Chapter 2

Review of Literature
Diabetes mellitus (DM) is well acknowledged as a chronic metabolic disease causing death and disability globally. The problem has reached pandemic proportions (Suja K et al., 2014). DM is a metabolic disorder characterized by chronic hyperglycemia and disturbances of carbohydrate, protein and fat metabolism associated with absolute or relative deficiency in insulin secretion and/or insulin action (Ozougwu JC et al., 2013). DM is often referred to as “starvation in the midst of plenty” because the intracellular levels of glucose are low, although the extracellular levels may be extremely high (Burnstock G et al., 2013). Uncontrolled hyperglycemia leads to life threatening complications that may result from acute metabolic decomposition, while long standing metabolic derangements are frequently associated with permanent and irreversible functional and structural changes in the cellular level itself (Abbas EK et al., 2009).

Global scenario – The rapidly increasing prevalence of DM worldwide is one of the most serious and challenging health problems in the 21st century. The number of people with diabetes had grown faster than expected. In 2007, 246 million people (roughly 6%) were affected worldwide and it is estimated that this will increase to 380 million or 7.35% by 2025. Furthermore, it is estimated that there are even more people (308 million or 8.1%) with impaired glucose tolerance (IGT). These people have a significant risk of developing type II diabetes mellitus. (Inge AMV et al., 2010). Type II diabetes is the commonest form of diabetes constituting almost 90% of diabetic population. There will be 42% increase i.e., from 51 to 72 million, in the developing countries. Countries with the largest number of people with diabetes are India, China, and the USA. In developing countries, the majority of people with diabetes are in the age group of 45-64 years and in developed countries around 65 years (Kesavadev JD et al., 2003). DM is the seventh leading cause of death in the United States and is the major cause of heart disease and stroke, and leading cause of kidney failure,
non traumatic lower limb amputations, and new cases of blindness among adults. It affects 25.8 million people of all ages (8.3%) of the US population. DM is the leading cause of kidney failure.

**Indian scenario** – Currently, India ranks highest with its largest number of diabetic subjects in any given country. WHO has already declared India as ‘global capital of diabetes’ (Jali MV et al., 2006). In 1970’s the prevalence of diabetes among urban Indian population was reported to be 2.1%, this has now risen to 12.1% (Apoorva SM et al., 2013). It has been estimated that presently, 19.4 million individuals are affected by DM and these numbers are expected to increase to 57.2 million by the year 2025 (Mohan V., 2004) according to WHO.

**2.1. History of Diabetes mellitus:**

The history of DM probably dates back to the beginning of human kind encompassing centuries, generations and civilizations. A historical review of the events surrounding the evolution of our current knowledge of DM must examine the oldest civilization including Babylonians, Assyrians, Egyptians, Chinese and Japanese as well as the centuries of the Greeks, Romans, Europeans and Americans.

Before Christ, Egyptians (1500 BC) had described an illness associated with the passage of much urine. Charaka and Sushruta (600-400 BC) recognised many aspects of this disorder and called it ‘Madhu meha’ (rain of honey) after noticing the sweet taste of urine. It was Greek physician: Aretacus of Cappadocia gave the name ‘Diabetes’.

The earliest of the Chinese medicine texts is based on the works of Haug-ti of 2697 BC. Records were preserved on Lacquer on strips of bamboo or palm leaves. The Chinese and Japanese also recognized the symptoms of diabetes, but were even less restrained with their description and wrote “The urine of diabetes was very large in amount and it was so sweet that it attracted dogs”.
Before Christ, Egyptians (1500 BC) had described an illness associated with the passage of much urine. Egyptians used strips of papyrus, reeds fastened together and shaped into rolls, on which they inscribed information. These strips subsequently became permanent records (Saima YQ et al., 2012). The most interesting of the papyri is papyrus ebers, written about 1500 BC, which records medical knowledge of ancient days. This contains a record of abnormal polyuria now believed to be related to diabetes. This probably represents the first recorded reference to the symptoms of diabetes.

The Hindu Medical writings of Charaka and Sushruta (600-400 BC) refer to diabetes as honey urine (madhu meha) (Ritu Lakhtiakia, 2013). It describes diabetes as “A disease of the rich and one that is brought about by gluttony or never indulgence in flour and sugar. This disease is ushered in by the appearance of the morbid secretions about the teeth, nose, ears and eyes. The hands and feet are very hot and burning. The surface of the skin is shiny as if oil had been applied to it, this accomplished by the thirst and the sweet taste in the mouth. The different varieties of this disease are distinguished from each other by the colour of the urine”.

Although DM had always been present, it was Aretaeus who is credited for naming this medical illness. He made first complete clinical description of diabetes, describing it as “a melting down of flesh and limbs into urine” (Neils M et al., 2008). The term ‘Diabetes Mellitus’, is a Greek word meaning ‘Siphon Sweet’ (Dia-cross, biano-go, mellitus-sweet i.e., sweet is being siphoned). It is a wasting disease, because energy giving glucose is being filtered across the body as sweet urine.

In 1674, Thomas Willis, a physician, anatomist and a professor of natural philosophy at Oxford, discovered (by tasting) that the urine of diabetic patient was sweet. This was actually a rediscovery, for unbeknownst to him, an ancient Hindu document by Sushruta in
India in about 400 BC had described the diabetic syndrome as characterized by a “honeyed urine” (Madhu meha). Dabson in 1755 demonstrated the presence of sugar in urine. Matthew Dobson of Manchester of England demonstrated in 1776 that diabetics actually excrete sugar in urine. In 1778 Cawley reported (without particular comment) that he observed a shrivelled pancreas with stones in a diabetic patient at autopsy. This may have been the first published reference to the pancreas in relation to human diabetes. John Rollo noted that the amount of sugar excreted depends primarily on type of food ingested. Foods containing grains and fruits increased glycosuria whereas meat (protein and fat) resulted in comparatively lower excretion of sugar. In 1815, Chevreuil showed that blood sugar behaved as if it is grape sugar (i.e., dextrose/glucose). Later, specific methods of analysis were devised and used to measure glucose as the major “reducing substance” in the plasma and urine. Thus Rollo’s predictions were confirmed that in diabetes a rise in blood sugar level causes the excretion of sugar and that the ‘seat’ of diabetes was outside of the kidneys.

Bouchardat in 1850 proved that sugar in diabetic urine was in fact glucose. The part of pancreas not involved in digestion was identified by Langerhans in 1869 and was named after him. In 1888 Cawley diagnosed diabetes for the first time by demonstrating the presence of sugar in urine. He observed that the disease may result from injury to pancreas as had already been observed in experimental animals in 1682 by Brunner. In 1889 Von Merin and Minkowiski accidentally discovered that pancreatectomised dogs became diabetic in addition to developing digestive disorders. In 1891, Minkowski demonstrated clearly that the pancreas was a gland of internal secretion and that a small portion of the gland, when implanted under the skin of freshly depancratised dog, prevented the appearance of hyperglycaemia until the implanted tissue was removed or had degenerated spontaneously. In 1909, de Meyer identified that substance from the islets of Langerhans that prevented diabetes and named it
‘Insulin’ from the Latin word insulae or islands. It was only in 1921 that Banting and Best were able to purify Insulin from pancreas and showed that it lowered the blood glucose levels in diabetic dogs.

Sanger worked out the complete amino acid sequence of Insulin and was awarded the Nobel Prize in 1960. Dorothy Hodgkin, another Nobel laureate worked on the crystal structure of Insulin.

The following chart gives chronological order in the understanding of the disease - diabetes mellitus (Leo PK et al., 1994).

**Table – 1 Important milestones in diabetes mellitus**

<table>
<thead>
<tr>
<th>Date</th>
<th>Source</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>15th Century BC</td>
<td>Ebers papyrus (Egypt)</td>
<td>Clinical Description of Polyuric condition resembling diabetes.</td>
</tr>
<tr>
<td></td>
<td>Areteaus (Cappadocia)</td>
<td></td>
</tr>
<tr>
<td>5th Century</td>
<td>Sushrutha and Charaka (India)</td>
<td>Clinical description including sugary urine complication including gangrene and obese thin patient distinguished.</td>
</tr>
<tr>
<td>10th Century</td>
<td>Avicenna (Arabic)</td>
<td>Clinical description including sugary urine complication including gangrene and impotence.</td>
</tr>
<tr>
<td>19th Century</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1810-20</td>
<td>William pront (England)</td>
<td>Diabetic coma described.</td>
</tr>
<tr>
<td>1850</td>
<td>Bouchardat</td>
<td>Presence of sugar in diabetic persons is Glucose.</td>
</tr>
<tr>
<td>1869</td>
<td>Paul Langerhans (Germany)</td>
<td>Pancreatic Islands identified.</td>
</tr>
<tr>
<td>1888</td>
<td>Cawley</td>
<td>Presence of sugar in the urine due to injury to pancreas.</td>
</tr>
<tr>
<td>20th Century</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1909</td>
<td>Demeter</td>
<td>Identified the substance from pancreas and called</td>
</tr>
</tbody>
</table>
Review of literature

<table>
<thead>
<tr>
<th>Year</th>
<th>Event/Inventor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1922</td>
<td>Fredrick G. Banting, Charles H. Best, James B. Collip</td>
<td>Isolation and first clinical use of Insulin.</td>
</tr>
<tr>
<td>1955</td>
<td>Frank and Fuchs</td>
<td>Hyperglycemic drugs (sulphonyl - urea introduced).</td>
</tr>
<tr>
<td>1957</td>
<td>Unger</td>
<td>Hypoglycemic drug Phenformin is introduced.</td>
</tr>
<tr>
<td>1971</td>
<td>Pierre Freychet (USA)</td>
<td>Identified Insulin receptors.</td>
</tr>
<tr>
<td>1993</td>
<td>Diabetes Control and complications trial (USA)</td>
<td>Strict glycemic control reduces the risk of diabetic microvascular complications in IDDM.</td>
</tr>
</tbody>
</table>

2.2. Classification of Diabetes mellitus:

National diabetes data group (NDDG) in 1974 proposed a systemic classification of diabetes mellitus. This was adopted by WHO in 1980 and later modified in 1985, further lot of research has taken place in the field and information has accumulated, this necessitated a revision of WHO classification.

International expert committee, working under the sponsorship of the American Diabetes Association was established in May 1995 to review the classification and diagnosis of diabetes based on etiology (American Diabetes Association, 2010).

According to American Diabetic Association (American Diabetes Association, 2010), DM is classified into four clinical classes.

- Type I Diabetes – Results from β-cell destruction, usually leading to absolute insulin deficiency.
• Type II Diabetes – Results from a progressive insulin secretory defect on the background of insulin resistance.

• Other specific types of Diabetes – Due to other causes like genetic defects in β-cell function, genetic defects in insulin action, diseases of the exocrine pancreas (such as cystic fibrosis) and drug or chemical induced diabetes (such as treatment of AIDS and or after organ transplantation).

• Gestational Diabetes – Diabetes diagnosed during pregnancy.

2.2.1. Type I diabetes (absolute insulin deficiency):

• Immune mediated – it accounts for only around 10% of those with diabetes. Previously known by the term insulin-dependent diabetes or juvenile – onset diabetes, results from cell–mediated autoimmune destruction of the pancreatic β-cells, here auto antibodies to islet cells can be detected that about 85-90% among individuals with fasting hyperglycemia.

• Idiopathic – Only a minority of patients with type I diabetes fall into this category mostly of African or Asian origin. It is an inherited autoimmune β-cell destruction with no immunological evidence.

2.2.2. Type II diabetes (insulin resistance, relative insulin deficiency):

It accounts for ~90-95% of those with diabetes, previously referred to as non-insulin-dependent diabetes or adult – onset diabetes. It is a metabolic disorder that is characterized by insulin resistance, relative insulin deficiency and hyperglycemia. In Type II diabetes, pancreas usually produces enough insulin. However, the body does not use it effectively. The condition known as “Insulin resistance” occurs when the cells do not respond to insulin’s attempt to enter with glucose. The pancreas responds by producing more and more insulin. When the cells do not respond, high levels of glucose build up in the blood, leading to type II
diabetes. In type II DM, cells are always present regardless of the duration and severity of the disease, but lack any signs of functional activity. It was primarily seen among adults over age of 40 and now increasingly seen in children and adolescent. Obesity is found in approximately 55% of patients diagnosed with type II DM. People with type II DM often need to take prescription drugs to lower blood sugar levels along with dietary and lifestyle changes to control the problem.

2.2.3. Other specific types of diabetes:

i. **Genetic defects of beta cell function** – This form of diabetes can be seen at early age (before 25 years) which is also called as maturity-onset diabetes of the young (MODY) leading to hyperglycemia with deficiency in secretion of insulin and no defects in insulin action.

ii. **Genetic defects in insulin action** – Any mutations to the insulin receptor can lead to hyperinsulinemia and hyperglycemia which in turn can lead to metabolic abnormalities.

iii. **Diseases of the exocrine pancreas** – Any injury to the pancreas can cause diabetes, the processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma.

iv. **Endocrinopathies** – Several hormones (e.g., Growth hormone, cortisol, glucagon and epinephrine) in excess are known to antagonize insulin action and cause diabetes.

v. **Drug or chemical-induced diabetes** – Many drugs can impair insulin secretion and cause diabetes in individuals with insulin resistance.

vi. **Infections** – Certain viruses are also associated with the β-cell destruction.
vii. **Uncommon forms of immune-mediated diabetes** – Binding of anti-insulin receptor antibodies to the insulin receptor blocks the binding of insulin to its receptor in target tissues which in turn can lead to diabetes.

viii. **Other genetic syndromes associated with diabetes** – Several genetic syndromes are accompanied with the increased incidence of diabetes mainly the chromosomal abnormalities (American Diabetes Association, 2010).

### 2.2.4. Gestational diabetes mellitus (GDM):

GDM has been defined as any type of glucose intolerance diagnosed during pregnancy. In most cases, spontaneous resolving after delivery is common. GDM definition applied whether or not the condition persisted after pregnancy and did not exclude the possibility that unrecognized glucose intolerance may have begun concomitantly with the pregnancy. This definition supports the classical detection and classification of GDM. The number of pregnant women with undiagnosed type II diabetes has increased.

