Chapter No – 2

Literature review
2.0 LITERATURE REVIEW

2.1 Liver:

2.1.1 Anatomy

2.1.2 Structure (Histology)

2.1.3 Blood Supply

2.1.4 Functions Of Liver

2.2 Liver Diseases

2.2.1 Acute Liver Failure

2.2.2 Chronic Liver Failure

2.2.3 Cirrhosis

2.2.4 Alpha1 – Antitrypsin Deficiency

2.2.5 Neoplasm Of Liver

2.2.6 Congenital Liver Diseases:

2.2.7 Circulatory Disorders

2.2.8 Viral And Other Infective Hepatatis

2.3 Mechanisms Of Hepatotoxicant Action

2.3.1 Direct Hapatotoxicity

2.3.2 Indirect Hapatotoxicity

2.3.3 Carbon Tetrachloride Induced Hepatotoxicity

2.3.4 Paracetamol Induced Liver Damage In Rats

2.4 Signs And Symptoms Of Liver Diseases

2.4.1 Jaundice: (Icterus)

2.4.2 Effects Of Portal Hypertension
2.4.3 Hepatic Encephalopathy

2.4.4 General Symptoms And Signs:

2.5 Liver Function Tests

2.6 Parameters Reflecting Liver Condition And Their Interpretation

2.6.1 Transaminases

2.6.2 Alkaline Phosphatase

2.6.3 Acid Phosphatase

2.6.4 Total Proteins

2.6.5 Total Bilirubin

2.6.6 Liver Weight And Volume

2.7 Drugs Used In The Treatment Of Liver Diseases

2.7.1 Some Ancient Ayurvedic Formulations In The Treatment Of Liver Disorder

2.7.2 Some Polyherbal Formulations Verified For The Antihepatotoxicity Against Toxic Chemical Induced Liver Damage In Experimental Animals

2.8 Livrin

2.9 Silymarin (Positive Control)

2.9.1 Medicinal Uses

2.9.2 Chemistry

2.9.3 Mode Of Action

2.10 Aims & Objectives
2.11 Plant Profile

2.12 Plan of Work
2.0 LITERATURE REVIEW:

2.1 Liver:

2.1.1 Anatomy:

The liver is the largest gland of the body enclosed within the right lower rib cage beneath the diaphragm. It is almost completely covered by visceral peritoneum as well as completely covered by a dense irregular connective tissue layer that lies deep to the peritoneum. Liver is divided into two principal lobes, a large right lobe, and a smaller left lobe separated by falciform ligament. The right lobe is considered by many anatomists to include an inferior quadrate lobe and a posterior caudate lobe. (Tortora G.J. et al.).

Liver has five surfaces as Anterior, posterior, superior, inferior, and right.

![Diagram of liver anatomy](image)

Dig 1 External Anatomy of liver (Inferior (visceral) surface of liver)

2.1.2 Structure (Histology):

The lobes of liver are made up of many functional units called lobules. A lobule consists of specialized epithelial cells called hepatic (liver) cells or hepatocytes
arranged in irregular, branching, interconnected plates around the central vein. Rather than capillaries liver has larger spaces lined by endothelium called sinusoids through which blood passes. The sinusoids are also partly lined with Stellate reticuloendothelial (Kuffer’s) cells. These phagocytes destroy worn out white and red blood cells, bacteria, and toxic substances. Bile secreted by hepatic cells enters bile capillaries or canaliculi that empty into small bile ducts. These ducts eventually merge to form the larger right and left hepatic ducts, which unites and exit the liver as the common hepatic duct. Further this common hepatic duct joins the cystic duct from the gallbladder form the common hepatic duct. The common hepatic duct and pancreatic duct enter the duodenum in a common duct called the hepatopancreatic ampulla (ampulla of vater). (Tortora G.J. et al.)

2.1.3 Blood supply:

The liver receives blood from two sources, from hepatic artery it obtains oxygenated blood, and from hepatic portal vein it receives deoxygenated blood containing newly absorbed nutrients. Branches of both the hepatic artery and the hepatic portal vein carry blood into liver sinusoids, where oxygen, most if the nutrients, and certain poisons are excreted by hepatic cells. The reticuloendothelial (Kuffer’s) cells lining the sinusoids phagocytize microbes and bits of foreign matter from the blood. (Tortora G.J. et al.)
Branches of hepatic portal vein, hepatic artery, and bile duct typically accompany each other in their distribution through the liver, collectively; these structures are called a “Portal triad”.

2.1.4 Functions of liver: (Guyton A.C et al., Parakrama Chandrasoma et al., and Godkar P.B. et al.)

- Secretion and excretion of bile:

  Bile is partially an excretory product and partially a digestive secretion. Each day the hepatic cells secrete 800-1000 ml of bile, a yellow, brownish or olive green liquid. It has pH of 7.6-8.6.

  Bile mainly consists water, bile salts, cholesterol, a phospholipid called lecithin, bile pigments, and several ions. The principal bile pigment is bilirubin. When worn-out red blood cells (RBCs) broken down, iron, globin, and bilirubin (derived from hem) are released.

- Metabolic functions: (Parakrama Chandrasoma et al.)

  - Carbohydrate metabolism:

    Liver maintains the normal blood glucose level. e.g. It can convert glucose to glycogen (Glycogenesis) when blood sugar level is high and breakdown of glycogen to glucose (Glycogenolysis) when blood sugar level is low. Also liver can convert amino acid and lactic acid to glucose (Gluconeogenesis) when sugar level is low.

  - Lipid metabolism:

    Liver stores some triglycerides (neutral fats) break down fatty acids into acetyl coenzyme A, this process is called as beta-oxidation, and converts excess acetyl coenzyme A into ketone bodies. (Ketogenesis). It synthesizes lipoproteins. Hepatic cells synthesize cholesterol and use cholesterol to make bile salts.
Protein metabolism:

The liver deaminates (removes the amino group, NH₂ from) amino acids so that they can be used for ATP production. It converts the resulting toxic ammonia (NH₃) into the much less toxic urea for excretion in urine.

