INTRODUCTION

Diabetes’ is the one of the leading cause of death in many countries”. There is huge increase in the number of cases with diabetes in India. There are so many proposed medications have been given in the treatment of diabetes in ancient times. Nature given plants is the best source of medicine to treat diabetes. Now-a day’s there is increase in the number of plants that are used for producing decrease in sugar levels. Research towards pharmacological screening with prior chemical tests should be emphasized. Many investigations have been undertaken to explore the possibilities of using plants available to treat human ailments such as, diabetes which is a chronic, progressive disease and requires lifelong treatment. The usage of synthetic drugs which are used concomitantly in some individuals may cause drug interactions when used to treat many types of ailments. Screening the plants which are having antidiabetic activity which are available easily to reduce the cost and to reduce the side effects which are generally seen in case of synthetic drugs.

1.1: Diabetes Mellitus

Definition: The term diabetes a clinical condition with an increased blood sugar levels” with a gradual reduction in the levels of insulin. The cells of the body are starved due to inadequate secretion of insulin so that there is increase in blood glucose concentration with insulin resistance.

1.2: HISTORICAL BACKGROUND

Diabetes mellitus has been known since ancient times. Aretaeus described the disease as a melting down of flesh and limbs into urine. Foods as such should not show any effectiveness with reduction in the levels of sugar.

During last decades of the 20th century, research has led to the recognition that diabetes mellitus is a syndrome and comprises of a heterogeneous collection of disorders and different types of diabetes mellitus have different etiologies, although their pathologic effects after onset of disease may be similar. In the mid 1930, Himsworth
named different clinical types of DM, insulin deficiency and insulin dependent. Condition of his clinical observations came with Bornstein and Lawrence’s development of a bioassay for insulin, and when radioimmunoassay for insulin became available a decade later Bornstein and Lawrence’s observations were conformed

1.3: Prevalence

The “World Health Organization recognizes three main forms” of diabetes mellitus: type- I formerly known as insulin dependent diabetes mellitus (IDDM)” type- II formerly known as non-insulin dependent diabetes (NIDDM)” and gestational diabetes (occurring during pregnancy), which have different causes and population distributions.

1.4: Epidemiology

Type-I diabetes mellitus account for up to” 10 % of all cases of diabetes and results from an autoimmune destruction of the pancreatic β-cells” the prevalence of β-cell autoimmunity appears proportional to the incidence of “type-I diabetes mellitus in various populations type-II diabetes mellitus” is heterogeneous disorder of glucose metabolism. Type-II diabetes mellitus occupy a major percentage of known cases of diabetes. Diabetes mellitus usually results from defects in insulin sensitivity and a relative defect in insulin secretion.
Table 1: Top ten countries for estimated number of adults with diabetes mellitus

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Country</th>
<th>1995</th>
<th>Country</th>
<th>2025</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>20.8</td>
<td>India</td>
<td>58.2</td>
</tr>
<tr>
<td>2</td>
<td>China</td>
<td>17.0</td>
<td>China</td>
<td>38.6</td>
</tr>
<tr>
<td>3</td>
<td>U.S.A</td>
<td>14.8</td>
<td>U.S.</td>
<td>22.5</td>
</tr>
<tr>
<td>4</td>
<td>Russian Federation</td>
<td>7.8</td>
<td>Russian Federation</td>
<td>13.4</td>
</tr>
<tr>
<td>5</td>
<td>Japan</td>
<td>5.3</td>
<td>Japan</td>
<td>11.3</td>
</tr>
<tr>
<td>6</td>
<td>Brazil</td>
<td>3.8</td>
<td>Brazil</td>
<td>11.4</td>
</tr>
<tr>
<td>7</td>
<td>Indonesia</td>
<td>3.4</td>
<td>Indonesia</td>
<td>10.6</td>
</tr>
<tr>
<td>8</td>
<td>Pakistan</td>
<td>3.4</td>
<td>Pakistan</td>
<td>10.8</td>
</tr>
<tr>
<td>9</td>
<td>Mexico</td>
<td>3.1</td>
<td>Mexico</td>
<td>8.3</td>
</tr>
<tr>
<td>10</td>
<td>Ukraine</td>
<td>2.9</td>
<td>Ukraine</td>
<td>8.4</td>
</tr>
<tr>
<td>11</td>
<td>All other countries</td>
<td>48.6</td>
<td>All other countries</td>
<td>102.8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>130.9</td>
<td>Total</td>
<td>296.3</td>
</tr>
</tbody>
</table>