The following are the major changes proposed in the new classification:

* The term insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) are eliminated.

* The terms type-I and type-II diabetes retained with Arabic numerals being used rather than Roman numerals.

* The stage impaired glucose tolerance (IGT) has been retained. A new entity named impaired fasting glucose (IFG) has been introduced.

* Gestational diabetes mellitus (GDM) is retained as defined by World Health Organization (WHO) and National Diabetes Data Group (NDDG). But more selective rather than universal screening during pregnancy is now recommended.
2.3. Diagnostic criteria for diabetes mellitus:

The National Diabetes Data Group and World Health Organization have issued diagnostic criteria for diabetes mellitus as follows-

1. Symptoms of diabetes plus casual random plasma glucose concentration $\geq 200$ mg/dl (11.1 mmol/L). Casual refers to the plasma glucose levels at any time in a day. The classical symptoms of diabetes include polyuria, polydipsia and unexplained weight loss.

2. Fasting plasma glucose $\geq 126$ mg/dl (7.0 mmol/L). Fasting is defined as no calorie intake for at least 8 hours.

3. Two hour plasma glucose $\geq 200$ mg/dl (11.1 mmol/L) during an oral glucose tolerance test. The test is done by giving 75g of anhydrous glucose in water as per WHO.

4. In a patient with classic symptoms of hyperglycemia, a random plasma glucose $\geq 200$ mg/dl (11.1 mmol/L) (American Diabetes Association, 2010).

2.4. Diagnosis and monitoring of diabetes:

A large number of diabetics are as yet unidentified despite the global awareness about diabetes. Most of the diabetics are detected on the strength of their postprandial glycosuria. Some of the methods are used for detection and diagnosis of hyperglycemia is as follows -

1. **Urine analysis:** It is one among the diagnostic tests firstly performed in patients with suspected diabetes. Glycosuria is the first symptom associated with diabetes and which provides the first clue to the development of the disease. However, urinary glucose may also be found in non-diabetic subjects. Absence of glycosuria, on the other hand cannot be taken to indicate absence of diabetes.
The finding of ketonuria in the presence of high urinary glucose or ketonuria in the absence of glucose excretion and concomitant low blood glucose also indicates diabetes. Detection of proteinuria or microhematuria is suggestive of diabetic nephropathy.

2. **Blood analysis:** Fasting and post-prandial blood glucose levels are the second set of diagnostic tests for hyperglycemia. Where these tests are not conclusive, a glucose tolerance tests is also performed which clearly indicates the borderline range and pathological range. Other tests include the detection of insulin and insulin-antibodies in the diabetic’s blood. In type II diabetes mellitus however, the insulin level would be normal or slightly elevated. Currently, C-peptide analysis is also carried out to find out whether insulin is being synthesized and released or not.

3. **Glycosylated haemoglobin:** HbA\(_{1C}\) forms normally 4 – 6 % of the total haemoglobin. In diabetes, this value increases to above 8%. This glycosylated haemoglobin provides an index to the average glucose level in diabetes.

2.5. **Metabolic complications of diabetes:**

Conventional treatment of diabetes with diet, insulin and oral hypoglycemic drugs have been highly successful in controlling hyperglycemia, thereby prolonging life, unfortunately it has been not able to prevent other complications associated with diabetes. These complications include acute and chronic complications described below:-

2.5.1. **Acute complications include:**

1. **Diabetic Ketoacidosis:**

Diabetic Ketoacidosis (DKA) was formerly considered as the hall mark of type I diabetes mellitus. Insulin deficiency leads to breakdown of triacylglycerols in the
adipose tissue thus elevating the free fatty acid (FFA) content of the plasma. Increased glycogen or increased glucagon/insulin ratio (caused by a decrease in insulin) stimulates the degradation of FFA via β-oxidation. As are results of these increased ketone bodies leading to ketoacidosis and related complication of diabetes (Kasper DL et al., 2005).

2. Hyperglycemic hyperosmolar state (HHS):

This state is in elderly individuals with type 2 DM, with a week history of polyuria, weight loss and diminished oral intake that leads to mental confusion, lethargy or coma. The physical examination reflects profound dehydration and hyperosmolality along with hypotension, tachycardia and altered mental status. This may also lead to serious illness such as myocardial infarction or stroke (Kasper DL et al., 2005).

2.5.2. Chronic Complications include:

1. Heart diseases:

Heart diseases occur in diabetics and it is one of the major causes of mortality among them. The co-existence of hypertension and diabetes contributes to the development of heart diseases.

2. Eye diseases (Ophthalmologic complications):

Diabetes mellitus is the leading cause of blindness between the ages of 20 and 74 in the United States. The data from U.S. study has revealed that 10% of new blindness at all ages and 20% of new blindness between the ages 45 and 74 are because of diabetic retinopathy. These incidences may be even higher in India.

Diabetic retinopathy is classified into non-proliferative and proliferative types. The primary effect of diabetes on the retina for the development of diabetic retinopathy appears to be on its capillaries. The exact mechanisms leading to damage are still largely unknown. But there may be alterations in retinal blood flow and breakdown in the blood – retinal barrier
Review of literature

resulting in abnormal leakage from retinal blood vessels appear to be the major cause of events (Ismail GM., 2014).

3. Kidney diseases (Diabetic Nephropathy):

Diabetic nephropathy is also one of major complication associated with diabetes. During this condition, number of functional abnormalities is present in the kidney in early diabetic. At this stage there will be 20 – 30% increase in the glomerular filtration rate without a proportional rise in renal plasma flow. In long term diabetics the most striking alteration are found in the glomeruli and the blood vessels (Allah RSA et al., 2007).

4. Diseases of the nervous system (Diabetic Neuropathy):

It is also one of the chronic complications associated with diabetes. Till the middle of the 19th century, diabetes was believed to be a disorder of the central nervous systems. Involvement of the peripheral nervous system by diabetics is referred to as diabetic neuropathy and the metabolic aspects of diabetes remains unknown (Jennifer AT., 2008).

In addition to these are a wide variety of other disorders associated with diabetes. However at the present time, a direct metabolic connection is not known in almost all of these disorders.

2.6. Insulin:

2.6.1. Chemistry of Insulin:

Insulin is solely synthesised in beta cells of islets of langerhans situated in pancreas with 200 units or 8 mg in adult human and adult rat with 10 micro grams of insulin. It is a polypeptide consisting of two chains, A and B, linked by two interchain disulfide bridges that connect A7 cysteine to B7 cysteine and A20 to B19. A third intrachain disulfide bridge connects residues 6 cysteine and 11 cysteine of the A chain. The location of these three disulfide bridges is invariant, and the A and B chains have 21 and 30 amino acids,
respectively, in most species/ the covalent structure of human insulin (molecular mass 5.734 KDa) and a comparison of the amino acid substitutions found in a variety of species. Substitutions occur at many positions within either chain without affecting bioactivity and are particularly common at positions 8, 9 and 10 of the A chain. Thus, this region is not crucial for bioactivity (Murray k et al., 2000).

**Figure – 1** Structure of human proinsulin.

Insulin and C – peptide molecules are connected at two sites by dipeptide links. An initial cleavage by a trypsin – like enzyme (open arrows) followed by several cleavages by a carboxypeptidase – like enzyme (solid arrows) results in the production of the heterodimeric (AB) insulin molecule (light blue) and the C – peptide.

Several positions and regions are highly conserved, including

1. The positions of the three disulfide bonds.
2. The hydrophobic residues in the carboxyl terminal region of the B chain, and
3. The amino and carboxyl terminal regions of the A chain.
Insulin is synthesised as a preprohormone of molecular weight ~15,000 which is later converted to proinsulin of molecular weight ~9000. The conversion of proinsulin involves cleavage of peptide bonds at specific sites that result in the formation of equimolar amounts of insulin and C – peptide occurs in the immature clathrin coated secretory granules (Murray k et al., 2000).

2.6.2. Regulation of Insulin secretion:

The human pancreas secretes 40-50 units of insulin daily, which represents 15-20% of the human stored insulin in the gland. In the fasting state, insulin concentration is 5-10 U/ml maintained by secretion of about 0.25-1.5 Units of insulin per hour into the portal vein. Insulin secretion is an energy requiring process that involves the microtubule – microfilament system in the β cells of the islets. The main character of beta cell is to function as ‘fuel sensor’ capable of adapting the rate of insulin secretion to the variations in plasma glucose levels and other energetic substrates (amino acids, ketone bodies, fatty acids). The threshold concentration for secretion is the fasting plasma glucose levels (80-100 mg/dl), and the maximal response is obtained at glucose levels between 300-500 mg/dl. It is accepted that an increase of the ATP/ADP ratio results in the inhibition of ATP – sensitive K+ efflux channels. This causes depolarization of the β-cells and activation of voltage sensitive Ca^{2+} channels. The Ca^{2+} influx results in insulin secretion (Murray k et al., 2000).

2.6.3. Metabolism of Insulin:

Insulin circulates in blood as free monomeric hormone. Insulin half life is about 5-6 min in humans. Pro-insulin half life is 17 min, C-peptide half life is 30 min, major organs involved in insulin metabolism are liver, kidneys and muscle (Pedro I et al., 2008). About 50% of the insulin that reaches the liver via portal vein never reaches the general circulation.
Proteolytic degradation of insulin in the liver occurs both at the cell surface and after receptor mediated internalization. Insulin with insulin receptors are internalized called endosomes the site of initiation of degradation. Some insulin is also delivered to lysosomes or lysosome related vesicle near the golgi for degradation. Mainly two enzyme systems are responsible for the metabolism of insulin. The first is Insulinase an endopeptidase, which acts at several sites preferentially A_{13} – A_{14} and B_{9} – B_{10}. Insulinase is inhibited by sulphydryl inhibitors and chelators such as EDTA and phenantrolene. A second insulin degrading enzyme is hepatic glutathione – insulin transhydrogenase (Arun V et al., 2014). This enzyme reduces the disulfide bonds and the individual A and B chains are rapidly degraded. In addition to insulin degradation, the signal created by insulin at the cellular level is reversed by phosphotyrosine phosphatase.

2.6.4. Insulin Receptor:

Insulin action begins after the binding of the hormones to a specific glycoprotein receptor on target cells. The insulin receptor is a heterodimer consisting of two subunits α and β in the configuration of α_{2} β_{2} linked by disulfide bonds. Both subunits are glycosylated.

The α subunit (135 KDa) is entirely extracellular, binds insulin via cysteine rich domain. The β subunit (95 KDa) is a transmembrane protein and a cytosolic domain. The insulin binding domain of the mature receptor is primarily in the α subunit. Proteolysis of β subunit does not influence insulin to α subunit appreciably (Murray k et al., 2000).
The α subunit contains 26 cysteine residues between residues 155 and 312. This cysteine rich region may be involved in forming disulphide bonds with the β subunit as well as with another α subunit. This region may also be important in insulin binding. The insulin receptor is constantly synthesized and degraded and its half-life is 7-12 hours. The precursor of the human insulin receptor has 1382 amino acids with a molecular weight 1,90,000. And the gene for insulin receptor is located on chromosome 19 (Murray k et al., 2000).

2.6.5. Mechanism of action of Insulin:

The first event that takes after the binding of insulin to its receptor is auto-phosphorylation of tyrosine residue of the β subunit. Insulin is known to share its receptor with other growth hormone receptors by the intrinsic tyrosine kinase activity (Antti V et al., 1999).
Insulin is known to regulate enzymes controlling intermediary metabolism within minutes of its binding to the receptor. Insulin is capable of altering the concentration of some critical proteins and thereby exerting profound effects on various cellular processes.

It affects mRNA translation and in general protein synthesis. First it affects the rate of protein synthesis in selected tissues like liver, adipose tissue, skeletal and cardiac muscle at the level of mRNA translation. Secondly, it has positive and negative effects on the expression of specific genes.

**Table – 2 Insulin regulated enzymes/proteins (O’brien RM et al., 1991)**

<table>
<thead>
<tr>
<th>Enzymes/Protein</th>
<th>Effect</th>
<th>Enzymes/Protein</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intracellular Enzymes</strong></td>
<td></td>
<td><strong>Intracellular Enzymes</strong></td>
<td></td>
</tr>
<tr>
<td>Pyruvate Kinase</td>
<td>↑</td>
<td>ATP Citrate Lyase</td>
<td>↑</td>
</tr>
<tr>
<td>Serine Dehydratase</td>
<td>↓</td>
<td>Ornithine decarboxylase</td>
<td>↑</td>
</tr>
<tr>
<td>Fatty Acid Synthase</td>
<td>↑</td>
<td>Aspartate aminotransferase</td>
<td>↓</td>
</tr>
<tr>
<td>Glutamine Synthase</td>
<td>↑/↓</td>
<td>Fructose 1.6 bisphosphatase</td>
<td>↓</td>
</tr>
<tr>
<td>Try amino transferase</td>
<td>↑/↓</td>
<td>Carbamoyl phosphate synthetase</td>
<td>↓</td>
</tr>
<tr>
<td>Protein disulphide isomers</td>
<td>↓</td>
<td>Glucose 6 phosphate Dehydrogenase</td>
<td>↑</td>
</tr>
<tr>
<td>PEP Carboxykinase</td>
<td>↓</td>
<td>Glyceraldehyde 3 P Dehydrogenase</td>
<td>↑</td>
</tr>
<tr>
<td>Glycerol 3 Phosphodehydrogenase</td>
<td>↑</td>
<td>Aldolase B</td>
<td>↑</td>
</tr>
<tr>
<td>PFK/Fructose 2,6 bisphosphatase</td>
<td>↑</td>
<td>Malic Enzyme</td>
<td>↑</td>
</tr>
<tr>
<td><strong>Integral Membrane Proteins</strong></td>
<td></td>
<td><strong>Secreted Proteins/Hormones</strong></td>
<td></td>
</tr>
<tr>
<td>Insulin Receptor</td>
<td>↑/↓</td>
<td>Prolactin</td>
<td>↑</td>
</tr>
</tbody>
</table>
2.6.6. Physiological action of Insulin:

Insulin is the primary regulator of the blood glucose level. It also plays an important role in regulating fat and protein metabolism. Its effects on muscle and liver tissues primarily concerned with the regulation of glucose metabolism.