Hepatic cells synthesize plasma proteins such as alpha and beta globulins, albumin, prothrombin and fibrinogen.

Hematological functions: (Godkar P.B et al.)

(Hematopoeisis and coagulation)

• Blood formation in the embryo (and in some abnormal states in the adults.)

• Production of fibrinogen, prothrombin, heparin and other clotting factors VII, VIII, IX and X.

• Destruction of erythrocytes. (At the end of their respective life span)

Circulatory functions: (Godkar P.B et al.)

• Transfer of blood from portal to systemic circulation.

• Activity of its reticuloendothelial system. (Kupffer cells) in immune mechanism.

• Blood storage. (Regulation of blood volume)

Detoxication & Protective functions: (Godkar P.B et al., Prakarama Chandrasoma et al.)

• Kupffer cell activity in removing foreign bodies from blood (Phagocytosis)

• Detoxication by conjugation, methylation, oxidation and reduction.

• Removal of ammonia from blood, particularly that absorbed from the intestine by way of the portal vein.
Drug metabolism:

Liver plays a vital role in biotransformation of drugs. It converts drug molecule from nonpolar to polar. These nonpolar drugs can be conjugated with more polar compounds, which makes it water soluble for the urinary excretion.

2.2 Liver Diseases:

2.2.1 Acute Liver Failure:

Acute liver failure most commonly results from acute massive liver cells necrosis caused by viral hepatitis and toxic drugs and chemical also it follows acute fatty change of the liver

Acute liver failure is characterized by

1) Jaundice
2) Hypoglycemia
3) Electrolyte and acid base disturbances (hypokalemia is most dangerous)
4) Hepatic encephalopathy
5) Hepatorenal syndrome
6) Elevation of serum enzymes (LDH, AST, ALT)

There are three types of acute liver failure

a) Fulminant hepatic failure (FHF) – It is a syndrome and abrupt onset, characterized by a progressively severe encephalopathy consequent on massive hepatocellular necrosis.

b) Acute or Chronic Hepatocellular Failure – This may result from protein overload sepsis, or intervention with drugs or surgery.

c) Sub-acute Hepatic Failure – It is defined as acute failure occurring in patients without pre-existing liver disease, in whom the signs of encephalopathy develops more than eight weeks after the onset of illness.
2.2.2 Chronic Liver Failure:

It results from cirrhosis, which associates of liver cells, fibrosis, noduler regeneration.

The effects of chronic liver failure are:

✓ Portal hypertension
✓ Hepatic encephalopathy
✓ Hepatorenal syndrome

Fetor hepaticus – A breath like that of ‘A freshly opened corpse’ believed to be due to deficient methionine metabolism. (Parakrama Chandrasoma et al., Robins and Cotran et al.)

2.2.3 Cirrhosis:

Cirrhosis is premalignant lesion. It is an irreversible and progressive disease that ultimately causes death. Cirrhosis of liver is a pathologic entity characterized by:

1) Necrosis of liver cells, causing liver failure and death.
2) Fibrosis, which involve both central vein and portal areas.
3) Regenerative nodules, a result of hyperplasia of surviving liver cells.
4) Distortion of normal hepatic lobular architecture.
5) Diffuse involvement of the whole liver.

Cirrhosis is classified according to its causes

• Cryptogenic cirrhosis

Hepatic cirrhosis is said to be cryptogenic when complete evaluation of the patient has failed to identify the cause. It may include cirrhosis following immune mediated chronic active hepatitis or following injury due to drugs or chemicals because there is no way to identify these causes.
• **Alcoholic Cirrhosis**

It is frequently associated with evidence of fatty change or acute alcoholic's hepatitis. It is typically fatty micro nodular cirrhosis. The disease is irreversible and causes death.

• **Virus Induced Cirrhosis**

Cirrhosis may follow chronic active hepatitis resulting from infection with hepatitis B and C viruses.

Typically virus induced cirrhosis is macro nodular. Virus induced cirrhosis may tend to progress rapidly with death due to chronic liver failure, portal hypertension, or hepatocellular carcinoma. Cirrhosis caused by hepatitis B virus may be identified by the presence of HBsAg in serum and liver cells.

• **Biliary cirrhosis**

Two types of Biliary cirrhosis are

- Primary Biliary cirrhosis – It causes portal fibrosis. It can occur mostly in middle aged female with long history of obstructive jaundice.

- Secondary Biliary cirrhosis – It occurs in patients with prolonged large bile duct obstruction (gallstones, stricture, tumor, cholangitis). Biliary cirrhosis causes fine nodularity (micronodules).

• **Hemochromatosis**

It results from iron overload in the body by increased serum iron, ferritin and saturation of iron binding protein, increase iron stores in bone marrow and presence of iron in liver cells.

• **Wilson disease**

Wilson disease is an autosomal recessive disorder characterized by

1) Defective excretion of copper into bile.

2) Increase in total body copper.
3) Accumulation of copper in cytoplasm of liver cells, complex to an abnormal protein.

4) Decrease ceruloplasmin level in plasma.

5) Increase “free” copper in plasma.

The primary defect is in the liver cell and is corrected by liver transplantation.

2.2.4 **Alpha₁ - Antitrypsin Deficiency:**

Severe Alpha₁ - antitrypsin deficiency (alpha – antiprotease) occurs in homozygous PiZZ individuals and is a rare cause of cirrhosis, usually with onset during childhood. *(Parakrama Chandrasoma et al., Robins and Cotran et al.)*

2.2.5 **Neoplasm Of Liver:** *(Robins and Cotran Et Al.)*

- **Benign** –
  - Hemangiomas
  - Adenomas (bile duct and hepatic cells)
  - Focal nodular hyperplasia

- **Malignant** -
  - Hepatocellular carcinoma (Hepatomas)
  - Cholangiocarcinoma
  - Malignant vascular neoplasm
    - Metastatic neoplasm
    - Liver neoplasm in infancy
  - Infantile hemangioendothelioma
  - Mesenchymal hamartoma
  - Hepatoblastoma
2.2.6 Congenital Liver Diseases:

- **Cystic liver disease** - It is due to mal development of bile duct, it is associated with the polycystic kidney.