1.5: Classification of diabetes mellitus

Clinically diabetes has been classified into following types

1. Insulin dependent diabetes mellitus (IDDM).

2. Non insulin dependent diabetes mellitus (NIDDM).

3. Maturity onset diabetes (MOD).

1.6” Insulin dependent diabetes mellitus”

Type-I diabetes mellitus identified due to decrease in the “insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to deficiency of insulin the underlying cause of this β cell loss is a T-cell mediated autoimmune attack” 15 the
destruction of β-cells may be due to drugs, genetic defects or environmental factors. Infection with Coxsackie virus B or encephalo-myocarditis virus can also cause destruction of β-cells.

1.7 ” Non insulin dependent diabetes mellitus ” (NIDDM)

“Type- II diabetes is increasing worldwide at an explosive rate this epidemic is largely driven by concomitant obesity” but in industrializing countries concomitant with the rapid change toward western life-style patterns worldwide.  

It is also called maturity onset diabetes or type- II diabetes.  

<table>
<thead>
<tr>
<th>Table .2: (Type -I Vs Type -II diabetes mellitus)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical</strong></td>
</tr>
<tr>
<td>Type I (IDDM)</td>
</tr>
<tr>
<td>Onset &lt; twenty years</td>
</tr>
<tr>
<td>Decreased blood insulin</td>
</tr>
<tr>
<td>Anti-islet cell antibodies</td>
</tr>
<tr>
<td>Ketoacidosis common</td>
</tr>
<tr>
<td>Type II (NIDDM)</td>
</tr>
<tr>
<td>Onset &gt; Thirty years</td>
</tr>
<tr>
<td>Obese</td>
</tr>
<tr>
<td>No anti-islet cell antibodies</td>
</tr>
<tr>
<td>Ketoacidosis rare</td>
</tr>
<tr>
<td><strong>Genetics</strong></td>
</tr>
<tr>
<td>Type I (IDDM)</td>
</tr>
<tr>
<td>Fifty% concordance in twins</td>
</tr>
<tr>
<td>HLA-D linked</td>
</tr>
<tr>
<td>Type II (NIDDM)</td>
</tr>
<tr>
<td>90% to 100% concordance</td>
</tr>
<tr>
<td>In twins No. HLA association</td>
</tr>
<tr>
<td><strong>Pathogenesis</strong></td>
</tr>
<tr>
<td>Type I (IDDM)</td>
</tr>
<tr>
<td>Auto immunity</td>
</tr>
<tr>
<td>Immunopathologic mechanism</td>
</tr>
<tr>
<td>Severe insulin deficiency</td>
</tr>
<tr>
<td>Type II (NIDDM)</td>
</tr>
<tr>
<td>Insulin resistance</td>
</tr>
<tr>
<td>insulin deficiency</td>
</tr>
<tr>
<td><strong>Islet cells</strong></td>
</tr>
<tr>
<td>Type I (IDDM)</td>
</tr>
<tr>
<td>Early Insulitis</td>
</tr>
<tr>
<td>atrophy and fibrosis</td>
</tr>
<tr>
<td>β-cell degeneration</td>
</tr>
<tr>
<td>Type II (NIDDM)</td>
</tr>
<tr>
<td>Focal atrophy and amyloid deposits mild</td>
</tr>
<tr>
<td>β-cell depletion</td>
</tr>
</tbody>
</table>
Table 3: Aetiological classification of diabetes mellitus

<table>
<thead>
<tr>
<th>Type - I (damage to β-cells as a result it leads to insulin deficiency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Autoimmune</td>
</tr>
<tr>
<td>• Idiopathic</td>
</tr>
<tr>
<td>Type- II</td>
</tr>
</tbody>
</table>

Other specific types
- Genetic defects of β-cell function
- “Drug or chemical induced” for example, nicotinic acid, glucocorticoids, high-dose thiazides, pentamidine, interferon-α infections
- Uncommon forms of immune-mediated diabetes

1.8: ORAL HYPOGLYCEMIC AGENTS USED IN DIABETES MELLITUS

They are beneficial” in the treatment of patients who have non-insulin dependent diabetes mellitus” but cannot be managed by diet alone. Type- I diabetes has to be treated with insulin and no other hypoglycemic agent should be given.