2.6.6.1. Effects of insulin on carbohydrate metabolism:

Insulin secreted by the β-cells of islets of langerhans of pancreas directly into the hepatic portal blood. In response of hypoglycaemia, hyperinsulinemia and in certain circumstances to increased catecholamines, plasma insulin levels fall. Basal amount of insulin present after an overnight fast has an inhibitory effect on glucose production. As the insulin concentration doubles it inhibits the glucose production by 80% in the liver. Insulin action on muscle and adipose tissue is slow. Insulin modifies glucose uptake by the α-cell and thus can decrease glucagon secretion. Basal amount of insulin exerts a restraining effect on gluconeogenesis. Insulin decreases the free fatty acid uptake by the liver, which increases the oxidation rate of glucose within the liver. Basal amount of insulin inhibits glycogenolysis by about 60%. Though insulin is very important regulator of glucose production it acts in concert with glucagon to control the blood glucose level. Glucagon does the action quickly by stimulating hepatic glycogenolysis and gluconeogenesis. Insulin increases glucose production.
utilisation by transport across cell membrane and converting the glucose to glycogen and also oxidation (Sylvain JLM et al., 2010).

2.6.6.2. Effects of insulin on fat metabolism:

Insulin plays a central role in the regulation of adipose tissue metabolism and in the storage, mobilization of adipose tissue triacylglycerols. The integrated physiologic effects of insulin on lipid metabolism are summarized as follows:

1. Inhibition of free fatty acid mobilization from adipose tissue.
2. Suppression of adipose tissue lipolysis.
3. Stimulation of intra adipocyte free fatty acid re-esterification (Meena A et al., 2010).
4. Inhibition of plasma free fatty acid uptake and oxidation and shunts fatty acid to triacylglycerols (McGarry JD et al., 1980).
5. Suppression of circulating ketone body concentration.
6. Reduction in supply of free fatty acid substrate in the liver for ketogenesis.
7. Inhibition of intrahepatic ketogenesis.
8. Acceleration of peripheral ketone body clearance and catabolism.
10. Increased clearance of triacylglycerols rich lipoprotein by peripheral tissues.
11. Stimulation of lipogenesis.

2.6.6.3. Effects of insulin on protein metabolism:

Insulin has an anabolic effect on protein metabolism, it stimulates protein synthesis and retard protein degradation. Hence insulin decreases amino acid concentration in plasma. Insulin decreases proteolysis of skeletal and cardiac muscle (Pidaran M et al., 2007).
Basal insulin level in muscle decreases free cathepsin-D activity hence stops the
degradation of polyribosomes. A decrease in insulin level in muscle enhances the muscle
ribosomal activity and protein breakdown (Kettelhut IC et al., 1988). During prolonged
insulin deficiency in IDDM, amino acid oxidation increases. Insulin decreases transaminase
activity and therefore limits the conversion of leucine to its alpha ketoacid in skeletal muscle.
But in adipose tissue it promotes leucine oxidation (Hutson SM et al., 1980).

2.6.7. Insulin resistance:

The down regulation of insulin receptor is one of the common causes of insulin
resistance. This is a normal metabolic event associated with diabetes mellitus. Pathological
desensitization of peripheral target tissues to the action of insulin is the major manifestation
of NIDDM. Insulin resistance in most NIDDM patients is due to defects that lie distal to
insulin binding in the insulin action pathways (Flier JS., 1983). Although the exact
mechanism is not known, a variety of post-receptor binding abnormalities have been
identified including impaired tyrosine kinase function, reduced activity of the glucose
transport system and diminished enzyme activities involved in intracellular glucose
metabolism (Marshall S et al., 1991). There appears to be a regulatory triangle between
dietary intake of metabolic substrate, the release of insulin from the endocrine pancreas and
regulation of insulin sensitivity and responsiveness. Specifically, circulating levels of glucose
and amino acids would modulate cellular metabolism through two independent but integrated
control systems. The first system is the classical one in which glucose and amino acids act as
potent secretagogues for the release of insulin. The second control system would act in
tandem with the first by enabling insulin target tissues to continuously monitor circulating
levels of glucose and amino acids. However, despite vigorous research into abnormalities
in elements of the signalling pathway in the insulin resistance in obesity and NIDDM, the
specific intrinsic defect remains un-clear, for example, the mechanism causing insulin resistance in skeletal muscle are not the result of a simple defect of insulin action.

2.7. Glucose transporters:

Glucose in diet is transferred from the lumen of the small intestine, both the dietary and synthesised glucose to be transported to the target cells, this occurs with the help of transport proteins called glucose transporters (GLUT). The mechanism of uptake of glucose has been shown in the 1930’s, but the details about this were given in 1980’s. Glucose transport was categorized into three –

1. The Na\(^+\) dependent glucose co-transporters (SGLT).
2. The facilitative Na\(^+\) independent sugar transporters (GLUT family).
3. Hormone sensitive transporters.

Particularly GLUT has implications for the controlling the delivery of glucose to mammalian cells. Different GLUTs have different affinity which is as follows –

**The Glucose Transporter**

**Table - 3** Glucose transporters

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Class</th>
<th>Main tissue location</th>
<th>Transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT 1</td>
<td>I</td>
<td>Erythrocyte, brain, ubiquitous</td>
<td>Glucose</td>
</tr>
<tr>
<td>GLUT 2</td>
<td>I</td>
<td>Liver, pancreas, kidney, intestine</td>
<td>Glucose (low affinity), fructose</td>
</tr>
<tr>
<td>GLUT 3</td>
<td>I</td>
<td>Brain</td>
<td>Glucose (high affinity).</td>
</tr>
<tr>
<td>GLUT 4</td>
<td>I</td>
<td>Heart, muscle, white adipose tissue, brown adipose tissue, brain.</td>
<td>Glucose (high affinity).</td>
</tr>
<tr>
<td>GLUT 5</td>
<td>II</td>
<td>Intestine, testes, kidney</td>
<td>Fructose, glucose (very low)</td>
</tr>
</tbody>
</table>
2.7.1. The SGLT transport glucose (and galactose) via secondary active transport mechanism provided by Na\(^+\)-K\(^+\) ATPase pump against a concentration gradient. In small intestine and the proximal tubules, glucose is transported across the luminal cells by SGLT. SGLT 1 is limited to certain tissues such as apical membrane of small intestine absorptive cells and renal proximal tubules.

A second type called SGLT 2, is of low affinity and is expressed on the apical membrane of renal convoluted proximal tubules.

2.7.2. Facilitative glucose transporters (GLUT): Utilize the diffusion gradient of glucose across plasma membranes and have different substrate specificities, kinetic properties and

---

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>affinity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT 6</td>
<td>III</td>
<td>Brain, spleen, leukocytes</td>
<td>Glucose</td>
</tr>
<tr>
<td>GLUT 7</td>
<td>II</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>GLUT 8</td>
<td>III</td>
<td>Testes, brain and other tissues</td>
<td>Glucose</td>
</tr>
<tr>
<td>GLUT 9</td>
<td>II</td>
<td>Liver, kidney</td>
<td>n.d</td>
</tr>
<tr>
<td>GLUT 10</td>
<td>III</td>
<td>Liver, pancreas</td>
<td>Glucose</td>
</tr>
<tr>
<td>GLUT 11</td>
<td>II</td>
<td>Heart, muscle</td>
<td>Glucose (low affinity)</td>
</tr>
<tr>
<td>GLUT 12</td>
<td>III</td>
<td>Heart, prostate, muscle, small intestine, white adipose tissue</td>
<td>n.d</td>
</tr>
<tr>
<td>HMIT</td>
<td>III</td>
<td>Brain</td>
<td>H(^+) -myo-inositol</td>
</tr>
<tr>
<td>SGLT 1</td>
<td></td>
<td>Small intestine</td>
<td>Glucose</td>
</tr>
<tr>
<td>SGLT 2</td>
<td></td>
<td>Kidney</td>
<td>Glucose</td>
</tr>
</tbody>
</table>

tissue expression. In facilitative glucose transporter there are thirteen members named GLUT 1 – 12 and HMIT (H⁺ Coupled myo-inositol transporter).

The facilitative sugar transporters have twelve membranes spanning regions with intracellular located amino and carboxyl-termini. These consist of conserved glycine and tryptophan residues which are essential for general facilitative transporter function. Based on dendrogram there are 3 subclasses (I-III) in GLUT family.

![Dendrogram of the glucose transporter (GLUT) family.](image)

**Figure** – 3 Dendrogram of the glucose transporter (GLUT) family. The three classes of GLUT proteins are colour – blue, class I: red, class II: green, class III and HMIT, H⁺ - coupled myo-inositol transporter.

**2.7.2.1. Class I facilitative transporters:**

This class contain GLUT 1-4, and these have been comprehensively characterized in terms of structure, function and tissue distribution.
GLUT – 1: The red cell transporter:

GLUT – 1 is expressed particularly in the brain (including blood – brain barrier) and erythrocytes. Moderate levels of expression observed in adipose tissue, muscle and the liver.

The band 4.5 glycoprotein on SDS-PAGE of erythrocyte membrane was identified as a glucose transporter (Baly DL et al., 1988). A similar glucose transporter from the rat brain expression library was found to have 98% homology with the red cell transporter in its amino acid sequence (Birnbaum MJ et al., 1986).

![Figure 4 Structure of GLUT – 1](image)

It has 12 helical membrane spanning domains, with amino and carboxy termini on the cytosolic side of the membrane, a highly charged cytoplasmic domain consisting of 65 hydrophilic amino acids between the helices M6 and M7, and a 33 amino acid long extracellular domain between M1 and M2, with one asparagine linked oligosaccharide on Asn 45 (Meuckler M et al., 1985).

GLUT – 2: The liver glucose transporter:

GLUT – 2 is expressed primarily in pancreatic β-cells, the liver and the kidney. In the β-cells, GLUT – 2 is involved in the glucose sensing mechanism. While in the liver it is expressed on the sinusoidal membrane of hepatocytes and it allows for the bidirectional transport of glucose. GLUT – 2 is also found in enterocytes and proximal renal tubules,
involved in glucose and fructose transport across the cells. It also had 55.5% homology with GLUT – 1.

**GLUT – 3: The brain glucose transporter:**

It has a high affinity for glucose, it is present where there is demand for glucose as a fuel particularly brain which disposes more than 50% of the glucose after a meal. GLUT – 3 has 64% homology with GLUT - 1 and 52% with GLUT – 2. The membrane topology was found to be similar to that of GLUT – 1. This is principally expressed in brain hence called brain glucose transporter.

**GLUT – 4: The insulin responsive glucose transporter:**

GLUT – 4 is found in the heart, skeletal muscle, brain and adipose tissue, it is responsible for the postprandial rise in plasma glucose levels. Insulin causes 20-30 fold increase in the rate of glucose transport across the plasma membrane of adipocytes. The effect of insulin on glucose transports are immediate occurring with a half time of 2-3 minutes. Various animals and human models of ‘diabesity’ exhibit reduced expression levels of GLUT 4 in adipose tissue, but not in muscle. The insulin stimulated glucose transport is due, at least in part to the translocation of an intracellular pool of glucose transporter to the plasma membrane (Suzuki K et al., 1980). Detailed studies indicated that the number of GLUT – 1 molecules on the plasma membrane increased approximately 3 fold. The rate of deoxyglucose transport increased 12 – 15 folds indicating that the translocation of GLUT – 1 alone was inadequate to explain the observed increase in the rate of glucose transport (Calderhead DM et al., 1988).

GLUT – 4 has 65%, 54% and 58% homology with GLUT – 1, 2 and 3 respectively. GLUT – 4 is stored in the intracellular storage vesicles. GLUT – 4 differs from other glucose
transporter in that about 90% of it is present in intracellular storage vesicles in the absence of insulin or other stimuli.

2.7.2.2. Class II facilitative transporter:

The class II facilitative transporter is headed by the fructose transporter GLUT – 5, GLUT – 7, GLUT – 9 and GLUT – 11.

GLUT – 5: Is expressed predominately in the small intestine, testes and kidney.

GLUT – 7: Least known member of the family, uncharacterized gene, the sites of expression are currently not known.

GLUT – 9: Expressed in the liver and the kidneys.

GLUT – 11: There are two splice variants – long and short forms consisting of 503 and 493 amino acid residues respectively. These two forms are expressed in a tissue specific manner. The short form has low affinity for glucose transport and expressed in heart or skeletal muscle. The long form of GLUT – 11 detected in liver, lung, trachea and brain, was shown to increase fructose transport (Stuart Wood I et al., 2003).

2.7.2.3 Class III facilitative transporter:

The class III facilitative transporters comprise five members – GLUT- 6, GLUT – 8, GLUT – 10, GLUT -12 and HMIT. One feature of this class is there is a characteristic glycosylation site on loop 9. The functionally important glycosylation site is found on loop 1 in other two classes. A second feature is the presence of targeting motifs.


GLUT – 8: Is present in the testis, brain, adipose tissue and skeletal muscle.

GLUT – 10: Is reported it’s expressed in the insulin – sensitive tissues of skeletal muscle, heart, liver and pancreas.
GLUT – 12: Expressed in heart, small intestine, prostate, skeletal muscle, adipocytes and insulin – sensitive tissues.

HMIT: \( \text{H}^+ \) - coupled myo-inositol transporter, expressed predominantly in the brain (Stuart Wood I \textit{et al.}, 2003).

2.7.3. Glucose transporter and diabetes:

In IDDM, auto immune mediated destruction of beta cells occurs gradually over a variable period of time with an average of about 3 years of duration. However clinical manifestation of the abnormality is generally not observed until about 80% of the beta cells have been destroyed. Immunoglobulin from patients with new onset of IDDM interfered with the high \( K_m \) glucose transport of rat islet cells and with glucose stimulated insulin secretion (Bell G., 1991), suggesting an involvement of GLUT – 2 or a factor that influences the function of GLUT – 2.

