REVIEW OF LITERATURE

The liver is the second-largest organ of the body and accessory digestive gland. In normal adult liver weighs about 1.5 kg; representing 2.5% of the body weight.[37] The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from exogenous and endogenous challenges like xenobiotics, drugs, viral infection and chronic alcoholism. It is the key organ regulating homeostasis in the body and involved in almost all biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction.[38] Majority of the reported work on hepatoprotective activity are either by oral administration or by injection of CCl_4, both are acute models. CCl_4 vapor induced method is a chronic model and long-term administration of the toxin by the experimental animals produces liver cirrhosis, which is similar to human alcoholic cirrhosis both histological and systemically.[39]

The liver has the capacity to recover from acute injury by hepatocellular regeneration with the production of new cells, which restore liver function and normal tissue structure. Acute injury is manifested as depletion of cellular energy, disturbance in ion and impaired stimulation of tissue repair. Chronic liver injury however often leads to fibrosis, scar formation and distortion of normal tissue architecture. [40]

DISEASES OF LIVER: [41]

Diseases that occur in liver are

Infections: - Viral hepatitis

Toxic: - Alcoholic liver disease, Drug-induced liver disease

Genetic: - Alagille Syndrome, Biliary Atresia, Crigler-Najjar Syndrome, Dubin-Johnson Syndrome, Gilbert Syndrome, Hemochromatosis and Wilson Disease

Immune: - Autoimmune Hepatitis, Primary Biliary Cirrhosis

Neoplastic: - Hepatocellular Carcinoma (HCC)

Hepatocellular Necrosis:

Necrosis may be classified in many ways, including location (Zonal, periportal, perivenular and so on), mechanism (lytic and coagulative), amount of hepatocytes (submassive versus focal), cellular type (lymphocytotoxic veruses hyline necrosis) and various patterns are associated with different etiologic factors. Zonal necrosis is a common pattern of injury after an acute hepatic injury. Sharply demarcated perivenular (zone 3) coagulation necrosis is typical of several anoxic injury or acetaminophen injury and may be explained by differences in oxygenation and activity of drug-metabolizing enzyme. Periportal necrosis (Zone 1) is not common and is noted in eclampsia. Midzonal injury is reported for yellow fever.

Hepatocellular necrosis (hepatocytolysis) of the lytic type, with the associated macrophage activity that is so complete and rapid that dead hepatocytes are rarely noted. Such necrosis is common in viral hepatitis, alcoholic liver disease also in many hepatotoxic and inflammatory reactions. Coagulative necrosis in the liver is characterized by dying hepatocytes that retain some staining of the cytoplasm and the nuclei lose basophilia and gradually disappear. The cells become shrunken and slowly disappear because of the action of inflammatory cells.

Acidophilic injury usually occurs in an isolated hepatocyte and is similar to coagulative necrosis except that the cytoplasm becomes more eosinophilic and waxy and the nucleus may be retained. These bodies are common in acute viral hepatitis, chronic hepatitis, severe burns and other liver disorders. Confluent necrosis is attributable to fusion of focal or zonal necrosis and may result from intensive necrosis

that bridge between different zones. Submassive hepatic necrosis is recognized by confluent necrosis that usually involves many perivenular areas and occurs most commonly in severe acute viral hepatitis, drug injury etc. Massive hepatic necrosis is distinguished from submassive hepatic necrosis by the presence of a thin rim of viable-appearing hepatocytes around each portal tract.

Regeneration:

The liver has a remarkable capacity for regeneration during recovery from submassive hepatic necrosis. In normal adult rat liver, the hepatocytes have an annual turnover of one mitosis per year; weight doubles in 48 hours and returned to normal weight at 3 to 6 days after partial hepatectomy. In man, liver regeneration occurs rapidly even in cirrhotic liver. After major hepatic resection for tumor, regeneration of normal hepatic volume occurs by 3 to 6 months and liver function appears normal at 2 to 3 weeks after surgery.

Fibrosis:[42, 43]

Hepatic fibrosis is the most important factor of chronic liver disease and leads to cirrhosis and irreversible physiologic changes in the liver that account for many clinical manifestations of fatal liver disease. Fibrosis of the liver is a common response to chronic inflammatory conditions. Experimental cirrhosis in the CCl_4 treated rat and mouse have served as models, but the adequacy of the morphologic and biochemical lesions has been doubled as compared to human cirrhosis.

Cirrhosis:[44]

Cirrhosis is a chronic disease of the liver represents the end stage of disease, in which diffuse destruction and regeneration of the hepatic parenchymal cells have

occurred and a diffuse increase in connective tissue resulted in disorganization of the lobular and vascular architecture.

Characteristics:[45]

Parenchymal nodules containing proliferating hepatocytes encircled by fibrosis, with diameters varying from very small to large. The parenchymal injury and consequent fibrosis are diffused, extending throughout the liver; focal injury with scarring does not constitute cirrhosis. Moreover, the fibrosis once developed is generally irreversible, although regression is observed in rare instances.

Production of cirrhosis:[46, 47]

The responses of the liver to necrosis are limited; the most important are collapse of hepatic lobules, formation of fibrous septa and nodular regrowth of liver cells. Thus irrespective of the etiology, the ultimate histological pattern of the liver is the same. In all the patients with cirrhosis, regardless of the presence, absence or nature of individual clinical manifestation, the triad of parenchymal necrosis, regeneration and scarring is present.

Fibrosis follows hepatocellular necrosis. This may follow interface hepatitis in zone 1 leading to portal-portal fibrosis bridges. Confluent necrosis a zone 3 leads to central-portal bridging and fibrosis. Focal necrosis is followed by focal fibrosis. The cell death is followed by nodules which disturb the hepatic architecture and full cirrhosis develops.

Fibrogenesis:

The transformation of liver to fibrotic liver and eventually cirrhosis is a complex process involving several key components in particular stellate cells, cytokines, proteinases and their inhibitors. The amount and composition of the extra-

cellular matrix changes. The normal low density basement membrane is replaced by high density interstitial type connective tissue containing fibrillary collagens. These changes owes as much to reduced degradation as to increased synthesis of connective tissue.

There is interaction between stellate cells, adjacent sinusoidal, parenchymal cells, cytokines, growth factors, proteases, their inhibitors and extra cellular matrix. The formation of fibrous tissue depends not only on the synthesis of excess matrix but also changes in its removal. This depends upon the balance between enzymes that degrade the matrix and their inhibitors.

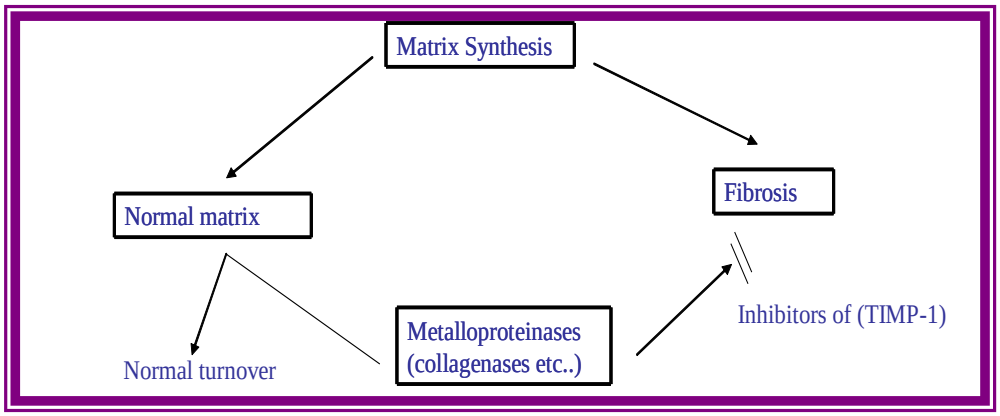


Figure 1: Mechanism of normal and abnormal connective tissue production
TIMP, tissue inhibitor of matrix metalloproteinase's

Normal liver has connective tissue matrix which includes type IV collagen (non-fibrillary), glycoprotein's (including fibronectin and laminin) and proteoglycans. These comprise the low density basement membrane in the space of disse. Following hepatic injury there is a three to eight fold increase in the extra-cellular matrix which is of a high density type, connecting fibril-forming collagens (type I and type III) as well as cellular fibronectin, hyaluronic acid, matrix proteoglycons and glycoconjugates. There is loss of endothelial cell fenestrations, hepatocyte microvilli

and capillarization of sinusoids, which impedes the metabolic exchange between blood and liver cells.

The hepatic stellate cell (also called lipocyte, fat storing cell, Ito cell and pericyte) is the principle cell involved in fibrogenesis. It lies in the space of disse and makes surface contact with hepatocytes, endothelial cells and nerves fibers.