The major oral hypoglycemic agents are:

I. Sulfonyl ureas:
These drugs are structurally and chemically related to sulfonamides. They are divided into 2 groups. They are:

1. “First generation eg: - tolbutamide, chlorpropamide, acetohexamide” tolazamide.
2. “Second generation eg:- glyburide, glimepiride” glipizide, gliclazide.
   Repaglinide and sodium glymidine.

“Mechanism of action”

“The hypoglycemic effect of sulfonyl ureas is thought to” be due to their ability to cause the release of insulin from pancreas. The administration of one of these agents is altered by an altered plasma levels in the insulin content of the pancreas. In the doses
usually employed, these compounds do not produce hypoglycemia in the absence of pancreas; larger doses produce a direct influence on the liver utilization mechanism of glucose.

II. Biguanides:
Eg: :- Metformin, Phenformin

Due to potentially fatal lactic acidosis, Phenformin was withdrawn

Mechanism of action:
Metformin shows a decrease intestinal glucose absorption, there is an increase in uptake of glucose by the intestines and erythrocytes, which results in an increased lactate formation. The formation of glucose from lactate and lack of an insulin tropic effect explain the absence of clinical hypoglycemia during metformin treatment. Metformin potentiates insulin action by a post receptor mechanism. Apart from the glucose lowering effect, metformin improves the blood lipo-protein profile in diabetic and non-diabetic subjects with hyper lipo-proteinemia.

III. Intestinal glucosidase inhibitors:
Eg: - Acarbose

Mechanism of action:
Acarbose is a \(\alpha\)-glucosidase inhibitor that decreases carbohydrate digestion and absorption of disaccharides by interfering with intestinal glucosidase activity. Used as mono therapy, acarbose does not cause hypoglycemia. The drug improves concentration of injected insulin” in insulin dependent diabetes mellitus patients” and partially compensates for delayed” insulin secretion in patients with non insulin dependent diabetes mellitus”

IV. Aldose reductase inhibitor:
Eg: - Tolrestat, Alrestatin

Aldose reductase is the enzyme which converts glucose to fructose and subsequently to sorbitol, which leads to glucose toxicity in tissues such as kidney, nerve trunk and eye. The aldose reductase inhibitor inhibits these enzymes and reduces the glucose toxicity in these tissues.
V. Thiazolidinedione derivatives:
Eg: - Pioglitazone, En格litazone, Troglitazone, Rosiglitazone etc

Mechanism of action:

Thiazolidinedione derivatives act as an insulin sensitizer, with concomitant insulin actions in the liver and skeletal muscle. They reduce hepatic output of glucose and promote insulin dependent glucose utilization in skeletal muscle. This counteracts insulin resistance.

1.8: Maturity – onset diabetes of the young

When present in the youngsters’ non-insulin dependent diabetes mellitus is often referred to as maturity onset diabetes” of youth. This is a type of spontaneous diabetes and may be due to genetic defects of β-cell function. In this group a ketosis-resistant, non-insulin dependent, generally asymptomatic form of diabetes is present in individuals before age of 25.

*Table 4: Pathogenesis of Type-I diabetes mellitus*
1.9: Pathogenesis of Type-I diabetes mellitus

Three interlocking mechanisms are responsible for the islet cell destruction:

1. Genetic susceptibility
   - Concordance in identical twins 50%
   - Susceptibility gene on HLA region in chromosome 6
2. Auto-immunity
   - Viral infections
   - Experimental induction with chemicals
   - Geographic and seasonal variations
   - Bovine milk proteins
3. Environmental
   - Islet cell antibodies
   - Insulitis
4. CD8 + T-lymphocyte-mediated selective destruction of β-cells.
1.10: Pathogenesis of “Type-II diabetes mellitus”

“The two metabolic defects that characterize type-II diabetes mellitus” are:

1. A “derangement in β-cell secretion of insulin”
2. A decrease response of peripheral tissue” to respond to insulin (Insulin resistance)"\(^{18,19}\)

1.11: COMPLICATIONS OF DIABETES MELLITUS
Life threatening complication of diabetes mellitus includes diabetes Ketoacidosis and hyperglycemic-hyperosmolar-non-ketonic coma. Hypoglycemia, another acute complication usually stems from drug therapy. Diabetes mellitus” are associated with a high risk for” number of chronic illnesses. Diabetic nephropathy and renal failure is one of the most complication of diabetes. Epidemiological data identified increased number of patients with renal failure caused by diabetic nephropathy.

