PERMEABLE

INTRODUCTION

The expedition of Indian medicine starts with the improvement of medical concepts as a visions and empiricism up to the awareness of the basics of a science of life (Valiathan MS, 1998). In *Rigveda*, 67 plants for medicinal purpose between 4500-1500 BC, while in *Yajurveda* enlisted 81 plants. The *Athravaveda* during 1200 BC, reports 290 plants of therapeutic values while *Ayurveda* between 900-600 BC explores 736 medicinal plants (Handa SS et al, 1982). "Ayurveda is the spine of the ancient medical science of India (Kapoor LD, 2005). Several therapeutic as well as Rasa, Guna, Vipaka, and Virya properties of various variety of medicinal plants through proper shlokas are described in the *Ayurveda* (Valiathan MS, 1998, Joshi SG, 2000). “Charaka Samhita” is text of *Ayurveda* that deals with method of drug administration while another text “*Susruta Samhita*” explored several surgicial methods by means of instruments and appliances (Lele RD, 1986). *Ayurveda* has primarily 8 subjects that deals by altered characteristics of art of healing (Kapoor LD, 2005, Lele RD, 1986):

- *kaya chikitsa* or oral medicine
- *salya tantra* or toxicology
- *salakya tantra* or head and neck disease treatment
- *agada tantra* or toxicology
- *bhuta vidya* or the convulsive disorders managent by evil spirit
- *bala tantra* or pediatrics
- *rasayana tantra* or geriatrics
- *vajikarana tantra* or aphrodisiacs art.

The “*Ayurveda*” is arrangement of a Sanskrit words where *Ayu* stands as a “Life” and *Veda* stands as a “Knowledge or science” (Lele RD, 1986). The name *Ayurveda*, states the science of healthy living which is not limited only to treatment of illness but also associated with complete health of patient including diet, habits and positive thinking (Pole S et al, 2006). This awareness of medicines gathered by generations is termed as “Ethno medicine” or “Traditional medicine” including *folk/tribal* medicines (Pushpangadan P, 1996).
PROBLEM IN HAND
LIVER TOXICITY:
In animals, various vital physiological processes like metabolism, secretion (e.g. bile for digestion), storage and detoxification happens in liver. Some of Hepatic diseases like acute or chronic disease (inflammatory), hepatosis (non-inflamattory) and cirrhosis (degenerative) diseases are amongst maximum serious sicknesses. Various types of Infections, autoimmune disorders, higher doses of antibiotics, different types of chemotherapy and excessive alcohol consumption are responsible for a number of liver diseases by peroxidation and oxidation cells (Smucker RA, 1975). Almost 90 % of the acute hepatitis is originate from infections through Hepatitis -A, -B, -C, -D, -E and -G viruses. Many therapeutic drug adverse events are responsible for Drug-induced liver diseases and to overcome this adverse events is a major health challenge to researchers. Various drugs leads to either direct hepatotoxicity or chemically reactive metabolites responsible for DNA damage, protein dysfunction, lipid peroxidation and oxidative stress. A vaccine available for immunization against Hepatitis B virus (WHO, 1997). But in spite of lot of research for synthetic hepatoprotective medicine, there was no any potent hepatoprotective drug are able to come in market from last decades. Since long time medicinal plants and around more than 100 phytochemicals which can cure hepatic disease and claimed to have hepatoprotective activity. Hence it is necessary to evaluate these plants for their hepatoprotective potential (Handa SS et al, 1990, Doreswamy R et al, 1995).
DIABETES MELLITUS (DM):
Diabetes mellitus is a common disease in which deficiencies in impaired metabolism of carbohydrate leads to decrease in insulin secretion, insulin action, or both which produced chronic high blood sugar levels in the body (Zhang F et al, 2006). This metabolic disorder affected a large population around the globe with noticeable increased morbidity and mortality rate and one of the major reason in maximum developed countries for death. High blood sugar level complications leads to macrovascular and microvascular injuries like nephropathy, retinopathy, peripheral neuropathy, kidney failure, high blood pressure and heart diseases (Vinik AI et al, 2003). The World Health Organisation (WHO) forecasts the present diabetic population of 177 million population may increase up to 370 million population 2030, while in India, diabetic population which may increase up to more than 90 million by 2030. Insulin and many antidiabetic agents (oral) like biguanides, sulfonylureas and α – glucosidase inhibitors are available to control high blood sugar levels in diabetes (Bjork S et al, 2003). But all of these have high or less toxicity and side effects on chronic use. Hence, alternative treatments for such chronic diseases are popular, promising and in the demand (Beatriz L et al, 2001). In the ethno botanical information more than 800 plants claimed to have anti-diabetic potential (Bandole S et al, 2006).

IMPORTANCE & SCOPE:
The present study was undertaken to evaluate the ability of *Piper cubeba* fruits to produce hepatoprotective activity, decrease blood glucose level, total cholesterol, and triglyceride level and increase in HDL cholesterol due to phytoconstituents presence in the fruits such as saponins, glycoside, alkaloids and terpenoids responsible for significant antidiabetic activity and may have future scope to relace allopathic madications, hence reduces side effects, cost and enhance patient compliance.
The expedition of Indian medicine starts with the improvement of medical concepts as a visions and empiricism up to the awareness of the basics of a science of life (Valiathan MS, 1998). In *Rigveda*, 67 plants for medicinal purpose between 4500-1500 BC, while in *Yajurveda* enlisted 81 plants. The *Atharvaveda* during 1200 BC, reports 290 plants of therapeutic values while *Ayurveda* between 900-600 BC explores 736 medicinal plants (Handa SS et al, 1982).

*Ayurveda* is the spine of the ancient medical science of India (Kapoor LD, 2005). Several therapeutic as well as Rasa, Guna, Vipaka, and Virya properties of various variety of medicinal plants through proper shlokas are described in the *Ayurveda* (Valiathan MS, 1998, Joshi SG, 2000). “*Charaka Samhita*” is text of *Ayurveda* that deals with method of drug administration while another text “*Susruta Samhita*” explored several surgical methods by means of instruments and appliances (Lele RD, 1986). *Ayurveda* has primarily 8 subjects that deals by altered characteristics of art of healing (Kapoor LD, 2005, Lele RD, 1986):

- *kaya chikitsa* or oral medicine
- *salya tantra* or toxicology
- *salakya tantra* or head and neck disease treatment
- *agada tantra* or toxicology
- *bhuta vidya* or the convulsive disorders management by evil spirit
- *bala tantra* or pediatrics
- *rasayana tantra* or geriatrics
- *vajikarana tantra* or aphrodisiacs art.

The “*Ayurveda*” is arrangement of a Sanskrit words where *Ayu* stands as a “Life” and *Veda* stands as a “Knowledge or science” (Lele RD, 1986). The name *Ayurveda*, states the science of healthy living which is not limited only to treatment of illness but also associated with complete health of patient including diet, habits and positive thinking (Pole S et al, 2006). This awareness of medicines gathered by generations is termed as “Ethno medicine” or “Traditional medicine” including *folk/tribal* medicines (Pushpangadan P, 1996).

From the historical period, medicinal plants are playing a crucial role in mortal health care. The survey done by WHO, use of traditional medicine for primary
health care deprived of substantial allusions to current systematic philosophies by about 80% of the world populations. The more than 7000 medicinal plants used in various tribal regions of India. Surprisingly most of these plants are rarely known for their wide-ranging therapeutic profile (Slator TF, 1984). The uses of plants in the form of extracts, tinctures or powder and also in paste form for local uses requires strong safety, efficacy and quality data (James ER, 2002). Hence it is decided to search such therapeutically important plants and establish complete profile of their scientific values.