Unlike the IDDM where auto-immune mechanisms were involved, in NIDDM the beta cell number is not affected (Leighton B \textit{et al.}, 1990). However, there appears to be a defect in the high \( K_m \) glucose transporter. In the rat model of NIDDM, using glucose intolerant Zucker fatty rats (Clark JB \textit{et al.}, 1983), GLUT – 2 was normal in prediabetic Zucker rats, but became virtually undetectable in animals with late severe diabetes (Johnson JH \textit{et al.}, 1990). However, even where GLUT – 2 was not completely absent, it was able to severely affect glucose stimulated insulin secretion suggesting the role for another glucose specific point in the glucose response pathway. Glucokinase was found to be the ideal partner for the GLUT – 2 dependent glucose response pathways (Iynedjian PB \textit{et al.}, 1989). Insulin independent glucose transport regulates insulin sensitivity. Insulin resistance is dependent on whether glucose is entering through GLUT – 2 or GLUT – 4 (Ebeling-pertti \textit{et al.}, 1998).
To generate signals for insulin secretion stimulatory concentrations of D – glucose (>7.5mM) is to be metabolised by aerobic glycolysis (Holz GG et al., 1992), where glucose is converted to glucose-6-phosphate, this is referred to as the glucose sensing mechanism.

Although the nature of the signals that mediate insulin release remain controversial, alterations in the cellular phosphate potential, cytosolic redox state, generation of phospholipid metabolites and others can induce insulin secretion. In fact all these may be required for a normal response (Holz GG et al., 1992). The latest signal identified was L – Arginine derived nitric oxide (Schmidt HH et al., 1992).

2.8. Lipids:

Lipids, a heterogeneous group of compounds act as energy rich fuels of our diet and a major stored fuel of our body also helps the internal organs of our body by acting as a coating substance around the organs.

The tissue and plasma lipids in humans, generally comprises of triacylglycerols, phospholipids, cholesterol, cholesteryl esters and free fatty acids, the important physiological functions of these lipids are as follows –

- Triacylglycerols are a major energy store of the body.
- Phospholipids form the structural constituent of cell membrane (Subhankar Chowdhury, 2002).
- Cholesterol a structural constituent of cell membranes, precursor of steroid hormones and bile acids.

Fats that are absorbed from the diet (exogenous source) and the lipids synthesized from the liver (endogenous source), adipose tissues for utilization and also storage. These lipids being water insoluble carried in plasma as lipoproteins. Lipoproteins are complexes of macromolecular with hydrophobic lipids like cholesterol esters and triacylglycerols in plasma.
with a central core of non-polar lipids – cholesteryl esters and triacylglycerols with a surface layer of polar lipids – phospholipids, apolipoprotein (Apo) and free cholesterol.

Based on density plasma lipoproteins are of four classes, with different compositional and functional properties (Refer table – 4)

1. Chylomicrons – (Lowest in density, largest in size, contain most percent of lipid, less percent of protein) derived from intestinal absorption of triacylglycerols.
2. Very low density lipoproteins (VLDL or pre β – lipoproteins) – derived from the liver for triacylglycerols export.
3. Low density lipoproteins (LDL or β – lipoproteins) – represent the final stage in the VLDL catabolism.
4. High density lipoproteins (HDL or α – lipoproteins) – involved in chylomicron and VLDL metabolism and in transport of cholesterol (Murray k et al., 2000).

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Lipoprotein</th>
<th>Triglyceride</th>
<th>Protein</th>
<th>Phospholipids</th>
<th>Cholesterol and Cholestryl esters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chylomicron</td>
<td>90%</td>
<td>2%</td>
<td>3%</td>
<td>5%</td>
</tr>
<tr>
<td>2</td>
<td>VLDL</td>
<td>60%</td>
<td>5%</td>
<td>15%</td>
<td>20%</td>
</tr>
<tr>
<td>3</td>
<td>LDL</td>
<td>8%</td>
<td>20%</td>
<td>22%</td>
<td>50%</td>
</tr>
<tr>
<td>4</td>
<td>HDL</td>
<td>5%</td>
<td>40%</td>
<td>30%</td>
<td>25%</td>
</tr>
</tbody>
</table>

Apolipoprotein associated with lipoprotein particle have a number of diverse functions

- Serving as structural components of the particles.
- Providing recognition sites for cell surface receptors and
- Serving as activators or coenzymes for enzymes involved in lipoprotein metabolism.
These apolipoproteins are divided on the basis of structure and function into classes A to H, with most classes having sub classes. (refer table – 5) (Murray k et al., 2000).

**Table – 5 Apolipoprotein of lipoproteins**

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>Lipoprotein</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo A-I</td>
<td>HDL, Chylomicrons</td>
<td>Activates LCAT</td>
</tr>
<tr>
<td>Apo A-II</td>
<td>HDL, Chylomicrons</td>
<td>Inhibits LCAT</td>
</tr>
<tr>
<td>Apo A-IV</td>
<td>Secreted with chylomicrons but transfers to HDL</td>
<td>Involved in the formation of triacylglycerols rich lipoprotein, synthesized by intestine.</td>
</tr>
<tr>
<td>Apo B-100</td>
<td>LDL, VLDL, IDL</td>
<td>VLDL secretion from liver. Ligand for LDL receptor.</td>
</tr>
<tr>
<td>Apo B-48</td>
<td>Chylomicrons, Chylomicron ruminants.</td>
<td>Chylomicron secretion from intestine.</td>
</tr>
<tr>
<td>Apo C-I</td>
<td>VLDL, HDL, Chylomicrons</td>
<td>Activator of LCAT</td>
</tr>
<tr>
<td>Apo C-II</td>
<td>VLDL, HDL, Chylomicrons</td>
<td>Activator of lipoprotein lipase</td>
</tr>
<tr>
<td>Apo C-III</td>
<td>VLDL, HDL, Chylomicrons</td>
<td>Inhibits lipoprotein lipase also Apo C – II</td>
</tr>
<tr>
<td>Apo D</td>
<td>Sub fraction of HDL</td>
<td>May act as lipid transfer protein</td>
</tr>
<tr>
<td>Apo E</td>
<td>VLDL, IDL, HDL, Chylomicron remnants.</td>
<td>Triggers clearance of VLDL and chylomicron remnants.</td>
</tr>
</tbody>
</table>
2.8.1. Lipoprotein metabolism:

Chylomicrons are formed in the enterocytes, and they contain mainly the newly absorbed fatty acids as triacylglycerols with smaller amounts of cholesterol esters. The major protein component is apo B-48, these acquire apolipoproteins C and E from HDL. These particles are transported via the lymph into the blood, where they bind to lipoprotein lipase on the surface of capillary endothelial cells, thus leading to rapid hydrolysis of most of the triacylglycerols. Some phospholipids and the apolipoproteins A and C are transferred to HDL resulting in a residual particle called the chylomicron remnant. These remnants are cleared from the blood to the liver by several mechanisms. Thus, virtually all cholesterol absorbed from the intestine is delivered to the liver. The cholesterol in hepatocytes can enter metabolic pathways leading to formation of bile acids, can be incorporated into nascent lipoproteins or be stored within the cell.

VLDL is mainly formed in hepatocytes, and provides a pathway for the export of excess triacylglycerols from the liver cells. Triacylglycerols can be derived from hepatic de novo production, from plasma free fatty acids taken up by liver or from chylomicron remnants. The VLDL particle consists of a large amount of triacylglycerols and smaller amounts of cholesterol and phospholipids. The major protein component of the nascent VLDL is Apo B-100, and it also contains C and E apolipoproteins. In the blood, the triacylglycerols of VLDL are hydrolyzed in extra hepatic tissues by lipoprotein lipase leading to smaller, remnant particles including particles IDL (intermediate density lipoprotein). The surface components of the remnant particle, including phospholipids, free cholesterol and soluble apolipoproteins, are transported to HDL facilitated by plasma phospholipids transfer proteins (PLTP). VLDL remnants can then interact with LDL Apo B-receptors on hepatocytes via Apo E. The remnant particles, which contain several molecules of Apo E,
bind effectively to the LDL Apo B – receptors on hepatocytes via Apo E. The remnant particles, which contain several molecules of Apo E, bind effectively to the LDL Apo B-receptors and are rapidly taken up from the blood to the hepatocytes for catabolism. Particles with smaller amounts of Apo E remain longer in the blood. These are transformed to IDL and with further processing by hepatic lipase and the loss of the rest of Apo C and E they can form LDL. In most mammals, the majority of VLDL remnants are rapidly taken up by the liver, and a smaller amount is converted via IDL to LDL. In humans a much greater fraction of the remnants, perhaps even 50%, is converted to LDL.

LDL is mainly produced as an end product of the metabolism of VLDL, and it contains predominantly cholesterol esters added in small amounts of triacylglycerols, phospholipids and free cholesterol. LDL cholesterol is the main carrier of cholesterol in blood since LDL cholesterol normally accounts for about two-thirds of plasma total cholesterol. The exclusive apolipoprotein of LDL is Apo B-100, one LDL particle containing one Apo B molecule. LDL can be taken up from the circulation into hepatocytes by LDL Apo B – receptors on hepatocytes or extra hepatic cells. The binding to the receptors is mediated via recognition of Apo B-100. Due to the relatively low affinity of LDL for the hepatic LDL Apo B – receptors, as compared to the respective affinity of VLDL remnants, LDL circulates in the blood for about three days. Therefore, an appreciable fraction of blood LDL is taken up by many extra hepatic tissues via their LDL Apo B-receptors. Thus, LDL is the major particle responsible for transporting cholesterol to peripheral tissues.

Nascent HDL particles are either secreted by the liver or the intestine, or are assembled in the plasma from products of the catabolism of triglyceride rich lipoprotein (TRL). During the lipolysis of TRL in peripheral tissues, their surface components, phospholipids, cholesterol and apolipoproteins, are transferred to HDL. This is facilitated by
PLTP. These (Babu PS et al., 1997) components give rise to new HDL, or may be incorporated into pre-existing HDL particles. The major apolipoproteins of HDL are Apo A-I and Apo-II. In addition to being transferred from VLDL and chylomicrons, apolipoproteins may be secreted as free apolipoproteins, which then acquire lipids via an interaction with the cellular ATP binding cassette transporter (ABC). In both the mechanisms, discoidal, prebeta – HDL particles are formed. The plasma cholesterol – esterifying enzyme lecithin:cholesterol acyl-transferase (LCAT) circulates bound to these nascent and discoidal HDLs, and generates cholesterol esters from free cholesterol. These cholesteryl esters form the core of the spherical, now mature HDL particle. HDL cholesteryl esters may be transferred to Apo-B containing lipoproteins by cholesteryl ester transfer protein (CETP) in exchange for triacylglycerols. The triacylglycerols of HDL are hydrolyzed by hepatic lipase. The transfer of triacylglycerols and other surface components form the Apo-B containing lipoproteins and the elevation in the core cholesteryl ester amount due to the function of LCAT both increase the size of the HDL particle. Conversely the transfer of cholesteryl esters out of HDL by CETP and hydrolysis of HDL triacylglycerols and phospholipids by hepatic lipase will reduce the HDL size. Large HDL particles are often called HDL 2 and the smaller HDL particles are called HDL 3.

HDL is an important mediator of the reverse cholesterol transport, in which cholesterol from peripheral tissues is delivered to the liver, pre-beta HDL particles are specially adapted for mediating free cholesterol efflux from peripheral cells. Cholesterol is then esterified, generating larger cholesteryl ester rich-HDL particles. Next, the cholesteryl esters can be removed from the circulation to the liver with Apo-B containing lipoproteins, through selective uptake of special scavenger receptor B1 (SR_B1), or as a part of an HDL particle uptake mechanism. The action of the different enzyme affecting and remodelling the
HDL composition contributes to the conversion of the mature HDL back to the pre-beta HDL, which is then capable of re-entering the HDL metabolism circle; thus the removal of cholesterol from the extra hepatic cells and the flow of the cholesterol to the liver are maintained.

**Figure – 5 Lipoprotein metabolism**
2.8.2. Lipid disorders in diabetes:

The abnormalities associated with diabetes termed as dyslipoproteinemia or dyslipidemia. Where there may be changes in both the quality and quantity of the lipoproteins and these changes depends on the type of diabetes and the degree of glycemic control. Dyslipidemia is more frequent in type 2 diabetes and it also contributes to the high risk of coronary heart diseases (CHD) (Suzuki K et al., 1980).

**Quantitative changes:** The frequent quantitative change associated with type 2 diabetes is increased triglyceride and low HDL cholesterol concentration. Along with these there may also be elevation in the level of plasma total cholesterol in diabetes. The Multiple Risk Factor Intervention Trial has shown that the incidence of coronary mortality increases with increasing concentrations of plasma cholesterol in both those with or without diabetes.

**Qualitative changes:** Changes associated in diabetic subjects been found to have greater glycation of LDL particle which are more susceptible to oxidation, thus leads to increased oxidized LDL in diabetic subjects (Subhankar Chowdhury, 2002).

2.8.2.1. DYSLIPIDEMIA AND DIABETES MELLITUS:

**Type I or Insulin-Dependent DM (T I DM):**

The levels of lipids are generally higher in patients with type I diabetes mellitus with poor control over plasma glucose can be seen, because of the accumulation of chylomicrons and very low density lipoprotein (VLDL) which indicates the influence of elevated blood glucose level resulting in the alteration in the lipid metabolism. Abnormality in the lipoprotein composition can be evidenced by the elevated plasma triglyceride, elevated cholesteryl ester in VLDL was found in diabetic patients. But low density lipoprotein (LDL) may remain normal but the triglyceride levels in LDL or small dense LDL (sd LDL) are known to increase frequently, whereas the high density lipoprotein(HDL) cholesterol or the
ratio between cholesterol and triglyceride in HDL reduces in type I diabetes mellitus. But the compositional changes in lipoprotein can be brought back to normal by the use of hypoglycemic agents (Goldberg JI,. 2001).