Diabetic retinopathy occurs in majority of diabetics after 20 years of the” disease diabetes is the second leading cause of blindness” another complication, neuropathy may result from the sorbitol pathway or from ischemia resulting from vascular disease. Diabetic neuropathy most frequently involves the peripheral nerves, but can involve any nerve. There is a higher incidence of coronary atherosclerosis among diabetic than non-diabetic. Macro-vascular complications of diabetes mellitus include coronary heart disease, hypertension, congestive heart failure and erectile dysfunction. Cholesterol absorption is decreased and biosynthesis is increased in diabetes. The increased cholesterol synthesis is reduced by insulin.
1.12: DIAGNOSIS OF DIABETES

1. Urine test

Urine is tested for reducing sugars like glucose, galactose, sucrose etc and is also tested for ketone bodies. The principle involved in sugar testing in urine is reduction of cupric ions in alkaline solution by reducing sugars to reddish orange insoluble cuprous oxide. Ketone in urine undergoes reaction between sodium nitro prusside and aceto-acetate as acetone under alkaline condition, a lavender colour is produced.

2. Fasting blood glucose level

Fasting blood sugar level in the early morning is normally 50-90 mg/dl and 110 mg/dl is considered to be the upper limit of normal. The principle involves conversion of blood sugar” to gluconic acid and hydrogen peroxide by glucose” Peroxidase. The H₂O₂ is then estimated by iodometric procedure. Blood collection in sodium fluoride bulb retains the sugar value for several hours at room temperature and several days if kept in refrigeration with 10% loss. In diabetes, arterial and venous difference is lost, meaning glucose is not utilized by tissues.

3. Insulin assay

Plasma insulin can be measured by radio immune assay or enzyme immuno assay.

4. Oral glucose tolerance test

When a normal fasting person is orally given with one gram glucose per kg, the blood sugar level rises from about 90mg/dl to 120-140 mg/dl and fall back to normal in about 2hrs. In a diabetic patient, the fasting blood glucose is always above 110mg/dl often above 140 mg/dl. On digestion of glucose the blood glucose level rises abnormally and the glucose level falls back to normal value only after 4-6 hrs or sometimes it may fail to fall down to normal value.

5. Glycated hemoglobin (HbA₁c) test
The most effective review of diabetic patients is identification of glycated hemoglobin through the estimation of glucose modified hemoglobin. When the red blood cells become older fraction of native hemoglobin(HbA) will be converted to glycated form Hb\textsubscript{1c}.

1.13: INSULIN

Insulin, the hypoglycemic anti-diabetic factor” is a polypeptide hormone secreted by the β-cells of islets of Langerhans” of pancreas. Insulin was the first hormone to be isolated from animal sources in pure enough form to be administrated therapeutically and it was the first mammalian peptide hormone whose biosynthesis by recombinant-DNA technology was achieved.\textsuperscript{31}

Human insulin has a molecular weight of 6000.\textsuperscript{32} Insulin is composed of two open polypeptide chains that are linked together by disulfide (-S-S-) bridges. Destruction of the bridges separates insulin into its two chains, an A chain and a B chain.\textsuperscript{33} Each codes for unique pro-insulin that is processed into two distinct active insulin Molecules. Insulin is derived from pre-pro-insulin. Pre-pro-insulin is cleaved to pro-insulin which gets packed into secretary granules. Pro-insulin is a precursor of insulin and includes the A and B chains and the connecting 31 residue C- peptide in a single polypeptide chain” the A chain of insulin is composed of 21 amino acids and B chain 30 amino acids.\textsuperscript{34}

Types of insulin preparations are regular insulin, lente insulin, ultra lente insulin, semi lente insulin etc.\textsuperscript{35} Insulin can be prepared from bovine, porcine and human sources.

