CHAPTER 3: REVIEW OF LITERATURE

"Genetics loads the Gun, Life style pulls the trigger". There are many diseases that are caused due to genetical disorders and is one of the cause for diabetes mellitus\textsuperscript{60}.

3.1 DIABETES MELLITUS

Diabetes mellitus (DM) was recognized as early as 1500 B.C. by Egyptian physicians who described it as a disease associated with "the passage of much urine". The term "diabetes" was coined by the Greek physician Aretaeus, who noticed that patients with diabetes had a disease that caused the siphoning of the structural components of the body into the urine\textsuperscript{61}.

DM afflicts about 5\% of the general population. Diabetes is a mysterious illness, a statement made in antiquity by the physician Aerates of Cappadocia (81-138 AD) is still valid today. At first Galen suspected that this illness was caused by a kidney complaint. Avicenna alone has been credited with two additional discoveries, first, the mention of further symptoms– besides the triad (polydypsia, polyuria and marasmus) known to antiquity– namely physical, mental, sexual weakness, occurrence of carbuncles, gangrene and secondly the alleged discovery of the sweetness of diabetic urine\textsuperscript{62}. The study suggest that for the world as a whole, between the years 1995 and 2025, the adult population will increase by 64\%, prevalence of diabetes in adults will increase by 35\% and the number of people with diabetes will increase by 122\%. For the developed countries, there will be an 11\% increase in the adult population, a 27\% increase in the prevalence of adult diabetes and a 42\% increase in the number of people with diabetes. For the developing countries, there will be an 82\% increase in the adult population, a 48\% increase in the prevalence of adult diabetes and a 170\% increase in the number of people with diabetes\textsuperscript{63}. 
In recent years, developed nations have witnessed an explosive increase in the prevalence of DM predominantly related to lifestyle changes and the resulting surge in obesity. The metabolic consequences of prolonged hyperglycemia and dyslipidemia, including accelerated atherosclerosis, chronic kidney disease and blindness, pose an enormous burden on patients with diabetes mellitus and on the public health system. The number of patients with DM is markedly increasing worldwide. DM is associated with impaired glucose metabolism that leads to an increase in free radical production and increase in triglyceride and lipoprotein levels. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of antioxidant enzymes and play a role in the long-term complications of diabetes. Therefore, among the various therapeutic strategies, combination of antihyperglycemic, antihyperlipidemic and antioxidant activity can be beneficial in the prevention of DM and its complications.

3.1.1 Disease profile

a) Definition

Diabetes is defined as a state in which homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. In diabetic condition, dyslipidemia, lipid abnormalities are the unbalanced metabolic states of diabetes. DM may present with characteristic symptoms such as polyphagia, polydypsia, polyuria, blurring of vision and weight loss. In its severe forms, ketoacidosis or a non-ketonic hyperosmolar state may develop and lead to stupor, coma and in the absence of effective treatment to death.

b) Prevalence

There are two types of diabetes- Type-1 diabetes mellitus formerly known as insulin dependent diabetes mellitus (IDDM) and Type-2 diabetes mellitus formerly...
known as non-insulin dependent diabetes (NIDDM). The vast majority of diabetic patients are Type-2 diabetes mellitus.

Diabetes patients are 25 times more prone to blindness, 2 times more prone to heart attacks, 2-6 times more prone to stroke and 17 times more prone to kidney damage as compared to non diabetics.\textsuperscript{68}

c) Epidemiology

DM in humans is undergoing a remarkable upsurge in prevalence in the India. Historically, the usual ratio for Type-1 to Type-2 diabetes has been 1:20. Classically, Type-1 diabetes is described as an autoimmune disease in which a foreign protein is incorporated into islet β cells, perhaps via viral infection. In response, the patient's lymphocytes attack the foreign protein and inadvertently destroy the patient's β cells as collateral damage. This leads to a state of absolute insulin deficiency.

The pathogenesis of Type-2 diabetes is less well defined, however, it is invariably associated with defective sensing of glucose signals by the β cell. It is often associated with a state of insulin resistance, which means insulin that is secreted by the β cell and bound to liver, muscle and fat cells is sub normally efficacious in carrying out its metabolic actions.\textsuperscript{69}

The WHO has predicted that the global prevalence of Type-2 diabetes will be more than from 135 million in 1995 to 300 million in 2025 and that this increase will affect both industrialized and developing countries expecting the greatest increase in India, from 19.4 to 57.2 million.\textsuperscript{70}
d) Classification of diabetes mellitus

Table No. 1: Classification of diabetes mellitus

<table>
<thead>
<tr>
<th>Classes</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type-1 diabetes</td>
<td>Islet β-cell destruction, autoimmune, idiopathic</td>
</tr>
<tr>
<td>Type-2 diabetes</td>
<td>Insulin resistance, insulin deficiency</td>
</tr>
<tr>
<td>Genetic defects of β-cell function</td>
<td>Chromosome 20, HNF 4α, chromosome 7, glucokinase</td>
</tr>
<tr>
<td>Genetic defects in insulin action</td>
<td>Type A insulin resistance, lipoatrophic diabetes</td>
</tr>
<tr>
<td>Disease of the exocrine pancreas</td>
<td>Pancreatitis, neoplasia, cystic fibrosis, pancreatectomy</td>
</tr>
<tr>
<td>Endocrinopathies</td>
<td>Cushing’s syndrome, hyperthyroidism</td>
</tr>
<tr>
<td>Drug- or chemical-induced</td>
<td>Nicotinic acid, thiazides, glucocorticoids</td>
</tr>
<tr>
<td>Infections</td>
<td>Congenital rubella, cytomegalovirus</td>
</tr>
<tr>
<td>Uncommon forms of immune-mediated diabetes</td>
<td>Insulin auto immune syndrome, Anti-insulin receptor antibodies</td>
</tr>
<tr>
<td>Other genetic syndromes</td>
<td>Down’s syndromes, Huntington’s chorea</td>
</tr>
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</table>

The clinical staging reflects that diabetes progresses through several clinical stages during its natural history. Moreover, individual subjects may move from stage to stage in either direction. Persons who have, or who are developing, DM can be categorized by stage according to the clinical characteristics, even in the absence of information concerning the underlying etiology. The classification by etiological type results from improved understanding of the causes of DM.

e) Different forms of Diabetes mellitus

- **General: Type- 1 Diabetes mellitus (formerly called insulin dependent diabetes mellitus or IDDM)**
  - Autoimmune Type -1 diabétès mellitus (Type- 1A).
  - Non-autoimmune or idiopathic Type- 1 diabétès mellitus (Type- 1B)
Type-2 Diabetes mellitus (formerly called non-insulin dependent diabetes mellitus or NIDDM)

- Specific: Defined gene mutations
  - Maturity-onset diabetes of youth (MODY)
  - MODY 1, chromosome 20 – hepatic nuclear factor $4\alpha$ gene mutations
- MODY 2, chromosome 7-glucokinase gene mutation
- MODY 3, chromosome 12-hepatic nuclear factor 1α gene mutations
- MODY 4, chromosome 13-pancreatic determining factor X-gene mutations
- MODY X, unidentified gene mutation (s)
- Maternally inherited diabetes and deafness-mitochondrial leucine tRNA gene mutations

❖ Other Specific forms of Diabetes:

1) Diseases of the exocrine pancreas
   Fibrocalcaneous pancreatopathy, Pancreatitis, Trauma, Pancreatectomy, Neoplasia, Cystic fibrosis, Haemochromatosis and others

2) Endocrinopathies
   Cushing's syndrome, Acromegaly, Pheochromocytoma, Glucagonoma, Hyperthyroidism, Somatostatinoma etc.

3) Infections
   Congenital rubella, Coxsackie B, Cytomegalovirus, Mumps, Adenoviruse etc.

4) Drug or chemical induced diabetes mellitus
   Nicotinic acid, Glucocorticoids, Thyroid hormone, α-Adrenergic agonists, β-adrenergic agonists, Thiazides, Dilantin, Vacor, Interferon-α therapy etc.

5) Other genetic syndromes sometimes associated with diabetes
   Down's syndrome, Friedreich's ataxia, Huntington's chorea, Klinefelter's syndrome, Laurence – Moon – Biedel syndrome, Porphyria, Prader willi syndrome, Turner's syndrome, Wolfram's syndrome etc.

6) Associated with Pregnancy
   Gestational Impaired Glucose Tolerance (GIGT)
   Gestational Diabetes Mellitus (GDM)
3.1.2 Insulin

The DM has been well known as a wasting disease due to insulin deficiency in human beings. The pancreas secretes insulin. Carbohydrate metabolism is primarily under the control of insulin. Insulin deficiency occurs in a person due to the functional disorder of the pancreas.

a) The Endocrine part of the Islets of Langerhans:

The normal human adult pancreas contains on an average some 500,000 islets of langerhans, distributed in scattered manner within the gland, comprising 1 to 3% of the total tissue. Each group of cells of the endocrine part is surrounded by the acini of the exocrine part, they look like islands and are hence termed as islets. The distribution of islets is maximum in the tail and minimum in the head of the gland.

Three types of cells are found in the islets. These are called the α (alpha), β (beta) and δ (delta) types. The α cells are fewer in number about 20% and they exist peripherally in the islets, while the most numerous β cells (about 75% to 80%) are situated centrally in the form of lumps.

The synthesis of two hormones insulin and glucagon takes place in the β cells and α cells respectively in the islets of Langerhans. Both hormones play an important role in carbohydrate metabolism. The function of the δ cells (about 5% in number) is not clearly known. It is assumed that they may secrete serotonin but some others believe that gastrin is secreted by these cells.

b) Chemistry:

It has minimum molecular weight of 5734. Insulin from different sources (e.g., pig, cattle, sheep and horses) shows minor differences in amino acid composition and immunological activity. The nearest to human insulin in structure is insulin from pig. Insulin is destroyed by action of digestive enzymes and is hence inactive.
when given (administered) by mouth. The biological action of the hormones can be prolonged by combining it with protamine or globin (protamine zinc insulin and globin insulin) or by altering the size of the crystals (ultralente insulin; large crystals and slow acting)\textsuperscript{74}.

c) **Metabolism of Insulin:**\textsuperscript{74}

Insulin is believed to be transported in the plasma bound to a specific insulin transporting protein. Insulin is degraded primarily in the liver and kidney by the enzyme, "Glutathione insulin transhydrogenase". The half life of plasma insulin is only 7-15 minutes.

d) **Mode of action of Insulin:**\textsuperscript{74}

1. Muscle, adipose tissue and liver are the major sites of its action
2. It is active on the lens and leukocytes
3. It has minor action on the metabolism of renal tissue, erythrocytes and GIT

e) **Extrahepatic tissues:**\textsuperscript{74}

- It facilitates the transport of glucose across the cell membrane.
- Insulin promotes metabolic pathways like glycogenesis, glycolysis and HMP pathways.
- Insulin stimulates intracellular transport of all sugars eg. arabinose, xylose and galactose.
- Insulin stimulates uptake of amino acids by the cell.
- Insulin stimulates the activity of enzymes hexokinase and glycogen synthetase.
- Insulin stimulates oxidative phosphorylation in mitochondria of muscle.
- Insulin stimulates the entry of Na+, K+ & PO\textsubscript{4}\textsuperscript{2-} into adipose tissue.
- Liver: Insulin is an anabolic hormone causing increased carbohydrate metabolism, glycogen formation, lipid synthesis, amino acid uptake and protein synthesis.
Glucose Homeostasis.75

A carbohydrate, particularly glucose, is an important source of fuel for living organisms. It has been found that glucose homeostasis contributes to two kinds of hormones, including insulin and anti-insulin or counter-regulatory hormones (glucagon, growth hormones, cortisol and catecholamines). Maintenance of serum glucose concentrations within a normal physiological range is primarily accomplished by two pancreatic hormones, insulin and glucagon. Derangements of glucagon or insulin regulation can result in hyperglycemia or hypoglycemia. Glucose penetrates most tissues slowly unless, insulin is present to facilitate its uptake; however, central nervous system (CNS) cells, capillary endothelial cells, gastrointestinal epithelial cells, pancreatic cells and renal medullary cells are freely permeable to glucose.