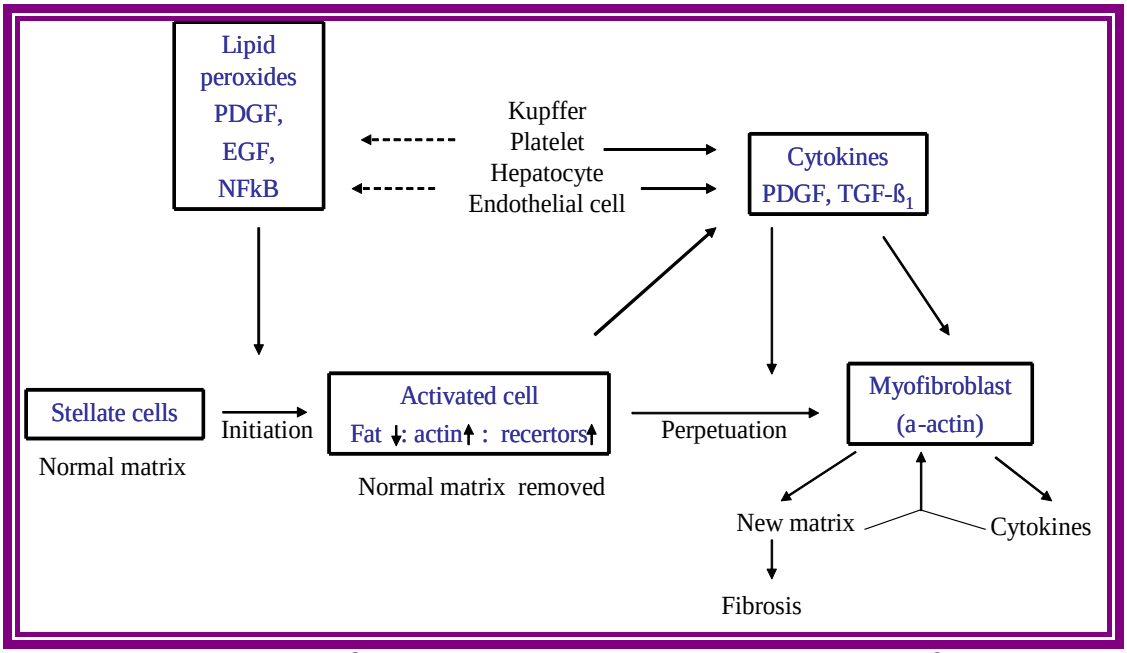


Figure 2: Activation of hepatic stellate cells in Fibrogenesis. Myofibroblasts probably also produce inhibitors of collagenases, enhancing Fibrogenesis

Classification of cirrhosis:[48, 49]

There is no satisfactory classification of cirrhosis for specification of the presumed underlying etiology, which varies both geographically and socially. The following is the approximate frequency of etiologic categories in the western world:

- Alcoholic liver disease- 6 to 70%
- Viral hepatitis- 10%
- Biliary disease- 5 to 10%
- Genetic hemochromatosis- 5%

- Wilson's disease- rare
- α_1 - antitrypsin deficiency- rare
- Cryptogenic cirrhosis- 10 to 15%

The classification proposed here is based on the one recommended at the Fifth Pan-American Congress and modified by World Health Organization.

- **Morphologic:** Micro nodular, Macro nodular and Mixed.
- **Histologic:** Portal, Post necrotic, Post hepatitic, biliary obstruction and Venous out flow obstruction.
- **Etiologic:** Alcohol and viral hepatitis, biliary obstruction, hemochromatosis, Venous out flow obstruction and hepatolenticular degeneration (Wilson's disease).

Micro nodular cirrhosis applies to the liver where nearly all the nodules are less than 3 mm diameter, though it has been used as 1.5 mm as the minimum diameter because that is the diameter of a normal lobule, e.g. malnutrition, alcoholic cirrhosis, biliary tract obstruction and hepatic venous obstruction.

Macro nodular cirrhosis applies if most of the nodules are greater than 3mm diameter and it occur in two forms. The more common form has nodules divided by thin septa that are often incomplete and have linking portal tracts. The median time interval for conversion of micro nodular cirrhosis to macro nodular type is 2.25 years and majority of patients have such progression. Alcoholic cirrhosis often contains fats within the hepatocytes and the parenchyma often increases in weight. Etiologic factors related to cirrhosis include alcoholism, hepatitis B virus and various metabolic diseases such as hemochromatosis, Wilson's disease, etc.

Cirrhosis without recognizable cause is called as "Cryptogenic cirrhosis". The irreversibility of cirrhosis has been emphasized in several experimental and clinical studies. If cirrhosis is reversible, it must be very rare, though the most convincing patients have biliary obstruction that has been corrected surgically. Functionally more important than nodular size in cirrhosis is the size of the entire hepatic mass, which can be estimated with radioisotope scans.

BASIC HEPATIC HISTOPATHOLOGY:[49]

Hepatocellular changes:

Hydropic change is a descriptive term applied to the hepatocytes with pale, watery cytoplasm and a normal nucleus. A wide variety of conditions produce this relative lack of cytoplasmic staining. Increased eosinophilia may occur with drug-related hydroperplasia of the smooth endoplasmic reticulum. Active regeneration of hepatocytes after necrosis as in several viral hepatitis or recovery phase of fatty liver produces a widespread hepatocellular hydropic change as well as a cobblestone pattern of the liver cords. Hydropic change is also an indicator of hepatocellular damage and is noted in acute viral hepatitis and drug induced hepatic injury including alcohol injury.

Fatty liver occurs because of

- Sudden increase in mobilization of fat from the periphery to the liver.
- Relative lack of protein necessary for hepatocellular fat release.
- Increased hepatocellular fat formation by metabolic changes.
- Decrease hepatocellular fat degradation.

Fatty liver is common in alcohol ingestion, parenteral nutrition, tuberculosis, starvation, certain drugs, diabetes mellitus and obesity. Electron microscopy shows that the cytoplasmic fat is not membrane bound and lysosomes are greatly increased.

Enzymes that detect hepatocellular necrosis, fibrosis and cirrhosis:[50, 51, 52]

The liver contains thousands of enzymes, some of which are also present in serum in very low concentrations. These enzymes have no known function in serum and behave like other serum proteins. They are distributed in plasma and intestinal fluid and have characteristic half lives of disappearance, usually measured in days. The elevation of a given enzyme activity in serum is thought to primarily reflect its increased rate of entrance into serum from damaged liver cells. Serum enzyme tests can be grouped into two categories: enzymes whose elevation in serum reflects generalized damage to hepatocytes and enzymes whose elevation in serum primarily reflects cholestasis.

Aminotransferases: Alanine transaminase and Aspartate transaminase-

The serum aminotransferases (formerly called transaminases) are sensitive indicator of liver cell injury and helpful in recognizing acute hepatocellular diseases such as hepatitis. ALT (SGPT) and AST (SGOT) activities in serum are the most frequently measured indicators of liver disease. These enzymes catalyze the transfer of the α-amino group of alanine and aspartic acid respectively to the α-keto group of keotglutaric acid. This results in the formation of pyruvic acid and oxaloacetic acid. Of the numerous methods developed for measuring ALT and AST activity in serum, the most specific method couples the formation of pyruvate and oxaloacetate, the products of the aminotransferase reactions and their enzymatic reduction to lactate and malate. The reduced form of nicotinamide-adenine dinucleotide (NAD), the

cofactor in this reduction is oxidised to NAD. Because NADH, but not NAD, absorbs light at 340 nm, the event can be followed spectrophotometrically by the loss of absorptivity at 340 nm. Both aminotransferases normally are present in serum in low concentrations, less than 30 to 40 IU/L.

AST is found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes and erythrocytes, in decreasing order of concentration. ALT is present in highest concentration in the liver. The increase in ALT and AST serum values are related to damage or destruction of tissue rich in the aminotransferases or changes in cell membrane permeability that allows ALT and AST to leak into serum.

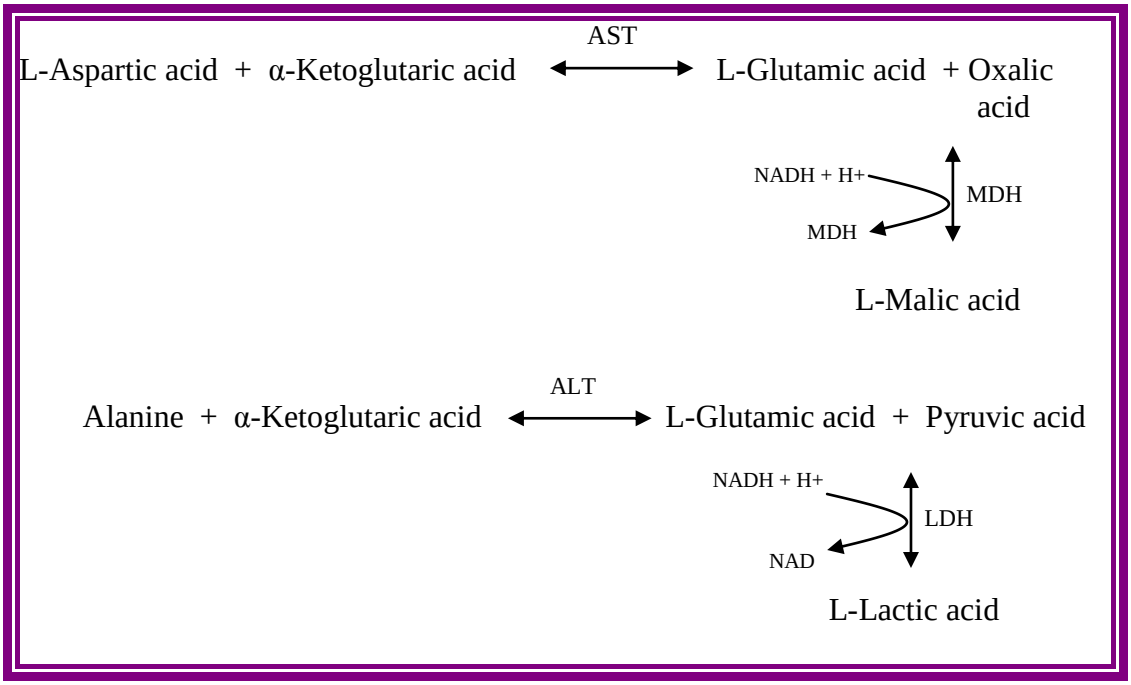


Figure 3: Role of AST and ALT in enzymatic reduction of Alanine and Aspartic acid