![Structure of Human Insulin](image)

**Fig. 3: structure of human insulin**
1.14: GLUCOSE HOMEOSTASIS AND INSULIN SECRETION

The major source of energy for all the cells is glucose. Insulin and glucagon are the most important hormones that maintain glucose homeostasis. \(^{36}\) The specific stimulus for insulin secretion involves elevations in circulating levels of glucose. The β-cell membrane contains specific glucoreceptors that recognize D-glucose. Stimulation of these receptors activates an adenyl cyclase system and causes an influx of calcium ions. This altered intra-cellular ionic balance stimulates a contraction of a sub-cellular microtubule-microfilament system, which” is involved in the transport and fusion of insulin containing” secretory granules with the cell membrane. Fusion of the granule and cell membrane permits the granule content to be released. The cell membrane of islet cells contains five coupled systems. These are the substrate carriers, a receptor transducer complex, a calcium gate that controls Ca\(^{2+}\) entry, an adenyl cyclase system and the secretory complex. These systems are involved in specific stimulus for insulin secretion.

![Diagram of Glucose Homeostasis](image-url)

**Fig.4: Glucose homeostasis**
Fig. 5: Schematic diagram of insulin receptor

1.15: MECHANISM OF INSULIN ACTION

Insulin is a major anabolic hormone. It catalyses the synthesis of various macromolecules and prevents undue breakdown of proteins, carbohydrates and fat. Thus it promotes cell growth by deposition of carbohydrates, lipids and proteins. The most clearly measurable insulin action is to enhance the penetration of amino acids and simple sugars through the cell membrane of skeletal and heart muscle which otherwise have low permeability to these substances. Thus reduction of blood glucose is brought about by:

1. Increasing glycogenesis.

2. Increasing glucose utilization into insulin sensitive cells such as myocytes, hepatocytes and adipocytes.

3. Inhibiting breakdown of lipids.

4. Increasing the rate of protein synthesis.

5. Stimulating some cell ion transport mechanisms such as Na⁺/K⁺/ATPase.

6. Decreasing gluconeogenesis.
Insulin interacts with the target cells by binding to insulin receptor which is composed of two glycoprotein subunits, α and β. In the mature α₂ & β₂ receptor, the extra cellular” α-subunit confers high affinity insulin binding” whereas transmembrane” β- subunit is responsible for transducing the signal of insulin binding to the interior of the cell receptor bound insulin” triggers a cascade of intracellular responses. Due to the “stimulation of tyrosine kinase activity some compounds in the β-subunit” are phosphorylated (auto phosphorylation). This leads to phosphorylation of some enzymes in the cytosol. Activation of these enzymes causes movement of transport proteins (glucose transporters) leading to the pharmacological action.³⁸

*Table 6: Relationship of insulin with glycogen, triglycerides and proteins*
1.16: Relationship of insulin with glycogen, triglycerides and protein:

Insulin stimulates glucose storage in the liver as glycogen and in adipose tissue as triglycerides and amino acid storage in muscle as protein” it also promotes utilization of glucose in muscle for energy. Insulin” inhibits the breakdown of triglycerides, glycogen, and the conversion of amino acids to glucose” (gluconeogenesis)” the conversion of amino acids to glucose” and of glucose to fatty acids occurs primarily in the liver.

1.17: INSULIN RESISTANCE

Biological activity of insulin is due to union with spanning-membrane tyrosine kinase receptor which shows specific substrate interaction with specific substrate molecules which are commonly known as docking proteins, along with insulin receptor substrate proteins so that it transport the signals to produce mitogenic and metabolic phenomenon . Tyrosine phosphorylation produces reaction so that signals from insulin undergoes transduction with major pathways occurring in” intracellular lipid and serine threonine kinases known as phosphatidyl inositol 3-kinase/Akt and extracellular regulated kinase (Erk) signaling” which shows specific biological responses.

1.18: MECHANISM OF INSULIN RESISTANCE

Insulin resistance after high fructose ingestion is due to:

1. Decrease in both binding and sensitivity of insulin.
2. Increased nonesterified fatty acid concentrations, which may reduce insulin sensitivity by increasing the intramyocellular lipid content and increased concentrations of nonesterified fatty acid concentrations, may have a deleterious effect on β cell function.
3. Fructose induced hypertriacylglycerolemia and reduced insulin binding may be one mechanism by which fructose diets promote insulin resistance.
5. Fructose feeding shows reduced autophosphorylation of insulin-stimulation.
1.19: Insulin effect on the targets

Insulin shows profound effect on the metabolism of the fat and carbohydrates, though there is marked effect on the specialized target cells which has promoted a wide variety of cellular growth and functioning of different tissues. Insulin stimulates synthesis of the circulating nutrients in the blood.
1.10: Insulin Endocrine effects

**Action on liver:**

- There is a change in the catabolism reversal of decreased insulin secretion.
- There is a decrease level of stored glycogen due to inhibition process.
- Degradation of inhibitory phenomenon in the interconversion of amino acids and fatty acids to ketoacids.