The endocrine portion of the pancreas, called the islets of Langerhans, consists of cordlike groups of cells arranged along pancreatic capillary channels. These pancreatic cells monitor changes in the availability of small calorigenic molecules, namely glucose and to a lesser extent amino acids, ketone bodies and fatty acids. Pancreatic β-cells appropriately alter their rates of insulin secretion in response to fluctuations in the levels of these calorigenic molecules, with glucose playing the dominant role in regulation of insulin secretion. Pancreatic β-cells secrete glucagon in response to increases in amino acid and fatty acid levels; however, glucose inhibits glucagon secretion. If blood glucose levels fall (e.g., during hypoglycemia or fasting), glucagon secretion is augmented, providing a counter regulatory hormonal response that stimulates gluconeogenesis in the liver and other tissues to avoid hypoglycemia. Circulating glucose levels are determined by the balance among absorption, storage, production and use (metabolic rate). Glucagon and insulin are the two most important hormones that maintain glucose homeostasis when blood concentrations are disturbed.
3.1.3 Insulin deficiency and its effects: In simplified terms, they can be described as stimulation of glucose utilization and inhibition of gluconeogenesis. In addition, the transport of glucose from the blood into most tissues is also insulin-dependent (exceptions to this include the liver, CNS and erythrocytes).

a. Fat metabolism:

The presence of insulin favors the production of triglycerides from free fatty acids (FFAs). When insulin deficiency causes an energy deficit, FFAs are oxidized to β-hydroxybutyric acid, acetoacetic acid and acetone. β-Hydroxybutyric acid can be used as an energy source, but in the absence of insulin the production of the keto acids eventually is greater than their metabolism and excretion. If insulin is not given to the patient, metabolic ketoacidosis ensues. The keto acids cause the blood pH to decline. The body’s neutralizing factors eventually are depleted and the patient continues to deteriorate to the point of coma and possibly death.
b. Protein metabolism:

The presence of insulin favors the production of structural proteins from constituent amino acids. When glucose is present intracellularly in sufficient quantities for needed energy production, most structural proteins retain their integrity. In the absence of insulin, structural protein production is not favored and intracellular glucose levels are insufficient to match energy demands. In attempt to produce energy, skeletal muscle converts its structural proteins to constituent amino acids. The liberated amino acids are transported to the liver, where they are converted to glucose via gluconeogenesis. In patients with diabetes, glucose enters the blood but is not taken up by tissues because of a true or relative lack of insulin. Thus, hyperglycemia is escalated and structural proteins are wasted.

![Fig. No. 4: Insulin deficiency and its effects](image)
3.1.4 A) Pathogenesis of Type-1 Diabetes mellitus

Three interlocking mechanisms are responsible for the islet cell destruction:

1. Genetic Susceptibility
2. Auto-Immunity
3. Environmental

![Diagram of the pathogenesis of Type-1 diabetes mellitus](image)

**Fig. No. 5: Overview of the pathogenesis of Type-1 diabetes mellitus**
1. Genetic Susceptibility:

At least one of the susceptibility gene for Type-1 diabetes resides in the region that encodes the class II antigens of the Major Histocompatibility Complex (MHC) on chromosome GP21 (HLA-D). The HLA-D region contains three classes of genes (DP, DQ and DR). The class II molecules are highly polymorphic and each has numerous alleles. About 95% of white patient with Type-1 diabetes have either HLA-DR3 or HLA-DR4 alleles or both where as in the general population the prevalence of these antigens is only 45%.

It is thought that genetic variations in the HLA class II molecules may alter recognition by the T-cell receptor, or may modify the presentation of the antigen because of variations in the antigen-binding cleft, thus, class II HLA gene may effect the degree of immune responsiveness to a pancreatic β-cell autoantigen or a β-cell autoantigen may be presented in a manner that promotes an abnormal immunologic reaction.

2. Auto-Immunity:

Clinical onset of Type-1 diabetes is abrupt; this disease in fact results from a chronic auto-immune attack of β-cells that usually exists for many years before the disease becomes evident. A lymphocyte with rich inflammatory infiltrate (Insulitis) is observed in the islets of patients in early diabetes. The infiltration consists mostly of CD8 T- lymphocytes. CD4 T cell from animals with auto immune diabetes can transfer diabetes to normal animals, thus establishing the primary of T-cell auto-immunity in Type-1 diabetes.

The Insulitis is associated with increase expression of class I MHC molecules and aberrant expression of class II MHC molecules on the β-cells. This aberrant expression is mediated in part by locally produced cytokines [eg. Interferon-gamma (IFN-γ) derived from activated T-cells]. Genetic dysregulation of a cytokine that induce IFN-γ production...
promotes the development of diabetes in a mouse model. About 70%-80% of patients with Type-1 diabetes have islet cell auto antibodies against intracellular islet cell antigens, such as Glutamic Acid Decarboxylase (GAD) “islet auto antigen 2” (1a-2a tyrosine phosphatases), insulin and gangliosides.

3. Environmental Factors:

Viruses

A viral infection has long been noted in the diagnosis of new cases and has the association between coxsackie viruses of group B and pancreatic diseases including diabetes. Other implicated viral infections include mumps, measles, cytomegalovirus, rubella and infections mononucleosis.

It has been postulated that one of these viruses causes mild β-cells injury, which is followed by an auto-immune reaction against previously sequestered antigens in virally altered β-cells in persons with HLA-linked susceptibility. Another is that an immune response develops against a viral protein that shares amino acid sequences with a β-cell protein (molecular mimicry).

Others

Antigenic exposure may also come from other sources. Children who ingest cow’s milk products early in life (before age of 4 months) have a 1.5 fold increase risk for Type-1 diabetes relative to those who do not, raising the spectrum of a cross-reacting antigen in cow’s milk.

3.1.4 B) Pathogenesis of Type-2 diabetes mellitus

The two metabolic defects that are characterizing Type-2 diabetes mellitus are:

1. A derangement in β-cell secretion of insulin
2. A decrease response of peripheral tissue to respond to insulin (Insulin resistance)
Fig. No. 6: Overview of the pathogenesis of Type-2 diabetes mellitus
1. Deranged β-cell Secretion of Insulin:

A modest hyperinsulinemia may be observed, attributed to β-cell hyperresponsiveness to physiological elevations in blood glucose, with the development of overt disease. The pattern of insulin secretion exhibits a subtle change. Early in the course of Type-2 diabetes, insulin secretion appears to be normal and plasma insulin levels are not reduced.

However, the normal pulsatile oscillating pattern of insulin secretion is lost and the rapid first phase of insulin secretion triggered by glucose is obtunded. Collectively, these and other observations suggest derangements in β-cell response to hyperglycemia early in Type-2 diabetes, rather than deficiencies in insulin synthesis per se. Later in the cause of Type-2 diabetes a mild to moderate deficiency of insulin develops which is less severe than that of Type-1.

2. Insulin Resistance:

Insulin resistance (IR) is a common pathological state in which target cells fail to respond to ordinary levels of circulating insulin. It results in inability of insulin to provide normal glucose and lipid homeostasis. Insulin resistance is also a feature of a number of other health disorders, including obesity, glucose intolerance, dyslipidemia and hypertension clustering in the so-called metabolic syndrome (also commonly referred to as syndrome X).

a) Symptoms of insulin resistance:

- Feeling agitated, jittery, moody, nauseated, or having a headache is common in insulin resistance, with almost immediate relief once food is eaten.
- Intestinal bloating.
- Sleepiness.
- Weight gain.
- Fatigue.
- Increased triglycerides.
- Increased blood pressure.

b) Causes and associated conditions of insulin resistance:  
A number of factors increase the risk for insulin resistance, including genetic predisposition, obesity and inactivity, aging, medications, polycystic ovary syndrome and rare disorders such as partial lipodystrophy. Concomitant conditions that are associated with insulin resistance include Type 2 diabetes, hypertension, dyslipidemia, atherosclerosis and polycystic ovarian syndrome.

![Insulin Resistance Diagram]

**Fig. No. 7: Insulin resistance and associated conditions.**

### 3.1.5 Pharmacological therapy

A) For Type- 1 Diabetes mellitus: Principal types of insulin preparations include-

1) Rapid-acting insulins – Insulin lispro and insulin aspart.
2) Short-acting insulin – Regular humulin, velosulin BR.
3) Intermediate-acting and long-acting insulins – Lente humulin, NPH (neutral protamine hagedorn) humulin, ultralente insulin and insulin glargine-lantus.
B) For Type- 2 Diabetes mellitus: Oral Hypoglycemic agents -

1) \(\alpha\)-glucosidase inhibitors (AGIs): Acarbose and miglitol.

   An enzyme in the brush border of proximal small intestinal epithelium \(\alpha\)-glucosidase serves to breakdown disaccharides and more complex carbohydrates. By competitive inhibition of this enzyme, the AGIs delay intestinal carbohydrate absorption. Their greatest effect is on post-prandial glucose levels and effect on fasting blood glucose level is small.

   Adverse effects: Flatulence, abdominal discomfort, diarrhea.

2) Sulfonylureas (SUs):

   They have been available in United States since 1954.

   First generation SUs: Chloropropamide, tolbutamide, acetohexamide and tolazamide.

   Second generation SUs: Glyburide, glipizide, glimepiride, glibenclamide.

   SUs bind to the SU receptor found on the surface of pancreatic \(\beta\)-cells. This interaction leads to a closure of voltage-dependent \(K_{ATP}\) channels, facilitating cell membrane depolarization, calcium entry into the cell and insulin secretion. The possibility that such agents may also directly enhance peripheral glucose disposal (i.e. decrease insulin resistance) has also been raised.

   Adverse effects: Weight gain, hypoglycemia. They must be used cautiously in hepatic or renal impairment.

3) Biguanides:

   Over 30 years ago, biguanides like metformin, phenformin, buformin were used for treatment of diabetes.

   Metformin’s major action is to decrease hepatic glucose output primarily by decreasing gluconeogenesis, but it may also increase glucose uptake by skeletal muscles. Metformin activates hepatic and muscle AMPK, a cellular signal for increased energy
requirements. Activation of hepatic AMPK results in phosphorylation and inhibition of acetyl-coenzyme A carboxylase, which catalyzes the rate-limiting step of lipogenesis. This block in fatty acid synthesis promotes fatty acid oxidation. In addition, activation of hepatic AMPK decreases expression of SREBP-1, a transcription factor implicated in the pathogenesis of insulin resistance, dyslipidemia and diabetes. Results of earlier studies suggest disruption of coupled oxidative phosphorylation in mitochondria. Whether this underlies increase in AMPK activity remains unclear.

Adverse effects: Gastrointestinal, lactic acidosis (rare). Contraindicated in liver, cardiac, renal dysfunction.

4) **Non-sulfonylureas:** Nateglinide, repaglinide.

The mechanism of action of these drugs is similar to that of SUs (closure of \(K\text{\textsubscript{ATP}}\) channel leading to calcium-dependent insulin secretion). However they bind to the SU receptor at a different site and with different kinetics than SUs. Their onset of action is faster and half-life is shorter, which results in brief stimulation of insulin release.

Adverse effects: Hypoglycemia, weight gain, contraindicated in liver, kidney dysfunction and concomitant use of repaglinide with gemfibrozil is avoided.

5) **Insulin sensitizers (Thiazolidinediones):**

The Currently available thiazolidinedione is pioglitazone. Troglitazone an earlier introduced thiazolidinedione was removed from market because of risk of hepatic failure.

Thiazolidinediones function as ligands for the PPAR\(_\gamma\), which is most highly expressed in adipocytes. These nuclear receptors, which are ligand-activated transcription factors, play an integral part in the regulation of the expression of a variety of genes involved in carbohydrate and lipid metabolism.

Thiazolidinediones improve insulin sensitivity, particularly in the peripheral tissues. In the adipocyte differentiation is enhanced, lipolysis is reduced, adipokines are altered,
namely a decrease in TNF-α and free fatty acid levels and increased adiponectin levels. These effects enhance insulin sensitivity.

Adverse effects: Weight gain, edema, anemia, pulmonary edema, congestive heart failure, contraindicated in liver dysfunction.

6) **Intestinal lipase inhibitor:** Orlistat

   It is an antiobesity agent that acts as a selective inhibitor of gastric and pancreatic lipases and thereby inhibits the hydrolysis of dietary fat into absorbable free fatty acids and monoglycerides.

   Adverse effects: Flatulence, oily spotting, fecal urgency, increased frequency of defecation and fecal incontinence. Absorption of fat-soluble vitamins can be adversely affected. Contraindications are chronic malabsorption syndrome, cholestasis and known hypersensitivity.

7) **Herbal Drugs:**

   Diabetes mellitus is a common chronic endocrine disorder. Since ancient time a number of herbal medicines were used in the treatment of DM. Many studies have been carried out in search of a suitable plant drug that would be effective in DM.

   Herbal medicines for diabetes can be classified into four categories according to their mode of action:

   i) Drugs acting like insulin

   ii) Drugs acting on insulin secreting beta cells

   iii) Drugs acting by modifying glucose utilisation

   iv) Drugs acting by miscellaneous mechanisms.