- **Dubin – Johnson syndrome and rotors syndrome** - Genetic defect in excretion of the conjugated bilirubin.

- **Crigler – Najjar syndrome** - It is very rare autosomal recessive disease characterized by complete absence of the enzyme in the homologous patient (Type A disease).

A less caviar form of Crigler- Najjar syndrome (Type B disease) in which enzyme deficiency is partial and compatible with more prolonged survival.

2.2.7 Circulatory Disorders:

- **Impaired blood flow into the liver** - Banti syndrome – In this syndrome extra hepatic portal vein obstruction is there and also sub clinical occlusion of the portal vein presents as variceal bleeding and ascites years later.

- **Impaired blood flow through liver** - Passive congestion and centrilobular necrosis.

- **Hepatic venous outflow obstruction**

  - **Budd – chiari syndrome** – In this syndrome the obstruction of to or more hepatic veins produced liver enlargement, pain, and ascites.

  - **Veno – occlusive disease (sinusoidal obstruction syndrome)** – It is characterized by obstruction of hepatic vein radicals by varying amounts of sub endothelial swelling and fine reticulated collagen.
2.2.8 Viral And Other Infective Hepatitis:

(Parakrama Chandrasoma et al., Robins and Cotran et al.)

Hepatitis means inflammation of liver. The causes of hepatitis are varied and include viruses, bacteria, protozoa as well as drugs and toxins.

- **Viral hepatitis:**

  The viruses mostly infect the liver includes hepatitis A, hepatitis B, hepatitis C, hepatitis E and delta hepatitis. Although other viral infection may present with symptoms of liver disease these are not classified as viral hepatitis. These agents includes Epsein-Barr virus, Cytomegalovirus, Varicella-Zoster virus, Herpes virus, Yellow fever virus.

- **Bacterial hepatitis:**

  Pyogenic liver abscess – Many bacteria as Escherichia coli, other gram negative bacilli, anaerobic bacilli, staphylococcus aureus and streptococci are the causative agents of bacterial hepatitis.

  Bacteria may reach the liver in the course of a systemic bacteremia in the hepatic artery or from the intestine along the bile duct or portal vein.

- **Protozoal hepatitis (Hepatic amoebiasis)**

  It is caused by the entry of amoebic protozoites into portal venous radical in the colonic submucosa; they are carried to the liver. The amoeba caused focal enzymatic necrosis of hepatocytes. Moderate increase in serum bilirubin and SGPT observed.

2.3 Mechanisms Of Hepatotoxicant Action:

As the drug metabolizing and detoxifying organ in the body, the liver is subject to potential damage from an enormous array of Pharmaceutical and Environmental chemicals.

The injury may result:

- From Direct toxicity
• Via hepatic conversion of a xenobiotics to an active toxin

• Through immune mechanism,

Usually by a drugs or a metabolite acting as a hapten to convert a cellular protein into an immunogen.

Also these hepatotoxins are recognized as direct and indirect hepatotoxins.

(Vishnu Ji Ram, 2001)

2.3.1 Direct Hepatotoxicity:

These are the agents that damage the membrane of hepatocytes directly resulting in interference of cell metabolism.

The most common chemo toxicants and drugs that impair the function of liver are CCl₄, thioacetamide, paracetamol, galactosamine, fulvine, phalloidin, ethyl alcohol, aflatoxin, lanthanium chloride and pyrrolizidine alkaloids.

2.3.2 Indirect Hepatotoxicity:

These are the agents that produce hepatic injury as a result of selective interference with metabolic pathways or selective binding to or alteration of a specific component. The occurance of hepatitis by indirect hepatotoxic drug reaction is infrequent and unpredictable. Short exposure of the liver to such drugs results in hepatic disorder.

Following drugs that affect normal liver and brings about certain morphological alterations as valproic acid, phenytoin, methyl-Dopa, chlorpromazine, phenylbutazone, allopurinol, etc.

2.3.3 Carbon tetrachloride induced hepatotoxicity:

Carbon tetrachloride is commonly used experimentally as hepatotoxin which is biotransformed by the cytochrome p-450 system to produce the trichloromethyl free radical which in turn covalently binds to cell membrane and organelles to elicit lipid peroxidation disturbs calcium haemostasis and finally results in death of cell.
Lipid peroxidation is a complex and natural deleterious process. The significant increase observed in levels of lipid peroxides in liver of CCL₄ intoxicated shows free radical induced liver damage. (Ilavarasan R et al., 2001)

The hepatotoxic effect of CCl₄ results in intense centrilobuler necrosis and vacuolization with significant no of swollen hepatocytes which ultimately leads to accumulation of fat in liver and kidney. (Unnikrishnan Latha et al., 1999) fatty degeneration was also observed in centrilobuler areas.

The AST, ALT and ALP are found higher in concentration in cytoplasm, when the liver cell membrane damaged by CCl₄ administration, these enzymes which are normally located in cytosoles are released into the blood stream. AST will be released into the cytosole also by the injury of the organelles such as mitochondria. The activity of these enzymes in serum is useful quantitative marker of the extent and type of hepatocellular damage. The elevation of alkaline phosphatase in serum due to toxic effect of CCl₄ is the result of defective excretion of bile by the liver. This ALP activity is related to functioning of hepatocytes. Increase in its activity is due to the increase synthesis in presence of increase biliary pressure (Chandrashekhar V.M. et al., 2004). The increased level of serum bilirubin is conventional indicator of liver injury.

One of the most important distinguishing features observed in experimental hepatic damage is the pronounced depression in the level of serum total protein. It is evident that CCl₄ poisoning leads to cessation of movement of large amount of triglycerides from the liver to the plasma. So the lipid profile of CCl₄ intoxicated group showed considerable degree of elevation in the concentration of serum, total lipid, cholesterol and triglycerides. Also the above parameters were increased in the tissue such liver, kidney, heart and lungs. (Unnikrishnan Latha et al., 1999)

The injury and dysfunction of liver caused by the toxic effect of CCl₄ in experimental animals simulated the human viral hepatitis model. In CCl₄ induced toxic hepatitis a toxic reactive metabolite trichloro methyl radical was produced by the microsomal oxidase system. These activated radicals bind
covalently to macromolecules of the lipid membrane of endoplasmic reticulum and causes peroxidative degradation of lipids. As a result fats from the adipose tissues were translocated and accumulated in the liver.