Liver Toxicity:
In animals, various vital physiological processes like metabolism, secretion (e.g. bile for digestion), storage and detoxification happens in liver. Some of Hepatic diseases like acute or chronic disease (inflammatory), hepatosis (non-inflammattory) and cirrhosis (degenerative) diseases are amongst maximum serious sicknesses. Various types of Infections, autoimmune disorders, higher doses of antibiotics, different types of chemotherapy and excessive alcohol consumption are responsible for a number of liver diseases by peroxidation and oxidation cells (Smucker RA, 1975). Almost 90 % of the acute hepatitis is originate from infections through Hepatitis -A, -B, -C, -D, -E and -G viruses. Many therapeutic drug adverse events are responsible for Drug-induced liver diseases and to overcome this adverse events is a major health challenge to researchers. Various drugs leads to either direct hepatotoxicity or chemically reactive metabolites responsible for DNA damage, protein dysfunction, lipid peroxidation and oxidative stress. A vaccine available for immunization against Hepatitis B virus (WHO, 1997). But in spite of lot of research for synthetic hepatoprotective medicine, there was no any potent hepatoprotective drug are able to come in market from last decades. Since long time medicinal plants and around more than 100 phytochemicals which can cure hepatic disease and claimed to have hepatoprotective activity. Hence it is necessary to evaluate these plants for their hepatoprotective potential (Handa SS et al, 1990, Doreswamy R et al, 1995).
Diabetes Mellitus (DM):
Diabetes mellitus is a common disease in which deficiencies in impaired metabolism of carbohydrate leads to decrease in insulin secretion, insulin action, or both which produced chronic high blood sugar levels in the body (Zhang F et al, 2006). This metabolic disorder affected a large population around the globe with noticeable increased morbidity and mortality rate and one of the major reason in maximum developed countries for death. High blood sugar level complications leads to macrovascular and microvascular injuries like nephropathy, retinopathy, peripheral neuropathy, kidney failure, high blood pressure and heart diseases (Vinik AI et al, 2003). The World Health Organisation (WHO) forecasts the present diabetic population of 177 million population may increase up to 370 million population 2030, while in India, diabetic population which may increase up to more than 90 million by 2030. Insulin and many antidiabetic agents (oral) like biguanides, sulphonylureas and α – glucosidase inhibitors are available to control high blood sugar levels in diabetes (Bjork S et al, 2003). But all of these have high or less toxicity and side effects on chronic use. Hence, alternative treatments for such chronic diseases are popular, promising and in the demand (Beatriz L et al, 2001). In the ethno botanical information more than 800 plants claimed to have anti-diabetic potential (Bandole S et al, 2006).
1.1. ANATOMY OF LIVER:

In human body, liver is present at below diaphragm and in right upper side of abdomen covered with thick capsule of connective tissue known as glisson’s capsule (Tortora GJ et al, 2003). The weight of liver is around 1.8 kg in male and 1.4 kg in females. The junction of two ducts i.e. right hepatic duct and left hepatic duct forms excretory apparatus known as common hepatic duct. Gallbladder is reservoir for the bile which enters the cystic duct and stored in the gallbladder (Mohan H, 2000).

1.1.1. Lobes:

The liver is six-lobed organ it is subdivided into hefty right section and a slighter left section as shown in Fig 1.1. Each lobe is again subdivided into lobules and these are the actual functioning units of the liver. Around 1 million lobules available in lobe and it consist of hexagonal row of hepatic cells known as “hepatocyte” which secretes bile and performs a variety of metabolic functions. Small cavities present among each row of hepatocytes are called “sinusoids” which are lined with kupffer cells, phagocytic cells that detoxifies body by removing amino acids, metabolites, sugars, old and defective red blood cells, bacteria and debris (Ross and Wilson, 2001).

Photograph 1.1: The liver lobule with portal triad (PT) (hepatic artery, portal vein and bile duct), hepatocyte (H), and sinusoids
From posterior view, surrounded to the porta hepatis, lobes are separated by the ligamentum venosum and ligamentum teres and divides the caudate from the quadrate lobe. Quadrate lobe is directly inferior to the porta hepatis and Caudate Lobe is closest to the vena cava (Guyton AC 2006).
Figure 1.1: Anatomy of liver
1.1.2. Supply of blood to Liver:

The liver receives its blood supply from two different sources:

- **Hepatic portal vein:** This is poorly oxygenated venous blood rich with nutrients at flow rate 1000 mL/min, drains from stomach, small intestines, large intestines, pancreas and spleen via portal vein.
- **Hepatic artery:** This is highly oxygenated arterial blood at flow rate 500 mL/ min drain into the liver capillaries (sinusoids) and into a central vein in each lobule and further into hepatic vein and inferior vena cava (Corwin EJ, 2000).

1.1.3. Histology:

![Figure 1.2: Histology of liver](image-url)
Histology of liver shows presence of many hexagonal shaped parenchyma cells (hepatocytes) in the form of lobules having 0.5 to 2 mm diameter radiates from central vein to the portal tracts. Each lobule lined via sinusoids on two sides wherein blood supply from portal space to central vein and portal vein, branches of bile duct and hepatic artery. Functionally, liver is divided into 3 major zones on the basis of oxygen supply,

**Zone 1** enclosing portal area with good oxygen and blood supply which able to burn of all forms of toxic injury.

**Zone 3** around the central vein and with poor oxygenation and blood supply highly susceptible to hepatic injury.

**Zone 2** area is situated in between above two areas.

The hepatocytes along with round single nucleus and a prominent nucleolus have a significant capability to undergo mitosis and regeneration. A hepatocyte has 3 surfaces:

1) In front of the sinusoids and space of Disse,
2) In front of the canaliculus,
3) In front of neighbouring hepatocyte.

Irregular endothelial and scattered flat Kupffer cells (liver macrophage situated in sinusoidal space) forms linings of blood-containing sinusoids. Space among hepatocytes and a sinusoidal lining is known as “space of Disse”. The liver arteriole and a bile duct has little mononuclear cells with slight connective tissue as Glisson’s capsule. The biliary system contains bile canaliculi which merely channels amongst contact surfaces of hepatic cells with microvilli coverings (Mohan H, 2000).
1.2. PHYSIOLOGY OF LIVER:
Liver is one of the vital sites of metabolism and also has a chief role throughout its various activities. Liver can divide fats and proteins into smaller substances which is used for the cells of other tissues for energy or for synthesizing specifically essential biomolecules. Liver also synthesizes molecules required for blood coagulation, transport of fats conferring immunity to infection and many other purposes. Liver has own storage capacity for large quantity of carbohydrates, fats and proteins and as per requirements releasing these nutrients into the tissue. Therefore, any liver disease can cause number of physiological problem with critical consequences. The liver is responsible for number of functions, details as per given below (Tortora GJ et al, 2003, Guyton AC 2000, 2006, Corwin EJ, 2000, Davidson’s. 1999),

- Carbohydrate Metabolism
- Protein Metabolism
- Lipid Metabolism
- Bile metabolism
- Synthetic functions
  - Plasma Proteins
  - Bile salts
  - Vitamins
  - Enzymes
  - Coagulation factors
  - Reticuloendothelial system
  - Secretion
1.3. PATHOPHYSIOLOGY OF LIVER AND CLASSIFICATION OF LIVER DISEASES:

A number of diseases start and spread in the liver. Liver diseases are classified into primary or secondary. Secondary liver disease is more serious than primary liver disease. For example, hepatitis is a primary liver disease but if hepatitis spreads into colon it leads to secondary diseases i.e. colon cancer (Mohan H, 2000).

CLASSIFICATION OF LIVER DISEASES:

1) **Congenital Defects**: If the deficiencies at the time of Birth in the liver generally affect the bile ducts and involves following:
   - Biliary atresia – bile ducts are present or absent in abnormal form.
   - Choledochal cyst – flow of bile is obstructed due to abnormality of the hepatic duct (Boon NA et al, 2006).

2) **Portal Hypertension**:

![Portal Hypertension](image)

*Figure 1.3: Portal Hypertension*
If the obstruction during flow through or out of the liver is known as portal Hypertension. If the Obstruction during flow through the liver which leads to fibrosis and scarring of the liver.

In portal hypertension when pressure is greater than 9 to 10 mm Hg, blood generally enters liver via a portal vein, initiates to bypass the liver in search of alternative routes that leads less resistance to flow, and finally, third spacing results (Corwin EJ, 2000).

3) **Splenomegaly:** The enlargement of the spleen is a Splenomegaly. Blood flow enters spleen through splenic vein with portal hypertension. Some additional blood can be stored in the spleen, leading to its enlargement. The unavailability of stored blood to the general circulation leads to anemia, thrombocytopenia, and leucopenia can occur (Mohan H, 2000, Corwin EJ, 2000).

4) **Jaundice:** High bilirubin in the blood leads to yellowish discolouration of skin and sclera of eyes is known as Jaundice. There are 3 main types of jaundice:-

   - **Hemolytic jaundice**- This is a prehepatic cause of jaundice in which excessive red blood cells lysis, and the factors not necessarily related to the liver.