3.1.6 **Animal models for experimental diabetes mellitus**

   There are many advantages of using animals models in research work on diabetes as various aspects of the disease like the etiology, its multifactorial genetics,
pathogenesis of the disease and its complication can be explicitly understood. Secondly, it also helps in the development and evaluation of newer agents for the treatment of diabetes. However, there are some limitations in the use of animal model for studies on diabetes.

Induction of diabetes in animals can be carried out by various ways—by using different chemical diabetogenic agents, surgically by partial Pancreatectomy, by viral induction and genetic manipulation by selective in breeding.

**Various diabetic chemicals—**

Induction of diabetes by various chemical diabetogenic agents is also dependent on the species, the strain, sex and the diet of the animals. Variations in susceptibility have also been observed amongst male and female mice of same strain, males being more susceptible to insulin dependent diabetes mellitus (IDDM) than females. Types of diabetes produced depend on the amount of diabetogenic agent used.

1. **Alloxan**

Diabetogenic action of alloxan is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic Ca\(^{2+}\) concentrations causes rapid destruction of β-cells. The action of alloxan in the pancreas is preceded by its rapid uptake by the β-cells. Since alloxan exhibits a high affinity to the SH-containing cellular compounds, reduced glutathione (GSH), cysteine and protein bound sulfhydryl groups (including SH-containing enzymes) are very susceptible to its action. The reaction between alloxan and dialuric acid is a process in which intermediate alloxan radicals (HA\(^{•}\)) and an unidentified “compound 305” (maximum absorption at 305
nm) is formed. Alloxan is converted into unstable dialuric acid which is then reoxidised back to alloxan. This reaction establishes a redox cycle for the generation of superoxide radicals and also accompanied by reduction of oxygen to the OFR, O₂ and H₂O₂. The latter, through a Fenton type reaction in the presence of transition metals generates the highly toxic OFR, OH. Increased production of OFR in the islets, together with inadequate defense makes the β-islet cells susceptible to alloxan. In normal non fasted animals, the blood glucose level after alloxan injection fluctuates in a triphasic pattern.

**Triphasic response of alloxan**

1. Early hyperglycemia of short duration (about 1-4 h) due to a sudden short lasting decrease or cessation of insulin release and a direct glycogenolytic effect on the liver.

2. Hypoglycemia phase lasting up to 48 h and often resulting in convulsion and death (which may be prevented by treatment by glucose) due to uncontrolled leakage of insulin from the damaged cells.

3. Chronic diabetes phase, consequence of insulin lack histologically only a few β-cells if any, are detectable in animals with fully developed alloxan diabetes. Exogenous insulin readily restores normal blood glucose level.

**2. Streptozotocin**

Streptozotocin [2-deoxy-2-{3-(methyl-3-nitrosoureido)-D-glucopyranose}] is synthesized by streptomycetes achemogenes and is used to induce both Type-1 and Type-2. It is freely soluble in water, unstable at room temperature and has to be stored below -20°C.

Streptozotocin induces diabetes in almost all the species. Diabetes dose varies with the species and the optimal dose required to produce diabetes in rat was found to be (50 – 60 mg/kg i.p. or i.v.), in mice (175-200 mg/kg i.p. or i.v.) and in dogs (15 mg/kg,
for 3 days). Due to its low stability the rapid i.v. injection appears to be the best route of administration. STZ induces diabetes in hamster, monkey and guinea pigs. STZ diabetes can be induced by two ways either by single injection of STZ or by multiple low dose injection of STZ. Like alloxan, it shows triphasic fluctuation pattern in diabetes. Initial hyperglycemia is observed by 1 h after the injection followed by hyperglycemia and again a hyperglycemia state at 48 h, the elevated blood glucose level is observed by 48-72 h (peak effect) and is maintained thereafter. Different mechanism of action on the β-cells destruction by STZ has been proposed. It mainly acts through free radical generation. Other report proposed that STZ exerts lethal damage by alkylating DNA or its phosphate backbone as well as glycolytic or mitochondria enzyme. STZ also influence the immune system by suppressing the T-cell function associated with atrophy of the thymus and peripheral lymphoid tissue. Like alloxan, STZ also induces OFR induced lipid peroxidation and DNA strand breaking in pancreatic islet cell Streptozotocin enters the β-cell via a glucose transporter (GLUT 2) and cause alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation leads to depletion of cellular NAD⁺ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage.

3. Other diabetogenic agents

1. Dehydroascorbic acid 650 mg/kg for three days in rat
2. Dehydroisoascorbic acid 1.5 mg/kg in rat
3. Dehydroglucoascorbic acid 3.5-3.9 gm/kg in rat
4. Methyl Alloxan 53 mg/kg in rat
5. Ethyl Alloxan 53-130 mg/kg in rat
6. Oxime & Dithizone 53 mg/kg in rabbit
7. Sodium Diethylthiocarbamate 0.5-1 g/kg in rabbit
8. Potassium Xanthate 200-350 mg/kg in rabbit

4. Non-insulin dependent diabetes mellitus (NIDDM) resembling animal models

By altering the dose and the day of the STZ injection, the n-STZ models exhibit various stages of Type-2 diabetes mellitus, such as impaired glucose tolerance, mild, moderate and severe hyperglycemia. Neonatal STZ-induced rat (n-STZ) model of Type 2 diabetes mellitus model is generated by injecting Wistar rats on the day of their birth (n0=birth) intravenously (sapheneous vein) or intraperitoneally with 100 mg/kg of STZ. Also, the n-STZ rat model is developed by varying the day of the STZ injection after the birth, such as 2\textsuperscript{nd} day or 5\textsuperscript{th} day of the birth and these are alternatively called n2-STZ and n5-STZ models respectively. The rats treated with STZ on the day of birth, exhibit insulin deficient acute diabetes mellitus 3-5 days after birth. They showed high plasma glucose and about 93% decrease in plasma insulin and high plasma glucagon content. It was found that only by 8 weeks of age and thereafter n0-STZ rats showed mild hyperglycemia.

Sprague-Dawley pups were injected intraperitoneally on the 2\textsuperscript{nd} day after birth with 90 mg/kg STZ and on 1.5 days after birth with 120 mg/kg STZ. By 6 weeks of age these animals showed basal hyperglycemia and abnormal glucose tolerance. The above two animal models are based mainly on β-cell deficiency and these models are useful for evaluating the effect of β-cell deficiency in the development of NIDDM.

NIDDM animal models can also be prepared by neonatal alloxan induced diabetes by injecting alloxan 200 mg/kg body weight i.p. to neonates of 6 days old.
5. Hormone induced diabetes

*Growth hormone induced diabetes:* In intact adult dogs and cats repeated administration of growth hormone induces an intensively diabetic condition with all symptoms of diabetes including severe ketonemia and ketonuria.

*Corticosteroid induced diabetes:* Hyperglycemia, glucosuria are observed in forced fed rats treated with cortisone. In guinea pig and rabbit, experimental corticoid diabetes could be obtained without forced feeding.

6. Insulin deficiency due to insulin antibodies

Bovine insulin (1mg) is injected subcutaneously to guinea pigs at monthly intervals and is bleed by cardiac puncture two weeks after the second and subsequent doses of antigen. Intravenous injection (0.25 – 1.0 ml) of guinea pig anti-insulin serum to rats induces a dose dependent increase of blood glucose. This effect is due to neutralization by insulin antibodies secreted by the injected animal.

7. Virus induced diabetes

Type- 1 diabetes mellitus may be due to virus infection and β-cell specific autoimmunity. The D-variant of the encephalomyocarditis virus (EMC-D) selectively infects and destroys the β-cells in the male ICR Swiss mice similar to the human insulin-dependent diabetes.

8. Genetically diabetic animals

Several animal species, mostly rodents have been described to exhibit spontaneous diabetes mellitus on a hereditary basis.

E.g. * Spontaneously diabetic rats like BB rat, WBN/ KOB rat etc.

* Spontaneously diabetic mice like KK-AY mouse, NOD mouse etc.

Other prone strains to Type- 1 diabetes mellitus include New Zealand white rabbit, Kreesbond dog, Chinese hamster and Celebes black ape.
9. **Models of diabetes accelerated atherosclerosis**

Accelerated cardiovascular disease is a leading cause of both morbidity and mortality in diabetic patients. Aggressive therapy of dyslipidemia is necessary, since the risk of myocardial infarction is the same as in nondiabetic patients with previous myocardial infarction. Currently, rats and mice are the most widely used models to study diabetes and atherosclerosis.

10. **Pancreatectomy**

The technique of complete Pancreatectomy in the dog has been used by many scientists as a relevant animal model for diabetes mellitus in man. Polyuria, polydipsia, polyphagia and severe glucosuria were noted following removal of the pancreas in dogs.

Precise evaluation of consequences of reduced β-cell mass in rats can be achieved by partial Pancreatectomy. After 90% of the pancreas is removed, animals maintain moderate hyperglycemia in fed state but show no differences in body weight and plasma insulin concentrations as compared with sham-operated control animals. Loss of glucose-stimulated insulin secretion was documented in the animal after oral or intravenous glucose challenge. No glucose stimulated insulin release can be seen in perfused pancreases in these animals. In contrast the reaction to other secretagogues is retained.

3.1.7 **Diabetes and oxidative stress**

It is accepted that oxidative stress results from an imbalance between the generations of oxygen derived radicals and the organism’s antioxidant potential. Various studies have shown that diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential. Due to these events, the balance normally present in cells between radical formation and protection against them is disturbed. This leads to oxidative damage of cell components such as proteins, lipids and nucleic acids.
In both insulin dependent (Type-1) and non-insulin-dependent diabetes (Type-2) there is increased oxidative stress\textsuperscript{89}.

During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation. The increase in the level of ROS in diabetes could be due to their increased production and/or decreased destruction by nonenzymic and enzymic catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) antioxidants. The level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes. Also this is particularly relevant and dangerous for the beta islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses\textsuperscript{90}. The peroxidation of lipoproteins is believed to play an important role in atherosclerosis. First, aldehyde products of lipid peroxidation are believed to react with the amino groups of low density lipoprotein (LDL), causing it to become modified and prone to uptake by scavenger receptors. Secondly, accumulation of oxidized phospholipids in the various fractions of lipoprotein may cause inappropriate, pathophysiological, responses within the cell types with which they come in contact. Precise measurement of lipid hydroperoxides would appear critical to the scrutiny of this oxidative stress hypothesis of atherosclerosis\textsuperscript{91}.

Oxidative stress has been related to the etiopathogenesis of several chronic diseases and plays a paramount role in the aging process. Of the many biological targets of oxidative stress, lipids are the most involved class of biomolecules. Lipid oxidation gives rise to a number of secondary products. These products are mainly aldehyde, with the ability to exacerbate oxidative damage. Longevity and high reactivity allow these molecules to act inside and outside the cells, interacting with biomolecules such as
nucleic acids and proteins, often irreversibly damaging the delicate mechanisms involved in cell functionality. Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation. Since the 1960s several methods have been developed to assess this molecule in order to quantify the level of oxidative stress in vivo and in vitro\(^2\).

Various studies have shown that diabetes mellitus is associated with oxidative stress, leading to an increased production of ROS, including superoxide radical (\(O_2^-\)), hydrogen peroxide (\(H_2O_2\)) and hydroxyl radical (\(OH\)) or reduction of antioxidant defense system. Implication of oxidative stress in the pathogenesis of diabetes mellitus is suggested not only by oxygen free radical generation but also due to non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired antioxidant enzyme, and formation of peroxides. Lipid peroxidation (LPO) is a key marker of oxidative stress. It is a free radical-induced process causing oxidative deterioration of polyunsaturated fatty acids that eventually results in extensive membrane damage and dysfunction. The significant extent of LPO products that was measured as thiobarbituric acid reactive substances (TBARS) has been reported in diabetes\(^3\).

Free radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, etc. and compounds that can scavenge free radicals have great potential in ameliorating these disease processes. Oxygen free radical activity can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alterations in the structure and function of collagen, basement and other membranes and play a role in the long term complications of diabetes. Oxidative stress in diabetes coexists with a reduction in the antioxidant status, which can increase the deleterious effects of free radicals\(^4\).
Antioxidants have been shown to reduce the risk of diabetes onset, improve glucose disposal and improve some of the associated complications. It is possible that a population prone to diabetes using sources of antioxidants kept diabetes in a preclinical state and reduced the occurrence of diabetic complications that may have arisen with fluctuating glucose levels\textsuperscript{95}.