Aminotransferases are typically elevated in all liver disorders. These include all types of acute and chronic hepatitis, cirrhosis, infectious mononucleosis, alcoholic liver diseases etc. Elevations of serum aminotransferases, eight times the upper limit of normal are non-specific and may be found in any of the mentioned disorders. The

highest elevation occurs in disorders associated with extensive hepatocellular injury such as drug and viral hepatitis, exposure to hepatotoxins such as CCl_4 and phalloidin.

Total Protein (TP):

The liver is the main source of most of plasma proteins. The parenchymal cells are responsible for the synthesis of albumin, fibrinogen, other coagulation factors and most of α-and β-globulins. Measure of total protein in the serum is useful in liver disorders.

Albumin (Alb):

Alb is quantitatively the most important plasma protein, synthesized exclusively in the liver and can be measured cheaply and easily. It is the main constituent of total protein; the remaining fraction is called globulin (including eg. immunoglobulins). Albumin levels are decreased in chronic liver disease, such as cirrhosis and are also decreased in nephrotic syndrome, where it is lost through the urine. Poor nutrition or states of protein catabolism may also lead to hypoalbuminaemia. The half-life of albumin is approximately 20 days and is not considered to be an especially useful marker of liver synthetic function; coagulation factors are much more sensitive for this purpose.

Alkaline phosphatase:

Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP is also present in bone and placental tissue, so it is higher in growing children. ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver.

Total bilirubin (TB):

Bilirubin is a breakdown product of heme (a part of hemoglobin in red blood cells). The liver is responsible for clearing the blood from bilirubin. It does this by the following mechanism: bilirubin is taken up into hepatocytes, conjugated (modified to make it water-soluble) and secreted into the bile, which is excreted into the intestine.

Liver function tests typically measure Total bilirubin (TB) and direct bilirubin. Indirect bilirubin (unconjugated bilirubin) is obtained by subtracting direct bilirubin from total bilirubin.

Increased/decreased of total bilirubin causes,

- Increased bilirubin production: This can be due to a number of causes, including hemolytic anemias and internal hemorrhage.
- Problems with the liver, causes decreased in hepatocyte uptake, impaired conjugation of bilirubin, and reduced hepatocyte secretion of bilirubin e.g. Cirrhosis and viral hepatitis.
- Obstruction of the bile ducts, reflected as deficiencies in bilirubin excretion. (Obstruction can be located either within the liver or outside the liver).

Looking at the levels of direct bilirubin narrows the diagnosis down further. If direct (i.e. conjugated) bilirubin is normal, then the problem is an excess of unconjugated bilirubin and the location of the problem is upstream of bilirubin excretion. Anemia, viral hepatitis, or cirrhosis can be suspected. If direct bilirubin is elevated, then the liver is conjugating bilirubin normally, but is not able to excrete it. Bile duct obstruction by gallstones or cancer should be suspected.

LYSOSOME:[53, 54]

The lysosome is the cell's main digestive compartment to which all sorts of macromolecules are delivered for degradation. The structure is variable and depends on the cell type and the actual conditions. In terms of function and cytochemistry, the lysosome is identified by the following criteria: acid pH, hydrolases with acid pH optimum, specific highly glycosylated membrane-associated proteins and the absence of the mannose-6-phosphate receptor. One of the primary sites of intracellular digestion are organelles known as the lysosomes, which are membrane-bounded compartments containing a variety of hydrolytic enzymes.

Lysosomes are organelles that contain digestive enzymes (acid hydrolases) to digest macromolecules. They are found both in plant and animal cells which are built in the golgi- apparatus. At pH 4.8, the interior of the lysosomes is more acidic than the cytosol (pH 7). The lysosome single membrane stabilizes the low pH by pumping protons (H+) from the cytosol, protects the cytosol and therefore the rest of the cell, from the degradative enzymes within the lysosome. The digestive enzymes need the acidic environment of the lysosome to function correctly. For this reason, should a lysosome's acid hydrolases leak into the cytosol; their potential to damage the cell will be reduced, because they will not be at their optimum pH. All these enzymes are produced in the endoplasmic reticulum and transported and processed through the golgi apparatus. The golgi apparatus produces lysosomes by budding. Each acid hydrolase is then targeted to a lysosome by phosphorylation. The lysosome itself is likely safe from enzymatic action due to having proteins in the inner membrane which has a three-dimensional molecular structure that protects vulnerable bonds from enzymatic attack.

The lysosomes are used for the digestion of macromolecules from phagocytosis (ingestion of cells) from the cell's own recycling process (where old components such as worn out mitochondria are continuously destroyed and replaced by new ones, and receptor proteins are recycled) and for autophagic cell death, a form of programmed self-destruction or autolysis of the cell, which means that the cell is digesting itself. Other functions include digesting foreign bacteria that invade a cell and helping repair damage to the plasma membrane by serving as a membrane patch and sealing the wound.

There are a number of illnesses that are caused by the malfunction of the lysosomes or one of their digestive proteins, eg Tay-Sachs disease or Pompe's disease. These are caused by a defective or missing digestive protein, which leads to the accumulation of substrates within the cell, resulting in impaired cell metabolism. Broadly, these can be classified as mucopolysaccharidoses, GM2 gangliosidoses, lipid storage disorders, glycoproteinoses and mucolipidoses.

Biogenesis of Lysosomes:

On the basis of the sub cellular distribution of acid phosphatase as demonstrated by enzyme cytochemistry, Novikoff and coworkers proposed the concept of GERL (golgi apparatus – endoplasmic reticulum – lysosome). It implied (a) that lysosomal enzymes, after biosynthesis in the rough endoplasmic reticulum (rough ER) are packaged into vesicles (primary lysosomes) budding off from tubules which are continuous with the endoplasmic reticulum ER and intimately related to the golgi apparatus, (b) that the vesicles are conveyed to pre-existing lysosomes which have already been engaged in a digestive process (secondary lysosome).

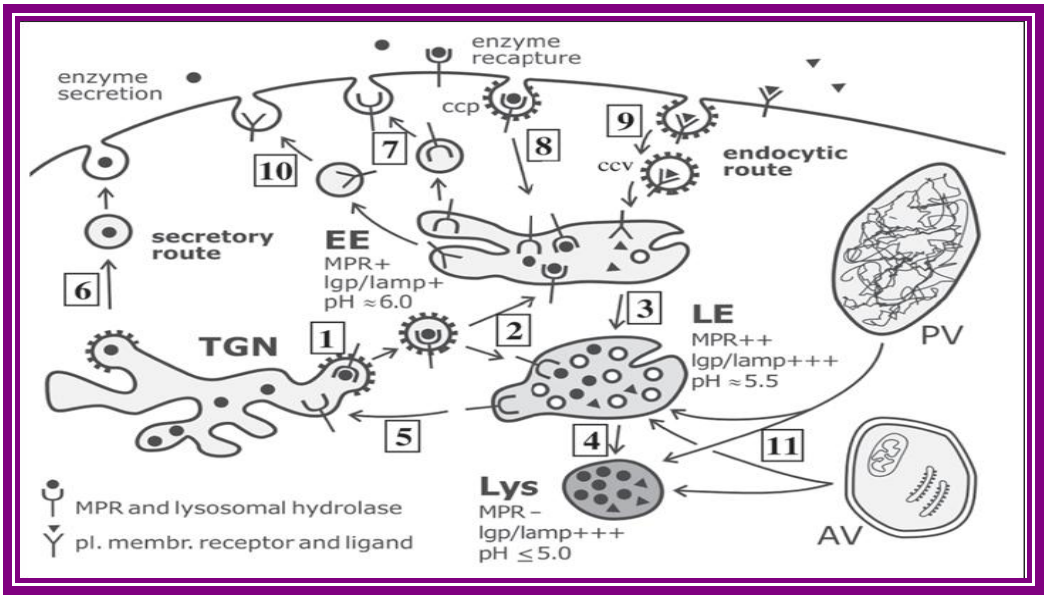


Figure 4: Diagrammatic summary of the endosome/lysosome system, with special reference to the biogenesis of lysosomes

This concept was later replaced by the concept of the trans golgi network as the common exit site for all products including lysosomal enzymes, secretory proteins and membrane proteins. Data indicating that the intracellular transport of lysosomal enzymes is receptor-mediated and has to pass through a prelysosomal compartment induced the most important modifications of the previous GERL concept.