**Type II or Non-Insulin-Dependent DM (T II DM):**

The elevations of triacylglycerols in type II DM with suboptimal glucose control are not evident as that in type I DM. The levels of total and LDL-cholesterol in type II DM patients are often increased or subnormal. Elevated levels of small dense LDL have frequently been detected in type II DM patients. A decreased level of HDL-cholesterol is often detected in patients with type II DM. Those are associated with elevated levels of apolipoprotein B (Apo B) and decreased levels of Apo A1. Hypoglycemic therapy alone usually does not normalize the dyslipidemia in type II diabetic patients (Goldberg JI,. 2001).

**2.8.3. Lipoprotein metabolism in diabetes:**

Diabetes mellitus may lead to many complications, one of the significant complication is dyslipidemia – alteration of lipid metabolism in uncontrolled glycemic levels. Dyslipidemia is a major risk factor for the development of macro vascular complications in type II diabetes patients. The features of dyslipidemia are the elevated levels of triacylglycerols, reduced levels of HDL cholesterol and increased levels of LDL-cholesterol (Howard BV,. 1987). These altered lipid profile can be seen only primarily with insulin resistance (Krishnaswami V,. 2010).

Many factors are involved in the development of dyslipidemia during diabetes including insulin resistance, disturbed fatty acid metabolism and hyperglycemia. The composition and amount of the lipoproteins are altered. Impaired action of insulin in adipocytes known to suppress intracellular hydrolysis of triacylglycerols with the release of free fatty acids into the circulation. This increased flux of free fatty acid into the liver leads to
the synthesis of triacylglycerols and its assembly and secretion of large VLDL, thus leads to hypertriglyceridemia.

There will be increased production of triglyceride rich – VLDL with decreased lipoprotein lipase activity and decreased catabolism of VLDL. There is an increased lipid exchange between triglyceride – rich VLDL and both HDL and LDL, possibly due to increased activity of CETP (cholesterylester transfer protein) and the excess VLDL pool (Ginsberg HN., 1987). This leads to the decrease of HDL cholesterol and the formation of triglyceride – rich HDL and LDL particles. In addition, the catabolism of HDL is increased because of the over activity of hepatic lipase. The finding has lead to the inhibition of CETP may increase the HDL – cholesterol levels (refer figure – 6).

**Figure – 6** Atherogenic dyslipidemia and changes in lipoprotein metabolism associated with type II diabetes mellitus (Krishnaswamy V., 2010).

Insulin resistance generally observed in type 2 diabetes mellitus is associated

i) With increased production of very low density lipoprotein (VLDL).
ii) With a reduction in the of intermediate – density lipoprotein (IDL) and small dense low density lipoprotein (sd LDL) catabolism.

iii) With increased production of high density lipoprotein (HDL) outweighed by increased catabolism.

One of the hallmarks of type II diabetes mellitus dyslipidemia is over production of very low density lipoproteins (Goldberg JI., 2001).

Defects in insulin action and hyperglycemia known to lead a change in plasma lipoprotein in diabetes with abnormalities in lipids. In uncontrolled diabetes hypertriglyceridemia and reduced HDL commonly occurs but these can be reversed by insulin therapy. These lipid abnormalities are more common in type 2 diabetes along with low density lipoprotein (LDL) converted to smaller low density lipoprotein.

Lipoprotein particles are also known to be modified by glycosylation in the presence of hyperglycemia (American Diabetes Association, 1993). The clearance of glycated LDL particles is prolonged and is susceptible to oxidation (Subhankar Chowdhury, 2002).

Insulin has a very important role in maintaining production of lipoprotein but the deficiency known to enhance the production of apolipoprotein B (apo B) major component of VLDL and LDL. Lipid known to regulate apo B production, apo B being a protein get degraded from translation but the interaction with lipid prevents. During diabetes lipolysis increases in adipocytes, releases more amount of free fatty acids because of poor insulinization. Thus increases the influx of free fatty acids to the liver a common abnormality associated with insulin resistant diabetes, this way cause an increase in VLDL secretion (Goldberg JI., 2001).

Insulin has a direct effect on production of apo B from liver and other proteins involved in the degradation of circulating lipoproteins and apo B. But the deficiency of
Review of literature

Insulin/resistance may lead to increased production of apo B and other proteins such as apo C III – a small apoprotein, may increase VLDL by preventing the actions of LPL and inhibits uptake of lipoprotein via LDL receptor related protein. Hepatic lipase – an enzyme synthesized by hepatocytes hydrolyzes phospholipids and triacylglycerols on HDL and remnant lipoproteins. But the deficiency of insulin known to reduce the activity of this enzyme, thus affects the clearance of remnant lipoproteins.

Lipoprotein lipase (LPL) – major enzyme responsible for conversion of lipoprotein triglyceride into free fatty acids, synthesized by adipocytes, interacts with circulating triglyceride – rich lipoproteins such as VLDL and chylomicrons. But the activity of LPL reduced in diabetes, can be stimulated by insulin therapy (John D et al., 1994). But the enzyme hormone sensitive lipase (HSSL) play a role in the release of fatty acids from adipocytes, HSSL is inhibited by insulin.

LDL usually not always increases in diabetes, there is a chance that imbalance may occur, the production of LDL depends on the hydrolysis of the VLDL by LPL. Deficiency of LPL and poor glycemic control may lead to increased LDL that can be further reduced in diabetes after treatment.

Insulin normally inhibits hormone sensitive lipase in adipose tissue, but resistance of insulin is involved in evoking the changes in lipoprotein metabolism. The anti-lipolytic effect of insulin is reduced in adipose tissue leading to increased release of fatty acids. Because of which liver is exposed to a large amount of free fatty acid load, which could induce hepatic insulin resistance (Subhankar Chowdhury, 2002) and free fatty acids acts as substrates for increased production of VLDL. As a matter of fact, abnormal VLDL production and a deranged activity of lipoprotein lipase have been linked to insulin resistance. In addition to
these small dense LDL particles have been shown to be closely related to hypertriglyceridemia in insulin resistance (Carey DG et al., 1996).

The combination of hypertriglyceridemia, and increased numbers of LDL particles are associated with increased levels of HDL cholesterol, is now-a-days called hypertriglyceridemic hyperapoB.

Cholesterol which is found in lipoprotein also plays important role, which is substrate for the synthesis of steroid hormones and bile acids. Increased synthesis of cholesterol can be seen in liver during diabetes mellitus, this is mainly because of the increased activity of the lipogenic enzyme HMG CoA reductase, a rate limiting enzyme in cholesterol biosynthesis with the increased availability of NADPH, starting with the acetyl CoA.

There are four important enzymes that play a major role in controlling lipoprotein metabolism – Lipoprotein Lipase (LPL), Hepatic triglyceride lipase (HTGL), Lecithin cholesterol acyltransferase (LCAT) and cholesterylester transfer protein (CETP) defects in any of these mentioned enzymes leads to disturbances in the plasma lipids (Subhankar Chowdhury, 2002).

Lipoprotein abnormalities associated with diabetes can be summarised as below:

- Slow chylomicron clearance from the blood after diet, takes several steps after the entry of chylomicrons from blood stream via thoracic duct, apo C II, activator of LPL is transferred from HDL.
- Deficiency of lipases, triglyceride enriched lipoproteins converted to small denser forms.
- Development of hypertriglyceridemia because of the lack of LPL activity.
- Increased VLDL production.
- Reduced HDL in diabetes occurs because of the defective lipolysis.
In poorly controlled diabetes, decrease in the LDL receptors can be seen.

Very poor glycemic control may lead to increased LDL with the lack of LDL receptors for clearance.

2.9. TREATMENT OF DYSLIPIDEMIA:

Dyslipidemia is one of the complications associated with diabetes mellitus with uncontrolled blood glucose level. It is associated with elevated levels of total cholesterol, triacylglycerols, LDL cholesterol and decreased HDL cholesterol. If the lipid levels are not maintained may lead to coronary heart diseases, so it becomes essential to lower the lipid levels during diabetes mellitus. This condition can be treated by lifestyle change, glycemic control and by lipid modifying drugs. In order to achieve decreased levels of lipids some of the drugs have been given are called as lipid modifying drugs.

2.9.1. Lipid modifying drugs:

The major classes of drugs used to modify lipids in diabetic dyslipidemia are as follows

1. Statins (HMG CoA Reductase inhibitors)
2. Fibrates (Fibric acid derivatives)
3. Nicotinic acid
4. Bile acid sequestrants
5. Miscellaneous

Of these the first three are considered the first line therapy against hypercholesterolemia, with fibrates having effective action in lowering the triacylglycerols. Among these the most commonly used groups are the statins and fibrates.

Mechanism of action:

1. Statins – These drugs are structurally similar to hydroxyl methyl glutaryl coenzyme A (HMG CoA), a precursor of cholesterol and also competitive inhibitors of the rate limiting
enzyme in cholesterol biosynthesis, namely HMG CoA reductase and are best administered in the evening. Statins are the most effective drugs against lowering the LDL and triglyceride levels and can be utilized for both primary prevention (prevention in subjects have not yet suffered from the myocardial infarction) and secondary prevention (prevention of progression of CHD in those who have already sustained a myocardial infarction). Thus by the administration of statins coronary artery diseases can be reduced by about 30%.

2. Fibrates – These resemble short chain fatty acids and increase the β-oxidation of fatty acids, with diminished triglyceride synthesis and VLDL secretion from the liver, also increase the LPL activity in muscle and adipose tissue. Fibrates are thus effective in lowering triacylglycerols by 25-60%, with the beneficial increase in HDL cholesterol concentration along with LDL cholesterol lowering action. It also reduces hepatic VLDL production and increase hepatic LDL uptake.

3. Nicotinic acid – Very effective against dyslipidemia, it inhibits hepatic triglyceride production and VLDL secretion. Thus, decreases LDL cholesterol, triglyceride and increases HDL cholesterol to a significant extent.

4. Bile acid sequestrants – Are used as adjuncts to statins to further lower the cholesterol. These sequestrants results in decreased absorption of exogenous cholesterol and increased metabolism of endogenous cholesterol into bile acids. Which leads to increased expression of LDL receptors on liver cells results in increased removal of LDL from the blood, thus a reduced concentration of LDL cholesterol in plasma occurs.

5. Miscellaneous –

Orlistat – A pancreatic lipase inhibitor, known to cause significant reduction in total cholesterol, LDL cholesterol, triacylglycerols and apolipoprotein B in obese type II diabetes.
**Acipimox** – a nicotinic acid congener, causes significant decrease in triacylglycerols, total cholesterol and apolipoprotein B, but does not have an effect in elevating HDL cholesterol.

**Fish oils** – contain eicosapentaenoic acid and docosahexaenoic acid (n-3 polyunsaturated fatty acids) known to decrease triacylglycerols.

Above stated drugs can individually help in lowering the lipid levels in a dyslipidemic condition. Sometimes there need to treat dyslipidemia with a combination of different drugs depending on the severity of the lipid profile and also to overcome the adverse effects of some drugs alone (Subhankar Chowdhury, 2002.). These are summarised in table – 6.

**Table – 6 Lipid modifying drugs**

<table>
<thead>
<tr>
<th>Type</th>
<th>Mechanism</th>
<th>Effect of lipid profile</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMG CoA reductase</td>
<td>↓ Cholesterol synthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>inhibitor (statin)</td>
<td>↑ LDL receptor</td>
<td>↓ LDL cholesterol 25-40%</td>
<td>Lovastatin 10-80 mg/dl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Simvastatin 5-80 mg/dl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Provestatin 10-40 mg/dl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Atorvastatin 5-80 mg/dl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fluvastatin 10-40 mg/dl</td>
</tr>
<tr>
<td>Bile acid sequestrant</td>
<td>↓ Reabsorption of bile acids in intestine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑↑↑ Synthesis of new bile acids and ↑↑ LDL receptor</td>
<td>↓ LDL cholesterol 20-30%</td>
<td>Cholestyramine 8-12 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ HDL cholesterol</td>
<td>BD or TD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Triglyceride</td>
<td>Colestipol 10-15 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BD or TD</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>↓ Hepatic triglyceride synthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Secretion of apoB 100 containing lipoprotein:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ LDL VLDL conversion ≥ LDL LDL LDL conversion</td>
<td>↓ LDL cholesterol 15-25%</td>
<td>50-100 mg TD; gradually increased to 1 - 2.5g TD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ VLDL cholesterol 25-35%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Lp (a) 30%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ HDL cholesterol</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Triglyceride 25-85%</td>
<td></td>
</tr>
<tr>
<td>Fibrate</td>
<td>↑ Fatty acid oxidation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Hepatic triglyceride</td>
<td>↓ Triglyceride 25-40%</td>
<td>Gemfibrozil 600mg BD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ HDL cholesterol</td>
<td>Bezafibrate 200mg TD</td>
</tr>
</tbody>
</table>
2.10. Hyperglycemia Induced Oxidative Stress and Diabetic Complications:

Diabetic complications are not just because of hyperglycemia instead it is the chronic hyperglycemic glucose toxicity mediated through oxidative stress leads to diabetic complications. Hyperglycemia causes various pathological changes in arteries, peripheral nerves and small vessels. Vascular endothelial cells become primary vulnerable targets for hyperglycemic damage as glucose continuously flows through them leading to the production of ROS inside the aortic endothelial cells. The possible biochemical mechanisms to explain diabetes induced organ/tissue damage includes -hyperglycemia induced activation of protein kinase – C (PKC) isoforms, elevated formation of glucose derived advanced glycation end products and increased glucose flux mediated through aldolase reductase pathway (Brownless M., 1995).

The raised reactive oxygen species (ROS) increases the generation of tumour necrotic factor - α (TNF – α) and aggravates oxidative stress. Increased liberation of cytokines like TNF – α and interleukins has been implicated in the pathogenesis of insulin resistance. TNF – α is putative inhibitor of tyrosine phosphorylation of insulin receptor and post receptor signalling intermediates (Hotamisligil GS et al., 1994). TNF – α is a pleiotropic cytokine involved in many metabolic responses in both normal and pathophysiological states (Bonet MB et al., 1999). It has central role in obesity, modulating energy expenditure, fat deposition and insulin resistance. TNF – α may produce insulin resistance by a decrease in autophosphorylation of insulin receptor tyrosine substrate-1 into an inhibitor of insulin.
receptor tyrosine kinase activity, decrease in circulating fatty acids, altering β – cell function and also increase in triacylglycerols and decrease in high density lipoprotein. TNF – α injection to healthy individuals reduces insulin sensitivity by inducing hyperglycemia without lowering plasma insulin levels. Adipocytes exposed to TNF – α become insulin – resistant, since insulin is not able to stimulate hexose transport. This appears to be a consequence of deregulation in expression of GLUT – 4, the insulin stimulable glucose transporter (Tiwari AK et al., 2002).