1.21: EXPERIMENTAL MODELS TO SCREEN ANTIDIABETICS

**ANIMAL MODELS**\(^{42,43,44}\)

There are many advantages of using animal models in research works on diabetes as various aspects of the disease like the etiology, its multifactorial genetics, pathogenesis of the disease and its complication can be explicitly understood. Secondly it also helps in the” development and evaluation of newer agents for the treatment” of diabetes.
However, there are some limitations in the use of animal model for studies on diabetes’’
induction of diabetes in animal can be carried out by various ways by using different
chemical diabetogenic agents, surgically by partial pancreatectomy, by viral induction
and genetic manipulation by selective inbreeding.

Classification:

- “Non insulin dependent diabetes mellitus (NIDDM)” resembling animal models.
- “Insulin dependent diabetes mellitus (IDDM)” resembling animal models.
- Diabetes induction by pancreatectomy in dogs and rats.
- Diabetes induced by virus.
- Diabetic genetic animals.
- Spontaneously diabetic animals.
- Diabetic transgenic animals.

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

1.22: Non Insulin dependent diabetes mellitus (NIDDM) resembling animal models

1. NIDDM animal models can be prepared by injecting Streptozotocin i.v. at a dose of
100mg/kg to 5 days old neonatal Wistar rat. New born rats are injected with
Streptozotocin (100mg/kg in 25 ml of citrate buffer, pH 4.5) through sephenous vein
directly accessible by transcutaneous puncture. The blood sugar level is elevated and
remains at diabetic levels for 3-4 months.⁴⁵

2. In another model of NIDDM injection of streptozotocin (90mg/kg i.p) to 2 days old
Sprague Dawley rats resulted in transient hyperglycemia followed by recovery. The
above two animal models are based on β-cells deficiency and these models are useful for evaluating the effect of β-cells deficiency in the development of NIDDM.

3. NIDDM animal models can also be prepared by neonatal alloxan induced diabetes by injecting alloxan 200mg/kg body weight intraperitoneally to neonates of 6 days old. Non fasted blood sugar is determined after 8 weeks. The hyperglycemia produced is stable up to 6 months.

4. NIDDM animal models can also be prepared by single intraperitoneal injection of 60mg/kg streptozotocin 15min after the intraperitoneal injection administration of 120mg/kg” in overnight fasted rats.46

1.23: Insulin dependent diabetes mellitus (IDDM) resembling animal models

IDDM both in human beings and in animal models of IDDM’ is due to the destruction of β-islet cells ultimately leading to” an absence or decreased secretion of insulin , the destruction of the β-cell can be brought about by viral induction , injection of diabetogenic or by introduction of transgenes . Streptozotocin and alloxan were found to be more selective in β-cell destruction than other chemicals like ascorbic acid and its derivative and uric acid. These substances have also been referred to as β-cytotoxic substances with the implication that the actions are restricted to the β-cell of the islet of Langerhans.

1.24: The mechanism of alloxan action47,48

Diabetogenic action of alloxan is enhanced by oxygen reactive mediated species. Alloxan undergoes reduction reaction with the formation of dialuric acid, so that there is a rapid speed up in the redox cycle generating superoxide radicals. Spontaneous reaction with dismutation leads to the formation of hydrogen peroxide” finally hydroxyl radicals are generated by fenton reaction reactive oxygen radical leads to increase in the concentration of cytosolic Ca\(^{2+}\) which cause rapid destruction of β-cells the action of alloxan in the pancreas is preceded by its rapid uptake by the β-cells” SH containing cellular compounds shows high affinity with reduction in the glutathione, cysteine and sulphydryl bound proteins are more prone to their action. Alloxan radicals are formed by
reaction of dialuric acid and alloxan with absorption maximum 305 nm. Alloxan is converted into unstable dialuric acid which is then reoxidised back to alloxan.