Cell Digestion and Secretory Pathway:

In endocytosis, macromolecules and particles from outside the cell are taken up by the cell via a progressive invagination (inpouching) and eventual pinching off of a region of the cell membrane, forming a membrane-bound vesicle (bubble) within the cytoplasm of the cell.

The vesicle then fuses with the lysosome and the lysosomal enzymes carry out their appointed task of destruction (by hydrolysis). Quite amazingly, the lysosomal enzymes do not normally damage the cell itself.

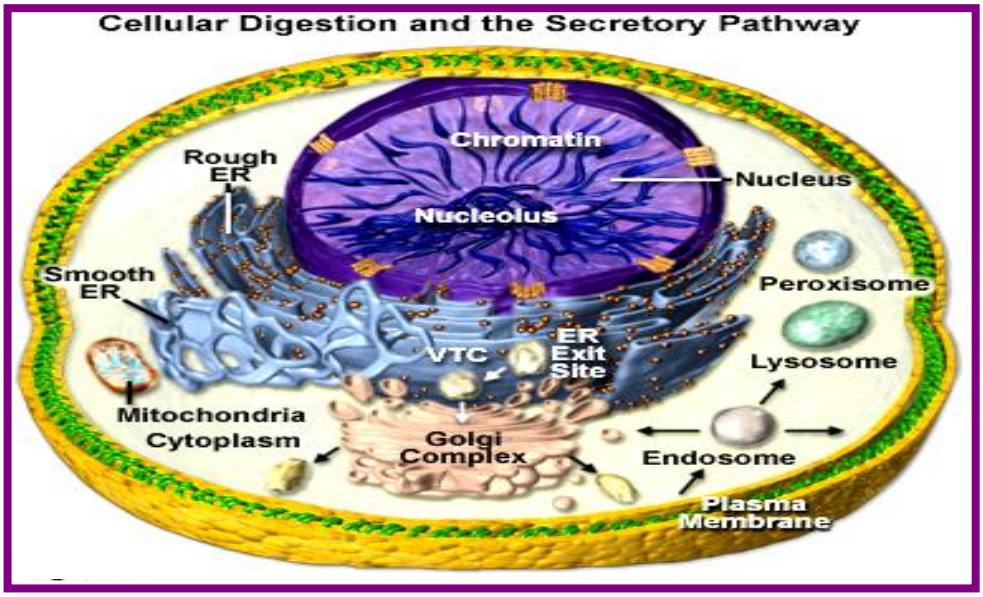


Figure 5: Cell Digestion and the Secretory Pathway

Lysosomes maintain an internal acidic environment through the use of a hydrogen ion pump in the lysosomal membrane that drives ions from the cytoplasm into the luminal space of the organelles. The high internal acidity is necessary for the enzymes contained in lysosomes to exhibit their optimum activity. Hence, the integrity of a lysosomal membrane is compromised and the enzymatic contents are leaked into the cell, little damage is done due to the neutral pH of the cytoplasm. If numerous lysosomes rupture simultaneously, however, the cumulative action of their enzymes can result in auto digestion and the death of the cell.

Lysosomal hydrolytic enzymes are manufactured in the rough ER, from whence they are transferred in a transport vesicle to the cis face of the golgi apparatus or complex. Inside of the golgi complex, the enzymes undergo additional processing and are transformed from an inactive to an active state. As outlined in figure lysosomes may then bud from the trans face of the Golgi apparatus, though they may also form via other mechanisms such as the gradual transformation of endosomes.

Presented in Fig. 5 is a diagrammatic representation of the digestion organelles present in a typical eukaryotic cell along with the secretory pathways. The majority of

the membrane and secretory proteins involved in cellular digestion are synthesized on the surface of the endoplasmic reticulum (rough ER) or are translocated to the organelle after being produced in the cytoplasm. In addition to serving as a center for protein processing and secretion, the endoplasmic reticulum forms a portion of the nuclear envelope. Newly synthesized proteins are sorted at ER exit sites and enter vesicles for trafficking to intermediate compartments (VTCs), where proteins are sorted for transport to the Golgi complex or returned to the endoplasmic reticulum.

Several different varieties of macromolecules may be digested by lysosomes and arrive at the organelles by disparate pathways. As illustrated in figure, for example, many unicellular organisms and certain cells in multicellular organisms consume particles of food and other items via a process called phagocytosis. When the cell engulfs the food during this process, a vacuole forms around it from an invagination in the cell's plasma membrane that pinches off to completely encase the foreign material. Once released into the cell, the food vacuole is able to fuse with a lysosome. The action of the hydrolytic enzymes upon the contents of the vacuole digests them, breaking them down into monomers, such as simple sugars and amino acids that are able to traverse the lysosomal membrane via transport proteins and enter the cytosol, where they serve as cell nutrients.

In addition to providing nutrients, the phagocytic process is important to some cell types as a mode of defense. Certain components of the vertebrate immune system, for instance, utilize phagocytosis to fight off infections. Specifically, macrophages and neutrophils, which are often referred to as scavenger cells, actively ingest bacteria and other microorganisms they encounter in the body. These intruders are then digested with the help of lysosomes.

An alternate mechanism that materials may be acquired by lysosomes for digestion is strictly intracellular, termed autophagy; the process entails the removal of membrane-bounded organelles or other cytoplasmic components through the action of lysosomes. Similar to phagocytosis, the enzymes present in the lysosome disassemble the mitochondrial components into monomers and the breakdown products from autophagy are released into the cytosol.

Lysosomes are also important for their role in the programmed death of certain cells. As previously discussed, if the enzymes of a single lysosome are released into a cell, there is little change in the cytosol, but a massive enzymatic discharge by many lysosomes can be fatal to the cell. Through the coordinated release of lysosomal enzymes a number of important developmental changes occur in various multicellular organisms.

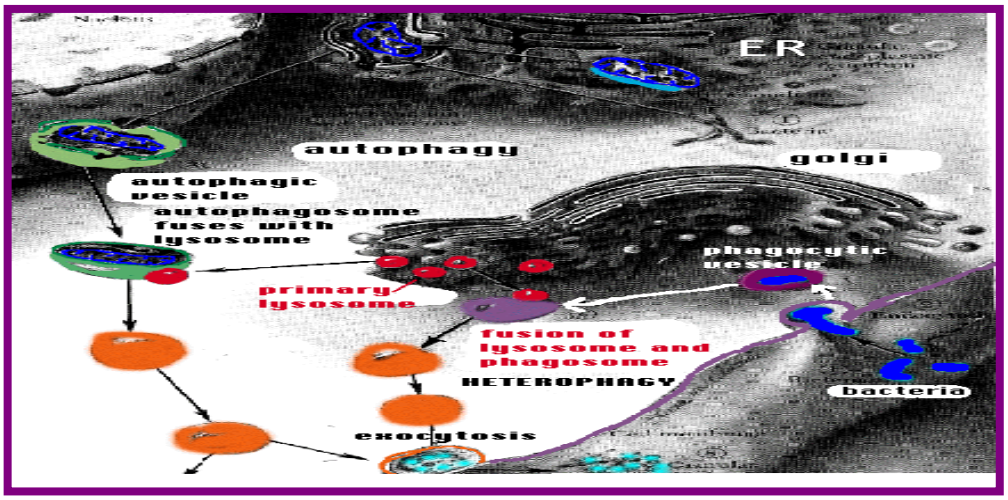


Figure 6: Autophagy

The term auto (self) phage (to eat) literally refers to a process whereby the cell digests its own contents. This process is most often referred to as “cellular turnover", it refers to a very important, normal cellular process. Many important cellular constituents, such as proteins, organelles and membranes are in a constant state of

flux. That is they are constantly being synthesized and degraded. Indeed, an interruption of this process can lead to a disease condition such as Tay Sach's disease. In the diagram below a cellular constituent such as mitochondria fuses with a primary lysosome. The enzymes of the lysosome digest the macromolecules and the digestion products can be resused by the cell or ejected by exocytotic process.