   - **Intrahepatic jaundice**- In intrahepatic jaundice decreased in hepatic uptake, conjugation and excretion of bilirubin leads to dysfunction of the hepatocytes or obstructions of the bile canaliculi.

   - **Extrahepatic obstructive jaundice**- If the bile flow is blockage through bile duct lead to obstructive jaundice. If the bile duct is blocked by gallstones or a tumor lead to Extrahepatic obstruction (Mohan H, 2000, Guyton AC 2006, Boon NA et al, 2006).
5) **Cirrhosis:** It is caused by liver scarring and fibrosis by replacing hard fibrous nodules in place of usual hepatic tissues. Normal liver architecture and function are disrupted.

![Figure 1.4: Liver Cirrhosis](image)

Repeated incidents of cellular injury in the liver and the resultant inflammatory reactions in the liver leads to Cirrhosis. The causes of cirrhosis includes infections like hepatitis, bile duct obstruction leads to bile accumulation in the canaliculi and rupture of the canaliculi subsequently and also toxin induced injury to the hepatocytes. The acetate formation due to alcohol causes injury and inflammation to liver cells (Mohan H, 2000, Boon NA et al, 2006).
6) **Hepatitis**: Hepatitis causes inflammation of the liver by infection or by toxins, through alcohol and or cancer. Signs and symptoms are similar for all types of hepatitis. Modes of transmission, if the cause is viral and final conclusions may be different.

![Hepatitis Diagram](image)

**Figure 1.5: Hepatitis**

- **Acute** - The acute inflammatory association of the whole liver is the most common consequence for all hepatotoxic viruses. All types of hepatitis means A, B, C, D and E show similar clinical change and also produce same pathological findings.

- **Chronic** – Continuation of more than 6 months of hepatic disease or repeat attack along with progressive evidences from serologic, biochemical and histopathologic tests of inflammation and necrosis. Generally chronic hepatitis are the result of infection with hepatotoxic viruses like hepatitis B or C and combined hepatitis B and D infection (Guyton AC 2006, Boon NA et al, 2006).
7) **Necrosis**: Cell necrosis is a degenerative process leads to cell death. Cell swelling and lipid accumulation leads to cell necrosis. Cell death along with rupture the plasma membrane, and this is predicted by a number of morphological changes like dilation of endoplasmic reticulum, cytoplasmic edema, accumulation of triglycerides, disaggregation of polysomes, swelling of mitochondria through disruption of cristae and dissolution of organelles and nucleus.

- Massive necrosis - large numbers of liver cells are killed.
- Focal necrosis - scattered liver cells are killed.
- Zonal necrosis - liver cells are killed within lobules (Boon NA et al, 2006).

8) **Circulatory Disturbances**: it includes 2 types:

- **Hepatic Venous Obstruction**: obstruction of the hepatic veins produced two uncommon liver diseases are hepatic veno occlusive and Budd Chiari disease.
  - Specially developing thrombosis.
  - Intimal thickening, stenosis and destruction of the terminal central veins and medium-sized veins shown in the Veno-occlusive disease.

- **Portal Venous Obstruction**: It may occur within the intrahepatic course or in extrahepatic site.
  - Intrahepatic reason of portal venous occlusion is hepatic cirrhosis is the commonest and most vital, followed in congenital hepatic fibrosis, decreasing frequency by tumor invasion and schistosomiasis.
  - Extrahepatic reasons of portal vein obstruction are intra-abdominal sepsis, intra-abdominal cancers, myeloproliferative disorders, direct invasion by tumor and upper abdominal surgical procedure followed by thrombosis.

9) Liver Failure:
Liver failure means any severe and unrelenting liver disease like onset of fulminating HBV and or low-grade HCV infection. Liver failure is a complex syndrome characterized by destruction of many body functions and different organs.  
Two types of liver failure are hepatic encephalopathy and hepatorenal syndrome.

![Figure 1.6: Stages of Liver Damage](image)

- **Hepatic encephalopathy**: Hepatic encephalopathy is a complex of CNS disorders in persons suffering from liver failure. The symptoms are memory lapses and personality changes. Development of a flapping tremor in the person.
- **Hepatorenal syndrome**: In Hepatorenal syndrome renal failure observed in association with advanced liver disease. Advanced liver disease individuals kidney frequently cease producing urine and fail to function, while the kidney appears to be physically capable of functioning (Corwin EJ, 2000).
10) **Liver Cancer:**

Chronic liver disease like cirrhosis or history of HBV or HCV infection or exposure to high levels of carcinogens (aflatoxins) leads to primary liver cancer. Primary liver cancers may be of the bile ducts (cholangiocarcinoma) or of the hepatocytes themselves (hepatocellular carcinoma) (Corwin EJ, 2000).

In Secondary liver cancer metastasis of cancers from part of the body (e.g. pancreas or intestine) that drain into the liver through portal vein. In primary and secondary liver cancers metastasize outside the liver, specifically to the heart and lungs, because these organs firstly encounters by the hepatic drainage.

All kinds of liver cancer have poor diagnoses, with only approximately 1% of afflicted persons surviving for longer than 5 years (Corwin EJ, 2000).

![Figure 1.7: Liver Cancer](image-url)
11) **Steatosis:**

Steatosis is a condition in which abnormal accumulation of triglycerides within the hepatocytes, steatosis is of 2 types-

- **Macovesicular steatosis:** In which single large cytoplasmic vacuole of triglyceride forms within the hepatocyte by increased hepatic steatosis fatty acids, increased mobilization of fatty acids, and removal of triglyceride from the hepatocyte through defective VLDL synthesis is incomplete.

- **Microvesicular steatosis:** Due to deficiency in mitochondrial β-oxidation and characterized by presence of Valproic acid, uncommon but severe lesion condition with inflammation, necrosis, bile duct injury through formations of multiple small droplets of triglyceride within the hepatocyte (Mohan H, 2000, Boon NA et al, 2006).
1.4. BIOCHEMICAL MARKERS FOR LIVER DISEASES:

Liver has a complex organ and its functions are multiple characteristics, overall defect in liver function is not detected by single one test. Therefore, multiple liver function tests are needed for accurate diagnosis, assessment of severity and proper therapy, (Vinik AI et al, 2003). General liver function tests (LFTs) measures concentration of enzymes and compounds in serum instead of hepatic functions (Walker R et al, 2003).

![Liver Function Test](image)

**Figure 1.8: Liver Function Test**

1.4.1. Tests for Bile:

Bile is secreted through biliary ducts in the duodenum. Bile is stored in the gall bladder contains biliary phospholipids, primary and secondary bile acids. Test used for assessment of the synthesis and elimination of bilirubin pigment, urobilinogen and bile acids, details are as follows:

- **Bilirubin:** The separation of Haemoglobin through degradation of RBC and transferred to liver, further Haemoglobin metabolised in to water soluble bilirubin through conjugation. For digestion of food this water soluble bilirubin excreted into bile.

At the time of liver damage Total bilirubin is increase, direct bilirubin fraction also increases outside the liver and part of bilirubin is metabolised by the liver. The high levels of a total bilirubin and Low levels of a direct
bilirubin leads to liver cell damage or bile duct damage. Bilirubin pigment detected in serum, urine and faeces.

- Assessment of Serum bilirubin done by spectrophotometrically by using Van Den Bergh diazo reaction method. Diazot reagent contains diazotized sulphamic acid. In hepatocellular jaundice, obstructive jaundice and hemolytic jaundice and Glibert’s diseases levels of Bilirubin is increases.

- Assessment of bilirubin in faeces by inspection of stools. In obstructive jaundice Clay- coloured stools due to absence of faecal excretion of the pigment.

- Assessment of conjugated bilirubin in urine by dipsticks method, Fouchet’s test, and foam test. Bilirubinuria seems in patients of hepatitis before the patient becomes jaundice.

- **Urobilinogen:**
Urobilinogen is commonly excreted in urine. The estimation of Urobilinogen in urine by “dipstick” method or by Ehrlich aldehyde reagent by preparing dilutions of the urine. In hepatocellular dysfunctions (e.g. cirrhosis, alcoholic disease and malignancy of the liver) urobilinogen is increases in the urine. Due to complete biliary obstruction Urobilinogen increases in pyrexia, hemolytic disease and cholestatic jaundice.

- **Bile Acid (Bile salts):**
The cholic acid and cheno-deoxycholic acid synthesised from cholesterol in the hepatocytes and is also known as primary bile acids. By enterohepatic circulation maximum bile acids is reabsorbed and goes to the liver. Normally 10% of the total bile acids (unabsorbable toxic lithocholic acid) excreted in the faeces.