1.25: The mechanism of streptozotocin action

Streptozotocin “(deoxy2-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose)” is prepared by *Streptomyces achromogenes* and induces both insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM and NIDDM, respectively)” it is freely soluble in water, unstable at room temperature and has to be stored below -20°C. Diabetes dose varies with the species and the optimal dose required to produce diabetes in rat was found to be (50-60 mg/kg “i.p” or i.v.)” in mice (175-200 mg/kg i.p. or i.v.) and in dogs (15 mg/kg, for 3 days). Due to its low stability the rapid i.v. injection appears to be the best route of administration. STZ induces diabetes in hamster, monkey and guinea pigs.

The action of Streptozotocin in β cells shows marked variation glucose concentration and blood insulin. Hyperglycemia is induced after two hour injection so that there in a decreased levels in blood insulin. Hypoglycemia is observed with increase in the insulin blood levels after 6 hours later, due to this there is a fluctuation in the abnormalities of β cell functioning.

Nitric oxide in the cytotoxic effect of STZ was confirmed in several experiments. Cells of pancreas when exposed to streptozotocin showed enhanced activity of cGMP and guanylyclase. Spontaneous donor activity is not shown by streptozotocin. Superoxide anions formation with streptozotocin action on increased activity of xanthine oxidase and mitochondria. It was demonstrated that Krebs cycle is inhibited by streptozotocin and there reduction in the levels of oxygen consumption by mitochondria so that it ultimately leads to reduction in ATP production of mitochondria and causes elevated levels of nucleotide.
1.26: SEIZURES/EPILEPSY/CONVULSION

DEFINITION

Epilepsy is a disease in which selectively effective drugs are still under clinical trials but in the case of convulsion there is a sudden violent involuntary skeletal muscle contraction. It is a chronic central nervous system disorder in which there is a brief sudden abnormal motor, sensory which results in sudden neuronal discharge. Epilepsy occurs due to many neurons are under high excited stage which delivers massive discharges suppressing the integrity of the brain” seizures occurs due to occasional sudden rapid excessive and local discharges of grey matter” as soon as this is initiated due to abnormal focus there is a attack of the neighboring cells of the brain which leads to generalized convulsions. Seizures are symptoms of a disturbance in brain function. Most cases of seizures have no immediate identifiable cause. Seizures occurs due to electrical stimulation in the brain as soon as this is initiated seizure is initiated by reentry of excitatory impulses .There is total depletion of neurotransmitter, accumulation of CO$_2$ & adenosine which leads to depletion of oxygen and energy rich phosphate intermediates 48,49.

1.27: PREVALENCE

Epilepsy affecting majority of the people in the world due to disturbances in the neurotransmitter levels in the brain. The percentage of the disease around the world is around one percent but it has been increased to around 2 percentages” the different forms of epilepsy is generally more prevalent in below the” age of twelve.50 The epileptic percentage in males has been more in females.51 The increase in ratio of epilepsy in increasing drastically every year.52 Epilepsy has been interfering with emotional thinking
and causing disturbances in study patterns which has been increasing to the suicidal tendencies.

1.28: PATHOPHYSIOLOGY

Various factors are involved in different forms of epilepsy. Nowadays there is alteration in the genes that is it may be due to autosomal changes in the brain more specifically in the frontal lobe and certain alterations in the neuronal discharges in the brain.\textsuperscript{53} The cause of different epileptic forms occurs due to variations in different factors” the exact cause of epileptogenesis is not known but probably is” due to different neurotransmitter levels in the brain i.e. is due to alterations in the GABA & glutamate levels in the different forms of epileptic conditions. The underlying mechanism of epileptogenesis has to understood with the invention of better novel drugs for a better known therapeutic action.\textsuperscript{54}

Changes of different sets of epileptogenetic behavior patterns of neuronal tissue is due to alterations in the excitability of neurons which is due to repolarization and depolarization.\textsuperscript{55, 56, 57, 58, 59, 60, 61}

1.29: CLASSIFICATION OF EPILEPTIC SEIZURES\textsuperscript{62}

Seizures classified based on the electrical discharges, depending on the symptoms the major forms of epilepsy has been categorized into two classes i.e. generalized & partial seizures.

1.30: DIAGNOSIS
The treatment involves in recognition of the different episodes of seizures whether the electrical stimulations are properly recognized. If it is not treated properly it may have serious adverse effects which will affect the social pattern of the life. Other major adverse effects that will affect the social pattern of life are unable to drive, income loss, frequent visits to the hospital. Epilepsy should not be confused with other disorders patterns the most frequent examples that we can see in the case of migraine, some movement disorders. In the case of children there may be syncope infantile, terrors in night and there may be prolongation in QT syndrome.