The estimation of total bilirubin confirms the intensity of jaundice. In viral and toxic hepatitis the degree of excretion of bilirubin from the intestine is very less and bilirubin present in the liver is excreted into the canaliculi and then regurgitated into the blood stream. Hence hyperbilirubinemia is most common in hepatitis patients. It is also known that liver synthesizes number of proteins. The change in serum protein level forms the bases for important laboratory aids to diagnose the depth of jaundice. (Krishna V., Shanthamma C., 2004)

Hepatocellular necrosis leads to very high level of GOT and GPT released from the liver in blood. Between the two GPT is better index of liver injury as liver GPT activity represents 90% of total enzyme present in the body. (Chandrashekhar V.M. et al., 2004)

2.3.4 Paracetamol induced liver damage in rats:

Paracetamol (N-acetyl p-amino phenol or acetaminophen) is well known antipyretic and analgesic which produces hepatic necrosis at higher doses. Indiscriminate ingestion can lead to accidental poisoning and potentially lethal hepatotoxicity. Paracetamol is mainly metabolizes by glucoronide and sulphate conjugation. A small amount is metabolized by the cytochrome P-450 system to a toxic metabolite. The cell is normally protected from injury by conjugation of this toxic metabolite with glutathione. As a dose increases the glutathione content of hepatocytes available for detoxification of the toxic metabolite is exhausted and the hepatocytes become vulnerable to the noxious effects of the metabolite resulting in liver cell necrosis. (Raj Kapoor B. et al., 2002)

Liver is the organ highly affected primarily by toxic agents. Paracetamol produces wide areas of frank necrosis of liver parenchyma and a picture of dilated vasculature and sinusoids around the necrotic zones (Chattopadhyay R.R. et al., 1992) Paracetamol produces hepatic necrosis in
high doses by covalent binding of its toxic metabolite N-acetyl-p-benzoquinone imine to sulphadryl groups of protein resulting in cell necrosis through lipid peroxidation induced by decreasing glutathione in the liver. (Shankar M. B., Parikh J.R., 2005)

Damage to the structural integrity of liver is reflected by an increasing in levels of serum transaminases because they are cytoplasmic in location and are release into the circulation after cellular damage. Free radicals cause damage in biological systems this in turn cause cellular damage that may lead to cancer, liver injury, heart disease etc. (Suja S.R., Latha P.G., 2004) The toxicity is medicated by a metabolite N-acetyl imidazole which binds covalently in endoplasmic reticulum and in protein of cytoplasm. (Gulati R., Agrawal S., 1995)

2.4 Signs And Symptoms Of Liver Diseases: (Chatterjea M.N. et al., Godkar P.B et al.)

They are categorized into-

1. Jaundice-
   - Pre-hepatic jaundice.
   - Hepatic jaundice.
   - Post-hepatic jaundice.

2. Pruritis & other skin signs-
   - Spider naevi.
   - Palmer erythema.

3. Ascites (Robins and Cotran et al.)

4. Encephalopathy (Neurological syndrome)

5. General signs & symptoms
2.4.1 Jaundice: (icterus):

The commonest system of liver disease is a yellow coloration of the skin and sclera of the eyes, mucous membrane owing to hyperbilirubinemia.

Jaundice is the sign of abnormal bilirubin metabolism and excretion. Jaundice is visible when serum bilirubin exceeds 2-4 mg/dl.

Classification of Jaundice:

- **Pre-hepatic (haemolytic) jaundice-**

  This is due to increased breakdown of Hb, so that the liver cells are unable to conjugate all the increased bilirubin formed.

  Since the liver of a newborn functions poorly for the 1st week and hence many babies experience a mild form of jaundice, called neonatal (physiological) jaundice that disappears as the liver matures.

- **Hepatic (Hepatocellular) jaundice-**

  This is the disease of parenchymal cells of liver. This jaundice occurs due to

  - Conditions in which there is a defective conjugation- There may be reduction in no of functioning liver cells eg. -Chronic hepatitis (all liver functions impaired)
  
  - Conditions such as viral hepatitis and toxic jaundice – In this extensive damage to liver cells associated with intra hepatic obstruction resulting in appreciable observation of conjugated bilirubin.
  
  - Cholestatic jaundice- This is due to drug induced eg. Chlorpromazine and steroids which cause intrahepatic obstruction, liver function being essentially normal.

- **Obstructive (Posthepatic) jaundice:**

  In this jaundice there is obstruction of bile flow in the extrahepatic ducts, eg. due to gallstones, carcinoma of head of pancreas, enlarged lymph glands pressing on bile duct etc.
2.4.2 Effects of Portal Hypertension:

- **Splenomegaly-**
  
  Splenic enlargement is caused by passive venous congestion.

- **Ascites-**

  Ascites is due to increased transudation of fluid across the peritoneal membrane over the surface of liver.

  The major factor leading to severe ascites in chronic liver disease is a decrease in serum albumin level, with portal hypertension playing only a contributing role. (*Parakrama Chandrasoma et al.*, *Robins and Cotran et al.*)

2.4.3 Hepatic Encephalopathy:

Hepatic encephalopathy is characterized by cerebral dysfunction (hypersomnia, delirium, flapping tremors of hands) leading to convulsions, coma and death. (*Parakrama Chandrasoma et al.*)

The pathogenesis of hepatic encephalopathy is unclear but it believed that nitrogenous products of intestinal bacteria accumulates in the systemic blood, having bypassed the liver through postsystemic anastomoses or having undergone deficient detoxification by failing liver cells.

The substances involved in the pathogenesis of hepatic encephalopathy are ammonia present high in plasma and cerebrospinal fluid in placenta with liver failure. The amides like octopamine act as a false neurotransmitter.