The serum bile acid levels may enhance in Hepatobiliary diseases with cholestasis which can cause pruritus. The serum bile acids excreted by the active transport and passive diffusion action by urine and this can be detected by Hay’s test and “dipsticks” methods (Mohan H, 2000, Blissitt CW et al, 1972).
1.4.2. **Serum Enzyme Assays:**

The estimation of specific enzymes like serum transaminase and alkaline phosphatase for the diagnosis of cholestatic liver injuries or hepatocellular or liver damage is continuously adequate.

- **Alkaline Phosphatase (ALP):**
  Many tissues like liver, bone, intestine and placenta excreted Alkaline Phosphatase (enzyme) in the bile. Increase in activity of Alkaline Phosphatase (enzyme) shows diseases of bone, liver (in hepatitis and cirrhosis, in biliary tract obstruction) and or pregnancy whereas absence of such diseases usually reflects hepatobiliary disease.

- **γ-Glutamyl Transpeptidase (γ-GT):**
  The liver is the chief source for the γ-GT enzyme in serum. The γ-GT serum level equivalents serum alkaline phosphatase. γ-GT serum level help in confirmation for high serum alkaline phosphates is of hepatobiliary origin. The levels of γ-GT enzyme is high in alcohol abuse patients, and also in cholestasis and hepatocellular disease.

- **Transaminases (Aminotransferases):**
  Liver cell necrosis assessment commonly done by estimation of Serum alanine aminotransferase (ALT) and Serum aspartate aminotransferase (AST).
  - ALT is a enzyme which is existing in liver and called as serum glutamic pyruvate transaminase (SGPT).
  - AST which is also called serum glutamic oxaloacetic transaminase (SGOT) is a mitochondrial enzyme excreted from liver, heart, skeletal muscle and kidney, AST.

Whenever damage to the tissues the ALT and AST levels are increased. ALT is chiefly for liver tissue, its elevations leads to liver cell injury, while elevations of AST leads to acute necrosis or ischemia of myocardium and also liver cell injury.
Estimation of Transaminase is basic for diagnosis of viral hepatitis. In acute hepatic necrosis (e.g. severe viral hepatitis and acute cholestasis), levels of Transaminase are very high. In cirrhosis and Alcoholic liver disease the elevation of transaminases is mild to moderate.

- **Other Serum Enzymes:**
  - 5’-Nucleotidase is a phosphatase derived from the liver. For differentiate of alkaline phosphatase of hepatic origin from that of bony tissue, 5’-Nucleotidase determination is beneficial.
  - In patients with metastatic liver involvement the levels of Lactic dehydrogenase (LDH) is elevated in serum.

**1.4.3. Tests for Metabolic Functions:**

Metabolism and synthesis of plasma proteins and amino acids, carbohydrates and vitamins, lipids and lipoproteins and also detoxification of drugs and alcohol in the liver. Liver is the chief most body part for metabolism and synthesis in the body.

- **Amino Acid and Plasma Protein Metabolism:**
  The amino acids releases through breakdown of the Tissue and nutritional component, in the liver these amino acids metabolized to the ammonia and urea. The plasma proteins and immunoglobulins releases into plasma by hepatocytes which consist of polyribosomes bounded to the rough endoplasmic reticulum and this is the main site for synthesis of number of plasma proteins and immunoglobulins. On the basis of above metabolic functions of liver, assessment of immunoglobulins, serum estimation of proteins, ammonia and aminoaciduria used for the diagnosis of liver damage.
1) **Serum Proteins:**
   
   The number of proteins like α fetoproteins, α-1-antitrypsin, transferrin, prothrombin, albumin, hepatoglobin, fibrinogen, ceruloplasmin and acute phase reactant proteins synthesize by Liver cells. Assessment of total amount of serum proteins, serum globulin, serum albumin and serum albumin/globulin (A/G) ratio for the diagnosis of liver damage. For the determination of proportions of α1, α2, β and γ globulins Electrophoresis method is used. Based on altered plasma protein components thymol turbidity and flocculation tests are discontinued due to availability of protein electrophoresis. In liver diseases hepatocytes are significantly destructed causes hypoalbuminaemia whereas Hyperglobinaemia grows due to chronic disorders such as cirrhosis.

2) **Immunoglobulins:**
   
   High levels of serum immunoglobulins like IgG, IgM and IgA shows nonspecific inflammatory or immune response instead of liver injuries. E.g., Increase in IgG leads to chronic active hepatitis, Increase in IgM leads to primary biliary cirrhosis and Increase in IgA level leads to cirrhosis.

3) **Clotting Factors:**
   
   The assessment of several clotting factors by prothrombin time and partial thromboplastin time in Hepatic synthetic function which are prolonged predominantly in hepatocellular disease patient’s. Prothrombin time is dependent on intestinal uptake of vitamin K and hepatic synthesis of clotting factors. Obstruction of bile duct and intrahepatic cholestasis due to diminished lipid absorption linked with prolonged prothrombin time leads to deficiency of vitamin K.

4) **Serum Ammonia:**
   
   In cirrhosis, acute fulminant hepatitis and hepatic encephalopathy blood levels of ammonia are high. In damaged liver the conversion of ammonia...
to urea is reduced leads to rise in level of serum ammonia. Therefore, in chronic liver disease urea synthesis is reduced.

- **Lipid and Lipoprotein Metabolism:**
  The synthesis of Lipids such as cholesterol and cholesterol esters, triglycerides and phospholipids in the liver. These Lipids are not soluble in water and goes in to circulation with lipoproteins and lipoproteins consist of apoproteins. Types of lipoproteins as per below,
  - High density lipoproteins,
  - Low density Lipoproteins and
  - Very low density Lipoproteins
  The Assessment of Blood lipids can be done by testing triglycerides, Total serum cholesterol and lipoproteins fractions in patients with hepatic problem. The total serum cholesterol levels increases and or Serum triglyceride also increases leads to Cholestasis whereas decrease in levels of total serum cholesterol and or Serum triglyceride leads to malnutrition or acute and chronic diffuse liver diseases.

- **Carbohydrate Metabolism:**
  The metabolism of Carbohydrate occur in liver, therefore due to any liver deficiency which shows changes in blood glucose levels. In fulminant acute hepatic necrosis Blood glucose level is decreased. Diminished glucose tolerance and relative insulin resistance occur in chronic liver disease (Mohan H, 2000, Blissitt CW et al, 1972).

1.4.4. **Immunologic Tests:**
Liver diseases accompanying with a number of immunologic abnormalities which may be antibodies against specific etiologic agents or nonspecific immunologic reactions.
• **Nonspecific Immunologic Reactions:**
  - In hepatic disorders with hepatic necrosis, formation of actin component of muscle from smooth muscle antibody. Proteins of hepatocytes which is immunologically similar to actin.
  - In primary biliary cirrhosis patients development of Mitochondrial antibody.
  - In some patients of chronic hepatitis presence of Antinuclear antibody. In these cases LE cell test may be positive.
  - Antibodies to specific etiologic agents
  - In cases of serum hepatitis, Diagnosis of Hepatitis B surface antigen (HBsAg) which will show positive value for hepatitis B infection.
  - In hepatitis-B suffering patients, Hepatitis-B core antibody (HBc) is detected.
  - In chronic varieties of hepatitis B Hepatitis Be antigen (HBeAg) is found.
  - In patients with amoebic liver abscess development of Amoeba antibodies to Entamoeba histolytica (Mohan H, 2000).
1.5. **HEPATOTOXICITY:**

The liver is a fundamental site of metabolism, during metabolism many toxic chemicals damages liver and produced hepatotoxicity. A number of chemicals produces liver injury is known as hepatotoxins. Hepatotoxicity also produced by chronic use and overdoses of drugs, few chemicals and herbal drugs. As per recent review, due to reason of hepatotoxicity more than thousand drugs are withdrawn from the market (Friedman SE et al, 2003) whereas due to acute liver failures about 50% patients are hospitalised (Ostapowicz G et al, 2002).

1.5.1. **DRUG INDUCED HEPATOTOXICITY:**

Parent compound and or its metabolites of various drugs produces hepatotoxicity in majority of cases due to its overdose or chronic use.