Diabetic patients are exposed to oxidative stress and complications of diabetes seem to be mediated by oxidative stress. Hyperglycemia is one of the main causes of oxidative stress in type 2 diabetes. Under hyperglycemia, the increased blood level of various reducing sugars promotes protein glycation and advanced glycation end products (AGEs). ROS are formed in this process and trigger tissue damage. Recently, the progressive deterioration of β cell function in type 2 diabetes has been accounted for in the oxidative stress-induced tissue damage. Due to a relatively low expression level of antioxidant enzymes, b-cells are implicated to be vulnerable to oxidative stress as compared with other tissues\textsuperscript{96}.

Many traditional plants treatments for diabetes are also used but most of the evidence for their beneficial effects is anecdotal. Traditional antidiabetic plants might provide new oral hypoglycemic compounds, which can counter the high cost and poor availability of the current medicines / present day drugs for many rural populations in developing countries. India is well known for its herbal wealth. Medicinal plants like \textit{Trigonella foenum graecum, Allium sativum, Gymnema slyvestre} and \textit{Syzygium cumini} have been studied for treatment of DM. In the indigenous Indian system of medicine good numbers of plants were mentioned for the cure of diabetes and some of them have been experimentally evaluated and active principle were isolated. WHO (1980) has also recommended the evaluation of the effective of plants in conditions where there are no safe modern drugs. The ethnobotanical information reports state that about 800 plants
may possess antidiabetic potential. Recently the medicinal values of various plants extracts have been studied by many scientists in the field of diabetic research\textsuperscript{97}.

### 3.2 HEPATOTOXICITY

#### 3.2.1a) Anatomy of liver\textsuperscript{98}

The liver is the second largest organ of the body and is located in the right upper quadrant (RUQ) of the abdomen, weighing 1400-1600 gm. in the males and 1200-1400 gm. in females. There are 2 main anatomical lobes – right and left, the right being about six times the size of the left lobe. The right lobe has quadrate lobe on its inferior surface and a caudate lobe on the posterior surface. The right and left lobes are separated anteriorly by a fold of peritoneum called the falciform ligament, inferiorly by the fissure for the ligamentum teres and posteriorly by the fissure for the ligamentum venosum.

The major functional unit of the liver is the hepatic acinus, which contains the portal vein, hepatic artery, bile duct and obviously the hepatocytes. The porta hepatis is the region on the inferior surface of the right lobe where blood vessels, lymphatics and common hepatic duct form the hilum of the liver. The liver has a double blood supply – the portal vein brings the venous blood from the intestines and spleen, and the hepatic artery coming from the coeliac axis supplies arterial blood to the liver. This dual blood supply provides sufficient protection against infarction in the liver. The portal vein and hepatic artery divide into branches to the right and left lobes in the porta. The right and left hepatic ducts also join in the porta to form the common hepatic duct. The venous drainage from the liver is into the right and left hepatic veins which enter the inferior vena cava. Lymphatics and the nerve fibres accompany the hepatic artery into their branchings and terminate around the porta hepatis.
b) Liver injury:

Although drugs are usually metabolized without injury to the liver, many fatal and near fatal drug reactions occur each year. Factors promoting the accumulation of hepatocyte toxins include genetic alterations in enzymes that allow the formation of the harmful metabolites, competition by another drugs and depletion of the substrates required to detoxify the metabolites.

A few compounds produce metabolites that cause liver injury in a uniform, dose dependent fashion. Injury to hepatocytes results in either directly from the disruption of intracellular functions or membrane integrity or indirectly from immune-mediated membrane damage.

c) Types of hepatotoxic agents:

Table No. 2: Hepatotoxic Agents

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INORGANIC AGENTS</strong></td>
<td>Metals and metalloids: antimony, arsenic, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, gold, phosphorous, selenium, tellurium, thallium, zinc, hydrazine derivative iodides.</td>
</tr>
<tr>
<td><strong>ORGANIC AGENTS</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Natural</strong>: Plant toxins</td>
<td>Albitocin, cycasin, nutmeg, tannic acid, icterogenin, pyrrolidizines, saferole, indospicine.</td>
</tr>
<tr>
<td><strong>Mycotoxins</strong>:</td>
<td>Aflatoxins, cyclochlorotine, ethanol, luteoskyrin, griseofulvin, tetracycline, and other antibiotics.</td>
</tr>
<tr>
<td><strong>Bacterial toxins</strong>:</td>
<td>Exotoxins(C.diphtheria, Clostridium botulinus, endotoxins, ethionine.</td>
</tr>
</tbody>
</table>
**Synthetic:** Non-medicinal

| Haloalkanes and haloolephins, Nitroalkanes, Chloroaromatic compounds, Nitroaromatic compound, organic amines, Azo compounds, Phenol and derivatives various other organic compounds. |

**MEDICINAL AGENTS:**

<table>
<thead>
<tr>
<th>Category of drugs</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Neuro psychotropics</td>
<td>Hydrazine, Tranylcypromine Anticonvulsants, Antidepressants.</td>
</tr>
<tr>
<td>2) Anti-inflammatory and anti-muscle spasm agents</td>
<td>Cinchopen, Cholchicine, Ibuprofen, Salicylates, Indomethacin.</td>
</tr>
<tr>
<td>3) Hormonal derivatives and other drugs used in endocrine disease</td>
<td>Acetohexamide, Azepinamide, Carbutamide, Tolbutamide.</td>
</tr>
<tr>
<td>4) Antimicrobials</td>
<td>Clindamycin, Novobiocin, Penicillin, Tetracycline, Sulfonamide, Amodiaquine, Isoniazid, Rifampin.</td>
</tr>
<tr>
<td>5) Antineoplastic</td>
<td>L-Asparaginase, Azacytidine, Methotrexate, 6-Mercaptopurine, Chlorambucil, Clavicin.</td>
</tr>
</tbody>
</table>

**d) Types of drug reactions:**

Although most hepatotoxic effects involve hepatocyte necrosis, some drugs injure bile ducts or canaliculi, causing cholestasis without marked damage of hepatocytes. Other therapeutic agents affect sinusoidal or endothelial cells or fat-storing Ito cells (causing Vitamin A toxicity, which leads to fibrosis) or cause a particular pattern of liver injury affecting multiple cell types.
I) Direct toxic reactions:

Acetaminophen is an example of an agent that causes direct toxic reaction. Two clinical scenarios account for most cases of acetaminophen-related hepatic necrosis i.e. the intentional suicidal overdose and the “therapeutic misadventure”. In the latter scenario, an alcoholic takes acetaminophen for pain relief in doses that exceed those recommended in the package insert (4 gm per 24 hrs). The result is a direct toxic reaction due to the enzyme-induction and glutathione depletion. Starvation may also play a part, presumably because of glutathione depletion. This alcohol-acetaminophen syndrome is the most common form of acute liver failure in the United States and Australia. Extremely elevated serum alanine and aspartate amino-transferase values (mean approx. 9000 units per liter in one study) distinguish this condition from viral or alcoholic hepatitis.

II) Idiosyncratic reactions:

Fifteen to twenty percent of patients receiving isoniazid as a single agent for prophylaxis against tuberculosis may have increased serum alanine and aspartate aminotransferase levels, but only 1 percent have hepatic necrosis severe enough to require the withdrawal of the drug. Several factors explain the relatively common toxic reaction observed. First, the simultaneous use of alcohol or rifampin may augment the toxicity of isoniazid. Second, elderly persons may be more likely to have toxic reactions than younger persons. Third, genetic differences are important, since person who is capable of rapid acetylation of isoniazid have an increased likelihood of toxic reactions resulting from the formation of acetylhydrazine, which is then transformed by cytochrome P-450 into a reactive metabolite. In the case of isoniazid and perhaps of other drugs causing idiosyncratic reactions, such reactions are not truly idiosyncratic but
occur when a series of genetic and environmental influences coincide to produce a significant quantity of one or more toxic metabolites.

**III) Combined toxic and allergic reactions:**

Halothane can induce a combination of toxic and allergic reactions leading to liver injury. Although there is usually no rash, fever and eosinophilia commonly observed and the histological features of liver-biopsy specimens are similar to those seen with idiosyncratic reactions. The initial elevations in serum alanine and aspartate amino transferase levels are delayed, but the interval between the drug administration and toxic reactions becomes shorter with each exposure. Protein adducts formed from the initial toxic reaction provide the hapten for the formation of antibodies, so that with subsequent exposure, antibody and cellular recognition of the halothane-protein-adduct antigen on the hepatocyte surface leads to cell injury.

**IV) Allergic hepatitis:**

Drugs such as phenytoin can cause a systemic allergic reaction characterized by fever, rash, lymphadenopathy, eosinophilia and the presence of eosinophils or granulomas in liver-biopsy specimens. This allergic reaction is accompanied by both hepatocyte necrosis and cholestasis. The mechanism responsible for the combined allergic and hepatotoxic reactions are unknown but the slow resolution of the illness suggests that the allergen remains on the hepatocyte surface for weeks or months. This drug-induced hypersensitivity hepatitis syndrome results in a mononucleosis-like illness that may be confused with viral illness or streptococcal pharyngitis, so that the agent is not withdrawn, despite signs of developing hepatitis. The result is often a severe form of the Stevens-Johnson syndrome, with fever lasting for weeks.
V) Cholestatic reactions:

The drugs that mainly affect bile flow, causing cholestatic injury, include estradiol, chlorpromazine, trimethoprim-sulphamethoxazole, rifampin, erythromycin, nafcillin and captopril. Typically, jaundice appears early, with associated purities but little alteration in the patient’s general well-being. A liver biopsy reveals engorgement of the canaliculi with bile and minimal hepatocellular injury. Eosinophils may be found in mildly inflamed portal tracts. The mechanism of cholestatic injury remains unclear. Estradiol and other estrogens have been shown to decrease bile flow and Na⁺/K⁺ ATPase, change tight junctions between cells, and alter the fluidity of hepatocyte membrane.

VI) Granulomatous reactions:

Noncaseating granulomas resembling sarcoidosis in the liver are caused by various drugs such as, Allopurinol, Isoniazid, Quinidine, Sulfonamides, Aspirin, Diazepam, Procainamide etc. The clinical picture is the same as that of other forms of granulomatous hepatitis i.e. low grade fever and chronic fatigue, with jaundice only in rare cases.

VII) Drug–induced chronic hepatitis:

Methyldopa and a number of other compounds like trazodone, nitrofurantoin, and acetaminophen have been found to cause a more indolent form of liver damage that closely resembles autoimmune chronic active hepatitis. Hyperglobulinemia may be present, with positive tests for antinuclear antibodies. The classic agent producing this reaction is oxyphenisatin, a laxative that has been withdrawn from the market. Early identification of drug-related chronic hepatitis is not easy, cirrhosis may develop before the hepatitis is diagnosed. Multiple prescription renewals may be a problem in the case of nitrofurantoin, which is used to control recurrent urinary tract infections.
VIII) Fatty liver and alcoholic hepatitis – like reactions:

Although fatty liver is most commonly related to obesity, diabetes, alcoholism or corticosteroid therapy, amiodarone and several other drugs can cause a disorder similar to alcoholic hepatitis, termed non-alcoholic steatohepatitis. This drug and some related compounds have been shown to cause severe liver toxicity, in an acute or chronic form, as a part of a multisystem syndrome. Patients typically have moderately elevated serum alanine and aspartate aminotransferase levels, with a characteristic lesion of steatohepatitis and cirrhosis can develop in just a few months. The presence of microvesicular fat within hepatocytes has a different meaning from that of the macro vesicular steatosis. Fine vesicles are associated with considerable cellular dysfunction but without cell death. This is the characteristic lesion of fatty liver caused by pregnancy, high doses of tetracyclines and Reye’s syndrome associated with aspirin.

IX) Indolent cirrhosis:

Of the several agents capable of causing a gradual progression to cirrhosis without any manifestation of clinical illness, methotrexate is the most frequently cited example. This agent is used in patient with severe psoriasis or rheumatoid arthritis, and toxicity may develop over a period of several years without any symptoms or evidence of hepatitis or other biochemical abnormalities. A liver biopsy is the only sure way to establish the diagnosis of indolent cirrhosis caused by a drug reaction. Methyldopa and Vitamin A have been reported to cause a similar syndrome.

3.2.2 Mechanism of hepatotoxicity: 101

There are numerous ways in which the structure and/or function of the liver can be altered. In view of this, the pathogenesis of hepatic injury requires consideration of at least several factors.
A. Pathogenesis of fatty liver:

The accumulation of abnormal amounts of fat within the liver may be due to either by extra hepatic causes that provoke a higher input of triglyceride (TG) precursors into the liver or as a consequence of changes in the function of the liver itself. In general, the mechanisms that can account for accumulation of TG include:

(i) the rate of synthesis of hepatic TG is normal, but the liver cells are unable to secrete the TG into the plasma (ii) the secretion of hepatic TG is normal, but the rate of synthesis is increased (iii) there is both an increase in the rate of synthesis and a block in the secretion of the synthesized TG and (iv) the TG synthesis takes place in a compartment of the cell other than the endoplasmic reticulum and thus this pool is not accessible to the normal secretory pathway.