2.4.4 General Symptoms And Signs:

Non-specific symptoms accompany early liver disease eg. anorexia, nausea, and vomiting. Fever is common, being mild to moderate in alcoholic hepatitis, though high fever with rigors may precede jaundice in acute viral hepatitis.
2.5) **Liver Function Tests**: (Chatterjea M.N. *et al.*, Godkar P.B *et al.*)

When the liver is diseased, one or more but not necessarily all of the functions are impaired. There can be no test for liver functions as a whole. The various “liver function tests” (LFTs) are tests of derangements of individual functions of the liver. Since many tests gives many similar abnormal results in a particular liver disease, it may be possible to extend a conclusion drawn from single test.

The liver biopsy results may not be comparable with the LFTs since many functional changes are not mirrored by obvious structural changes in the liver cells. For understanding various liver functions of the liver, following tests are performed.

- **Tests for bile pigments and bile salts excretion**-
  - Serum total, direct and indirect bilirubin.
  - Urine bile salts, bile pigments and urobilinogen.

- **Tests for plasma proteins**-
  - Thymol turbidity test
  - Determination of total proteins, albumin, globulin, and A/G ratio.

- Determination of plasma fibrinogen

- Various flocculation tests

- Amino acids in urine
• **Tests for carbohydrate metabolism**-
  ✓ Galactose tolerance test
  ✓ Glucose tolerance test

• **Tests for lipid metabolism**-
  ✓ Serum cholesterol and ester cholesterol and their ratio
  ✓ Faecal fats.

• **Tests for excretion of injected substances by the liver**-
  ✓ Bromsulphthalein test (BSP retention test)
  ✓ $^{131}$I Rose Bengal test

• **Tests based on detoxicating function of liver**-
  ✓ Hippuric acid synthesis test

• **Formation of prothrombin by liver**-
  ✓ Determination of prothrombin time and index.

• **Tests based on Amino acid catabolism**-
  ✓ Determination of ammonia.
  ✓ Determination of glutamine in CS fluid (indirect liver function test).

• **Investigative method**:
  ✓ Ultrasonography, Computerized Tomography and Magnetic Resonance Imaging discloses mass lesions in the liver or dilation of the biliary system.
  ✓ Arteriography and Isotope scans- Hepatic blood flow can be detected by this method.
  ✓ Gallium Scanning- Useful in detecting neoplasms and abscesses in the liver.
Liver Biopsy- It is the safe method of obtaining the tissue for histological examinations. Blind biopsy using a cutting needle indicated for diffuse lesions of the liver. Radiologically dissected fine needle and Aspiration biopsy is indicated when it is necessary to examine a localized lesion. (Parakrama Chandrasoma et al.)

2.6 Parameters Reflecting Liver Condition And Their Interpretation:

2.6.1 Transaminases:

The transaminase enzyme are involved in nitrogen transfer reactions from amino acids to other substrate for eventual disposal in the urea cycle.

- Serum Glutamate Oxaloacetate Transaminase (SGOT)-

  It is also known, as Aspartate Transferase (AST) is an enzyme present in the cytoplasm and mitochondria of most cells, because SGPT is found in many tissues, abnormalities are not specific for liver injury. SGOT is elevated in conditions as liver cell injury, myocardial infarction, muscle trauma, acute pancreatitis, intestinal injury, pulmonary infarction, cerebral infarctions, and renal infarctions. Levels may be falsely decreased during diabetic ketoacidosis, beriberi, severe liver disease and chronic liver failure. SGOT catalyzes the reaction

\[
\text{Aspartic acid} + \text{SGOT Oxaloacetic acid} \rightarrow \text{Alpha-Keto-Glutaric Acid} + \text{Glutamic acid}
\]

The amount of oxaloacetic acid are determined after incubation colorimetrically by the formation of hydrazone with dinitrophenyl hydrazine reagent (DNPH) which is highly coloured in alkaline medium and thus serum level of SGOT is determined by Reitman and Frankel method.

Levels of SGOT and SGPT normally parallel to each other.

Levels very high= acute viral hepatitis

High= Myocardial infarction or shock
If AST high, ALT normal = Alcoholic liver disease.

- **Serum Glutamate Pyruvate Transaminase (SGPT):**

  It is also known as Alanine aminotransferase (ALT). It is an enzyme found in mitochondria of many cells but most abundant in liver. Though it may increase in many conditions, an elevation of SGPT is more specific in liver injury, including hepatic necrosis and acute hepatitis. SGPT catalyzes the reaction
  \[
  \text{Alanine} + \overset{SGPT}{\rightarrow} \text{Pyruvic acid} + \overset{\alpha}{\rightarrow} \text{Alpha-Keto-Glutar Acid} \rightarrow \text{Glutamic acid}
  \]

  The amount of pyruvic acid can be determined after incubation colorimetrically by the formation of hydrazone with dinitrophenyl hydrazone reagent (DNPH) which is highly colored in alkaline medium and the serum level of SGPT was determined by Reitman and Frankel method. In case of post hepatic condition (Biliary obstruction) SGPT level show a steady elevation of 3-4 times if the upper normal limits and decline shortly after relief of obstruction.

2.6.2 **Alkaline Phosphatase:**

  This enzyme present in variety of tissues including bone, liver, intestine and placenta. Liver fraction of alkaline phosphatase is predominantly elevated by biliary system obstruction wheather in intrahepatic or extrahepatic. In viral hepatitis moderate rise in alkaline phosphatase was observed higher values are observed in toxic hepatitis, and very high values are observed in toxic hepatitis.

2.6.3 **Acid Phosphatase:**

  Acid phosphatase is frequently employed as a marker enzyme to assess the lysosomal changes in vivo because it is localized almost exclusively in the particles and its release parallels that of lysosomal hydrolases. *(Emmanuel et al. 2001)*
2.6.4 **Total Proteins:**

Serum total protein level decreases below normal range in different clinical conditions as Cirrhosis of liver and other liver diseases in which liver cells were severely damaged.