**Drug induced Liver Toxicity:**

In Figure 1.3 is expanding numerous mechanisms, which are discussed as follows:

![Figure 1.9: Drug induced Liver Toxicity](image-url)

**Figure 1.9: Drug induced Liver Toxicity**
1.5.1.1. **Interference with Bilirubin Transport and Conjugation:**

Quantity of drugs hinder through bilirubin transport and enhance plasma bilirubin which leads to hyperbilirubinaemia. For example: In neonats due to Novobiocin through inhibition of UDP glucuronosyl transferase of unconjugated bilirubin in plasma is elevated (Breen KJ et al, 1973). Additional example, Rifampicin (antibiotic antitubercular drug) which increases conjugated and unconjugated bilirubin in dose related manner (Kenright S et al, 1974).

1.5.1.2. **Cytotoxic Injury:**

A number of basic mechanisms hepatic parenchyma cells are damages by several drugs. The development of centrilobular hepatic necrosis due to overdose of paracetamol (Boyd EM et al, 1966, James O et al, 1975, Mitchell JR et al, 1975, Mitchell JR et al, 1974). The responsive metabolite extent is increases because of overdoses which reduces liver glutathione and formerly retorts cellular macromolecules. Derivatives of hydrazine such as Isoniazid and iproniazid produces hepatocellular, cytotoxic type of damage.

1.5.1.3. **Cholestasis:**

Cholestasis is a condition in which impaired bile salt secretion leads to hepatocytes become ineffective in secretion of bile (Popper H, 1968). These type of drugs induced cholestatic reactions by two types,

- Steroid-induced
- Sensitivity type

1.5.1.4. **Fatty Liver (Steatosis):**

An antibiotic tetracycline cause’s fatty liver at large intravenous doses upto 1.5 g/day, this toxicity is common in females than males. This toxicity is very uncommon after oral doses and dose dependent for intravenous doses. The toxicity of tetracycline may be as a result of fatty acid oxidation, raised fatty acid uptake or triglyceride uptake, lipid transport inhibition and protein synthesis beyond the hepatocyte (Breen KJ et al, 1973).
1.5.1.5. **Chronic Active Hepatitis, Cirrhosis and Sub Acute Necrosis:**
Liver diseases such as Chronic Hepatitis and Necrosis associated with various drugs like isoniazid oxyphenisatin a-methyldopa, and nitrofurantoin. Prolonged use of isoniazid (antitubercular) drug produced hepatic dysfunction in nearby 20% population and also goes to severe hepatic injury in around 1% population through production of toxic metabolite of isoniazid by acetylation to acetylisoniazid and acetylhydrazine which are tremendously hepatotoxic and generates centrilobular hepatic necrosis (Mitchell JR et al, 1975).

1.5.1.6. **Phospholipidosis:**
Various drugs produces this type of syndrome and also affects several organs. For example, Coralgil (coronary dilator) causes hepatic damage and consequently phospholipids accretion liver cells, explosion of bile duct and inflammation. The interaction of drug and phospholipids changes surface charge and accordingly damages capacity of phospholipases to break them down (Bahri AK et al, 1981). A number of therapeutic agents along with mechanism which produced hepatic injury listed in the Table 1.1.
<table>
<thead>
<tr>
<th>Type of Mechanism</th>
<th>Representative drug</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered lipid metabolism leads to Fatty liver</td>
<td>Tetracycline, Doxycycline, Amiodarone, Aminiptine, Tianeptine, Pirprofen</td>
<td>Letteron P et al, 2003</td>
</tr>
<tr>
<td>Decreased bile salt clearance leads to Cholestasis</td>
<td>Ethinyl estradiol, Estradiol-17 beta-d-glucuronide, Cyclosporine A, Taurolithocholate</td>
<td>Crocenzi FA et al, 2003</td>
</tr>
<tr>
<td>Formation of protein adduct leads to Immune reaction</td>
<td>Diclofenac, Sulfamethoxazole, Flucloxacillin, Tienilic Acid, Halothane</td>
<td>Crocenzi FA et al, 2003</td>
</tr>
<tr>
<td>Increased oxidative stress leads to Cellular injury</td>
<td>Diclofenac, Ketoconazole, Acetaminophen sodium, Diethyldithiocarbamate</td>
<td>Amin A et al, 2005</td>
</tr>
<tr>
<td>Decreased a mitochondrial function leads to Apoptosis or necrosis</td>
<td>Stavudine, Zidovudine, Nimesulide, Tamoxifen, Amiodarone</td>
<td>Begriche K et al, 2006</td>
</tr>
<tr>
<td>Cytotoxic T cells leads to Cell killing</td>
<td>Carbamazepine, Lidocaine, Sulfamethoxazole, Lamotrigine, Phenindione</td>
<td>Sanderson JP et al, 2006</td>
</tr>
</tbody>
</table>

Table 1.1: List of Therapeutic agents and their Mechanism cause Hepatotoxicity
1.5.2. CHEMICALLY INDUCED HEPATOTOXICITY:

1.5.2.1. ETHANOL:

Ethanol is also known as ethyl alcohol, Ethanol is a colorless volatile liquid, having inflammable in nature, Physical and chemical properties as per below,

- Melting point: $-114^\circ C$
- Boiling point: $78.37^\circ C$
- Molecular weight of ethanol: $46.080 \text{ g mol}^{-1}$
- Density of ethanol: $0.7890 \text{ g / cm}^3$
- Solubility in water: Completely
- Vapour pressure: $44 \text{ mmHg at } 20^\circ C$
- Vapour density: 1.49
- Specific gravity: 079

![Figure 1.10: Ethanol Induced Hepatotoxicity](image)

Consumption of excessive ethanol leads to levels of loosely coupled enzyme i.e. CYP2E1 increases which creates Reactive Oxygen Species like superoxide radical, $\text{H}_2\text{O}_2$ with hydroxyl radical, ferryl species, 1-
hydroxyethyl radical in presence of iron. Reactive Oxygen Species capable to cause toxicity through enzyme inactivation, protein oxidation, cell membranes damage through lipid peroxidation and reactive lipid aldehydes production specifically to mitochondria. Release of endotoxin increases by Ethanol from gut bacteria which stimulates Kupffer cells leads to release substances like TNF-alpha, eicosanoids and free radicals. This elements produce hypermetabolic state and hypoxia in liver and tissue are damage in oxygen-poor regions (Thurman RG et al, 1999). The ROS go into stellate cells leads to production of collagen is simulate and thus a fibrotic response is produced (Jaeschke H et al, 2002).

Figure 1.11: Mechanism of Ethanol toxicity
1.5.2.2. CARBON TETRACHLORIDE:

Carbon tetrachloride is a liquid chemical, colourless and sweetish odour having non-inflammable in nature. Physical and chemical properties as per below,

- Melting point : 23°C
- Boiling point : 76.5°C
- Molecular weight : 153.70 g mol⁻¹
- Water solubility : 800 mg / lit
- Vapour pressure : 91.3 mmHg at 20°C
- Vapour density : 5.32
- Specific gravity : 1.54

By huge number of investigators studied the toxic effect of CCl₄ on the liver, from long time ago such type of studies have been developed major concepts to our understanding regarding the cell injury by biochemical mechanisms.

The characteristics of CCl₄ as per given below:-

- CCl₄ is easily available in pure form
- In many species like rat, rabbit, mouse and man liver injuries induced by CCl₄ is constantly.
- Depending on method of application and the dose of administration of CCl₄, nature and severity of liver injury produced from triacylglycerol accumulation, cirrhosis through necrosis and finally cancer.
- The acute lesion means necrosis and fatty degeneration is altered by a number of pre-treatments that affect the activity of the NADPH-cytochrome P450 chain, and by numerous protective agents including many free radicals (Slator TF, 1984).

Mechanism:

Many investigators recommended that lipid peroxidation done with help of CCL₄ free radical derivatives leads to CCl₄ induced hepatic toxicity.

In the endoplasmic reticulum Cytochrome P450 enzyme facilitated activation of CCl₄ produces CCl₃, in presence of O₂ this trichloromethyl free radical (CCl₃) combines with proteins and cellular lipids which produce
trichloromethyl peroxyl radical. This produce morphological, structural and functional disruption, fatty acids autoxidation and lastly produced lipid peroxidation (Amin A et al, 2005). Due to influx of extracellular Ca\(^{2+}\) ions into the cell leads to Cell death (Slator TF, 1984).