1.31: TREATMENT

There are different drugs which are used to treat epilepsy with minimum side effects. The primary goal is to treat the disease with available anticonvulsant drugs. But some patients may not respond to the drugs and there may be reoccurrence in the disease. So a combination of the drugs has to be prescribed depending on the body conditions, but these may have possible drug interactions so selection of better combination is very important and fixation of the dose levels should be considered because epileptic drugs induce hepatic enzymes which leads to decrease in the serum levels of the drugs so it is very important to adjust the dose levels.

1.32: Anticonvulsants drug therapy:

The selection of the drug is based on the type of the epilepsy and probable Pathophysiology mechanism” because a single drug may have different mechanism it may produce its actions different receptors.

1.33: ANIMALS MODELS TO SCREEN ANTICONVULSANT ACTIVITY

<table>
<thead>
<tr>
<th><strong>In vivo</strong></th>
<th><strong>In vitro</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1” Electrical stimulation in mice”</td>
<td>1”[^3]H]-GABA receptor binding”</td>
</tr>
<tr>
<td>2”Pentylentetrazol induced in” mice and rats”</td>
<td>2”GABA receptor binding”</td>
</tr>
</tbody>
</table>
3” Strychnine-induced convulsion in mice” 3” $^3$H-GABA uptake in rat cerebral “
4”Picrotoxin-induced convulsions in mice” cortex synaptosomes”
5 “Isoniazid-induced convulsions in mice” 4” GABA uptake and release”
6”Bicuculline test in rats” in rat hippocampus slices”
7”4-aminopyridine-induced seizures in mice” 5.”Electrical recordings from isolated
8”Epilepsy induced by focal lesions” nerve cells”
9”Kindled rat seizure model” 6”Isolated neonatal rat spinal cord”
10”Posthypoxic myoclonus in rats” 7” Cell culture of neurons”
11”Genetic animal models of epilepsy”
1.34: FORMULATION

The most common drug delivery route of administration among the all available and explored for various active pharmaceutical products of dosage form is the “oral route of administration this route of administration is the commonly available method” and considered as natral, uncomplicated it very convenient to use with effective decrease in the cost.

1.35: Evaluation of tablets include
1. General appearance.
2. Size and shape.
3. Unique identification markings.
4. Organoleptic properties.
5. Hardness and friability.
6. Drug content and release.
7. Weight variation.
8. Disintegration.
10. Stability studies.

1.36: Methods of tablet manufacture include:
1. Direct compression.
2. Dry granulation.
3. Wet granulation.

1.37: Tablet Design and Formulation
1. Diluents.
2. Binders and adhesives.
3. Disintegrants.
4. Lubricants, antiadherents and glidants.
5. Colors, flavours and sweeteners.
Plant profile: *Madhuca Indica*

- *Madhuca indica* belonging to the family Sapotaceae is the most common tree seen in India. Traditionally it has been used against diabetes, rheumatism, ulcers, bleeding and tonsillitis.\(^{70,71}\)

![Plant bark of Madhuca indica](image)

*Fig.8: Plant bark of Madhuca indica*

- Traditionally, *Madhuca indica* bark has been used against diabetes, rheumatism, ulcers, bleeding and tonsillitis. The flowers, seeds and seed oil of Madhuca have great medicinal value.\(^{72,73}\)
**Plant profile: Vitex negundo**

*Vitex negundo* belongs to family verbenaceae. *Vitex negundo* comprising of about 75 genera and nearly 2500 species. *Vitex negundo* is distributed in East Asia, south west China, throughout India and cultivated in Pakistan. Chloroform extract of defatted seed of *Vitex negundo* showed anti-inflammatory activity. It also possesses potent mosquito repelling activity against *Aedes aegypti*, anti-tumor, gastro protective and analgesic activity. The present study was undertaken to study antiepileptic activity of vitex negundo, phytochemical tests reveal the presence of flavanoids, flavones, and terpenoids.

![Fig.9: Plant leaf of vitex negundo](image)

The present has been designed to evaluate antidiabetic activity of *Madhuca indica* bark extract and anticonvulsant activity & formulation of *vitex negundo* leaf extract.