2.6.5 **Total Bilirubin:**

Disordered bilirubin metabolism could be considered under

1) Increased formation of bilirubin.

2) Abnormal uptake of bilirubin in the liver cells.

3) Defective conjugation.

4) Failure of normal amounts of bile to reach the duodenum i.e. development of condition called cholestasis.

2.6.6 **Liver Weight And Volume:**

The weight and volume of liver increases due to accumulation of fats in liver disorders.

2.7 **Drugs Used In The Treatment Of Liver Diseases—**

**Table 2. Some Herbal Drugs Effective In Liver Disorder:**

<table>
<thead>
<tr>
<th>BOTANICAL NAME / FAMILY NAME / PARTS USED</th>
<th>ACTIVE CONSTITUENTS</th>
<th>BIOCHEMICAL AND PHARMACOLOGICAL PARAMETERS STUDIED</th>
<th>LIVER DISORDER PROTECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrus precatorius/ Leguminosae/ seeds</td>
<td>Agglutinins and lectins S.C.</td>
<td>Effectiveness in Hepatitis (Patented product)</td>
<td></td>
</tr>
<tr>
<td>Plant Name</td>
<td>Constituents</td>
<td>Activity</td>
<td>Effect</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>--------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Allium sativum/Liliaceae/Bulb</td>
<td>Volatile oil contains allicins allin-s-allylmethymercaptstein</td>
<td>In vivo and in vitro studies showed inhibitory activity in CCl₄ and galactosamine induced cytotoxicity in cultured rat hepatocytes.</td>
<td>Liver protecting</td>
</tr>
<tr>
<td>Allophyllus edulis var gracillis/Sapindaceae/Leaves</td>
<td>C-glucosy; flavones</td>
<td>Liver protection against CCl₄ and galactosamine induced cytotoxicity in cultured rat hepatocytes.</td>
<td>Antihepatotoxic activity</td>
</tr>
<tr>
<td>Angelica dahurica/Apiaceae/Aerial parts</td>
<td>Furonocoumarins viz.,phallopterin</td>
<td>Normalised SGPT enzymes levels followed by inhibition of microsomal lipid peroxidation by CCl₄ metabolites</td>
<td>Antihepatotoxic activity</td>
</tr>
<tr>
<td>Aphanamixis polystachya/Meliaceae/bark</td>
<td>Ester soluble fraction, 0.6mg/kg</td>
<td>Reversal of liver injury</td>
<td>Liver protecting</td>
</tr>
<tr>
<td>Azadirachta indica/Meliaceae/leaves</td>
<td>Leaf extract</td>
<td>SGOT, GPT, ACP, ALP reduction due to paracetamol induction</td>
<td>Heapatoprotectants,</td>
</tr>
<tr>
<td>Boerhaavia repanda/Nyctaginaceae/Roots</td>
<td>Petroleum ether, CHCl₃ and methanolic extract of roots</td>
<td>Liver protection against CCl₄ induced liver damage</td>
<td>Jaundice</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Bioactive Component</td>
<td>Effect</td>
<td>Outcome</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------------------</td>
<td>----------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Canarium manii/Burseraseae e/ Dry nuts</td>
<td>Bioflavonoid</td>
<td>Experimentally induced CCl₄ toxicity was inhibited</td>
<td>Antihepatotoxic activity</td>
</tr>
<tr>
<td>Carica papaya/Caricaceae e/ Fruits</td>
<td>Papain along with citric acid and starch</td>
<td>Mixture of 35-20 parts of citric acid and one part of papain useful in digestive tract disorders</td>
<td>Hepatitis</td>
</tr>
<tr>
<td>Glycyrrhiza spp./Leguminosae / roots</td>
<td>Saponins, Glycyrrhizin</td>
<td>Hepatitis C virus induced liver damage reversed</td>
<td>Antihepatitis activity</td>
</tr>
<tr>
<td>Garcinia cota/guttiferae/Seeds</td>
<td>Extracts 100mg/kg b.w.</td>
<td>SGOT, SGPT, were significantly reduced by reducing paracetamol lethality</td>
<td>Hepatoprotective agent</td>
</tr>
<tr>
<td>Phyllanthus amarus/Euphorbiaceae/whole plant</td>
<td>Alcoholic extract (100mg/kg) and butanol fraction (50mg/kg)</td>
<td>Protects from experimentally induced liver toxicity</td>
<td>Detoxify liver</td>
</tr>
</tbody>
</table>

2.7.1 Some Ancient Ayurvedic Formulations In The Treatment Of Liver Disorder:

Dantyadyarishtra, Rohitakarishtra, Kalyanka guda, Dani haritak, Dashmoolaharishta, Drakshavaleha, Manibhdra yog, Shiva ghutica, Patoladi Kawatch choorna, Triphala ghrita, Shatpaladi ghrita, Sukumara ghrita, Navays choorna, Sudarshan choorna, Chandraprabha vati, Suran vatica, Kasisa bhasma, loha bhasma, Mandura bhasma, Shankha bhasma, Pnarnavadi mandura, Kumar Kalyan ras, Lokhantha ras, Dhatri loha, Vidangadi loha, Rohitaka loha, etc.
2.7.2 Some Polyherbal Formulations Verified For The Antihepatotoxicity Against Toxic Chemical Induced Liver Damage In Experimental Animals: (Subramoniam A., et al., 1999).


2.8 Livrin:

The Polyherbal medicine used in this study is “Livrin” which used in liver dysfunction, hepatomegaly, jaundice viral hepatitis and alcoholic liver disorders.

Livrin Contains 10 different herbs having different Hepatoprotective activity but no scientific study of whole formulation prepared from these plants altogether had been reported.

Livrin Syrup- Each 5 ml contains-

Kalmegh  Andrographis paniculata  500 mg
Trifala  Terminalia chebula
  Terminalia belirica  300 mg
  Terminalia emblica
Maka  Eclipta alba  100 mg
Chitrak mool  Plumbago zeylanica  100 mg
Kutki  Picrorrhiza Kurroa  150 mg
Gulvel  Tinospora cordifolia  200 mg
Haritaki  Terminalia chebula  150 mg
Wawding  Embelia ribes  200 mg
Punamava mool  Boerhaavia diffusa  200 mg
Kalimirch’  Piper nigrum  50 mg
Literature review

Suger flavoured syrup base Q.S.