The term hetero (different) phagy (to eat) refers to a process whereby lysosmes aid in the intracellular digestion of material gathered from outside of the cell by some kind of endocytotic mechanism. The endocytotically ingested material is sequested in a membrane-bounded vesicle. In the diagram above bacteria are ingested by phagocytosis. The ingested bacteria are packaged in a phagocytic vesicle. This vesicle fuses with a primary lysosome forming a secondary lysosome. The enzymes of the lysosome digest the bacterial macromolecules.[55]

Introduction to hepatotoxicity:

In recent times lot of interest has been generated to find out a natural remedy for hepatic disorders caused by toxins like alcohol and hepatitis virus[48]. The agent should protect against such damage, especially one that facilitates regeneration by proliferation of parenchymal cells after damage and arrest growth of fibrous tissue.[56]

There are no remedies for liver diseases, which are so prevalent in the population. The treatment is mainly symptomatic.[57] Scientists and some industrialists deliberated on various prospective plant remedies for ailments of liver disorder management. In the decade 70s, the world scientific community concentrated on an herbal plant *Vinca rosea*. Then in 80s the attention was focused on *Panax ginseng*. Now the news of multifarious activities of the Neem tree indicates that it may become centre for research in 90s. Indian Council of Medical Research, New Delhi(ICMR), in its

revived research on traditional medicine, had adopted liver diseases as one among six thrust areas and for multidisciplinary study. Screening of active constituents from Kutki *(Picrorhiza kurroa)* and Bhoomyamalaki *(Phyllanthus niruri)* have shown marked protection against jaundice. Hepatitis continues to be a major health problem in urban areas in India, several studies in viral hepatitis were under investigation by the ICMR; for example, extracts of milk thistle *(Silybum marianum)* fruits under investigation for the treatment of alcoholic hepatitis. According to Indian Society of Gastroenterology, Mulethi *(Glycyrrhiza glabra)* prevents multiplication of viruses inside liver cells.

The disorder of liver may be acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory liver diseases) and liver cirrhosis (fibrosis of the liver). Liver enzymes act as an index of sub-clinical hepatic damage. The ALT, AST, ALP and Serum lactic dehydrogenase are reported as an index of hepatic injury and cholestasis.[55]

Environmental injury – drug and toxins:[58]

Water-soluble drugs and chemical substances are polar molecules excreted by the kidneys whereas, mainly the liver handles water insoluble i.e. non-polar molecules. The main drug metabolizing enzymes increase the polarity of the molecule in one of the three ways; Oxido-reduction, in which the cytochrome P-450 is important, hydroxylation transformation and conjugation reaction as shown in Fig 7.

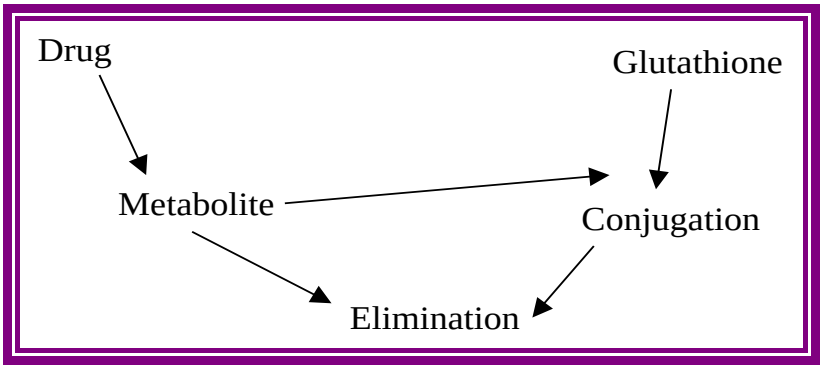


Figure 7: Hydroxylation and Conjugation Reaction.

Traditionally drug injury to the liver was classified into direct, where injury is predictable and dose-dependent and indirect where it is not dose-dependent and is unpredictable. It is now clear that this was a great oversimplification and most drugs undergo metabolism within the liver cell before causing injury. Substances such as tannic acid, ethionine, ferrous sulphate and white phosphorus all causes liver damage directly through cell injury. Paracetamol and halothane in overdose probably operate in similar way as shown in Fig 8.

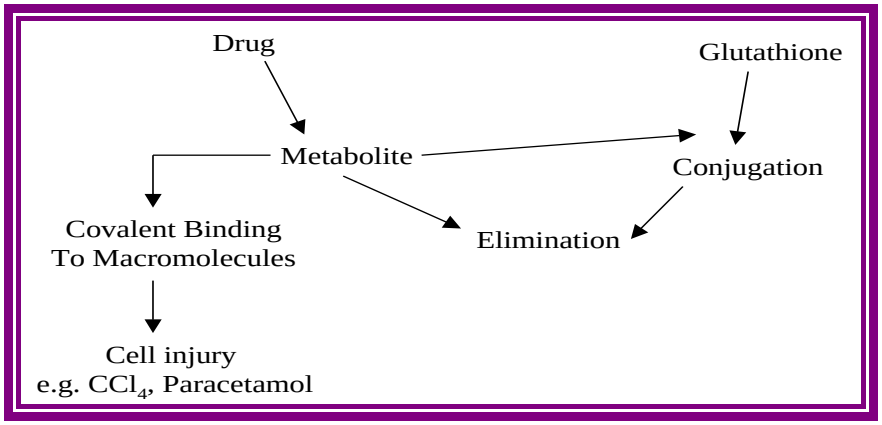


Figure 8: Mechanism of Cell injury by CCl_4 and Paracetamol.

Alcoholic liver injury may be the result of a reactive metabolite. Although both zonal necrosis and viral hepatitis-like liver cell necrosis caused by drugs and toxins usually have a mechanism similar to that outlined above, very occasionally

reactive metabolite may create new cellular antigens which elicit immunological response free drug hypersensitivity eg; Methyldopa.

Cell injury by CCl_4:[59, 60]

Carbon tetrachloride (CCl_4) is a powerful hepatotoxin that is used extensively to generate experiments to study necrosis and steatosis of the liver in rodents. This free radical is formed in cytochrome P-450 mediated reductive process. CCl_4 is converted into trichloromethyl radical ($CCl_3^{\cdot}$) and trichloromethyl peroxy radical ($CCl_3O_2^{\cdot}$). These radicals are extremely reactive and their duration of action is often short. Carbon tetrachloride induced necrosis is most severe in centrilobular hepatocytes (zone3) as the concentration of cytochrome P-450 is highest. As seen above, the free radicals initiate lipid peroxidation of biological membranes and bind covalently to lipids, proteins and nucleic acids. It is now assumed that the trichloromethyl radical forms covalent bonds and that trichloromethylperoxy radical is the initiator of lipid peroxidation as shown in Fig 9.

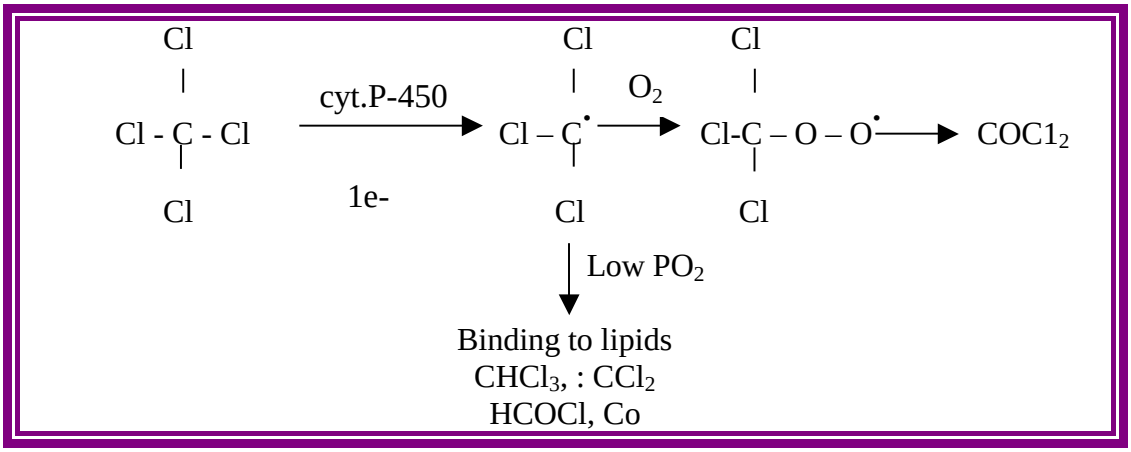


Figure 9: Formation of free radials from carbon tetrachloride

The reason is that, the conversion of trichlromethyl radical to the trichloromethylperoxy radical takes place much more rapidly than binding to cellular components. Lipid peroxidation leads to a cascade of events.