Impairment of lipid release:

The movement of fat from the liver may be blocked by either interference with the formation of VLDL or by defective movement of the VLDL across a damaged plasma membrane. Defective formation of the VLDL may be due to impairment of synthesis of apoprotein moiety or of the mechanism for assembly of its three components, such as destruction of cellular site of protein synthesis like, rough endoplasmic reticulum (RER) and its ribosome. For example, CCl₄ acts through destruction of RER, puromycin inhibits protein synthesis by attaching itself to the ribosome as the “P” site, tetracycline and its other congeners inhibit protein synthesis, by binding to t-RNA, ethionine inhibit protein synthesis by ATP depletion or interfering with other steps of the synthetic pathway. However, there are several agents which inhibit protein synthesis without producing fatty liver (e.g. cyclohexamide and actinomycin D) and others which produce fatty liver without affecting protein synthesis e.g. orotic acid.
Increased mobilisation of depot lipid:

The studies have clearly shown that increased mobilization of lipid from depot can provoke toxin induced catecholamine release which is responsible for the increased mobilization of fat from depots and thereby contribute to fatty liver.

B. Pathogenesis of necrosis:

Although many hepatotoxic substances that produce necrosis have been shown to cause similar morphological changes, the exact mechanism by which these agents lead to necrosis remains to be understood. Several studies in the past have focused attention on the organelles of hepatocyte as the probable sites of injury responsible for the necrosis in animals, exposed to hepatotoxins. Toxic damage of mitochondria, lysosomes, smooth (SER) and rough endoplasmic reticulum (RER) and the plasma membrane may be responsible for necrosis. It was suggested that injury to mitochondria might lead to loss of bioenergetics required to maintain cellular integrity and thus results in necrosis. The possibility that it plays a subsidiary role in the necrogenic process continues to be the subject of study. The lysosomes seem to play a little role in necrosis but the main role of lysosomes in injury seems to be that of scavenger of the debris.

An injury to the RER or to the synthesis of a protein for maintenance of cell integrity or inability to particular protein that might be essential for maintenance of cell integrity of inability to synthesize protein destroyed by the toxic agent might contribute to necrogenesis but damage to the SER may not contribute much in the development of necrosis.

Recently, much attention has been given on the plasma membrane and the molecular basis for the membrane injury and their role in the pathogenesis of the necrosis. On the basis of several studies it has been concluded that the offending
agent leads to injury to the plasma membrane which permits intra hepatic accumulation of calcium ion. The high concentration of calcium ions in turn enhances plasma membrane injury permitting even higher intracellular content of the ion which leads to necrosis. In addition, several other molecular mechanisms lead to necrosis through membrane injury and physico-chemical changes in hepatocyte mainly by peroxidation of lipids, by trapping and depletion of cellular uridine triphosphate (UTP), and by alkylation or arylation of key macromolecules. For example, dimethylnitrosamine alkylates purines, pyrimidines and proteins. Bromobenzene and large overdoses of acetaminophen lead to arylation of cell macromolecules.

C. Cholestatic reactions:

The interference with the bile flow induced by the hepatic injury can result from (i) damage to the bile ducts and ductules (ii) damage to the canicular membrane of the hepatocyte (iii) injury to its ATPase activity (iv) interference with the energy source required for the active transport of constituents of bile into the canaliculus (v) defects in the synthesis and transport of bile acids into bile and (vi) defects in the metabolic conversion of substances into the molecular form required for excretion. Besides these several physico-chemical changes produced in the micelles of the bile could result into cholestasis.

Some chemicals may block the transport of bile constituents (bilirubin and bile acids) from the sinusoidal blood into the hepatocyte or their conjugation in the hepatocyte or transportation of bile into the canaliculus for excretion. For example, saramycetin, mirex, kepone and rifampicin inhibit the transport of bile from sinusoidal blood into the hepatocyte perhaps by competing for binding proteins of the hepatocyte or by affecting changes in the plasma membrane or both. Novobiocin
inhibits glucuronyl transferase enzyme responsible for conjugation of bilirubin and thereby decrease the transport into the canaliculus for excretion. This in turn results decrease clearance of bilirubin from blood.

Chemicals like C-17 alkylated steroids cause damage to fibrillar network of the canaliculi and thus produce anatomic obstruction of the extra hepatic biliary tree which may prove to be an important factor in the production of cholestasis. Similarly, chemicals like manganese sulphate and norethindrolone lead to definite manifestations of intrahepatic cholestasis, such as hyperbilirubinemia of intrahepatic cholestasis and canicular bile plugs.

D. Pathogenesis of cirrhosis:

The hepatotoxins which produce necrosis in experimental animals can produce cirrhosis but little is known about the mechanism responsible for cirrhotogenesis. Several clinical cases which show steatosis do not lead to cirrhosis but so far the evidence is less than conclusive. The exact mechanism by which necrosis triggers cirrhosis or the difference between the steatosis that does not lead to cirrhosis and that which appears to do so is obscure. However, it is certain that factors liberated from injured tissue provoke the fibrogenesis which in turn lead to cirrhosis. The hepatotoxic chemicals which lead to chronic inflammatory response or injury to hepatocytes may contribute to fibrogenesis.

E. Hepato-carcinogenesis:

In most instances, induction of hepatic carcinoma requires prolonged administration of the carcinogens. Most hepatocarcinogens are also hepatotoxic, but all hepatotoxins are not carcinogens. The carcinogens are electrophilic reactants in their own right or must be converted so in vivo by metabolism and/or by chemical breakdown. The reactions so formed bind with cellular macromolecules and so
initiate a chain of events that lead to cancer. Thus, the hepatoma inducing action of aromatic amines resides in their ability to produce electrophilic radicals, formed during the course of the metabolism by the N-hydroxylation pathway. The crucial significance of these radicals in malignancy induction is increased by observations indicating a failure of non-carcinogenic analogues (e.g. several amines or anthracene) to generate free radicals in liver hepatocytes and mitochondria. The initial strong generation of radicals subsides to fluctuating changes during tumor genesis. Treatments with the noncarcinogenic counterparts do not lead to similar variation in free radical content of the liver.

The new precursor cell population produced, have distinctive biochemical properties, including the acquisition of one or more new antigens that appear early and persist in the different cell populations in the ultimate cancer.

**F. Hepatic injury due to host idiosyncrasy:**

Some drugs can produce hepatic injury unpredictably in a small proportion of recipients. The injury produced is an expression of unique, individual susceptibility instead of intrinsic toxicity of the offending agents. The mechanism is presumed to be that of drug allergy. Several other chemicals produce hepatic injury probably through a different mechanism, may be through an aberrant metabolic pathway of the drugs.

**G. Hypersensitivity:**

Indeed, no firm evidence for the role of hypersensitivity in chemical induced hepatic injury is available. The evidence available so far indicates that chemical-induced allergy as the cause of hepatic injury is incomplete because the antigen responsible for the presumed allergic state might be an unknown metabolite of the chemical. Despite lack of concrete evidence, chemical-induced allergy is probably
H. Biochemical mechanism of hepatic injury:

As discussed previously, changes produced by hepatotoxins are preceded or succeeded by functional or metabolic changes in the liver. Some biochemical changes which disturb the liver function are summarized as under:

i) Depletion of coenzyme:

Some chemicals disturb the liver function by depleting an essential metabolite or coenzyme followed by morphological changes in the particular cell concerned. For example, ethionine depletes ATP in rat liver which results changes in protein synthesis. Another chemical CCl₄ causes rapid depletion of NADPH, and antibiotic azaserine depletes the liver of NAD⁺ + NADH.

ii) Activation of insulting agents:

Now, a variety of hepatotoxic agents are known which require preliminary metabolism through an interaction with the NADPH-cytochrome P-450 chain before their toxic potential can become fully expressed. The process whereby a material is metabolized to a biologically more active and toxic form is called activation. Chemicals in this category include CCl₄, halothane, dimethyl-nitrosamine, trichlorethylene, vinyl chloride, paracetamol, aflatoxins etc.

iii) Lipid peroxidation:

During the last few years much evidence has accumulated showing that lipoperoxidation occurs in living tissues and is of importance in some pathological phenomena. CCl₄ was metabolized to chloroform and concluded that this transformation was caused by homolytic cleavage, yielding free radicals that could...
alkylate sulfhydryl groups of enzyme. It was reported that free radicals arising from
the homolytic cleavage of CCl₄ could attack the methylene bridges of unsaturated
fatty acid side chains of microsomal lipids, resulting in morphological alteration of
the endoplasmic reticulum, loss of drug metabolizing enzyme activity, loss of
protein synthesis, loss of the capacity of liver to form and excrete VLDL.

There is evidence in support of the role of lipid peroxidation as the cause of
hepatic injury and against the importance of alkylation or oxidation of thiol group
or other direct attacks on proteins, nucleic acid polymers, or nucleotides. According
to them the free radical leads to peroxidation of the unsaturated lipids of the ER
resulting in destruction of the membranes, and to the generation of secondary free
radicals derived from the lipids of the membrane-a form of chain reaction, and
damage to other organelles of the hepatocyte following exposure to these secondary
free radicals derived from the initial effect of the CCl₄ on the lipids of the ER.

3.2.3 Biochemical and functional manifestations of injury:

In toxicity studies much attention is devoted to the effect of drugs or chemicals
on liver function. Since the liver is an organ with such diverse functional activities,
no single parameter can be selected to be representative of “Liver Function”. The
development of the so-called liver function tests has largely followed the
development of new knowledge about the biochemistry of the liver. So, tests
indirectly become a measure of hepatic functions.

a. Transaminases:

The two transaminase enzymes, which are sensitive indicators of parenchymal
cell integrity, are:

- Aspartate aminotransferase (AST), formerly known as serum glutamate
  oxaloacetate transaminase (SGOT).
• Alanine aminotransferase (ALT), formerly known as serum glutamate pyruvate transaminase (SGPT).

There is little to choose better ALT and AST in terms of sensitivity and, though ALT has the greater specificity for liver damage, the tendency for AST to be elevated in primary skeletal muscle disease as well as in myocardial infarction seldom causes problems of interpretation. In alcoholic hepatitis, the increase in AST is usually more pronounced than that of ALT.

**Clinical significance:**

i) Hepatitis: AST and ALT both show their greatest elevations in acute hepatitis, in which the rise in enzyme activity begins in the prodromal phase, preceding the onset of jaundice and occurring also in those patients who remain anicteric. Levels may reach more than 20 times, or even 50 times, the upper reference limit, with AST and ALT reaching comparable levels. Declining values accompany clinical improvement, though falling enzyme levels accompanying clinical deterioration may result from widespread necrosis and signal impending liver failure.

ii) Other infections involving the liver: Infections such as infectious mononucleosis, cause elevated transaminase levels.

iii) Poisoning and drugs: AST and ALT levels similar to those seen in acute hepatitis may follow poisoning with chemicals or plant toxins (e.g. from mushrooms). Hepatotoxic or hepatitis-inducing drugs usually result in smaller enzyme increases.

iv) Chronic parenchymal disease: Transaminase levels may reach over five times the upper reference limit in chronic active or persistent hepatitis, but are generally much lower than those typical of acute hepatitis. In cirrhosis or fatty change, levels are usually only slightly or moderately elevated and in cirrhosis may fluctuate. A
mild, long-standing elevation of AST may be observed in the presymptomatic phase of Wilson’s disease.

v) Malignancy: Moderate increases of transaminase levels with occasional exacerbations, which presumably also reflect episodes of cell destruction, are seen in malignant disease of the liver. AST is more markedly increased than ALT.

vi) Reduced liver perfusion: The sensitivity of the transaminase is such that even minor degrees of parenchymal cell damage result in elevated transaminase levels in serum. Reduced perfusion of the liver in congestive cardiac failure leads to hypoxia, a potent cause of enzyme leakage from cells, and hence to the escape of transaminases and other enzymes from the centrilobular regions. Similarly, haemodynamic changes, such as the loss of fluid due to diarrhea and vomiting can result in small, transient elevations.

vii) Cholestasis: Transaminase levels are often normal in extrahepatic cholestasis, but may rise slightly or moderately in prolonged obstruction. Levels are also generally low in intrahepatic cholestasis. However, because of the frequent concurrence of cholestasis and hepatitis, many patients do not fall neatly into these classes.

b. Bilirubin and bile pigments: \(^{102}\)

The liver disposes of considerable quantities of bilirubin each day. This process involves several stages and malfunction at any stage can give rise to useful biochemical signs.