After single dose of CCl\(_4\) development of Centrilobular necrosis within 12 hours and blown necrosis within 24 hours. Growth in hepatic enzymes on First day of exposure and after continuously dosing of 2-4 days symptoms like enlargement or liver tenderness, dark urine, mud coloured stools and jaundice are observed. Death occurs due to hepatic failure after a week (Shah H et al, 1979).

Figure 1.12: Activation and reaction of carbon tetrachloride
1.5.2.3. HEPATOTOXICITY INDUCED BY PARASITIC AND FUNGAL INFECTIONS:

Schistosomiasis is a parasitic infections and caused by S. mansoni, Schistosomiasis chiefly produced hepatic fibrosis. By host reactions soluble egg antigen (SEA) or Schistosome eggs get swept into liver and starts mediated macrophage activation of T-cell and formation of granuloma around them (Mahmoud MR et al, 2002).

Kodo millet (Paspalum scrobiculum L.) is a basic food in north India and is most popular in human being of north India. This food is generally infected with Aspergillus tamari Kita. The cyclopiazonic acid (CPA), mycotoxin and fumigaclavin A produce by fungus in major amount which leads to development of Kodo poisoning. Cyclopiazonic acid (CPA) is highly toxic and in many organs produces focal necrosis (Antony M et al, 2003).

Aflatoxins is a type of fungal toxins and which is produced by fungi Aspergillus flavus and A. parasiticus. Hepatic cytochrome P450 enzyme facilitated stimulation of AFB₁ which is responsible for production of highly reactive AFB₁-8,9-epoxide and which converts to AFB₁-DNA adducts to induce hepatocarcinogenesis (Preetha SP et al, 2006).

1.5.2.4. CELLULAR MECHANISM OF TOXIC LIVER INJURY:

Due to drug metabolites or toxic substances change in biochemistry of the cell or immune response can result into hepatic disorder. Hepatitis is depending on the extent of mitochondrial involvement in cell death by cytotoxic T cells or intracellular stress. The organells are directly affect by different chemical reactions of drug metabolites. The organells like mitochondria, endoplasmic reticulum, nucleus, cytoskeleton, microtubules, enzyme kinase and also gene countenance profiles. Cytokines Toxic effect in immune structure produces apoptosis or necrosis in liver

The basic pathogenesis of clinical drug hepatitis is biochemical consequence of noxious metabolites leads viability cell loss and finally in cell death (Zimmerman HJ, 1999, Kaplowitz N, 2001). It also consist of death receptors
means Fas or porine-mediated death cascade (Kaplowitz N, 2000, 2001) which leads to apoptosis of hepatocytes.

Formation of Class-I complex of proteins and drug metabolites leads to sensitization due to liver is primary target of immune system (Robin M et al, 1997). Without association of extrinsic immune system some drug metabolites which lead to cell death which depends on drug, environmental factors, genetic factors, exposure time, defence, transport, regeneration and different genes (Kalpowitz N, 2000). The determination of specific risk is based on combinations of all this factor is the greater preference. The activation of innate immune system by Drug Induced Liver Injury (DILI) and Parenchymal cell damage is presented in Figure 1.6.

![Figure 1.13: Mechanism of toxic liver injury by DILI](image-url)
1.6. **DIABETES MELLITUS:**

Due to abnormality of insulin secretion, insulin action or together, prolonged hyperglycemia with impaired function in metabolism of carbohydrate, lipid and protein which lead to Diabetes mellitus (Zhang F et al, 2006). Diabetes mellitus a metabolic disorder and affects many organs especially heart, eye, foot, nerve, and kidney (Frank V, 1999).

![Figure 1.14: Diabetes Mellitus](image)
1.6.1. **History of Diabetes Mellitus:**

The unusual excessive urination with insatiable thirsting are the primary symptoms in Diabetes mellitus from ancient period. “Diabetes” was been resultant from Greek term *diabainein* which means to flow through or ‘siphon’ and this disease is invented by Graeco-Roman doctor Aretaeus of Cappadocia. In 1675, due to sweet taste of urine, Thomas Willis added the word “*mellitus*” which means honey. This sweet taste noticed by lot of peoples from different regions of world such as Indians, Chinese, Greeks, and Egyptians. In 1776, on the basis of sweet taste of urine, Matthew Dobson had proven sugar in urine and blood presence in diabetic people. In India, on the basis of attraction of ants to urine this disease is known as “*Madhumeha*” which means “sweet urine disease”. In 1889, role of the pancreas in diabetes by Joseph von Mering and Oskar Minkowski. In 1910, role of single chemical *insulin* (word from Latin *insula*, meaning island) producing from islets of Langerhans in the pancreas (Frank V, 1999).
1.6.2. Types of Diabetes:

1.6.2.1. Type 1 Diabetes:

It had known “Insulin dependent diabetes mellitus”. IDDM is uncommon with low degree of hereditary susceptibility. Destruction of β cells due to facilitation of autoimmune antibodies or circulating insulin levels is very low or juvenile onset diabetes mellitus leads Type 1 Diabetes. Generally detected in teenagers, kids and in early grownups. Sometimes there were β cells antibody is absent and this condition is known as idiopathic or type 2 B.

1.6.2.2. Type II Diabetes Mellitus:

It is called as “Non-insulin-dependent diabetes mellitus” (NIDDM) or “maturity-onset diabetes mellitus”. This type of diabetes is common with high degree of hereditary susceptibility. Its onset is late generally in past middle age. The main reasons for this type of diabetes is defective insulin secretion and reduced insulin sensitivity or insulin resistance. In which no or moderate loss in β cell mass, no anti-β-cell antibody, abnormality in circulation of insulin (Tripathi KD, 2003).

1.6.2.3. Type 3 Diabetes Mellitus:

The development of Type 3 Diabetes due to drug therapy or non-pancreatic disease (Katzung BG, 2004).

1.6.2.4. Type 4 Diabetes Mellitus:

Type 4 Diabetes is also known as “gestational diabetes”. During pregnancy in women due to hormonal imbalance or less insulin leads to development of this type of diabetes and after born of baby this type of diabetes goes away. Such type of woman has always risk for development type II diabetes later in life. (Tripathi KD, 2003, Diabetes mellitus – [online]).

1.6.2.5. Pre-Diabetes:

In this type of diabetes, blood glucose levels raises a little but not like great as in diabetes. In future it converts to type II diabetes in maximum peoples
along with high risk of heart disease and stroke. Type II diabetes can delay or prevent in people having moderate physical activity and weight loss.

1.6.2.6. Other Type Diabetes

The other types of diabetes are (Wong HL, 2005),

1. Diabetes due to mutant insulin
2. Diabetes due to insulin receptors
3. Diabetes due to mitochondrial DNA
4. Maturity Onset Diabetes

1.6.3. Symptoms of Diabetes:

In diabetes, glucose levels increase can cause number of problems such as excessive thirst, fatigue, weight loss, frequent urination, hunger and blurry vision. Due to slow development of type 2 diabetes, there were no experiences of symptoms at all in many hyperglycemia people (Diabetes mellitus – [online], Wong HL, 2005, Gennaro AR, 2000).

![Figure 1.16: Symptoms of Diabetes](image-url)
Symptoms of Type 1 Diabetes:
• Fatigue,
• Weight loss,
• Polyuria,
• Polyphagia,
• Polydipsia,
• Increased hunger,
• Irritability,
• Nausea and
• Vomiting

Symptoms of Type II Diabetes:
• Fatigue,
• Weight loss,
• Polyuria,
• Polyphagia,
• Polydipsia,
• Nocturia,
• Blurred vision,
• Anemia,
• Infections,
• Slow wound healing,
• Vascular complications and
• Impotency

1.6.4. Epidemiology:
More than billions of diabetic’s patients are identified worldwide with high frequency in relations of diabetics, obese people and adults (Davidson’s. 1999).

1.6.5. Etiology:
Pancreatic cells autoimmune destruction leads to Type 1 diabetes whereas due to less sensitive insulin tissues arises more genetic predisposition leads to
Type II diabetes. Even though the conditions like hyperthyroidism, hyperpituitarism, Cushing’s syndrome, pancreatitis, hemochromatosis or carcinoma also responsible for production diabetes. Also glucose intolerance develops by long term administration of oral contraceptives, glucocorticosteroids, thiazides, in Pregnancy or excessive stress.