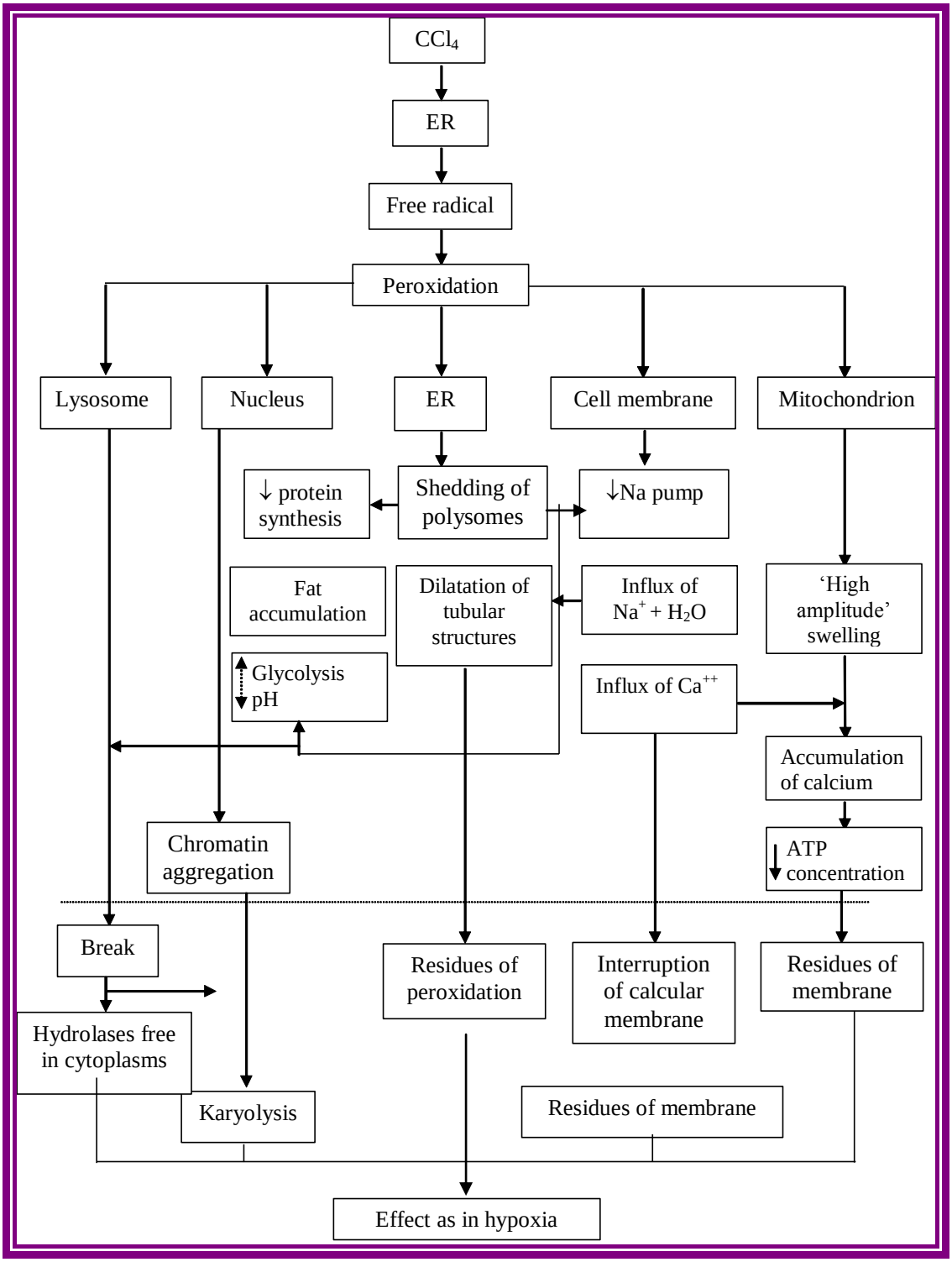


Figure 10: Sequence of hepatotoxic effects following lipid peroxidation induced by free radicals formed in carbon tetrachloride metabolism

Toxic injury of liver induced by CCl_4 is a model system and results in metabolic conversion by a complex of enzymes bound to membranes of the smooth-surfaced endoplasmic reticulum. Action of these enzymes is a major mechanism by

which toxic compounds are converted to less toxic ones. In some instance non-toxic substances are metabolized to toxic ones such as in case of CCl_4.

CCl_4 is converted by hemolytic cleavage to a highly reactive haloalkane free radical and a chlorine free radical in the following reaction:

$$CCl_4 \dashrightarrow CCl._3 + Cl.$$

These in turn reacts with a variety of intracellular molecules, notably the unsaturated fatty acids. eg. Polyenolic fatty acids are converted to organic free radicals which in turn react with molecular oxygen to form organic peroxides. These compounds are highly unstable and decompose spontaneously to form aldehydes, ketones and other products.

CCl_4 reacts with sulfahydryl groups, which mediate the function of the many cell proteins including a number of important enzymes, and this reaction leads to their alkylation and subsequent loss of function. The free radicals formed react rapidly with other molecules to form additional free radicals; such reactions are autocatalytic and tend to spread from a small focus to involve large areas of cytoplasm. The earliest change that has been detected in rat liver cells is a functional one that occurs 30 min after the intragastic administration of a single dose of 0.25 ml of CCl_4. It consists of rapid decrease in synthesis of the export protein albumin. The diminution of protein synthesis after intoxication appears to be linked to disaggregation of the polysomes and probably represents a physical disruption of their association with messenger RNA.

Significantly in this early phase of CCl_4 induced injury, mitochondria appear morphologically intact and are capable of normal oxidative phosphorylation and fatty acid oxidation among their many functions.

Within few hours after administration of CCl_4 neutral lipids (triglycerides) begin to accumulate in the cytoplasm making their first appearance as osmiophilic droplets ultimately fill the entire cytoplasm. Approximately 10 to 12 hours after CCl_4 administration the liver is grossly enlarged and pale because of accumulated fat. Lipid can also accumulate in the liver by mechanisms such as by increased mobilization of free fatty acids from depot fat.

Shortly after the ingestion of as little as 5 to 100 ml of carbon tetrachloride results in swelling and hydropic degeneration of the centrilobular hepatic cells develop. These changes progress to a diffuse fatty degeneration and necrosis in the centrilobular parenchyma with collapse of the reticulum network followed shortly by hemorrhage and leukocytic infiltration.

Auto radiographic studies have shown a rapid uptake of CCl_4 by the cytoplasm and nuclei of the cells of centrilobular areas. Auto radiographic avoidance shows that radioactive 14C and CCl_4 remain in the centrilobular areas as long as 2 days after ingestion. Endoplasmic reticulum is damaged 30 minutes of the administration of CCl_4, whereas the mitochondria survive unaltered for several hours. Protein synthesis is reduced within 2 hours of poisoning. Fatty acids are mobilized from peripheral fat depots to the liver. In the liver cell they are oxidized to triglycerides. Experimentally, CCl_4 has been widely used to study toxic hepatic necrosis and it is still a favoured model of cirrhosis, which regularly develops after repeated injection of CCl_4 into rats.

GROWTH HORMONE

Growth hormone (GH) is a protein-based polypeptide hormone. It stimulates growth and cell reproduction, regeneration in humans and other animals. It is a 191-amino acid, single-chain polypeptide hormone that is synthesized, stored and secreted

by the somatotroph cells within the lateral wings of the anterior pituitary gland. Somatotropin refers to the growth hormone produced natively and naturally in animals, whereas the term somatotropin refers to growth hormone produced by recombinant DNA technology [61] and is abbreviated "rhGH" in humans.

Growth hormone is used clinically to treat children's growth disorders and adult growth hormone deficiency. In recent years, replacement therapies with human growth hormones (HGH) have become popular in the battle against aging and weight management. Reported effects include decreased body fat, increased muscle mass, increased bone density, increased energy levels, improved skin tone and texture, increased sexual function and improved immune system function. At this time HGH is still considered a very complex hormone and many of its functions are still unknown.[62]

Regulation

Peptides released by neurosecretory nuclei of the hypothalamus (Growth hormone-releasing hormone and somatostatin) into the portal venous blood surrounding the pituitary are the major controllers of GH secretion by the somatotropes. However, although the balance of these stimulating and inhibiting peptides determines GH release, this balance is affected by many physiological stimulators[63] (e.g., exercise, nutrition, sleep) and inhibitors of GH secretion (e.g., Free fatty acids).

Stimulators of GH secretion include:

- peptide hormones [64, 65]
    - Growth hormone releasing hormone (GHRH also known as somatocrinin) through binding to the growth hormone releasing hormone receptor (GHRHR).
    - Ghrelin through binding to growth hormone secretagogue receptors (GHSR).
- Sex hormones[66]
    - increased androgen secretion during puberty (in males from testis and in females from adrenal cortex)
    - estrogen
- Clonidine and L-DOPA by stimulating GHRH release[67]
- Hypoglycemia, arginine [66] and propranolol by inhibiting somatostatin release[68]
- Deep sleep[69]
- Fasting[70]
- Vigorous exercise[71]

Inhibitors of GH secretion include:

- Somatostatin from the periventricular nucleus[72]
- Circulating concentrations of GH and IGF-1 (negative feedback on the pituitary and hypothalamus)[62]
- Hyperglycemia[67]
- Glucocorticoids[73]

In addition to control by endogenous and stimulus processes, a number of foreign compounds (xenobiotics such as drugs and endocrine disruptors) are known to influence GH secretion and function.[74]

Secretion patterns

HGH is synthesized and secreted from the anterior pituitary gland in a pulsatile manner throughout the day; surges of secretion occur at 3- to 5-hour intervals. The plasma concentration of GH during these peaks may range from 5 to even 45 ng/ml.[75] The largest and most predictable of these GH peaks occurs about an hour after onset of sleep[74]. Otherwise there is wide variation between days and individuals. Nearly fifty percent of HGH secretion occurs during the third and fourth REM sleep stages[76]. Between the peaks, basal GH levels are low, usually less than 5ng/ml for most of the day and night.[76] Additional analysis of the pulsatile profile of GH described in all cases less than 1ng/ml for basal levels while maximum peaks were situated around 10-20 ng/ml.[78, 79]

