *(Fahmi, 1982).* Kupffer cells, which occupy 33% of the hepatic sinusoids are larger than the endothelial cells. They have cytoplasmic projections called pseudopodia *(Boluin, 1977 and Arias, Popper, Schachter and shafritz, 1982).* With its great number of lyosomes and phagocytic vacuoles, the cytoplasm of Kupffer cells is similar to that of other macrophages. The most important function of Kupffer cells is phagocytosis. They also participate in various immunological reactions. The synthesis and catabolism of lipids, haemoglobin degradation and cleaning of senile erythrocytes are other functions *(Jones and Summerfield, 1982).* Ito cells (lipocytes, perisinusoidal cells, stellate cells, fat storing cells or vitamin A storing cells) are usually located in the perisinusoidal recesses within the space of Disse. They form 22% of sinusoidal cell volume. They are small in size and have a single small round to oval eccentrically placed nuclei. The most prominent characteristic is the presence of several large lipid vacuoles. They contain desmin *(Yokio, Akihisa, Kenichiro, Koh, Juncko, Harayo, Hiroyuki and Toshihiko, 1983).* They are known to play a role in maintaining the sinusoidal vascular tone. They produce reticulin fibres frequently seen in the space of Disse *(McGree and Patrick, 1972; Kent,*

Ionuye, Minik and Baha, 1977 and Leeu, McCarthy, Geerts and Knook, 1984). Pit cell is a newly described cell type. It is found in the sinusoids and displays certain ultrastructural characteristics which bear some resemblance to neuroendocrine cells. Pit cells have been rarely reported in humans. It is suggested that they may represent a variant of circulating lymphocytes, however, their significance in human liver requires further investigation *(Wisse, Vant, Noordende, Van DerMeulen and Daems, 1976).*

The perisinusoidal space of Disse separates the sinusoidal endothelium and hepatocytes. The space of Disse is usually occupied by the microvilli of heptocytes and small bundles of collagen fibers of variable length. The finely granular, slightly electron dense background observed in the space of Disse represents plasma proteins. Perisinusoidal recesses are triagnular expansions of the space between adjacent hepatocytes, and are often occupied by endothelial cell nuclei, lipoyctes or portions of Kupffer cell cytoplasm *(Meyer, Yancey and Revel, 1981).*

Fungal infection is a major and growing cause of disease throughout the world. Its incidence is increasing for a number of reasons, including a greater use of broad spectrum antibacterial and other chemotherapeutic agents *(Denning and Stevens, 1989).*

Although a number of antifungal drugs are available, there is considerable scope for improvement because most agents are not truly fungicidal, there is a danger of incomplete elimination of the infection followed by relapse *(Zaias and Serranol, 1989).*

Terbinafine (Lamisil) is a novel antifungal agent of the allylamine class. Its chemical name is E-N- (6,6- dimethy 1-2-hepten-4-ynyl) -N-methy -1-naphthalene- methenamine hydrochloride. It is the first directly fungicidal drug which is active by both the oral and topical routes ***(Rashid, 1996).*** The directly fungicidal action of terbinafine refers to its highly specific inhibitory effect on sequalene epoxidase which catalyses an early stage of fungal ergosterol an essential constituent of fungal cell membranes, as is cholesterol in mammalian cell memebranes systhesis. This leads to disruption of fungal cell membranes ***(Ryder, 1990).*** These properties are responsible for the excellent results obtained with terbinafine in clinical studies. Cures are produced with shorter courses of treatment than are needed with other drugs such as griseofulvin. In addition, follow-up of patients demonstrated that the cures are lasting, and the relapse or re-infection is unlikely ***(Julia & Diana, 1992).*** The recommended adult daily dosage o oral terbinafine is 250 mg for just two weeks for treatment of tinea corporis, four weeks to six weeks in cases of tinea pedis and may be longer up to twelve weeks in cases of onlychomycoses ***(Villaris & Jones, 1990 and De-Backer, De-Keyser, De-Vroey and Lesaffre, 1996).*** In patients with hepatic or renal dysfunction, dosage reduction is necessary. As terbinafine is secreted into breast milk, women receiving oral treatment should not breast feed. It should not be used during pregnancy and should be kept out of reach of children ***(Jensen, 1990).*** Approximately, 70 to 80% of an orally administered dose of terbinafine was absorbed from the gastrointestinal tract ***(Jensen, 1989);*** the bioavailability of the drug is not significantly affected by the presence of food ***(Nejjam, Zagula, Cabiac, Gusseous, Humbert and Lakhdar, 1995).*** Terbinafine is highly lipophilic and extensively distributed in humans ***(Schuster, Schaude, Schatz and Mieth, 1988).*** It is strongly and

nonspecifically bound to plasma proteins. Tissue distribution studies of orally administered terbinafine in the rat indicated that the highest concentrations were found in the liver ***(Back, Stevenson, Tjia, 1989).*** The drug appears to arrive at the body surface by two routes : firstly by diffusion from the vascular system through the dermis and epidermis, and secondly via sebum to hair follicles, ***(Lever, Dykes, Thomas and finlay, 1990).*** Terbinafine is extensively metabolised in the liver in both man and animals. This involves only a small fraction (< 50%) of the total cytochrome P-450 capacity of the liver. The major biotransformation pathways of terbinafine appear to be the same in humans and in animals ***(Schuster, 1985).*** Urinary excretion accounts for 80% of the dose with most of the remainder found in the faeces, 85% of the dose was recovered within 72 hours ***(Seyffer, Eichelbaum, Jensen and Klotz, 1989).*** High cure rates, rapid action, low relapse rates and excellent acceptability mean that the advent of terbinafine is an important advance in both oral and topical treatment of superficial fungal infections ***(Van Den Bossche, Willemsence, Cooles, Marichal and Lauwers, 1983).*** Clinical experience with terbinafine is limited at present and few puplished data regarding side effects are available ***(Haroon, Hussain and Mahmoud, 1992).*** In general terbinafine is well tolerated and side effects are mild to moderate and transient. The most common are gastro-intestinal symptoms (Fullness, loss of appetite, nausea, mild abdominal pain and diarrhoea) which generally appear during the first week of therapy and may be a result from the durg; delaying effect on gastric emptying and would cause distress in patients with hiatus hernia or peptic ulcer. Skin reactions or miscellaneous nonspecific symptoms such as malaise or lethargy may appear ***(Faergemann, Zehender, Jones and Maibach, 1991).*** Monitoring of the liver functions in terbinafine-treated patients revealed ocassional transient