1.6.6. Pathophysiology:

The overproduction of glucose and deficiency in secretion of insulin in the hepatic system leads to hyperglycemia. Due to Insulin glucose enter in to the cells of adipose tissue and muscle, synthesis of fat in cells and synthesis of protein is stimulates. The deficiency of glucose in muscle cells which lead to glycogenolysis and release of amino acids for gluconeogenesis. The glucose levels are high in adipose tissues with absence of insulin leads to diminishing of release of free fatty acids and synthesis of triglyceride. The free fatty acids metabolizes to ketones in the liver and this ketones are used by muscles for energy. Hepatic overproduction of glucose from glycogenolysis and gluconeogenesis due to insulin deficiency. Physiologically insulin oppose by Glucagon and this is another hormone increased in diabetes. The Hyperglycemia converts in to glycosuria after the levels of serum glucose exceed the renal threshold for reabsorption of glucose. The ketones are excreted in the urine is known as ketoacidosis. The osmotic diuresis which lead to polyuria and polydipsia and finally dehydration. Due to this urinary loss of sodium, potassium and bicarbonates. When glucose levels are increased the glycoprotein deposited in capillaries which lead to vascular complications of diabetes mellitus. In hyperglycemia, metabolism of glucose to sorbitol by aldose reductase produced neuropathy and cataracts, sorbitol produced osmotic swelling and damage.

The levels of blood glucose with moderate loss of pancreatic islet tissues and assessable plasma insulin concentration decreases in type-2 diabetes. The pathological changes in type 2 diabetes are depositions of amyloid and atrophy of islet epithelial cells. The collection of insoluble fibrils formed from islet amyloid polypeptide is called as Islet amyloid also known as amylin. Very common in elderly non-diabetic patients is the small quantities of islet amyloid, so the islet amyloid produced tentative type 2 diabetes. Amyloid
deposition is not a cause of diabetes, but relatively reproduces a pathological process which is increased in type II diabetes (Diabetes mellitus – [online]).

Due to insulin resistance reduction in beta cell is more than 30%, glucagons secretion is increased and unchanged alpha cell mass in Hyperglycemia of type II diabetes.

Spontaneous resistance after a highest secretory capacity of insulin accompanied by increase in levels of fasting blood glucose leads to failure in insulin generation due to a number of possible mechanisms of destruction of beta cells which lead to abnormality in insulin production pathway, chronic degranulation and glucotoxicity. In type II diabetes, the history of function of pancreatic beta cells, resistance of insulin insulin is compensated by increases in secretion but ultimately fails. The plasma insulin concentrations at fasting state related with the plasma glucose at fasting state, this profile is known as 'Starling curve of the pancreas', are shown in Figure 1.17.

![Figure 1.17: Insulin secretory capacity in type 2 diabetes](image.png)
Complications of diabetes:

<table>
<thead>
<tr>
<th>Complications</th>
<th>Causes</th>
<th>Signs and Symptoms</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Acute Complications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic Ketoacidosis (DKA)</td>
<td>The metabolism of fat into ketone bodies in absence of insulin in Liver, the blood pH is decrease by elevated ketone bodies levels in the blood leading to DKA.</td>
<td>Abdominal pain, breathing rapidly and deeply, Dehydration, lethargy, hypotension, shock and coma.</td>
<td>Diabetic retinopathy – [online]</td>
</tr>
<tr>
<td>B) Chronic Complications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic Retinopathy</td>
<td>Due to Hyperglycemia pericyte death, thickening and inability of vascular walls, retinal blood vessels are more permeable and tiny blood vessels damages in the retina</td>
<td>Blurred vision, Macular edema, bleed (haemorrhage)</td>
<td>Diabetic nephropathy – [online]</td>
</tr>
<tr>
<td>Diabetic Neuropathies</td>
<td>Generally observed with High levels of blood glucose and fat, chronic diabetes, mechanical injury to nerves, smoking or alcohol drinking and somewhat genetic factors</td>
<td>Nausea, vomiting, indigestion, constipation, diarrhoea, weakness, dizziness, numbness, pain in the toes, feet, legs or tingling, wasting of muscles of the hands or feet, frequently urination, in men erectile dysfunction and in women vaginal</td>
<td>Diabetic Neuropathies – [online]</td>
</tr>
</tbody>
</table>
### Complications of Diabetes

<table>
<thead>
<tr>
<th>Complications</th>
<th>Causes</th>
<th>Signs and Symptoms</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic Nephropathy</td>
<td>Glomerulus thickening, destruction of glomeruli and microalbuminuria.</td>
<td>headache, nausea, vomiting, poor appetite, fatigue, ill feeling, frequent hiccups Foamy and frothy urine, itching, edema, weight gain.</td>
<td>T. Sindhu et al, 2010</td>
</tr>
</tbody>
</table>

**Table 1.2: Complications of diabetes**

#### 1.6.7. Dyslipidemia (Hyperlipidemia):

The levels of plasma cholesterol and/or triglycerides are increased and level of HDL is decreased with primary i.e. genetic or secondary factors mediated dyslipidemia which leads to development of atherosclerosis. The diagnosis of atherosclerosis which can be measured by plasma levels of total cholesterol, triglycerides and individual lipoproteins.

The treatment for atherosclerosis is changes in diet, exercise, weight loss and use of lipid-lowering agents. The main reasons for atherosclerosis are overproduction or underproduction or defective clearance of triglycerides and low density lipoproteins (LDL), cholesterol or excessive clearance of high density lipoproteins (HDL).

- **Symptoms and Signs:**
  
  Dyslipidemia does not show any symptoms in several times, but dyslipidemia leads to vascular, peripheral artery and coronary artery disease. Increase in levels of triglycerides i.e. more than 1000 mg/dL which leads to acute pancreatitis whereas increase in levels of LDL which leads to eyelid xanthelasmas, arcus cornea and tendinous xanthomas. Increase in levels of triglycerides i.e. more than 2000 mg/dL leads to creamy white retinal arteries.

- **Diagnosis:**
  
  For the diagnosis of dyslipidemia serum lipids is measured.
A Fasting Lipid Profile:

Tests for secondary causes of Dyslipidemia – The conformation of severe changes in lipid profile with assessment of glucose in fasting condition, liver enzymes, Thyroid Stimulating Hormone (TSH), creatinine and urinary protein (DLD – [online], William TC, 2006).


- Rodent Models
  - Rat Models
  - Mouse Models
- Transgenic Models for Cell Pathology
- Swine Models
- Primate Models

1.6.8.1. Mouse Models:

In mouse models can easily studied for onset, development and course of diabetes accompanied by molecular mechanisms, the number of advantages such as short breeding span, ease of genetic manipulation, complete genome knowledge and physiological testing as well as invasive testing. The type of models are inbred, naturally mutated, genetically engineered and transgenic mouse. In the study of diabetes and obesity naturally mutated mouse are one of the special by researchers from many years. Now a days with all of the characteristics of Type 1 Diabetes, no single mouse models are present.

Now a day’s spontaneously developed diabetes in non-obese (NOD) mouse is one of the most useful animal model. The streptozotocin induced total destruction of pancreatic cells resembles phenotype of insulin-dependent Type 1 Diabetes similar hyperglycaemia and exogenous insulin. These type of models are very beneficial for investigations of diabetic microvascular complications like nephropathy, retinopathy and neuropathy, but in this type of model cell destruction is not autoimmune, therefore this type of models are very different from human diabetic conditions.
The mouse models of Type II Diabetes are considerable similarity to human condition such as insulin resistance, pancreatic dysfunction, obesity and cardiovascular disease. In Mutated mouse models for development of severe obesity with natural conditions, changes in leptin receptor or in leptin gene. In humans differentiation of insulin resistance diabetes and obesity facilitated diabetes is very difficult, but in mouse models this differentiation is very easy. In mouse models the sequence of diabetic actions are, within 2 weeks hyper-insulinemia, within 3 to 4 weeks obesity, within 4 to 8 weeks hyperglycemia with insulin resistance subsequently destruction of β-cell depending on the genetic make-up, sex, and age of the mice. Therefore, generally mouse models are broadly chosen animal model for Type II Diabetes study.