Bilirubin entering the plasma is normally derived mainly from the senescence of circulating erythrocytes, with a smaller contribution from the degradation of erythropoietic elements in the bone marrow. It is almost completely bound to
albumin and transported to the liver, normally the only organ that removes bilirubin from the circulation.

Uptake by hepatocyte is mediated by a carrier. The carrier also takes up bilirubin glucuronide from plasma as well as the exogenous dyes, such as bromsulphalein, sometimes used to test excretory capacity; such tests are useless, therefore, when plasma bilirubin is already raised.

Conjugation: The formation of bilirubin monoglucuronides and diglucuronides takes place in the hepatocyte. It is affected by the enzyme bilirubin uridine diphosphate glucuronyltransferase and converts bilirubin from a lipid-soluble to a water-soluble form.

Bile excretion of conjugated bilirubin: Some of the bilirubin glucuronide refluxes back into the plasma, but most is excreted in the bile by mechanisms that are still incompletely understood, but which limit the overall rate of transport of bilirubin.

Conjugated bilirubin in the plasma: Impairment of biliary excretion of conjugated bilirubin in intrahepatic or extrahepatic cholestasis increases its regurgitation into the plasma, raising both total and ‘direct’ bilirubin concentrations.

Conjugated bilirubin in the urine: Conjugated bilirubin is water-soluble and so can pass into the urine. Bilirubinuria can be detected by ‘stick-tests’ before the level of plasmabilirubin begins to rise; therefore, the fractionation of plasma bilirubin into its conjugated and unconjugated components is unnecessary in most cases. Bilirubin bound to albumin in this way cannot pass the glomerulus, and therefore may persist in plasma after bilirubinuria has disappeared.

Fate of bilirubin in the bile (urobilinogen formation): Bilirubin glucuronides excreted into the bile undergo hydrolysis by the glucuronidases of the intestinal flora followed by oxidation and reduction of bilirubin to the colorless urobilinogen.
Most urobilinogen is excreted in the faeces; oxidation of urobilinogen produces the faecal pigment, stercobilin. Some urobilinogen is reabsorbed from the intestine into the plasma, from which most is re-excreted by the liver into bile. However, re-excretion is not complete and some urobilinogen passes into the urine.

3.2.4 Histological assessment of the liver:

a. Normal liver

The normal portal tract contains a hepatic artery, portal vein and a bile duct, with a few inflammatory cells present. The interface between the hepatocytes and the portal tracts is known as the limiting plate. The hepatocytes are arranged as cell plates radially distributed down to hepatic venules. Blood flows from the hepatic artery and portal vein through the hepatic sinusoids to the venules. These sinusoids are lined by fenestrated endothelial cells and Kupffer cells. The space between the endothelium and the surface of hepatocytes contains a matrix of fibrillar and non-fibrillar collagens with proteoglycans, along with perisinusoidal cells. In contrast to the flow of blood, bile flows from the hepatocytes into canaliculi and then via the canalicular system to the bile ductules and ducts in the portal tracts.

b. Chronic active hepatitis

Two histological types (chronic active hepatitis and chronic persistent hepatitis) are defined, though there is a large, grey area between these two. In chronic persistent hepatitis, the inflammatory infiltrate is limited to the portal tract. Hepatocyte necrosis is absent. In chronic active hepatitis, the chronic inflammatory infiltrate crosses the limiting plate to cause piecemeal hepatocyte necrosis.

c. Granulomatous Inflammation

A granuloma is a small nodular collection of modified macrophages, which are often termed epithelioid macrophages. In this biopsy, there were numerous
granulomas scattered through the liver parenchyma and the portal tracts. The clinical history was suggestive of drug reaction (carbamazepine). Granulomatous inflammation is merely a descriptive term, with many factors causing such inflammation, viz:

- Sarcoidosis
- Infection
- Drugs
- Primary biliary cirrhosis
- Foreign body reactions (e.g. in drug addicts)

d. **Alcoholic Hepatitis**

   Alcoholic hepatitis is shown, with fatty change in the liver. Fatty change in readily reversible if alcohol consumption stops. Alcoholic hepatitis is recognized by liver cell necrosis with polymorph infiltration around hepatocytes (satellitosis) and the presence of Mallory hyaline bodies (aggregates of ubiquitin filaments) in the cytoplasm. It is almost always accompanied by some new collagen formation in perivenular sinusoids. Cirrhosis may result if alcohol ingestion is heavy and prolonged.

e. **Cholestasis**

   Cholestasis is defined as an accumulation of bile pigments within the liver. In this case, brown bile can be seen within canaliculi, and also within the cytoplasm of hepatocytes. Cholestasis can be caused by many different diseases.

f. **Cirrhosis**

   The liver parenchyma is composed of proliferating parenchymal nodules divided by interconnecting fibrous septa. Classifying cirrhosis into macro nodular or micro nodular forms is of little histological value. The causes of cirrhosis include
alcohol, chronic viral hepatitis, chronic active hepatitis, biliary cirrhosis and haemochromatosis. A significant proportion may be cryptogenic (idiopathic). Complications include portal hypertension and hepatocellular carcinoma.

g. **Hepatocellular carcinoma**

Hepatocellular carcinoma is also known as hepatoma; this is a misnomer – it is not a benign tumour and the term should be dropped. This lesion is composed of cords and trabeculae of pleomorphic hepatocytes, with no normal architectural features (e.g. portal tracts). The histological appearances can vary from solid to acinar, and sclerosing to papillary, with some lesions being well differentiated and others anaplastic.

h. **Metastatic adenocarcinoma**

The most common form of carcinoma in the liver is a metastasis. This patient presented with a single lesion in the right lobe of the liver, which was biopsied under ultrasound control. Histology revealed irregular gland structures lined by pleomorphic epithelial cells, reminiscent of bowel epithelium.

I. **Carbon tetrachloride induced hepatotoxicity:**

It is useful to divide the mechanism of CCl₄ into the following sequence.

- Initial events
- Secondary evoked mechanism
- End stage pathological consequences

The initial event involves carbon-halogen bond cleavage, probably by a one-electron reduction of CCl₄, by a particular ferrous cytochrome P-450, to form chloride anion and trichloromethyl radical (CCl₃). Trichloromethyl peroxy radical (OOCCl₃) is probably generated and small quantity of CO may appear, mostly through dichlorocarbene intermediate.
In next stage, CCl₄-carbon is covalently bound to microsomal lipids and proteins. This placed CCl₄ into a general class of xenobiotics, the toxicity of which appears to depend on their metabolism and subsequent covalent bindings to cellular macromolecules. However within the first hour, there is inhibition of movement of liver triglycerides to the plasma as VLDL, polyribosomal desegregation and findings of protein synthesis also set in well. Protein synthesis could not take place as the specific binding site is already occupied by cytochrome P-450 induced free radicals.

The peroxidative decomposition of lipids of the ER is initiated by CCl₄ metabolism. Lipid peroxidation generates a wide variety of more or less toxic products, not organic radicals, which presumably could migrate from membrane sites near cytochrome P-450 to the other parts of the cell. This states that CCl₄ hepatotoxicity is primarily a matter of lipid peroxidation rather than covalent binding of CCl₄ cleavage products.

Free radical reactions are implicated in the progression of cancer, inflammation, atherosclerosis, hepatocellular damage and the biological process of aging. The hepatoprotective action combined with antioxidant activity has a synergistic effect to prevent the process of initiation and progress of hepatocellular diseases.

3.2.5 Liver damage due to free radicals:

The pioneering studies on the role of free radical reactions in the genesis and the expression of cellular and tissue damage have been carried out mainly in the liver. Most recently, the hepatotoxicity of several free radical-generating compounds like paracetamol, halothane and iron overload has been reported.

It is now generally accepted that reactive free radicals can exert cellular damage through a variety of mechanisms e.g. lipid peroxidation, covalent bonding, depletion of
glutathione and protein thiols, derangement of intracellular free calcium homeostasis, DNA fragmentation etc with different relevance in the various conditions. An essential involvement of lipid peroxidation in the events leading to hepatocyte death has been proved in the *in-vitro* and *in-vivo* acute intoxication.

Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective action. However, numerous medicinal preparations have been advocated a traditional system of medicine, especially in Ayurvedic, for treating liver disorders. Only a small portion of the hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their efficiency.

Plant derived natural products such as flavonoids, terpenoids and steroids etc. have received considerable attention in recent years due to their diverse pharmacological properties including hepatoprotective and antioxidant activity. There has been growing interest in the analysis of certain flavonoids, triterpenoids and steroids stimulated by intense research in to their potential benefits to human health. Anti-oxidant plays an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative diseases\textsuperscript{106}.

### 3.3 FREE RADICALS IN BIOLOGICAL SYSTEM

A free radical is any atom or group of atoms capable of independent existence that contains one or more unpaired valence electrons. The unpaired electrons do not contribute to intramolecular bonding. That unpaired electron/s controls the properties of radicals\textsuperscript{107}. They are produced by oxidation/reduction reactions, in which there is a transfer of only one electron at a time, or when a covalent bond is broken and one electron from each pair remains with each group. Free radicals can be either highly
reactive species like hydrogen atom, hydroxyl radical, or they can be stable entities like nitric oxide, DPPH radical. In biological systems free radicals have a range of transitory existences depending upon their reactivity. Some are stable, e.g. melanin can have a long lifetime, moderately stable ones such as nitric oxide can have lifetimes of ~5 seconds and highly unstable ones such as hydroxyl radicals exist for only a hundredth of a microsecond. The importance of free radicals and reactive oxygen species (ROS) has attracted increasing attention over the past decade. ROS, which include free radicals such as hydroxyl radicals (‘OH), superoxide anion radicals (O2•-) and non free radical species such as H2O2 and singlet oxygen (‘O2), are various forms of activated oxygen. These molecules exacerbate cellular injury and aging process\textsuperscript{108}. In living organisms, various ROS can be formed in different ways. Normal aerobic respiration and the stimulation of polymorphonuclear leukocytes, macrophages and peroxisomes constitute prominent sources of ROS. These are major endogenous sources of cellular oxidants. Exogenous sources of ROS include tobacco smoke, certain pollutants, organic solvents and pesticides\textsuperscript{109}.

Many present day diseases are reported to be due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic phenomenon in the body\textsuperscript{109}. Pro-oxidant conditions dominate either on account of increased generation of free radicals caused by excessive oxidative stress, or due to poor scavenging in the body caused by depletion of the dietary anti-oxidants. ROS differ significantly in their interactions and can cause extensive cellular damage such as nucleic acid strand scission modification of polypeptides, lipid peroxidation etc\textsuperscript{110, 111}.

Free radicals are assumed to play an important role in aging, cancer, radiation injury, inflammation, atherosclerosis, ischemia of the heart, brain, small intestine, kidney and liver; neurodegenerative diseases, diabetes mellitus and disorders of prematurity.
Free radicals seem to be one of the final common pathways of cell damage and affect the cell membrane and the nuclear DNA. The cell membrane damage is by cross-linking of proteins and by critical alterations of lipids\textsuperscript{112}.

### 3.4 ANTI-OXIDANT

Antioxidant can be defined as “Any substance, present at low concentrations compare to those of an oxidizable substrate, significantly delays or prevents oxidation of the oxidizable substrate”\textsuperscript{113}. Antioxidants are the first line of defense against free radical damage, and are critical for maintaining optimum health. The need for antioxidants becomes even more critical with increased exposure to free radicals. As part of a healthy lifestyle and a well-balanced, wholesome diet, antioxidant supplementation is now being recognized as an important means of improving free radical protection.

The human body employs many antioxidant systems. The exact activity of an antioxidant depends on the reactive species involved, the area of the body affected by reactive species and the exact molecular target.

In general, an antioxidant in the body may work in one of the following five ways.

(i) The removal of or decrease in the local O\textsubscript{2} concentrations
(ii) The removal of catalytic metal ions
(iii) The removal of ROS such as O\textsubscript{2} and H\textsubscript{2}O\textsubscript{2}\textsuperscript{*}
(iv) Scavenging initiating radicals such as ’OH, RO’ and RO\textsubscript{2}\textsuperscript{*}
(v) Breaking the chain of an initiated sequence.