A number of factors are known to affect HGH secretion, such as age, gender, diet, exercise, stress, and other hormones. Young adolescents secrete HGH at the rate of about 700 μg/day, while healthy adults secrete HGH at the rate of about 400 μg/day.[80]

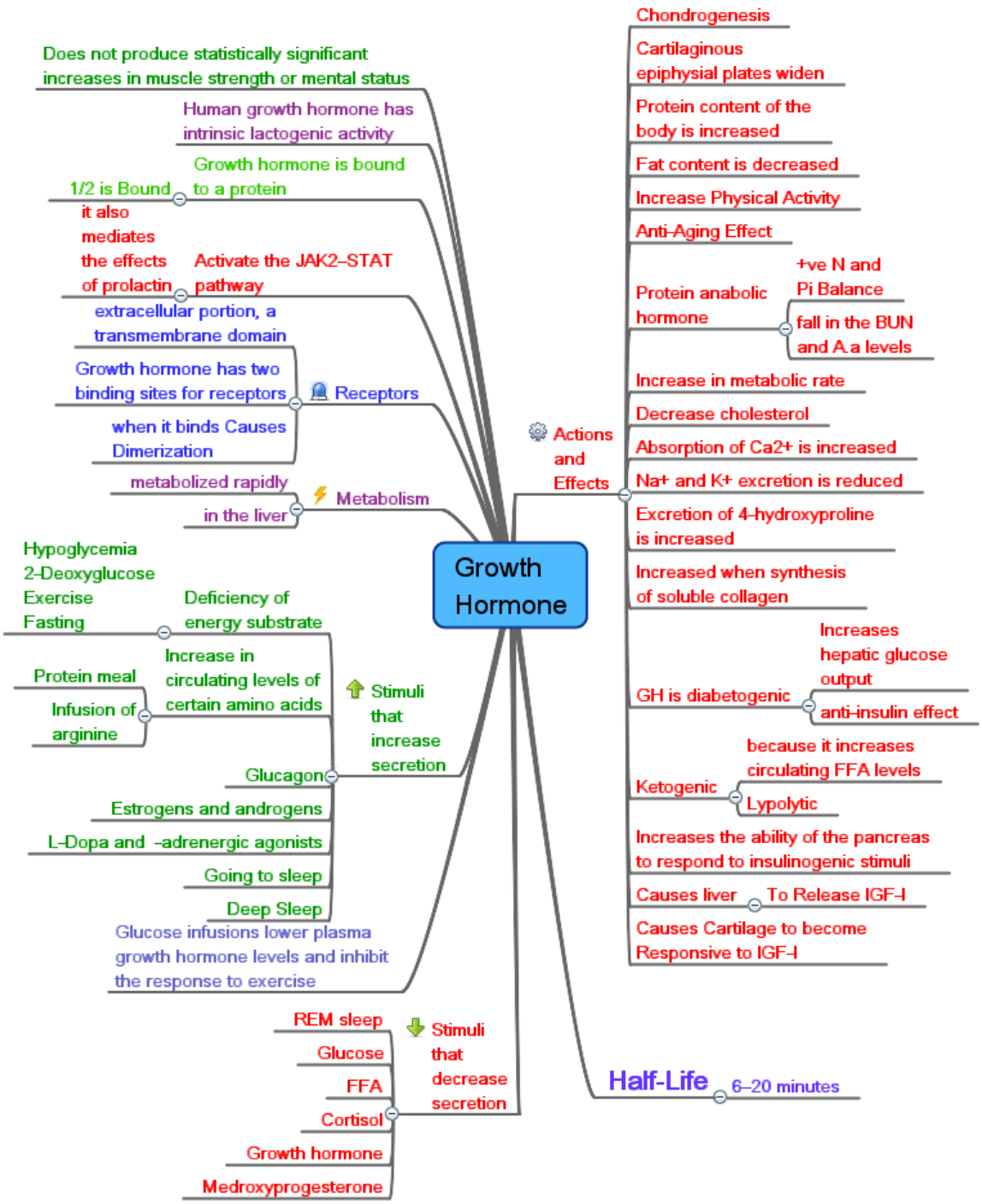


Figure 11: Physiology of growth hormone

Growth hormone and liver:

It has been well established that the liver can regenerate after resection. Liver regeneration occurs via a complex process that includes multiple signals and sequences of events. These signals drive hepatocytes from the quiescent G_0 state into the G_1 phase of the cell cycle and then through the restriction points of G_1 into the S phase, where commitment to division has been reached. [81, 82, 83]

Liver regeneration may be initiated by the activation of one or more cytokine receptors, whereas growth factors usually act later on the already primed hepatocytes.[84, 85] TNFα and IL-6 are thought to play major roles in initiating the process of liver regeneration. Mice lacking TNF receptor type 1 (TNFR-1) exhibited severely impaired liver regeneration after partial hepatectomy. This was found to be due to a defect in DNA synthesis and could be corrected by treatment with IL-6.[86] Similarly, IL-6 knockout mice showed impaired liver regeneration characterized by liver necrosis, liver failure, and a blunted DNA synthetic response in hepatocytes.[87] Hepatocyte growth factor and TGFα are potent hepatic mitogens *in vitro* that are highly expressed after hepatectomy.[84, 88, 89] In addition, epidermal growth factor (EGF) is a primary mitogen for hepatocytes in culture, and its expression increases after liver resection.[85, 90] After partial hepatectomy the activation of cytokine and growth factor signaling pathways leads to the induction of transcription factor complexes such as nuclear factor-κB, c-Myc, signal transducer and activator of transcription-3 (STAT3), cAMP responsive element modulator, and activating protein-1.[49] Mice with conditional knockouts of either STAT3[91] or c-jun[92], specifically in the liver, exhibited impaired DNA synthesis, but liver regeneration still occurred.

GH is a member of the cytokine superfamily of polypeptide regulators[18]. The growth-promoting effects of GH can be direct in selected target tissues, such as liver, or indirectly, via its endocrine mediator IGF-I. GH is the primary regulator of IGF-I synthesis and secretion in hepatocytes; in turn, IGF-I regulates GH secretion through a classical negative feedback loop.[19, 20] In the circulation, IGF-I is bound to specific IGF-binding proteins (IGFBPs). Approximately 70–80% of the circulating IGF-I is found within a large ternary complex composed of the acid-labile subunit (ALS) and IGFBP-3 [91]. A smaller proportion (15–20%) circulates as a binary complex with other IGFBPs, and less than 5% of IGF-I circulates in the free form.[93, 94]

Despite the fact that the liver is the main source of circulating IGF-I and IGFBPs, hepatocytes have not been considered to be a primary target for IGF-I, because they do not express the IGF-I receptor.[95] However, nonparenchymal cells within the liver, such as Kupffer cells and hepatic stellate cells, do express the IGF-I receptor and respond to IGF-I stimulation[96, 97] suggesting that IGF-I can act in a paracrine fashion in the liver. Interestingly, IGFBP-1 was shown to be one of the most rapidly induced and highly expressed proteins in regenerating liver[98]. Mice that lacked IGFBP-1 exhibited normal development, but showed abnormal liver regeneration after partial hepatectomy, characterized by liver necrosis and reduced and delayed hepatocyte DNA synthesis.[99, 100] Recent evidence shows that GH can regulate EGF receptor (EGFR) expression in the liver as well as suppressors of cytokine signaling (SOCS) and glycoprotein 130 gene expression.[101] It has also been shown that GH can stimulate tyrosine phosphorylation of the EGFR in cultured hepatocytes[102, 103] raising the possibility that cross-talk between the GH receptor (GHR) and EGFR could also be important in liver regeneration.

The growth promoting effect of GH can be direct in selected target tissues, such as liver, or indirectly, via its endocrine regulator IGF-I. GH is the primary regulator of IGF-I synthesis and secretion in hepatocytes; in turn, IGF-I regulates GH secretion through a classical negative feedback loop [19, 20] It has been reported that both pituitary and serum concentrations of GH were significantly reduced in CCl_4-induced cirrhotic rats and CCl_4- induced acute liver injury in rats can be restored by exogenous GH-treatment, and upregulation of IGF-I and HGF (hepatocyte growth factor) mRNAs may be involved in that phenomenon.[104, 105] It is also reported that GH was able to inhibit the release of lysosomal enzymes from resting polymorphonucelar leukocytes.[106]

CATECHOLAMINES

Catecholamines are sympathomimetic[107] "fight-or-flight" hormones that are released by the adrenal glands in response to stress.[108] They are part of the sympathetic nervous system. They are called catecholamines because they contain a catechol group, and are derived from the amino acid tyrosine.[109] The most abundant catecholamines are epinephrine (adrenaline), nor epinephrine (nor adrenaline) and dopamine, all of which are produced from phenylalanine and tyrosine. Catecholamines are water-soluble and are 50% bound to plasma proteins, so they circulate in the bloodstream. Tyrosine is created from phenylalanine by hydroxylation by the enzyme phenylalanine hydroxylase. (Tyrosine is also ingested directly from dietary protein). It is then sent to catecholamine-secreting neurons. Here, many kinds of reactions convert it to L-DOPA, to dopamine, to nor epinephrine, and eventually to epinephrine.[110]

Location

Mainly the chromaffin cells of the adrenal medulla and the postganglionic fibers of the sympathetic nervous system produce catecholamines. Dopamine, which acts as a neurotransmitter in the central nervous system, is largely produced in neuronal cell bodies in two areas of the brainstem: the substantia nigra and the ventral tegmental area.