The literature survey had revealed that all the ingredients in this formulation individually possess hepatoprotective activity.

- **Andrographis paniculata** (Kalmegh):

  Traditionally used for a variety of ailments including liver disorders and has also been shown to protect against toxin induced hepatotoxicity. The diterpenes of andrographis were shown to increase glutathione (GSH), which may decrease susceptibility of the tissue to oxidative damage. \( \text{(Kapil A., 1993)} \)

  Andrographolide the active principle showed choleretic action in rats. \( \text{(Tripathi G.S., Tripathi Y.B., 1991)} \) and prevention of CCl\(_4\) induced increase of ALT and AST. \( \text{(Handa S.S., et al. 1986)} \)

  The hepatoprotective and antioxidant activity of Kalmegh was reported against Carbon tetrachloride, BHC, Paracetamol and galactosamine. \( \text{(Trivedi N.P., Rawal U.M.2001, Visen P.K., et al. 1993, Handa S.S., Sharma A.1990)} \)

- **Trifala**:

  It is the powder consisting of equal parts of three botanical medicines namely as *Terminalia chebula, Terminalia belirica, Phylanthus Emblica*. The reported biological activities of *Trifala* are antioxidant, Immunomodulatory, cardiotonic, antifungal, antiviral and hepatoprotective activity.

- **Eclipta alba** (Maka):

  Mainly its leaf and roots were traditionally used as cholagogue (aids bile secretion) and deobstruent (removes functional obstructions in the body) in hepatic enlargement, for jaundice, and other ailments of the liver and gall bladder. Two active principles as Wedelolactone and demethyl-wedelolactone showed a significant stimulatory effect on liver cell regeneration. *Eclipta alba* exerts its protective action through a reduction in GSH depletion. Pretreatment with *Eclipta alba* reduced alcohol induced hepatic necrosis in rats in a CCl\(_4\) guinea pig model and caused significant decrease in AST, ALT and serum alkaline phosphatase, and improvement in parenchymal damages. \( \text{(Handa S.S., et al., 1986)} \).
Hepatoprotective activity of *Eclipta alba* leaves was reported. *(Singh B. et al., 2001, and Saxena A.K. et al., 1993).*

- **Plumbago zeylanica** (Chitrak mool):

  It is believed to increase the digestive power. It promotes the appetite and is said to be useful in dyspepsia, piles and skin diseases.

- **Picrorrhiza Kurroa** (Kutki):

  This plant is a constituent of many marketed formulations. In CCl₄ paracetamol and aflatoxin models *Picrorrhiza Kurroa* reduced liver transaminases and restored the levels of Na+/K+ ATPase normal. Used traditionally in Ayurveda for centuries as a general liver tonic and for liver cleansing, hepatitis, biliousness, fevers, and poisoning. It is also used as valuable bitter tonic, antiperiodic, stomachic and febrifuge. It is laxative in larger doses.

  It helps in the restoration of hepatic glycogen and total proteins.

- **Tinospora cordifolia** (Gulvel): *(Rege N. et al., 1984)*

  In a CCl₄ model, acute damage was enhanced by pretreatment with *Tinospora cordifolia*, but it is proved effective in chronic injury models in prevention of fibrosis and in stimulating regeneration of hepatic tissue. In Ayurveda text *Tinospora cordifolia* is considered as Rasayana, a plant, which promotes longevity, increases body resistance and imparts immunity against disease. Modulation of kupffer cell activity by *Tinospora cordifolia* in liver damage was reported. *(Nagarkatti, et al. 1994)*

- **Terminalia chebula** (Haritaki):

  Traditionally used in chronic diarrhoea and dysentery, flatulence, vomiting, colic, and enlarged spleen and liver.

- **Embelia ribes** (wawding):

  The berries of this plant were used in treatment of jaundice as claimed in Ayurveda. The reported activities are anthelmentic, antifertility and on
reproductive organs. Traditionally used for hepatic conditions and liver rejuvenation. (Nadkarni A.K, 1994)

- Boerhaavia diffusa (Punarnava mool):

  It is used as a diuretic in patients suffering from oedema and dropsy due to various causes. The Hepatoprotective activity of chloroform and methanolic extracts of roots and aerial parts of Boerhaavia diffusa against CCl₄ induced liver injury had been reported. (Rawal A.K. et al., 1997 and Chandan B. K. et al., 1991)

- Piper nigrum (Kalimirch):

  *Piper nigrum* is one of the constituent of Ayurvedic recipe called ‘Trikatu” (Sanskrit meaning “Three acids”). It consists of the dried, unripe fruits of the perennial climbing plant. Mostly the flowers are used for medicinal purposes. Volatile oil is the main constituent of *Piper nigrum*. It is mainly used as antiperiodic for malaria, postnatal complaints.

2.9 Silymarin (Positive Control):

**Introduction:**

Silymarin is the name applied to the specific extract made from the seed of the milk thistle (*Silybum marianum* L. *gaertn syn. Carduus marianum* L.) also known as St mary’s or variegated thistle. It is the member of the daisy family, Asteraceae (compositae). (Choksi S. et al., 2000)

2.9.1 Medicinal Uses:

Milk thistle had been used since 4th century for plague and for congestive conditions of the liver and spleen. In modern times the use of milk thistle and silymarin has been focused on liver conditions.

2.9.2 Chemistry:

Silymarin is a standardized seed extract rich in a type of flavonoid compounds known as flavonolignans. The main flavonolignans in silymarin are the isomers of silybin, silydianin, isosilybinand and silychristin.
2.9.3 Mode Of Action:

The main actions of silymarin can be described as hepatoprotective and hepatoregenerative. (Choksi S. et al., 2000). It protects the hepatocytes membrane against oxidative damage. It also inhibits the lipid peroxidation of hepatocytes, microsomal and erythrocytes membranes in rats. Silybin protects against iron induced oxidative liver damage in rats and it also protects hepatocytes against ethanol induced lipid peroxidation by increasing glutathione level.