#### 3.4.1 Mode of action of antioxidant

Antioxidant means "against oxidation." Under normal conditions the damaging actions of ROS and RNS are minimized by abundant protective and repair mechanisms that cells possess, including many enzymes and redox active molecules. The human body
has an elaborate antioxidant defense system. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken. After donating an electron an antioxidant becomes a free radical. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. Steric and electronic factors are also responsible for a chain breaking antioxidant. Antioxidants are manufactured within the body and can also be extracted from the food humans eat such as fruits, vegetables, seeds, nuts, meats, and oil. There are two lines of antioxidant defence within the cell. The first line, found in the fat-soluble cellular membrane consists of vitamin E, beta-carotene etc. Of these, vitamin E is considered the most potent chain breaking antioxidant within the membrane of the cell. Inside the cell water soluble antioxidant scavengers are present. These include vitamin C, glutathione peroxidase, superoxide dismutase, and catalase.

3.4.2 Classification of antioxidants

The antioxidant systems are classified into two major groups, enzymatic antioxidants and non enzymatic antioxidants.

- **Enzymatic antioxidants**

  Enzyme antioxidants are produced in the body and they act as body’s first line of defense against free radicals. They convert reactive free radicals into less reactive or inert species. Enzymatic antioxidant present in the body includes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

**Catalase**

Catalase is an enzyme, which can function either in the catabolism of H₂O₂ or in the peroxidase oxidation of small substrates such as ethanol, methanol, and quinine. Most of the aerobic cells have catalase activity. It was first crystallized from beef liver by
Sumner and Dounce\textsuperscript{118}. Catalase is present in all major body organs, especially in liver. The catalyse activity of animal and plant is largely located in sub cellular organelles known as peroxisome\textsuperscript{119}. Peroxisomes in animal cells are involved in the oxidation of fatty acids, synthesis of cholesterol and bile acids. Hydrogen peroxide is a byproduct of fatty acid oxidation. Catalase promotes the conversion of hydrogen peroxide into molecular oxygen and water without the production of free radicals\textsuperscript{120}.

Peroxisomes in plant cells are involved in photorespiration (the use of oxygen and production of carbon dioxide) and symbiotic nitrogen fixation (the breaking apart of the nitrogen molecule N\textsubscript{2} to reactive nitrogen atoms). Hydrogen peroxide is produced as an intermediate during these chemical processes removed by catalase to prevent damage to cellular machinery. White blood cells produce hydrogen peroxide to kill bacteria during which excess of hydrogen peroxide is removed by the catalase. Catalase is composed of four identical subunits, each containing a protoporphyrin ring and a central iron (Fe) atom that are very much like the familiar hemoglobins, cytochromes, chlorophylls and nitrogen-fixing enzymes in legumes\textsuperscript{121}.

- **Non-enzymatic antioxidants**

Endogenous non-enzymatic antioxidants such as GSH and total thiol were playing an important role in scavenging ROS. Low molecular weight non-enzymatic antioxidants such as carotenoids, and dietary phenolic compounds are not manufactured by cell itself so they are required to supplement through food and diet. In the absence of effective and affordable interventions for both types of diabetes, the frequency of the disease is expected to escalate worldwide, with a major impact on the population of developing countries\textsuperscript{122}. The preventing activity of allopathic drugs against progressive nature of diabetes and its complications has been modest and sub-optimal. Insulin therapy affords effective glycemic control, yet its drawbacks such as ineffectiveness on oral...
administration, short shelf life, requirement of constant refrigeration, and in the event of excess dosage leads to hypoglycemia etc, limits its usage. Treatment with sulfonylureas, biguanides and thiazolidinediones is also associated with side effects.

For such various reasons in recent years, the popularity of complementary medicine has increased. WHO (1980) has also recommended the evaluation of the plants in conditions where we lack safe modern drugs. This leads to increasing demand for herbal products with anti-diabetic activity with fewer side effects. Further, the selection of herbal products is easier because of supporting folklore claims and evidence gathered from traditional usage.

There has been growing interest in the analysis of certain flavonoids, triterpenoids, quinones and steroids, stimulated by intense research in to their potential benefits to human health. Plants provide a rich source of antioxidants, which include tocopherols, Vit.C, phenolic compounds, carotenoids, flavonoids, terpenoids, anthraquinones, steroids, strychnine and eugenol alkaloids etc.

Under normal circumstances, reactive oxygen species (ROS) such as O$_2^{•-}$, •OH, and H$_2$O$_2$ are detoxified by an efficient antioxidant system that includes enzymes such as superoxide dismutase, catalase and glutathione peroxidases. In case this defense system is inefficient, the cells experiences an oxidative stress which contributes in a variety of chronic inflammatory diseases such as arthritis and atherosclerosis as well as other ailments viz. cancer, diabetes, hepatitis, neurodegeneration and early aging. Likewise in liver injury, free radicals and lipid peroxidative metabolites also cause damages to hepatocytes leading to severe necrosis, sepsis or endotoxemia. Carbon tetrachloride (CCl$_4$) is a widely used hepatotoxin in rodents and its trichloromethyl radical (•CCl$_3$)-induced toxicity in rat liver closely resembles to human cirrhosis and hence is an acceptable animal model for analyzing hepatoprotective agents.
3.5.1 REVIEW OF BAUHINIA VARIEGATA PLANT

Botanical name  :  Bauhinia variegata  
Family  :  Caesalpiniaceae  

3.5.1 A) Description:

*Bauhinia variegata* Linn is a medium-sized, deciduous tree, found throughout India, ascending to an altitude up to 1800 meter in the Himalayas. The Hong Kong Orchid Tree, botanically known as genus Bauhinia. The origin of the Hong Kong Orchid Tree is China. The name Bauhinia was named after the Bauhin brothers who were sixteenth century herbalists.\(^ {131} \)

The plant is known by various names in different languages as under.

<table>
<thead>
<tr>
<th>Language</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>Mountain Ebony</td>
</tr>
<tr>
<td>Marathi</td>
<td>Rakta kanchan</td>
</tr>
<tr>
<td>Kannada</td>
<td>Kempu mandara</td>
</tr>
<tr>
<td>Hindi</td>
<td>Kachnar</td>
</tr>
<tr>
<td>Tamil</td>
<td>Shemmandarai</td>
</tr>
<tr>
<td>Telgu</td>
<td>Daevakanchanamu</td>
</tr>
</tbody>
</table>

3.5.1 B) Morphology:

Bark is grey with longitudinal cracks, pale pink inside. Leaves are rather broader than deep, rigidly sub-coriaceous, deeply cordate with two leaflets, connate for about two-thirds up, leaflets are ovate, rounded at apex, 10-15 cm long, pubescent beneath when young.

Its young stem Flowers are variously coloured, in few-flowered, lateral, sessile or short peduncled corymbs, the uppermost petal darker and variegated usually appearing before the leaves in short axillary or terminal racemes, stamens 5, staminodes absent, fruits flat; hard glabrous dehiscent pods, 10-15 seeded.\(^ {131,132} \)
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Fig. No. 8: *Bauhinia variegata* Linn. Plant

Fig. No. 9: Roots of *Bauhinia variegata* Linn. Plant
3.5.1 C) Medicinal Uses of Plant Parts:

Parts used: Bark, roots, buds, gum, leaves, seeds and flowers.

- The bark is astringent, tonic and anthelmintic and is used for ulcers and leprosy. A decoction of the bark is taken for dysentery. It is used to give tone and vitality to body. It is used against tuberculosis and skin ailments\textsuperscript{131,132}.

- The leaves contain Vitamin C (146mg \%). They are rich in reducing sugars and have good nutritive value. The infusion of the leaves is used as a laxative and for cure of diarrhoea, dysentery and piles\textsuperscript{132}.

- The dried buds are used for the treatment of diarrhoea, dysentery, worms, piles and tumours\textsuperscript{133}.

- A decoction of the buds is given in cough, piles, haematuria and menorrhagia. The flowers are laxative. Flower buds are pickled\textsuperscript{131-133}.

- An aqueous extract of the plant was found to be effective in induced goiter in rats.

- A gargle made from the bark with the addition of extract of acacia pods and pomegranate flowers is a remedy in salivation and sore throat\textsuperscript{133}.

- Bark rubbed into an emulsion with rice water and administered with the addition of ginger in scrofulous enlargement of the glands of the neck. A paste made of the bark together with dried ginger is also applied to scrofulous tumours\textsuperscript{134}.

- This plant is used in malaria and is also an antidote to snake poison\textsuperscript{135}.

- Both roots and bark are astringent, acrid, constipating and anthelmintic. They are useful in diarrhea, dysentery, cough, leprosy and diabetes\textsuperscript{135}.

- Bark is tonic to the liver\textsuperscript{136}.

- Plant is described as astringent to bowels, tonic to the liver and useful in treatment of leucoderma, leprosy, menorrhagia, asthma, wounds and ulcers\textsuperscript{137}.
3.5.1 D) Constituents Present in Various Part of *B. variegata* Linn:

- The stem-β-sitosterol, lupeol, kaempferol-3-glucoside and 5,7-dehydroxy and 5,7-dimethoxy flavanone-4-O-α-L-rhamnopyranosyl-β-D-glucopyranosides\(^{131}\).
- The pale violet flowers- Cyaniding-3-glucoside, maluidin-3-glucoside, maluidin-3-diglucoside, peonidin 3-diglucoside\(^{132}\).
- White flowers- Kaempferol-3-galactoside and kaempferol-3-rhamnoglucoside\(^{132}\).
- The bark yields a fibre\(^{133}\).
- The tree yields a gum similar to cherry gum\(^{134}\).
- Root and bark - Flavanone, (2S)-5,7-dimethoxy-3’,4’-methylenedioxyflavanone and a new dihydrodibenzoxepin, 5,6-dihydro-1,7-dihydroxy-3,4-dimethoxy-2-methylidibenzoxepin\(^{138}\).
- Root- Flavonol glycoside 5,7,3’,4’-tetrahydroxy-3-methoxy-7-O-α-L-rhamnopyranosyl (1→3)-O-β galactopyranoside\(^{139}\).
- Stem bark- hentriacontane, octacosanol, stigmasterol\(^{140}\) and sterols, glycosides, reducing sugars and nitrogenous substances\(^{141}\).
- Stem- Flavonone glycoside-5, 7- dihydroxyflavonone-4-O-α-L-rhamnopyranosyl- β-D– glucopyranoside\(^{142}\).
- Stem-β-sitosterol, lupeol, kaempferol-3-glucoside and a 5, 7-dimethoxy-flavonone-4-O-α-L– rhamnopyranosyl- β-D–glucopyranoside\(^{143,144}\).
- Stem-Flavonol glycoside-Kaempferol-3-glucoside\(^{144}\).
- Plant-Phenantraquinone-bauhinone-2, 7-dimethoxy-3-methyl-9,10-dihydro-phenanthrene-1, 4-dione\(^{145}\).
- Leaves-Two new long chain compounds- heptatriacontan-12, 13-diol and dotetracont-15-en-9-ol\(^{146,147}\).
• Leaves-Saponins, steroids, flavonoids, alkaloids, tannins and sugars\textsuperscript{148}.

• Volatile oil of leaves-Sesquiterpenes, β-caryophyllene, germacrene D and spathulenol along with δ - γ - cadinene\textsuperscript{149}.

3.5.1 E) Reported activity:

• Antitumor activity: Ethanolic extract of stem was evaluated against Dalton's Ascitic lymphoma in swiss albino mice. A significant enhancement of survival time of tumor bearing mice was found with respect to control group. Extract was able to reverse changes in haematological parameters protein and PCV consequent to tumor inoculation\textsuperscript{150}.

• Methanolic extract of leaves was tested for antimicrobial activity. Antifungal activity was shown against Aspergillus fumogalus, A.niger. The activity maxima was displaced by A.fumigatus, Bacillus anthracis, S.agalcties\textsuperscript{151}.

• Kanchanar (B. variegata) along with Manjishtha was given orally in non-healing diabetic foot ulcers and gangrene. 80% of patients showed improvement with 10% partial amputation\textsuperscript{152}.

• The Kanchanar guggulu is an ethical preparation advocated for the management of various glandular swellings like galgand, gandmala, granthi and arbuda etc\textsuperscript{153}.

• Oral administration of Kanchanara (B. variegata) bark and Ghanastava of Manjishtha (Rubia cordifolia) root to the patients of diabetic microangiopathy gave satisfactory results in newly formed ulcers\textsuperscript{154}.

• Solid extract of B.variegata in a polyherbal formulation has been found to be useful as a thyrocap in treatment of simple diffuse goiter with physical and biochemical improvement\textsuperscript{155}. 
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- **Anti-inflammatory activity**: Six flavonoids together with one triterpene caffestate were evaluated as inhibitors of some macrophage functions involved in the inflammatory process. These experimental findings suggest, use of the plant *B. variegata* in the management of inflammatory conditions\(^{156}\).