**Figure – 7 Alloxan Induced Oxidative Stress**

2.11. *Medicinal Plants and their use in diabetes mellitus:*

Over the centuries human beings are depending on plants for basic needs such as food, clothing and shelter, all obtained and manufactured from plant matrices (leaves, woods, fibers) and storage parts (fruits, tubers). Plants have also been utilized for additional purposes, namely as an arrow and dart poisons for hunting, poisons for murder, hallucinogens used for ritualistic purpose, stimulates for endurance, and hunger suppression, as well as inebriants and medicines. The plant chemicals used for these latter purposes are largely the
secondary metabolites (e.g., carbohydrates, amino acids and lipids) and are not directly involved in the growth, development or reproduction of plants. These secondary metabolites can be classified into several groups according to their chemical classes, such as alkaloids, terpenoids and phenolics. Secondary metabolites can be directly used as drug in their original form, these can also be used as drug precursors, templates for synthetic modification (Ramawat KG et al., 2008).

And the compounds derived from plants have been used as drugs, either in the original form or in semi-synthetic form which are mainly the secondary metabolites. These plant derived drugs called as plant extracts or ‘phytomedicines’ are been employed in the clinical trials for treatment of various diseases (Ramawat KG et al., 2008). Utilization of plants for medicinal purposes in India has been documented long back in ancient literature because they are essential for human survival (Manju paghal et al., 2010). Now a day’s, herbal renaissance is happening all over the globe, because these herbal products symbolize safety in contrast to the synthetics, that are regarded as unsafe to human and environment. Herbs are known for their medicinal, flavouring and aromatic qualities (PP Joy et al., 1998). Herbal traditional system of medicine has been practiced in many countries worldwide because of the beneficial effects. A World Health Organization (WHO) study shows that 80% of world population solely relies on medicinal plants for their primary health care needs (Ngugi MP et al., 2012).

There are many traditional systems of medicines in the world to treat the diseases, such as Ayurvedic, Siddha, Unani and Chinese traditional systems which are used in many areas of the world. Among these Ayurveda is the most widely practiced of the Indian traditional medicine systems, but there are also other systems such as Siddha and Unani which are also used in the Indian subcontinent (Maury Umashanker et al., 2011).
Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda is most developed and widely practiced in India. Ayurveda dating back to 1500-800 BC has been an integral part of Indian culture. The term comes from the Sanskrit root Au (life) and Veda (knowledge). Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and or reduced toxicity. The small fraction of flowering plant that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants (PP Joy et al., 1998).

There are more than 2,50,000 plant species on earth of which more than 80,000 are medicinal. India is one of the world’s 12 biodiversity centres with the presence of over 45,000 different plant species. India’s diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15,000-20,000 plants have good medicinal value. However, only 7,000-7,500 species are used for their medicinal values by traditional communities. In India, drugs of herbal origin have been used in traditional systems of medicines such as Unani and Ayurveda since ancient times. The Ayurveda system of medicine uses about 700 species, Unani 700, Siddha 600, Folk 600 and modern medicine around 30 species (PP Joy et al., 1998).

Hypoglycemic and hypolipidemic effects of plants:

Many plants and their extracts are used as therapeutic agents. Most commonly these are used extensively as hypoglycemic and hypolipidemic agent to treat diabetes induced hyperglycemia and hyperlipidemia in normal and experimental animals. Some of the commonly studied plants are Allium cepa, Aliu staivum, Momordica charantia etc., Active principles present in these are extracted and employed.
Table – 7 Plants having hypoglycemic and hypolipidemic activity (Kashikar VS et al., 2011)

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Name of Plant</th>
<th>Part used</th>
<th>Model used</th>
<th>Reported mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acacia Arabica(Lam.)</td>
<td>Seed, Powdered seed (2,3 and 4 mg/kg)</td>
<td>Normal rats, alloxan rats, rabbits</td>
<td>It helps in the release of insulin from β-cells of pancreas</td>
</tr>
<tr>
<td></td>
<td>Muhl. Ex Willd.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Common Name: Indian Gum Arabic tree [Family: Leguminosae]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Allium cepa L.</td>
<td>Ether soluble fraction of onion (0.25 mg/kg p.o)</td>
<td>STZ rats</td>
<td>Reduces blood glucose levels</td>
</tr>
<tr>
<td></td>
<td>Common name: Onion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Family: Liliaceae]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Allium sativum L.</td>
<td>Ethanol, petroleum ether and ethyl acetate extract (0.25 mg/kg)</td>
<td>Alloxan rabbits</td>
<td>Acts as antioxidant by reacting with the proteins thiol group which is responsible for hypoglycemic property</td>
</tr>
<tr>
<td></td>
<td>Common name: Garlic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Family: Alliaceae]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Aloe vera (L.)</td>
<td>Leaf pulp extracts</td>
<td>STZ rats</td>
<td>Helps in maintaining homeostasis of glucose by controlling the enzymes of</td>
</tr>
<tr>
<td></td>
<td>Burm.f. Common name: Aloe [Family: Aloaceae]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scientific Name</td>
<td>Part of Plant</td>
<td>Treatment</td>
<td>Effect on Glucose Metabolism</td>
</tr>
<tr>
<td>---</td>
<td>----------------</td>
<td>--------------</td>
<td>-----------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>5</td>
<td>Artemisia pallens wall</td>
<td>Aerial parts (100 mg/kg orally)</td>
<td>Alloxan rats</td>
<td>Increase the utilization of peripheral glucose</td>
</tr>
<tr>
<td></td>
<td>Common name: Davana [Family: Compositae]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Azadirachta indica A. Juss.</td>
<td>Hydro alcoholic plant extract, crude ethanolic extract of the plant</td>
<td>STZ rats, alloxan albino rats</td>
<td>Inhibits epinephrine action on glucose, promotes the peripheral glucose utilization</td>
</tr>
<tr>
<td></td>
<td>Common name: Neem [Family: Meliaceae]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Amaranthus esculents [Family: Amaranthaceae]</td>
<td>Whole plant, oil fraction</td>
<td>STZ rats</td>
<td>Insulin, Glucose</td>
</tr>
<tr>
<td>8</td>
<td>Biophytum sensitivum (L.)</td>
<td>Plant leaf extract</td>
<td>Alloxan rabbits</td>
<td>Helps in the insulin release</td>
</tr>
<tr>
<td></td>
<td>Common name: Life Plant [Family: Oxalidaceae]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plant Name</td>
<td>Extract Type</td>
<td>Test Animals</td>
<td>Effect</td>
</tr>
<tr>
<td>---</td>
<td>----------------------------------</td>
<td>-------------------------------</td>
<td>--------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>9</td>
<td><em>Caesalpinia bonducella</em> (L.) Roxb.</td>
<td>Aqueous and 50% ethanolic seed extracts</td>
<td>STZ rats</td>
<td>Increases the release of insulin from pancreatic cells</td>
</tr>
<tr>
<td>10</td>
<td><em>Coccinia indica</em> wight &amp; Arn.</td>
<td>Alcoholic leaf extract</td>
<td>Guinea pig, Alloxan dogs</td>
<td>Suppresses insulin, reduces enzymes of gluconeogenesis</td>
</tr>
<tr>
<td>11</td>
<td><em>Caesarea esculent</em> Roxb. Common name: Camilla Fruit [Family; Flacouriaceae]</td>
<td>Root extracts (300 mg/kg p.o)</td>
<td>STZ rats</td>
<td>Reduces blood glucose, hypoglycemic</td>
</tr>
<tr>
<td>12</td>
<td><em>Camellia sinensis</em> Kuntze Common name: Greentea [Family: Theaceae]</td>
<td>Hot water extract of green tea</td>
<td>STZ rats</td>
<td>Increase insulin activity</td>
</tr>
<tr>
<td>13</td>
<td><em>Eugenia jambolana</em> Lam. Common name: Indian black berry [Family: Myrtaceae]</td>
<td>Pulp extract of the fruits, alcoholic extract (100mg/kg)</td>
<td>STZ rats, alloxan rats</td>
<td>Releases insulin, exhibits normoglycemia, peripheral glucose utilization</td>
</tr>
<tr>
<td>No.</td>
<td>Common Name [Family]</td>
<td>Extract Type</td>
<td>Animal Model</td>
<td>Activity</td>
</tr>
<tr>
<td>-----</td>
<td>---------------------</td>
<td>--------------</td>
<td>--------------</td>
<td>----------</td>
</tr>
<tr>
<td>14</td>
<td>Egyptian Morus alba [Moraceae]</td>
<td>Alcoholic extract</td>
<td>STZ rats</td>
<td>Lipid peroxidation, insulin, glucose</td>
</tr>
<tr>
<td>15</td>
<td>Ficus bengalensis L. [Moraceae]</td>
<td>Bark extract</td>
<td>STZ rats, alloxan rats</td>
<td>Enhances insulin secretion, inhibits insulinase enzyme</td>
</tr>
<tr>
<td>16</td>
<td>Hibiscus rosa sinensis L. [Malvaceae]</td>
<td>Ethanol extract of the plant, alcoholic leaf extract</td>
<td>STZ rats</td>
<td>Insulin secretion stimulation, glucose uptake and utilization</td>
</tr>
<tr>
<td>17</td>
<td>Helicteres isora L. [Sterculiaceae]</td>
<td>Ethanolic root extract (300 mg/kg after 9 days of administration)</td>
<td>Mice</td>
<td>Acts through insulin-sensitizing activity</td>
</tr>
<tr>
<td>18</td>
<td>Mangifera indica L. [Anacardiacea]</td>
<td>Aqueous leaf extracts (1 g/kg p.o)</td>
<td>STZ rats</td>
<td>Reduces the reabsorption of glucose from intestine</td>
</tr>
<tr>
<td>19</td>
<td>Momordica charnita [Cucurbitaceae]</td>
<td>Methanolic extract, isolated compounds</td>
<td>STZ rats, STZ mice</td>
<td>Glucose</td>
</tr>
<tr>
<td>No.</td>
<td>Plant</td>
<td>Extract/ Plant Part</td>
<td>Rodent</td>
<td>Study Outcome</td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
<td>---------------------</td>
<td>--------</td>
<td>---------------</td>
</tr>
<tr>
<td>20</td>
<td>Mucuna pruriens (L.) Common name: Velvet bean [Family: Leguminosae]</td>
<td>Powdered seeds (0.5, 1, &amp; 2 g/kg), Plant extract, alcohol(200 mg/kg) extract of the plant (200 mg/kg)</td>
<td>Mice</td>
<td>Increases uptake of glucose</td>
</tr>
<tr>
<td>21</td>
<td>Murraya koenigii (L.) Spreng. Common name: Curry leaf tree [Family: Rutaceae]</td>
<td>Leaf powder</td>
<td>Normal rats</td>
<td>Decreases gluconeogenesis and glycogenolysis</td>
</tr>
<tr>
<td>22</td>
<td>Psidium guajava Linn. [Family: Myrtaceae]</td>
<td>Aqueous extract/whole plant</td>
<td>STZ rat</td>
<td>Glucose, lipids, insulin</td>
</tr>
<tr>
<td>23</td>
<td>Punica granatum L. Common name: pomegranate [Family: Punicaceae]</td>
<td>Ethanolic flower extract, plant extract (200 mg/kg for 30 days)</td>
<td>STZ rat</td>
<td>Anti-hyperglycemic</td>
</tr>
<tr>
<td>24</td>
<td>Raphanus stivus</td>
<td>Aqueous</td>
<td>STZ rat</td>
<td>Glucose, lipids,</td>
</tr>
</tbody>
</table>
The plants belonging to the genus Allium group have been extensively studied for their hypoglycemic and hypolipidemic effect. Studies into the physiological and therapeutic effects of garlic and onion, their products and essential oils have been conducted since the early part of this century and very probably even before that. But these were necessarily limited by the lack of knowledge about the nature and the interrelationship of the chemical components in

<table>
<thead>
<tr>
<th>(Brassicaceae)</th>
<th>extract/whole plant</th>
<th>insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 Salacia Oblonga Wal. [Family: Celastaceae]</td>
<td>Aqueous methanolic extract, aqueous methanolic extract</td>
<td>Zucker rat (OZR)</td>
</tr>
<tr>
<td>26 Viscum album L. [Family: Llorenthaceae]</td>
<td>Aqueous extract, ethanolic extract/whole plant</td>
<td>STZ rat</td>
</tr>
<tr>
<td>27 Sisyphus spinachristi [Family: Rhamnaceae]</td>
<td>n-butanol fraction, isolated compounds/leaves</td>
<td>STZ rat</td>
</tr>
</tbody>
</table>
the fresh tissue processed product or essential oil. Allium group of plants, specifically Allium sativum (garlic) been used for their hypoglycemic and hypolipidemic actions.

2.11.1. Chemistry of Garlic and its products:

Isolation and identification of active principles of garlic have been the long occupied interests of chemists and pharmacists. Subjecting the crushed cloves of garlic to steam distillation, Wertheim and Semmler obtained strong smelling oil with 0.1% and 0.2% yield which principally consisted of diallyl disulphide (DADS) together with smaller quantities of diallyl trisulphides and diallyl polysulphides and little of diethyl disulphide.