Silybin have the ability to accelerate the regeneration of hepatocytes following liver damage. Silybin stimulates protein synthesis and mitosis via activation of DNA dependent RNA polymerase-1. Silymarin has also demonstrated ulcer protective activity, hypocholesterolaemic potential, UV protective effect.

Main indications for Silymarin:

Acute liver diseases. (including hepatitis)

Chronic hepatitis.

Toxic liver damage.

Fatty liver degeneration.

Exposure to hepatotoxins.
2.10 Plant Profile:

Name : *Enicostema axillare Lam.*

Syno name : *Enicostima lithorale*

Family : Gentianacea

Synonyms : Nagajihva

Hindi : Mamajjakah

Sanskrit : Nahi

Marathi : Katvinayi

Tel. : Chevvu

Tamil : Vellaruku

Kan. : Karibandita,

Distribution:

It is widely distributed in India, Malaysia, Bangladesh and Shri Lanka.

Description:

A glabrous or procumbent perennial herb upto 50 cm in height from a thick root stock, stems and branches sub quadrangular; leaves simple, opposite, sessile, linear, linear-oblong or elliptic-lanceolate, glabrous, 3-nerved marginal-nerves often obscure; flowers white, tubular, in whorled axillary clusters; fruits ellipsoid capsules, narrowed at the base and rounded at the apex with the remains of the style.

Parts Used:

Whole plant.

Traditional Uses:

The plant is bitter, acrid, thermogenic, digestive canninative, stomachic, laxative, anthelmintic, anti-inflammatory, liver tonic urinary astringent, depurative, revulsive and antiperiodic and is useful in dyspepsia, flatulence, colic, helminthiasis,
abdominal ulcers, hernia, constipation, dropsy, swellings, vitiated conditions of kapha and vata, hepatopathy, glycosuria, leprosy, skin diseases, pruritus, intermittent fevers and malaise. The plant is locally applied in snake bite.
Plant photo:
Enicostema axillare belongs to family Gentianaceae. It is a perennial herb found throughout India and is common in coastal areas. The plant is used in folk medicine to treat diabetes mellitus, rheumatism, abdominal ulcers, hernia, swelling, itching and insect poisoning (Kirtikar and Basu. 1999), anti-inflammatory (Sadique et al., 1987), hypoglycemic (Jyoti, et al ., 2000, Murili, et al., 2002, Jyoti, et al.. 2003) and anticancer (Murili, et al., 2002) activities have been reported. These reported activities and many of the ethnobotanical used of the plant related to its hepatoprotective activity. Swertimarin, alkaloids, steroids, triterpenoids, saponins, flavonoids, xanthones, and phenolic acid were isolated from the plant (Kavimani, S. and Manisenthilkumar, 2000). Many such compounds have protective effects due to their pharmacological activities (Wargovich et al, 2001) Liver disease remains one of the serious health problems. Herbs play a major role in the management of various liver disorders. A number of plants possess hepatoprotective property (Heba et al, 2006).

Various indigenous plants are known to play a vital role in the management of liver disorders but the perusal of literature reveals lack of scientific validation for the use of much of the traditional medicine. Hence the present study “Evaluation of Indian medicinal plant (Enicostima lithorale) for hepatoprotective activity” was undertaken to fill the lacuna in this regard. Though the plant used for the treatment of liver disorders in Ayurveda, an ancient system of medicine (Nadakarni, 1976, Jain, 1991), a review of literature showed that these plants have not been subjected to systematic investigation to assess their hepatoprotective effects. Hence the present study was undertaken to explore the possible molecular level mechanisms involved in hepatocellular membrane protection of the above said plants against carbon tetrachloride (CCl₄) and paracetamol-induced hepatic damage in rats.

2.11 Aims & Objectives:

Liver disease is a leading cause of death in many countries and the causative factors are alcohol consumption, malnutrition, anemia, hepatotoxic drugs and infections etc. The liver, a vital organ instrumental in metabolism, detoxification and elimination, is responsible for protection of human body against adverse effects of drugs, chemicals, toxins, bacteria, viruses and parasites etc., but in the process liver itself is under threat and obviously needs protection.
So far, no effective measures are available for the treatment of liver diseases. The different medical, surgical and therapeutic methods used at present are inadequate and with generally poor results. Also, some of the modern drugs which are given to treat liver diseases may themselves cause liver damage. It is therefore, imperative to search alternative drugs for the treatment of liver diseases to replace the existing drugs of doubtful efficacy and safety. In this context the present study has been undertaken with the following objectives.

1. To study the hepatoprotective activity of the plant selected from traditional system of medicine, which are used for liver disorders.

2. To carry out the phytochemical studies of the plant showing promising hepatoprotective activity including activity guided fractionation and isolation of active principle(s) responsible for the hepatoprotective activity.

3. To quantify the active marker components responsible for hepatoprotective activity using HPTLC.

4. To standardize all the selected plants/extracts using HPTLC and various physico-chemical parameters.

5. To evaluate therapeutic efficacy, toxicity studies of the selected plant extracts as per WHO standards/guidelines.

2.12 Plan of Work:

For the present work, the protocol used for evaluating the Quality control parameters and hepatoprotective activity is as follows;

- Collection of plant material.
- Authentication of plant material.
- Drying and grinding of plant material.
- Physicochemical evaluation of crude plant material.
- Successive solvent extraction of plant material with various solvents.
• Preliminary phytochemicals screening of various extracts for the detection of different plant constituents.

• Chromatographic evaluation by HPTLC of different extracts.

• Evaluation of hepatoprotective activity of different extracts.

• Formulation and evaluation of tablet of potent extract of Enicostima lithorale.

REFERENCES:


5. Chattopadhyay R.R., Bandyopadhyay M. Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract against paracetamol induced hepatic damage in rats. *Indian J. of Pharmacol.* (2005); 3(37): 84-185.


23. Nadkarni A.K., Indian material medica, Popular Prakashan, 1985