1.6.8.2. Rat Models:
The Zucker diabetic fatty rat (ZDF1) is commonly used as a model for the study of T2D. Like the mouse model, the ZDF rat harbors mutations on leptin receptors, becomes obese, and presents with hyperglycemia within the first few months of age. Also similar to the mouse models, the ZDF rat appears to develop diabetes because of an inability to increase cell mass. The ZDF rat therefore lacks sufficient insulin secretion required to compensate for the insulin resistance as part of the obesity. The mechanism that is responsible for the failure of cell mass expansion is not fully understood but as is postulated in mouse models, may be secondary to gluco- and lipotoxicity. The ZDF rat does not display the same islet pathology as humans with T2D (islet amyloid). The Goto-Katazaki (GK1) rat is another model used for the study of T2D. The GK rat is nonobese and has a decreased cell mass. Although the decreased β-cell mass is noted at birth, it is believed to be secondary to defective cell proliferation. The GK rat displays abnormalities characteristic of human T2D in the presentation of liver and skeletal muscle insulin resistance. Due to impaired insulin secretion, fasting blood glucose levels appear to be only slightly increased. Therefore, although there are similarities between the characteristics of the ZDF rat and the human condition, the overwhelming majority of humans with T2D do not
have inadequate cell proliferation in early life. Thus, this characteristic of the GK rat model is a limitation as it relates to the human condition.

1.6.9. **Experimental Methods for Induce Diabetes Mellitus** (Ghosh MN, 2005)

- Diabetes induced by Streptozotocin
- Diabetes induced by Alloxan
- Diabetes induced by Hormone
- Pancreatectomy in dogs
- Diabetes induced by other diabetogenic compounds
- Insulin deficit because of antibodies of insulin
- Diabetes induced by Virus

1.6.9.1. **Alloxan and Streptozotocin - Induced Diabetes Mellitus in Animals:**

Since the initial findings in 1943 of alloxan (ALX) induced beta cell necrosis in rabbits, this compound has long been used for inducing experimental diabetes. Alloxan is a uric acid derivative and is highly unstable in water at neutral pH, but reasonably stable at pH 3. ALX acts by selectively destroying the pancreatic beta islets leading to insulin deficiency, hyperglycemia and ketosis. ALX causes diabetes in many rodent and non rodent animals and is most preferably used in case of rabbit because of the relative ineffectiveness of streptozotocin (STZ) in rabbits for induction of diabetes and development of well characterized diabetic complications.

Because of its low stability, relatively very shorter half-life (less than 1 min) and acidic nature of solution, intravenous route of administration of ALX (dose: 40-200mg/kg in rats & 50-200mg/kg iv or ip in mice) is preferred. The ALX treated animals exhibit severe hyperglycemia, glucosuria, hyperlipidemia, polyphagia, polydipsia and other symptoms of uncontrolled diabetes and do also develop various complications such as neuropathy, cardiomyopathy, well marked retinopathy and others. ALX is disadvantageous as the percentage incidence of diabetes is quite variable. Further, the incidence of ketosis and resulting mortality is high. The reversal of hyperglycemia due to pancreatic regeneration is early and common in
case of ALX treated animals. Because of these limitations, ALX is now almost replaced by STZ for induction of diabetes in laboratory animals. Streptozotocin is an antibiotic derived from *Streptomyces achromogenes* and structurally is a glucosamine derivative of nitrosourea. Like ALX, it causes hyperglycemia mainly by its direct cytotoxic action on the pancreatic beta cells. Its nitrosourea moiety is responsible for beta cell toxicity, while deoxyglucose moiety facilitates transport across the cell membrane. Like ALX, the involvement of free radicals generation and resulting alteration of endogenous scavengers of these reactive species have been reported in STZ diabetogenicity. Further, STZ causing alkylation’s or breakage of DNA strands and a consequent increase in the activity of poly-ADP-ribose synthetase, an enzyme depleting NAD in beta cells finally leading to energy deprivation and death of beta cells is reported. There is wide variety of reports available in the literatures on doses and development of hyperglycemia with STZ since the susceptibility of animals to STZ appear to depend on age, species and even within strain. STZ(dose:35-65mg/kg in rats & 100-200mg/kg i.v or i.p in mice) is a preferred agent to induce experimental diabetes since it has some advantages over ALX such as, relatively longer half-life (15 min), sustained hyperglycemia for longer duration and the development of well characterized diabetic complications with fewer incidences of ketosis as well as mortality. ALX and STZ diabetic animals are most widely used for screening the compounds including natural products for their insulinomimetic, insulinotropic and other hypoglycemic/antihyperglycaemic activities (Zhang BB et al, 2000).

1.6.9.2. **Alloxan Induced Diabetes Mellitus in Animals:** (Vogel HG, 2002):

**Purpose and rationale:**

The dosage and treatment schedule of alloxan facilitated hyperglycemia and glucosuria in various animals like rats, rabbits, dogs and more other species except guinea pigs which have resistant. The main purpose is development of hyperglycemia by Alloxan in animals.
Mechanism of action:
The uptake of Alloxan is rapidly by B cells when first time administration to animal, secretion of insulin is speedily increase in absence of glucose, but response of islet cells to glucose is entirely suppresses at repetitive administration. The development of ROS are decreased by alloxan level in presence of different types of falling agents like condensed glutathione (GSH) and cysteine.

The proper glucose-induced insulin secretion is maintain by two -SH groups of glucokinase, this both -SH groups of glucokinase reacts with Alloxan and inactivates the enzyme. The dialuric acid formed because of alloxan reduction re-oxidized back to generation of superoxide radicals which release ferric ions from ferritin and reduce them to ferrous ions. The alloxan radicals also reduced Fe3+. Additionally superoxide radicals go through dismutation to hydrogen peroxide.

\[
O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2
\]

In this reaction presence of Fe2+ and hydrogen peroxide, catalyzed by superoxide dismutase and by Fenton reaction forms highly reactive hydroxyl radicals:

\[
Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^-
\]

The mechanism of alloxan-induced reactive oxygen species generation in Beta cells of rat pancreas given in Figure 1.8.
Figure 1.18: Mechanism of action Alloxan Induced Diabetes

GKa - glucokinase active,  GKi – glucokinase inactive,  
HA– alloxan radicals,  [Ca2+] i – intracellular calcium concentration  
The DNA of pancreatic islets is targeted by Reactive Oxygen Species (ROS)  
and stimulates destruction of DNA which leads to development of diabetes.  
After the first administration of Alloxan insulin release is suddenly increase.  
At the start alloxan with free radicals of H₂O₂ induces voltage dependent  
calcium channels opening and thus calcium influx from extracellular fluid  
by depolarisation of B cells of pancreas and also restrictions on elimination  
of calcium from the cytoplasm.

Procedure:  
Rabbits administered with alloxan monohydrate via ear vein for 10 min, at  
dose of 150 mg/kg with concentration of 5 g/100 ml, having weight of about  
2.0 to 3.5 kg and the solution pH is 4.5 which leads to development of  
hyperglycemia and uricosuria in almost animals.  
Rats of Wistar or Sprague-Dawley strain are administered with alloxan via  
subcutaneous route at dose of 100–175 mg/kg having weight about 150 –  
200 gm.  
Male Beagle dogs are administered with alloxan via intravenous route at 60  
mg / kg dose.
After this, all the animals are administered with 1 Litre of 5% glucose solution, regular insulin (10 I.U.) for a week and nutrition ad libitum. Afterwards, lone insulin (28 IU) daily dose administered subcutaneously.

1.6.9.3. Streptozotocin Induced Diabetes (Ghosh MN, 2005):

**Purpose, rationale:**

The streptozotocin is an antibiotic developed diabetes via cytotoxic effect to beta-cells of pancreas (Rakieten et al. 1963).

**Procedure:**

The healthy male Wistar rats having weight about 150 gms – 220 gms kept with standard diet. The streptozotocin was given at 60 mg/kg dose via intravenous route to rats.

The reaction of streptozotocin can be known as three phase response means after 3 hours initial blood glucose levels increase up to 150 - 200 mg/dl, after 6 - 8 hours increase in serum insulin trailed by persistent hyperglycemia. The harshness and diabetes symptoms depends on dose streptozotocin. Development of hyperglycemia up to 800 mg and other symptoms like glucosuria and ketonemia within 48 hours at dose of streptozotocin is 60 mg/kg is administered intravenously. The degranulation and necrosis of pancreatic beta cells can be confirm by doing histological studies. The animals used only after 10-15 days for assessment of pharmacological activity when blood glucose levels are stable.

1.6.10. Method for Critical assessment:

In diabetes research many investigators use streptozotocin induced diabetes in laboratory animals like rats. The streptozotocin is more valuable agent in diabetes research.