Cavallito et al., (1944) reported the occurrence of the active principle of garlic as Allicin. It was Stoll and Seebeck who reported for the first time the formation of Allicin by the degeneration of a precursor substance called Allin by an enzyme called Allinase. Freshly peeled garlic has the following composition:

Table – 7a Chemical Contents of Garlic

<table>
<thead>
<tr>
<th>Chemical Contents</th>
<th>(g/100g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>61.3 – 86.3</td>
</tr>
<tr>
<td>Proteins</td>
<td>2.2 – 6.20</td>
</tr>
<tr>
<td>Fat</td>
<td>0.2 – 0.3</td>
</tr>
<tr>
<td>Ash</td>
<td>0.6 – 1.5</td>
</tr>
<tr>
<td>Energy (cal)</td>
<td>30 – 140</td>
</tr>
</tbody>
</table>
### Table – 7b Bulk elements of garlic

<table>
<thead>
<tr>
<th>Bulk element</th>
<th>(mg/100g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>50 – 90</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>390 – 460</td>
</tr>
<tr>
<td>Potassium</td>
<td>100 – 120</td>
</tr>
<tr>
<td>Sodium</td>
<td>10 – 22</td>
</tr>
<tr>
<td>Magnesium</td>
<td>43 – 77</td>
</tr>
<tr>
<td>Aluminium</td>
<td>0.5 – 1</td>
</tr>
<tr>
<td>Barium</td>
<td>0.2 – 1</td>
</tr>
<tr>
<td>Iron</td>
<td>2.8 – 3.9</td>
</tr>
</tbody>
</table>

### Table – 7c Trace elements of garlic

<table>
<thead>
<tr>
<th>Trace element</th>
<th>(mg/100g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strontium</td>
<td>0.1 – 0.7</td>
</tr>
<tr>
<td>Barium</td>
<td>0.3 – 0.6</td>
</tr>
<tr>
<td>Copper</td>
<td>0.02 – 0.03</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.8 – 3.1</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.2 – 0.6</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.3 – 0.5</td>
</tr>
<tr>
<td>Sulphur</td>
<td>65</td>
</tr>
<tr>
<td>Chloride</td>
<td>43</td>
</tr>
</tbody>
</table>
Table – 7d Vitamins of garlic

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>(mg/100g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine</td>
<td>0.25</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.08</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>0.5</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>5</td>
</tr>
<tr>
<td>Retinol</td>
<td>5 µg</td>
</tr>
<tr>
<td>Other B complex</td>
<td>Traces</td>
</tr>
</tbody>
</table>

Table – 7e In volatile Sulphur compounds of garlic

| S – methyl cysteine sulfoxide | +          |
| S – propyl cysteine sulfoxide | +          |
| S – allyl cysteine sulfoxide  | +++        |

Table – 7f Volatiles sulphur compounds of Garlic

| Allyl propyl disulphide       | 6%         |
| Diallyl disulphide            | 60%        |
| Diallyl trisulphide           | 29%        |
| Diallyl tetrasulphide         | 10.5%      |

Allicin:

Alliin (S – cysteine sulphoxide) constitute about 0.4% of the raw garlic. Allicinase – an enzyme involved in the conversion of alliin, which gives characteristic odour to garlic only after crushing. It can also be called as ‘Alliin lyase’ or Alliin alkyl sulphonate lyase’
(E.C: 4.4.4.4). This enzyme catalyse the conversion of allyl cysteine sulfoxide (alliin) to allyl sulphinic acid later on spontaneously changes to give diallyl thiosulphinate (allicin) finally get converted to diallyl disulphide (DADS) on warming/heating.

Later Mazelis and Crews (1968) showed purification of garlic allinase from that of the homogenate. Allicin on heating or upon steam get converted to disulphide i.e., Diallyl disulphide by losing oxygen. It is involved in inhibiting the sulphydryl enzymes and also non sulphydryl enzymes, which are associated. Some of the enzymes inhibited by garlic and are listed in table-8.

**Table – 8** Enzymes inhibiting activity of garlic and its fractions:

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Enzymes inhibited</th>
<th>Garlic fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alcohol dehydrogenase</td>
<td>Allicin</td>
</tr>
<tr>
<td>2</td>
<td>Alkaline phosphatase</td>
<td>Allicin</td>
</tr>
</tbody>
</table>

**Figure – 8** Garlic Sulphur compound transformation
2.11.2. Biochemical functions of garlic and its extracts:

The natural disulphides and sulphonides of garlic are known to possess hypoglycemic, hypolipidemic and fibrinolytic properties (Brahmachari HD et al., 1962).

The active principle of garlic oil is Diallyl disulphide (DADS)

\[
\text{CH}_2 = \text{CH} - \text{CH}_2 - \text{S} - \text{S} - \text{CH}_2 - \text{CH} = \text{CH}_2
\]

Seigers and Pentz (1990) showed that after an oral administration of alliin (60mg/kg body weight), only little unchanged alliin could be detected in the plasma and the bioavailability amounted to 16.5% within four hours. It has been concluded by the same authors that the removal or excretion of alliin as measured by the removal of thiol compounds is 15% in bile juice and about 50% in the urine in five hours and twenty four hours respectively.

The known nutritional and pharmacological properties of garlic can be attributed to their allyl compounds and to their broad variety of derivatives formed when these vegetables
cooked or processed. The biochemical and cellular functions of DADS, a natural component of garlic oil, can be summarized as follows:

1. Garlic products are hypoglycemic and insulinogenic agents and controls the side effects of diabetes like loss of weight, glycosuria, derangements in enzymes and metabolism of lipids and proteins (Augusti KT., 1996).

2. Lowering blood and tissue cholesterol & triacylglycerols levels, through its inhibitory effects on key cholesterol and fatty acids synthesizing enzymes and on the functions of thiol containing coenzymes (Augusti KT., 1977).

3. Reducing the platelets ability to aggregate the tendency of blood to clot and arteries ability to contract (Aptiz-Castro R et al., 1986).

4. Modulating the metabolic conversions of arachidonic acid to icosanoids through which garlic may have broad impacts on the immune functions, cell divisions/ multiplication, growth of normal and cancerous tissues, inflammatory responses, neurohormonal functions and on the behaviour of platelets and arteries.

5. To be an anti-cancer agent through its inhibitions of nitrosamine formation and its modulations of the metabolism of polyene carcinogens and perhaps through its effects on glutathione regulating enzymes and the enzymes involved in DNA replication, messenger RNA and protein synthesis.

6. Attenuating the toxic effects and subsequent liver damages caused by non-biological compounds (xenobiotics such as chemical pollutants and synthetic drugs).

7. To be an intestinal worm killer and a germicide/ pesticide when germs are brought into contact with raw garlic, onion or certain garlic allyl compounds (Moore GS et al., 1977).

8. One of garlic constituent azoene when administered in combination with conventional anti-HIV drugs may be a promising approach for treatment of AIDS (Aptiz-Castro R et al., 1986).
9. Serving as anti-oxidant, garlic thio-allyl compounds or related thiols have a solubility range between water soluble vitamins C and oil soluble vitamin D and have molecular configuration and active sites different from the vitamins. Thus they are complimentary to these two anti-oxidants (Block E et al., 1988).

10. Blood pressure reduction is observed (Augusti KT., 1996). This action of garlic is due to prostaglandin like effects.

2.11.3. Garlic and its phytosulphur compounds – hypolipidemic effect:

From ancient period many plants and their extracts which are known to have hypoglycemic and hypolipidemic effects have been employed to control diabetes mellitus. There are many herbal products which are proved to have the beneficial effect in significantly lowering the blood glucose and lipid levels in diabetes mellitus. Among those plants garlic is one which is known to have a beneficial action against diabetes.

Garlic a member of the Liliaceae family, is one of the popular herbs used worldwide to treat various diseases. It is known to contain a variety of effective compounds that exhibit anticoagulant, antioxidant, antibiotic, hypercholesterolaemic and hypoglycemic as well as hypotensive activities (Kathi JK., 2000).

It is known to contain many sulphur compounds – at least 33 sulphur compounds, several enzymes, 17 amino acids and minerals such as selenium. It contains a higher concentration of sulphur compounds than any other Allium species. The sulphur compounds are responsible both for garlic’s pungent odour and many of its medicinal effects. One of the most biologically active compounds, allicin (diallyl thiosulfinate or diallyl disulfide) does not exist in garlic until it is crushed or cut; injury to the garlic bulb activates the enzyme allinase, which metabolizes alliin to allicin (Kathi JK., 2000).
Alliin metabolism

Alliin (odourless)

Alliinase, activated by heat or cutting

Allicin (garlic odour)

Diallyl trisulphide

Diallyl disulphide

Ajoenes, Vinyl dithiines

The observed hypolipidemic actions of garlic disulphide, DADS, is attributed to its

i) NADPH lowering effects (Farva D et al., 1989) as well as to its

ii) Sulphhydryl exchange reactions that it undergoes with enzymes of lipid metabolism (Gilbert HF et al., 1981).

\[
\text{DADS + NADPH} \rightarrow \text{Allyl thiol} + \text{NADP}
\]

\[
\text{Enzyme-SH + DADS} \rightarrow \text{Enzyme-S-S-Enzyme} + \text{Allyl thiol}
\]

Alteration in carbohydrate metabolism during diabetes is also accompanied by disordered fat and protein metabolism. The characteristic features of diabetic dyslipidemia are a high plasma triacylglycerol concentration, low HDL – cholesterol concentration. Faulty glucose utilization causes hyperglycemia and mobilization of fatty acids from adipose tissue occurs for energy purpose. The lipid changes associated with diabetes are attributed to increased flux of free fatty acids into the liver secondary to insulin deficiency/ resistance. This results in excess fatty acid accumulation in the liver, which is converted to triacylglycerols (Nidhi Sharma et al., 2010). It has been shown that garlic can decrease plasma lipids, especially total cholesterol and low density lipoprotein cholesterol in patients with coronary artery disease (Kishu Tripathi, 2009). Yun – Yan Yeh et al., (1994) reported that garlic reduces plasma lipids by lowering hepatic cholesterol and triacylglycerols.
synthesis by inhibiting the lipogenic enzymes. Hence exhibit hypolipidemic effect by depressing the key enzymes of the cholesterol biosynthesis, HMG CoA reductase and reduce the LDL cholesterol concentration is mainly by active compound of garlic, DADS.

It has been reported by Chun-chen et al., (2004) that the water soluble organosulphur compounds present in garlic and other Allium group plants. These are cysteine containing compounds such as N-acetyl cysteine (NAC), S-allyl cysteine, S-ethyl cysteine, S-methyl cysteine and S-propyl cysteine. Several studies have indicated that these above mentioned compounds have the inhibitory action on triacylglycerols and cholesterol biosynthesis along with the decreased activity of lipogenic enzymes in cultured rat hepatocytes. Apart from these some polyherbal ayurvedic formulation do possess antihyperglycemic, antihyperlipidemic and antioxidant effects in diabetic rats (Snehal SP et al., 2009).

It has been shown by many workers that feeding these phytochemicals for their hypolipidemic actions could result in certain untoward or harmful effects because of misuse or overuse, along with the beneficial effects. Garlic oil is reported to cause biochemical toxicity like increases in plasma transaminases, increases in urea as well as 100 mg garlic oil fed to a fasted rat could be fatal and the biopsy reports show that it is due to massive pulmonary oedema (Joseph PK et al., 1989).

The liver plays a central role in the metabolism of glucose and lipid. The liver tissue is mainly involved in the lipid metabolism through uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids and secretion of lipoproteins. A profound alteration in the structure and composition of lipid profile occurs during diabetes (Umesh CSY et al., 2004). The accumulation of triacylglycerols leads to insulin to a reduction in insulin-mediated metabolic activity. There is a decrease in the lipogenic enzyme activity in liver and overall rates of hepatic lipogenesis. It has been shown
by Umesh CSY et al., (2004), sodium – orthovanadate and *Trigonella foenum – graecum* commonly called fenugreek seeds showed decreased activity of the hepatic lipogenic enzymes during diabetes as compared to control.

During diabetes altered lipoprotein metabolism occurs with elevations in plasma and tissue lipids. Along with this some alteration in enzyme activity do occur during diabetes, one such is decreased lipoprotein lipase activity associated with increased accumulation of lipids, especially triacylglycerols (Sarah Varghese et al., 1985). It is observed that serpasil (antihypertensive or hypotensive) treatment increased the lipolytic activity of lipase enzyme, also decreased the cholesterol in plasma and tissues of alloxan diabetic rats (Sarah Varghese et al., 1985). With all this, the agent also showed the hypocholesterolaemic effect may be due to its inhibitory effect on the enzyme HMG CoA reductase – regulatory enzyme in cholesterol biosynthesis. It also showed beneficial effect in decreasing the plasma and tissue phospholipids.

In diabetic condition altered lipid metabolism can be seen, indicated by the increased mobilization of free fatty acids from fat depots which occurs in the absence of insulin leads to hypercholesterolemia and hypertriglyceridemia. The antihyperlipidemic effect of Cassia Auriculata flowers may be due to the down regulation of NADPH, a cofactor in the fat metabolism (Pari L et al., 2002).

2.12. Different models to induce experimental diabetes mellitus:

The various animal models for inducing diabetes are:

1) Chemical induced diabetes
   a) Streptozotocin
   b) Alloxan
   c) Dithizone
d) Gold thioglucose

2) Surgical model of diabetes

3) Hormone induced diabetes

4) Genetic model of diabetes
   a) Spontaneously developed diabetic rats
   b) Genetically engineered diabetic rats

2.12.1. Chemical induced diabetes:

a) Streptozotocin:

Streptozotocin is a synthetic nitrosoureido glucopyranose derivative isolated from fermentations of streptomyces achromogenes that is classically an anti-tumor, antibiotic and chemically is related to other nitrosureas used in cancer chemotherapy. Streptozotocin sterile powder are provided and prepared as a chemotherapy agent. Diabetes develops gradually and may be assessed after a few days, usually four days for mice and seven days for rats. Usually, a plasma glucose level of about 180-500 mg/dl indicates the induction of diabetes mellitus. Sometimes diabetic animals are maintained on insulin if the experiments are not to commence immediately to prevent the animal deaths (Williamson EM et al., 1996). Although streptozotocin is the most commonly used drug for induction of diabetes in rats (Balamurugan AN et al., 2003), there are some disadvantages to its use in chronic experiments, especially spontaneous recovery from high blood glucose levels by the development of functioning insulinoma and high incidence of kidney and liver tumours. These problems are due to strongly oncogenic action of streptozotocin (Camila AMO et al., 2004). Non insulin dependent diabetes mellitus (NIDDM) was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg) and nicotinamide (120 mg/kg) to rats (Pellegrino et al., 1998). Nishiyama Y et al., (2013) reported that, injection of a single dose
of streptozotocin (200 mg/kg) induced rapid and permanent hyperglycemia in mice. Female mice are less susceptible to streptozotocin at both doses.

b) Alloxan:

\[
\text{Alloxan (2,4,5,6 – tertraoxypyrimidine; 2,4,5,6 – pyrimidinetetrone; 5,6 – dioxyuracil) is an oxygenated pyrimidine derivative. It is present as alloxan hydrate in aqueous solution. The IUPAC name is 1,3 – Diazinane – 2,4,5,6 – tetrone.}
\]

It was first originally isolated in 1818 by Brugnatelli and in 1838 by Wholer and Liebig synthesized a pyrimidine derivative, which they later called alloxan.