- **Hepatoprotective activity**: Alcoholic stem bark extract exhibited hepatoprotective activity in carbon tetrachloride (CCl\(_4\)) intoxicated Sprague-Dawley rats\(^{157}\).

- **Anti-arthritic activity**: Ethanol extract has significant antiarthritic effect\(^{158}\).

- **Immunomodulatory activity**: Ethenolic extract of the stem bark possesses immunomodulatory property\(^{159}\).

- **Antibacterial activity**: Aqueous and methanolic extract of plant shown remarkable antibacterial activity\(^{160}\).

- **Antihyperglycemic and anti-hyperlipidemic activity**: Aqueous and ethenolic extract of leaves has shown antihyperglycemic and anti-hyperlipidemic activity in normal and STZ induced diabetic rats\(^{161}\).

- **Antioxidant and antihyperlipidemic activity**: Alcoholic and aqueous extract of stem bark and root showed significant antioxidant and antihyperlipidemic activity in rats\(^{162}\).

- **Antiobesity effect**: Methanolic extract of bark has shown antiobesity action on female rats\(^{163}\).

- **Antinociceptive and anti-inflammatory activity** of Triterpene Saponin was found in leaves\(^{164}\).

- **Analgesic activity**: Aqueous and ethenolic extract of root has shown dose dependent analgesic activity\(^{165}\).

- **Anticarcinogenic and antimutagenic potential** of ethanolic and aqueous extract in Swiss Albino mice\(^{166}\).
3.5.2 REVIEW OF *TECTONA GRANDIS* PLANT

**Botanical name**: *Tectona grandis*  
**Family**: Verbenaceae

3.5.2 A) Description:

*Tectona grandis* Linn. (Verbenaceae) is a large deciduous tree. Branchlets are quadrangular, channeled and stellately tomentose. The tree is growing in higher situations, native to central India, Konkan, Western Deccan peninsula, South India and Burma\(^{167}\). Teak is a hardwood species of worldwide reputation\(^{168}\).

The plant is known by various names in different languages as under\(^{168,169}\):

- **English**: Teak  
- **Marathi**: Sag  
- **Kannada**: Tega, Jadi  
- **Hindi**: Sagvan  
- **Sanskrit**: Sakah  
- **Telgu**: Peddateku

3.5.2 B) Morphology:

This is an erect, large, deciduous tree growing up to 20 meters or more in height. It grows best in warm, moist tropical climates with 1,250 to 3,000 mm of mean annual precipitation and a marked dry season of 3 to 6 months. The branchlets are 4-angled. The leaves are large, elliptic or obovate, 20 to 30 centimeters in length, pointed at both ends, usually wedge-shaped at the base, and entire at the margins; the upper surface is rough, but without hairs, and the lower is densely covered with gray or yellowish hairs. The calyx is small, board, bell-shaped, and covered with stellate hairs, with subequal and spreading lobes. The corolla is white, and smooth, and less than 1 centimeter across, with subequal and spreading lobes. The fruit is somewhat rounded, about 1.3 centimeters in
diameter, and somewhat 4-lobed, the soft pericarp densely clothed with felted, stellate hairs\textsuperscript{167,170}.

![Fig. No. 10: Tectona grandis Linn. Plant](image)

![Fig. No. 11: Bark of Tectona grandis Linn.](image)
3.5.2 C) Medicinal Uses of Plant Parts:

Parts used: Bark, roots, leaves and flowers.

- The roots are useful in anuria\textsuperscript{167,171}.
- The bark is astringent, acrid, sweet, cooling, constipating, anthelmintic and depurative. It is useful in bronchitis, hyperacidity, diabetes, leprosy and skin diseases\textsuperscript{168}.
- Leaves are useful in inflammation, leprosy and in skin diseases\textsuperscript{171}.
- The flowers are acrid, bitter, refrigerant, diuretic and anti-inflammatory and are useful in leprosy, skin diseases, burning sensation and diabetes\textsuperscript{171}.

3.5.2 D) Constituents Present in Various Part of T. grandis Linn:

- Wood- Resin, silica, calcium, ammonium and magnesium phosphate\textsuperscript{169}
  Anthraquinone-2-carboxylic acid, anthraquinone-2-carboxaldehyde\textsuperscript{172}
  Triterpenic and hemiterpenic compound\textsuperscript{173}
  9, 10-dimethoxy-2-methyl-1, 4-anthraquinone, 5-hydroxy-2-methyl-9, 10-anthraquinone, 1-hydroxy-5-methoxy-2-methyl-9, 10-anthraquinone, 1, 5-dihydroxy-2-methyl-9,10-anthraquinone,tecomaquinone-I, tectoquinone, dehydro-a-lapachone\textsuperscript{174,175}.
  lapachol, 5-hydroxy-lapachol, methlyquinizarin, squalene\textsuperscript{176}
  Dehydro-a-isodunnione\textsuperscript{177}
  Lignins\textsuperscript{178}

- Root- Lapachol, tectol, dehydrolectol, tectoquinone, b-lapachone, dehydro-a-lapachone,b-sitosterol, new diterpene, tectgrandino\textsuperscript{172,179}
  Non-structural carbohydrates\textsuperscript{180}
  hydroxy-2-methyl anthraquinone, obtusifolina, betulinic acid\textsuperscript{179}

- Leaves- Tectoleafquinone\textsuperscript{173}
Tannins, dye-Tectoionols-B, tectoionols-A, monoterpenes, apocarotenoids\textsuperscript{181}

Protein (7.1%), crude fiber (22.3%), calcium (3%), phosphorous (0.46%)

Steroidal compound squalene, polyisoprene-a-tolymethyl ether and betulinic acid, a anthraquinonenapthaquinone pigment\textsuperscript{174,182}

- **Seed** - Seed oil contain fatty acids as caprylic (1.45%) myristic acid (2.86%), palmitic acid (12.12%), stearic acid (9.52%), oleic acid (23.33%) and linoleic acid (43.22%)(5). Xanthene\textsuperscript{183}

- **Bark** - Tannin (7.14%), quinone\textsuperscript{173}
  
  5-hydroxy-1,4-napthalenedione (juglone), sterols\textsuperscript{184}

  Ob tusifolina, Desidro-A-lapachona\textsuperscript{184}.

3.5.2. E) Reported activity:

- **Antifungal activity:** Teak (\textit{T. grandis}) sawdust extract inhibited the growth of \textit{Aspergillus niger}\textsuperscript{185}.

- **Antiulcer activity:** Lapachol, a naphthaquinone isolated from the roots was found to have an anti-ulcerogenic effect on subsequently induced experimental gastric and duodenal ulcers in rats and guinea-pigs\textsuperscript{186}.

- **Anti-anaemic activity:** Teak ethanolic extract increases significantly the concentration of haemoglobin, osmotic resistance of red blood cells and the number of reticulocytes after 7 days of Phenyl hydrazine administration. This study supports the use of \textit{Tectona grandis} in the treatment of anaemia\textsuperscript{187}.

- **Nitric oxide scavenging activity:** The plant extract exhibited a dose-dependent NO scavenging activity\textsuperscript{188}.

- **Wound Healing activity:** Leaf extract when applied topically (5% and 10% gel formulation) or given orally (250mg and 500 mg/kg body weight), promoted the
breaking strength, wound contraction and period of epithelization so it is used to promote wound healing\textsuperscript{189}.

- According Ayurveda, wood is acrid, cooling laxative sedative to gravid uterus and useful in treatment of piles, leucoderma and dysentery. It allays thirst and possess anthelmintic and expectorant properties. \textit{Tectona grandis} leaf extract are widely used in the folklore for the treatment of various kinds of wound, especially burn wound\textsuperscript{190}.

- \textbf{Antiasthmatic activity:} Ethyl acetate extract of bark showed significant antiasthmatic activity\textsuperscript{191}.

- \textbf{Antihyperglycemic activity:} Bark extract exerted antihyperglycemic activity in alloxan induced diabetic rats\textsuperscript{192}.

- \textbf{Antibacterial, cytotoxic and antioxidant activity:} Different extracts from leaves, leaf, bark and wood showed Antibacterial, cytotoxic and antioxidant activity\textsuperscript{193}.

- \textbf{Tocolytic effect:} Stem extract possess tocolytic effect on uterus of female albino wistar rats\textsuperscript{194}.

- \textbf{Wound healing activity:} Aqueous and methanolic extracts of leaves have significant wound healing activity\textsuperscript{195}.

- Methanolic extract (root)\textsuperscript{196}, ethenolic extract (bark)\textsuperscript{197} and water juice extract (leaves)\textsuperscript{198} showed \textbf{antioxidant activity}. 

3.5.3 REVIEW OF SCHREBERA SWIETENIOIDES PLANT

**Botanical name**: *Schrebera swietenioides* Roxb.

**Family**: Oleaceae

3.5.3 A) Description:

*Schrebera swietenioides* Roxb. (Weaver's Beam tree) belonging to family Oleaceae, is a moderate sized tree of 20 m height with thick grey bark growing in deciduous forests, to an altitude of 1200 m, throughout India. Mostly found in tropical and sub-tropical Himalayas, south and Central India, from Rajasthan to west Bengal.

The plant is known by various names in different languages as under.

<table>
<thead>
<tr>
<th>Language</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>Weaver’s beam tree</td>
</tr>
<tr>
<td>Kannada</td>
<td>Bula, Gante, Nagganti, Mogalingamara</td>
</tr>
<tr>
<td>Hindi</td>
<td>Moka, Banpalas</td>
</tr>
<tr>
<td>Tamil</td>
<td>Mogalingam</td>
</tr>
<tr>
<td>Telgu</td>
<td>Mogalinga, Tondamukkudi</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Muskakah</td>
</tr>
</tbody>
</table>

3.5.3 B) Morphology:

Leaves opposite, simple or imparipinnate; rachis usually winged. Inflorescence a paniculate cyme. Flowers heterostylyous and bisexual. Calyx campanulate, loosely enveloping the corolla, truncate or irregularly and obscurely lobed. Corolla salver-shaped, white, sometimes tinged with pink or puce; tube well developed, cylindrical; segments 6 or more, spreading to reflexed, each with a group of swollen brown to purplish hairs at the base. Stamens 2, inserted on the corolla; filaments short, anthers large, introrse. Ovary bilocular, small, truncate or obscurely bi-lobed at apex; ovules 4 in each loculus; style filiform; stigma included or excerted, subcapitate or oblong in outline.
Capsule bi-valved, woody with loculicidal dehiscence; seeds produced into a long solitary subapical wing\textsuperscript{200-202}.

\textbf{Fig. No. 12: Schrebera swietenioides Roxb. plant with fruit}

\textbf{3.5.3 C) Medicinal Uses of Plant Parts:}

Parts used: Bark, roots, Fruit, gum, leaves, seeds and flowers.

- Bark-used for treating boils and burns, Roots- used in Leprosy and also for killing worms in the wounds of cattle, Leaves-In treatment of urinary discharges and enlargement of spleen, and Fruits- useful for curing hydroccele\textsuperscript{203}.

- Root, bark, leaves, fruits which are used for medicinal purpose are bitter, acrid, appetizing, digestive, thermogenic, stomachic, depurative, constipating urinary astringent and anthelmintic\textsuperscript{204}.
• The roots, bark and leaves are bitter, acrid, appetizing, digestive, constipating and anthelmintic. They are useful in flatulence, skin diseases, leprosy, diarrhea, anemia and rectal disorders. The fruit is digestive, purgative and stomachic, and is useful in flatulence, anorexia, colic and diabetes\textsuperscript{205}.

3.5.3 D) Constituents Present in Various Part of \textit{S. swietenioides} Roxb:

• The tree exudes a grey gum which is sweet in taste. It consists of mannitol, fructose and a digalactoside named swietenose\textsuperscript{203, 206}.

• Fruit reported the presence of triterpenoids- Oleanolic acid, Betulinic acid\textsuperscript{207}.

3.5.3 E) Reported activity:

• \textbf{Antioxidant, Anti-Inflammatory and Antipyretic Activity:} Ethenolic extract of roots showed significant antioxidant, anti-inflammatory and antipyretic activities\textsuperscript{208}.

• Its stem bark is used in \textit{joint and body pains}, headache, itching\textsuperscript{209}.

• Its boiled fruit with ginger and sugar is used for \textit{haemorrhoids} by tribals of Saurashtra, Gujrat\textsuperscript{210}.

• Powdered leaf material methanol, ethanol and aqueous extracts were found significant \textit{antibacterial} activity\textsuperscript{211}.