Effects

Catecholamines cause general physiological changes that prepare the body for physical activity (fight-or-flight response). Some typical effects are increases in heart rate, blood pressure, blood glucose levels, and a general reaction of the sympathetic nervous system. Some drugs, like tolcapone (a central COMT-inhibitor), raise the levels of all the catecholamines.

Catecholamine and liver:

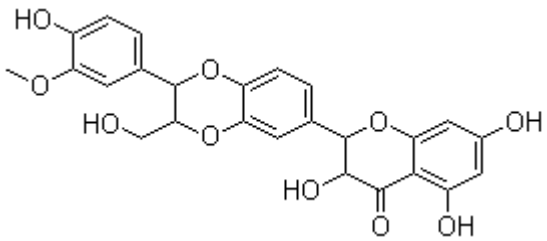
The autonomic nervous system influences many of the functions of the body, including those of cardiovascular system, kidneys, liver, pancreas, gastrointestinal tract and glands[111]. Detailed studies of the mechanisms that regulate liver growth and apoptosis have been done in animals subjected to partial hepatectomy or chemical injury.[112] Since the autonomic nervous system directly innervates the hepatic parenchyma and has a role in metabolic control[113] it seems likely that liver regeneration and apoptosis are cooperatively regulated by both humoral factors and the autonomic nervous system. Moreover, it is well known that autonomic abnormalities and neuropathy tend to increase with age.[114] The prevalence and severity of autonomic dysfunction appears to be related to the severity of liver disease and is associated with an increase in mortality.[115, 116] It is also reported that patients

with liver cirrhosis have parasympathetic hypofunction and sympathetic hyperfunction.[117, 118] In recent years, there has been increasing interest in the relationships between liver and autonomic nervous system.

Adrenergic modulation of chemical-induced hepatotoxicity has been recognized for several decades. Early studies performed by Calvert and Brody utilized the model hepatotoxin, CCl_4, and demonstrated the ability to diminish hepatotoxicity via adrenergic blockade and adrenalectomy. [119, 120, 121] This led to the hypothesis that catecholamines played a role in CCl_4 toxicity. The mechanism of adrenaline action on the structure and function of the lysosomal-vacuolar cell apparatus were studied in experiments on liver sections of Wistar rats. The adrenaline added to the incubation medium, were shown to produce a labilizing effect on lysosomal membranes, increasing free activity of acid phosphatase and osmotic sensitivity of Lysosomes. The previous studies have demonstrated that there is marked decrease in myocardial content of nor adrenaline in animals treated with rhGH (recombinant human growth hormone). Not only in myocardial content of nor adrenaline (NA) but also plasma was NA significantly lower in rats receiving rhGH.[28] Previous clinical studies indicate that GH may have pronounced effects in the regulation of sympathetic function, which is based on the fact that patients with GH deficiency have markedly increased activation of the sympathetic nerve fibers firing in skeletal muscle.[122] Catecholamines may induce oxidative damage through reactive intermediates resulting from their auto-oxidation, irrespective of their interaction with adrenergic receptors.[24] It has been reported that, the extracts of ginkgo biloba leaves primarily composed of Quercetin up regulates the GH in the cortex.[123] On the other hand Quercetin has been reported to inhibit catecholamine secretion from cultured bovine adrenal chromaffin cells, presumably through its inhibitory action on protein kinase C.

In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. More than 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity. At the same time, surprisingly, we do not have readily available satisfactory plant drugs/formulations to treat severe liver disease. Most of the studies on hepatoprotective plants were carried out using chemical-induced liver damage in rodents as models. Only a small portion of the hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their efficacy.

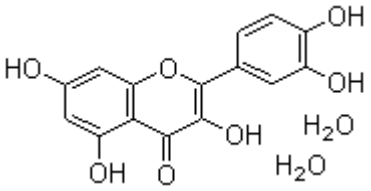
Silymarin:

Silymarin	
Identification	
Name	Silymarin
Synonyms	2-(2,3-Dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-1,4-benzodioxin-6-yl)-2,3-dihydro-3,5,7-trihydroxy-4H-1-benzopyran-4-one.
Molecular Structure	
Molecular Formula	$C_{25}H_{22}O_{10}$
Molecular Weight	482.44

Silymarin is obtained from *Silybum marianum* (milk thistle), an edible plant that has been used medicinally for centuries as a herbal medicine for the treatment of liver-related disorders. It is widely prescribed by herbalists and has almost no known side effects. The plant is native to the Mediterranean and grows throughout Europe and North America. [124,125] It also grows in India, China, South America, Africa, and Australia. This herb is approved for sale in Canada in 70 different products and generates an annual business of $180 million in Germany alone.[124]

Silymarin is a polyphenolic flavonoid, extracted using 95% ethanol, from the seeds of the milk thistle. The plant consists of approximately 70-80% of the silymarin flavonolignans and approximately 20-30% of a chemically undefined fraction, comprising mostly polymeric and oxidized polyphenolic compounds. The most prevalent component of the silymarin complex is silybin (50-60% of silymarin), which is the most active photochemical and is largely responsible for the claimed benefit of the silymarin. Besides silybin, which is a mixture of two diastereomers (A and B) in approximately 1:1 proportion, considerable amounts of other flavonolignans are present in the silymarin complex, namely silychristin (20%), silydianin (10%), isosilybin (5%), dehydrosilybin, and a few flavonoids, mainly taxifolin. The seeds also contain betaine, trimethylglycine, and essential fatty acids that may contribute to silymarin's hepatoprotective and anti-inflammatory effects.[124, 125, 126, 127, 128]

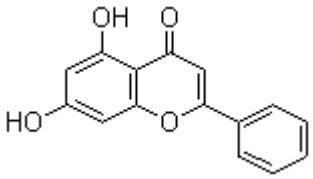
Quercetin:

Quercetin dihydrate	
Identification	
Name	Quercetin dihydrate
Synonyms	3,3',4',5,7-Pentahydroxyflavone dihydrate; 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one dihydrate
Molecular Structure	
Molecular Formula	$C_{15}H_{10}O_7 \cdot 2(H_2O)$
Molecular Weight	338.27

Quercetin is widely distributed in the plant kingdom and is the most abundant of the flavonoid molecules. It is found in many often-consumed foods, including apple, onion, tea, berries, and brassica vegetables, as well as many seeds, nuts, flowers, barks, and leaves. It is also found in medicinal botanicals, including *Ginkgo biloba*, *Hypericum perforatum* (St. John's Wort), *Sambucus canadensis* (Elder), and many others, and is often a component of the medicinal activity of the plant. Quercetin appears to have many beneficial effects on human health, including cataract prevention, cardiovascular protection, as well as anti-cancer, anti-ulcer, anti-allergy, antiviral, and anti-inflammatory activity. All flavonoids have the same basic chemical structure – a three-ringed molecule with hydroxyl (OH) groups attached. A multitude of other substitutions can occur, giving rise to more than 4,000 identified flavonoids. Flavonoids often occur in foods as a glycoside – with a sugar molecule (rhamnose, glucose, galactose, etc.) attached to the C ring. Quercetin is the aglycone (without the

sugar molecule) of a number of other flavonoids, including rutin, quercetin, isoquercetin, and hyperoside. These molecules have the same structure as quercetin except they have a specific sugar molecule in place of one of quercetin's hydroxyl groups on the C ring. This difference can dramatically change the activity of the molecule, as activity comparison studies have identified other flavonoids as often having similar effects as quercetin, but quercetin usually having the greatest activity.[129]

Chrysin:

Chrysin	
Identification	
Name	Chrysin
Synonyms	5,7-Dihydroxyflavone; 5,7-Dihydroxy-2-phenyl-4H-benzo[b]pyran-4-one
Molecular Structure	
Molecular Formula	$C_{15}H_{10}O_4$

Chrysin is a naturally occurring flavone chemically extracted from the blue passion flower (*Passiflora caerulea*).

Inflammation

Chrysin has been shown to induce an anti-inflammatory effect, most likely by inhibition of COX-2 expression and via IL-6 signaling.[130]

Anxiety

In rodent in vivo studies, chrysin was found anxiolytic.[130, 131] In herbal medicine, chrysin is recommended as a remedy for anxiety[132], but there are no controlled data in humans available. Many herbal remedies that contain chrysin promote their value as a libido-increasing supplement. There is some in-vivo evidence for chrysin's libido-enhancing effects in rats.[133] Chrysin demonstrated cell toxicity and inhibition of DNA synthesis at very low concentrations in a normal trout liver cell line.[134]