The compound was discovered by Justus Von Liebig and Friedrich wholer following the discovery of urea in 1928 and is one of the oldest named organic compounds that exist.

The alloxan model of diabetes was first described in rabbits by Dunn, Sheehan and McLetchie in 1943. Since then, alloxan diabetes has been commonly utilized as an animal model of insulin dependent diabetes mellitus (IDDM).

Alloxan is strongly acidic, hydrophilic and unstable substance with half life of 1.5 min at neutral pH and at 37°C (Szkudelski T., 2001). It exerts its diabetogenic action within 24-48 hours of administration by selectively destroying the beta cells of islets of Langerhans in many species of experimental animals, when administered parenterally intravenously, intraperitoneally or subcutaneously. The dose of alloxan required for induction of diabetes varies with species, route of administration and nutritional status. Too small dose is not diabetogenic and too large dose may cause serious kidney damage with uremia. Larger
animals are administered with I.V route. Subcutaneous route is preferred in small animals. Alloxan is effective when given intraperitoneally in rats but the degree of liver damage tends to be higher. So cutaneous or I.V route of administration is preferred. Human islets are considerably more resistant to alloxan than those of the rat and mouse (Eizirik DL., 1994).

Diabetogenic substances are hydrophilic and non-diabetogenic substances are liphophilic (Jorns A et al., 1997). Hydrophilicity of alloxan was identified as a factor essential for diabetogenicity. However, selective uptake of alloxan by GLUT – 2 of beta cell is not a prerequisite for the diabetogenicity of alloxan (Ashok DC et al., 2007). The diabetes is a result of degeneration and resorption of the beta cells of the pancreatic islets, the alpha cells and acinar tissue being unaffected. Alloxan acts directly, promptly and specifically on the beta cells (Weaver DC et al., 1978).

**Mechanism of action:**

Alloxan acts by binding to insulin receptor causes autophosphorylation of tyrosine residue of the β subunit (Rosen OM., 1987), it has two distinct pathological effects; it selectively inhibits glucose – induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the β-cell, and it causes a state of insulin-dependent diabetes through its ability to induce ROS formation resulting in the selective necrosis of the beta cells. These two effects can be assigned to the specific chemical properties of alloxan, the common denomination being selective cellular uptake and accumulation of alloxan by the β-cells.

It is a very unstable chemical compound with a molecular shape resembling glucose. Both alloxan and glucose are hydrophilic and do not penetrate the lipid bilayer of the plasma membrane. The alloxan molecule is structurally so similar to glucose (Boquist L et al., 1983) that the GLUT-2 glucose transporter in the β-cell plasma membrane accepts this
Review of literature

glucomimetic and transports it into the cytosol. Alloxan does not inhibit the function of the transporter, and can therefore selectively enter beta cells in an unrestricted manner (Hammartrom L et al., 1967).

Selective inhibition of glucose-induced insulin secretion is the major pathophysiological effect of the thiol group reactivity of alloxan. Alloxan has a central 5-carbonyl group that reacts with thiol groups. Glucose is the most sensitive thiol enzyme in the beta cell, with a half maximal inhibitory concentration in the 1-10 µmol range. Inhibition of glucokinase reduces glucose oxidation and ATP generation, thereby suppressing the ATP signal that triggers secretion. Inhibition of glucokinase is achieved within 1 min of exposure to alloxan (Konrad RJ et al., 2002).

The inhibition of glucose-induced insulin secretion is preceded by a very transient (1-2 min) stimulation of insulin secretion immediately after exposure to alloxan. This effect can be explained by an initial reduction of ATP consumption resulting from the blockade of glucose phosphorylation by glucokinase, which produces a transient increase in ATP in the β-cell and triggers a transient release of insulin (Weaver DC et al., 1978).

The inhibition of insulin secretion after exposure to alloxan is restricted to that induced by glucose, which induce insulin secretion through interaction with glucokinase. Glucose protects against alloxan-induced inhibition of glucose-induced insulin secretion because its binding to the sugar-binding site of glucokinase prevents the oxidation of the functionally essential thiol groups. The non-metabolisable seven carbon sugar mannoheptulose protects glucokinase through the same mechanism, but this alone is not sufficient to prevent alloxan-induced inhibition of insulin secretion. The glucose analogue 3-O-methylglucose, which is not a substrate of glucokinase, does however prevent inhibition. It
does this through competitive blockade of alloxan uptake into the beta cell via the GLUT-2 glucose transporter.

Alloxan can generate reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid, chemical redox cycling reactions between alloxan and dialuric acid and protective actions of cytoprotective enzymes. In the beta cells the toxic action of alloxan is initiated by free radicals formed in this redox reaction. Autoxidation of dialuric acid generates superoxide radicals and hydrogen peroxide, and in the Fenton reaction (Sakurai K et al., 1995), in the presence of a suitable catalyst (typically iron), hydroxyl radicals. The autoxidation of dialuric acid involves the intermediate formation of the alloxan radical (Malaisse WJ 1982; Winterbourne CC et al., 1989).

The reduction of alloxan to dialuric acid in the cell requires the presence of a suitable thiol, typically the tripeptide glutathione (GSH) to generate the redox cycling partner, dialuric acid, and oxidised glutathione. The triketone structure of alloxan is vitally important for this two-step reaction with glutathione, which generates the alloxan radical as an intermediate product. Alloxan can also generate ROS by reacting with thiol groups on proteins such as enzymes and albumin. During each typical redox cycle a small amount of ‘Compound 305’, an alloxan – GSH adducts is formed. The intracellular concentration of compound 305 increases in a time – dependent manner, which gradually decreases the amount of reduced GSH available in the cell for redox cycling. Thus producing a lower pro-oxidative ration between alloxan and GSH, rather than a higher antioxidative ratio (Sakurai K et al., 1991; Winterbourne CC et al., 1989).

The antioxidative enzyme SOD has a cytoprotective effect against both alloxan and dialuric acid in the presence of GSH by virtue of its ability to scavenge between dialuric acid and alloxan. The resultant suppression of dialuric acid autooxidation prevents the generation
of further ROS, although increasing the concentrations of the toxins can reinstate the toxic
effects of both compounds (Munday R., 1988; Winterbourne CC., 1989).

Apparently, the superoxide radical is not the species responsible for the cytotoxicity
of alloxan and dialuric acid. Several lines of evidence point to hydroxyl radicals as the
principal cause. Hydroxyl radical (Heikkila RE et al., 1976) is the ultimate toxic ROS
species, and its formation is prevented by the destruction of hydrogen peroxide by catalase.
Optimum protection against the cytotoxic action of alloxan and dialuric acid is provided only
by a combination of SOD plus catalase, which completely prevents redox cycling between
alloxan and dialuric acid, and thus generation of all ROS species in this reaction pathway.
Glucose also provides complete protection against all toxic effects of alloxan both in vivo and
in vitro. This universal protection is achieved through the prevention of glucokinase
inhibition and the preservation of the antioxidative defence mechanisms of the β-cell. The
non-metabolisable glucose analogue 3-O-methyglucose also provides protection, but does
this exclusively through the prevention of alloxan uptake into the beta cell via the GLUT-2
glucose transporter. Thus, it can be concluded that the pancreatic beta cell toxicity and the
resultant diabetogenicity of alloxan are due to redox cycling and the generation of toxic ROS.

Apart from diabetogenic action alloxan is also known to cause a serious disturbance
in intracellular calcium homeostasis by which alloxan elevates cytosolic free Ca^{2+}
concentration in pancreatic β-cells (Kim HK et al., 2006; Park BH et al., 1995). And this
effect is a result of many events such as – alloxan induced calcium influx from extracellular
fluid, exaggerated calcium mobilization from intracellular stores and its limited elimination
from the cytoplasm. This calcium influx is the result of the ability of the alloxan to depolarize
the pancreatic β-cells (Takasu N et al., 1991) and depolarization of the cell membrane opens
the voltage dependent calcium channels. Alloxan is also having a stimulatory effect on
mitochondrial Ca\(^{2+}\) efflux with inhibitory action on Ca\(^{2+}\) uptake by mitochondria (Lenzen S., 1992). Lastly alloxan is also involved in the inhibition of the liver plasma membrane Ca\(^{2+}\)-ATPase (Kim HR et al., 1994; Seckin S., 1993).

\[
\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2
\]

\[
\text{GK}_a \quad \text{GK}_i
\]

\[
-\text{SH} \quad \text{HS}^- \quad \text{S}^- \quad \text{S}^-
\]

\[
\text{Alloxan} \quad \text{Dialuric acid}
\]

\[
\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2
\]

\[
\text{Fe}^{3+} \quad \text{Fe}^{2+} \quad \text{OH}^+
\]

- Augmented Ca\(^{2+}\) influx from extracellular Fluid
- Exaggerated Ca\(^{2+}\) mobilization from intracellular stores
- Limited Ca\(^{2+}\) elimination from the cytoplasm

**Figure – 9 Alloxan action**

It is seen that alloxan administration leads to sudden release of insulin from β-cells (Kliber A et al., 1996) is due the alloxan induced augmentation in cytosolic Ca\(^{2+}\) concentration and the exaggerated concentration of this ion leads to supraphysiological insulin release, reactive oxygen species causes damage to pancreatic β-cells. It has been
demonstrated that the calcium channel antagonists can suppress hyperglycemia and the onset of alloxan diabetes in rats (Katsumata K et al., 1992).

Many investigators suggested that the diabetogenic dose of alloxan decrease –SH groups with a rise in glutathione peroxidase activity in the rat liver two minutes after administration (Szudelski T et al., 1998) with the elevation in the blood insulin level. It was also observed that alloxan also intensifies basal and epinephrine – induced biolysis in isolated rat adipocytes and insulin failed to restrict this effect (Kandulska k et al., 1999).

So, to induce diabetes by alloxan, animals should be examined at time intervals to minimize the adverse effects of alloxan action. The diabetogenic dose of alloxan is narrow, a slight overdose may lead to loss of animals. The loss of animals because of alloxan is likely due to the kidney tubular cell necrotic toxicity in the case of high dose of alloxan administration.

c) Dithizone:
Zinc chelating agent such as dithizone causes diabetes in laboratory animals. Dithizone has abilities to permeate membranes and to complex zinc inside liposomes with the release of protons, that can enhance diabetogenicity (Vineeta T et al., 2014).

d) Gold thioglucose:
Gold thioglucose developed obesity, induces diabetes in normal wistar strain rats. Gold thioglucose treated mice gained weight rapidly and significantly increase non fasting plasma glucose level within 8-12 weeks (Vineeta T et al., 2014).

2.12.2. Surgical model of diabetes:

Another technique used to induce diabetes is complete removal of the pancreas (pancreatectomy). Few researchers have employed this model in the previous years to explore effects of natural products with animal species such as rats, pigs, dogs and primates (choi SB
et al., 2004). Limitation to this technique include high level of technical expertise and adequate surgical environment, major surgery and high risk of animal infection, adequate post operative analgesia and antibiotic administration, supplementation with pancreatic enzymes to prevent malabsorption and loss of pancreatic counter regulatory response to hypoglycemia. More recently, partial pancreatectomy has been employed, but large resection (more than 80% in rats) is required to obtain mild to moderate hyperglycemia. In this case, small additional resection can result in significant hypoinsulinemia (Masiello, 2006).

2.12.3. Hormone induced diabetes:

Growth hormone has long distinguished history in diabetes, with possible participation in the development of renal complications (Vineeta T et al., 2014). Repeated administration of growth hormone in cats and adult dogs induces diabetes with all symptoms of diabetes including severe ketonemia and ketonuria. More prolonged administration of growth hormone produced permanent diabetes, there was loss of pancreatic islets tissue and of beta cells and only traces of insulin could be extracted from pancreas (Vineeta T et al., 2014).

2.12.4. Genetic model of diabetes:

a) Spontaneously developed diabetic rats:

These models permit the evaluation of the effect of a natural product in an animal without the interference of the side effects induced by chemical agents like streptozotocin, alloxan reported above. Several recent publications summarized the major advances in this field (Masiello, 2006). Example is the spontaneously diabetic Goto-Kakizaki rat which is a genetic lean model of type II diabetes originating from selective breeding over many generations of glucose-intolerant non diabetic wistar rats (Chen D et al., 2005). Mutant strains, obese diabetic mice are available such as the C57B/Ksj-db/db. With this model it is
possible to test for effects of plant extracts on blood sugar, body weight, insulin production and insulin resistant.

b) Genetically engineered diabetic mice:

In this case, rodents may be produced to over (transgenic) or under (knockout) express proteins, thought to play a key part in glucose metabolism (Masiello, 2006). Although significant advances in this field have arisen in recent years, especially with the advent of transgenic mice, there have been no studies carried out involving natural products on these models.

Chemical induction appears to be the most popularly used procedure in inducing diabetes mellitus in experimental animals. The best known drug induced diabetic model is the alloxan diabetes. It is capable of inducing both type I and type II diabetes mellitus with proper dosage selection. But the most commonly used drug is streptozotocin for reasons that are not well specified. The surgical, hormonal and genetic models require highly technical skills, may be associated with a high percentage of animal death and thus are rarely use. Alloxan induced diabetes model appears to be the most reliable and easily reproducible method of inducing diabetes mellitus in experimental animals.