Diabetes

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and postprandial blood sugar levels. The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025. WHO [1999] has predicted that the major burden will occur in developing countries.

Studies conducted in India in the last decade have highlighted that not only is the prevalence of diabetes high but also that it is increasing rapidly in the urban population. It is estimated that there are approximately 33 million adults with diabetes in India. This number is likely to increase to 57.2 million by the year 2025 [Ramachandram, 2002].

Diabetes mellitus is a complex metabolic disorder resulting from either insulin insufficiency or insulin dysfunction. Type I diabetes (insulin dependent) is caused due to insulin insufficiency because of lack of functional beta cells. Patients suffering from this are therefore totally dependent on exogenous source of insulin while patients suffering from Type II diabetes (insulin independent) are unable to respond to insulin and can be treated with dietary changes, exercise and medication. Type II diabetes is the more common form of diabetes constituting 90% of the diabetic population.
Symptoms for both diabetic conditions may include:

(I) High levels of sugar in the blood
(II) Unusual thirst
(III) Frequent urination.
(IV) Extreme hunger and loss of weight
(V) Blurred vision
(VI) Nausea and vomiting
(VII) Extreme weakness and tiredness
(VIII) Irritability, mood changes etc.

Though pathophysiology of diabetes remains to be fully understood, experimental evidences suggest the involvement of free radicals in the pathogenesis of diabetes [Diabetes Care, 2000] and more importantly in the development of diabetic complications [Oberlay, 1988]. Free radicals are capable of damaging cellular molecules, DNA, proteins and lipids leading to altered cellular functions [Baynes, 1997]. Many recent studies reveal that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models [Nazir oglu, 2001] as well as reducing the severity of diabetic complications [Lipinski, 2001].

For the development of diabetic complications, the abnormalities produced in lipids and proteins are the major etiologic
factors. In diabetic patients, extra-cellular and long lived proteins, such as elastin, laminin, and collagen are the major targets of free radicals. These proteins are modified to form glycoproteins due to hyperglycemia. The modification of these proteins present in tissues such as lens, vascular wall and basement membranes are associated with the development of complications of diabetes such as cataracts, microangiopathy, atherosclerosis and nephropathy [Glugliano, 1996]. During diabetes, lipoproteins are oxidized by free radicals.

There are also multiple abnormalities of lipoprotein metabolism in very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) in diabetes. Lipid peroxidation is enhanced due to increased oxidative stress in diabetic condition [Brownlee, 1996] Apart from this, advanced glycation end products (AGEs) are formed by non-enzymatic glycosylation of proteins. AGEs tend to accumulate on long-lived molecules in tissues and generate abnormalities in cell and tissue functions [Elgawish, 1999]. In addition, AGEs also contribute to increased vascular permeability in both micro and macrovascular structures by binding to specific macrophage receptors. This results in formation of free radicals and endothelial dysfunction. AGEs are also formed on nucleic acids and histones and may cause mutations and altered gene expression.

As diabetes is a multifactorial disease leading to several complications, and therefore demands a multiple therapeutic approach. For example, to manage post-prandial hyper-glycaemia at digestive level, glucosidase inhibitors such as acarbose, miglitol are used. These inhibit degradation of carbohydrates thereby reducing the glucose absorption by
the cells. To enhance glucose uptake by peripheral cells biguanide such as Metformin is used. Sulphonylureas like Glibenclamide is insulinotropic and works as secretogogue for pancreatic cells. Although several therapies are in use for treatment, there are certain limitations due to high cost and side effects such as development of hypoglycemia, weight gain, gastrointestinal disturbances, liver toxicity etc [Dey, 2002]. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes.

Diabetic patients are also at increased risk of cardiovascular, peripheral vascular and cerebrovascular diseases. Diabetic people can manage their disease and lower the risk of complications by dietary modifications and by maintaining their blood glucose level. The incidence of diabetes mellitus increases with age, and usually occurs in individuals older than 45 years [Vasudevan, 2006].

**Pancreas:**

Pancreas helps out in the digestive system. Pancreatic digestive enzymes help to further breakdown the lipids, proteins, and fats in the chime. The part of the pancreas with endocrine function is made up of approximately a million of cell clusters called islets of langerhans. Four main cell types exist in the islets.

They are relatively difficult to distinguish using standard staining techniques, but they can be classified by their secretion:

1. A cells – secrete glucagon (increase glucose levels)
2. B cells - secrete insulin (decreases blood glucose levels)
3. D cells- secrete somatostatin (regulates A & B cells)

4. PP cells- secrete pancreatic polypeptide

Reduction in number and size of islets is often seen in type 1 diabetes. In type 2 diabetes, there may be a subtle reduction in islet cell mass, which can be demonstrated only by special procedures [Datta, 2004].

**Insulin:**

Both type I and type 2 diabetes share one central feature: elevated blood sugar (glucose) levels are due to deficiency or dysfunction of insulin, a hormone produced by the pancreas. Insulin is a key regulator of the body metabolism. About 2-4 hours after a meal both blood glucose and insulin are at low levels, with insulin being slightly higher. The blood glucose levels are then referred to as fasting blood glucose.

**Homeostasis of glucose:**

Homeostasis of glucose is defined as an organism’s tendency to maintain the equilibrium of different internal systems. It relies on the balance and interactions - insulin and Glucagon to maintain a healthy blood glucose level.

Under normal circumstances, the body is able to balance the amount of glucose or sugar levels (80mg/dl) in the blood with the
amount of glucose that the cells need for fuel. Insulin facilitates the transport of glucose into the cells. Too little available insulin in the blood stream will raise the blood glucose level, which in turn stimulates the pancreas to release more insulin and more glucose absorption.

**Homeostatic imbalance:**

Many diseases are a result of disturbance of homeostasis, a condition known as homeostatic imbalance. As it ages, every organism will lose efficiency in its control systems. The inefficiencies increase the risk for illness leading to death. In ideal circumstances, homeostatic control mechanisms should prevent this imbalance from occurring, but, in some people, the mechanisms do not work efficiently enough or the quantity of the substance exceeds the levels at which it can be managed. In these cases, blood glucose levels raise $>125\text{mg/dl}$.

**EPIDEMIOLOGY**

The incidence rate of diabetes mellitus varies from country to country due to difference in genetic and environmental factors. Worldwide currently 170 million peoples are suffering from diabetes mellitus and this figure will double by the year 2025[Wild, 2004].

Nowhere is the diabetes epidemic more pronounced than in India as the World Health Organization reports show that 32 million
people had diabetes in the year 2000. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025. In USA, about 18 million people are affected by diabetes mellitus every year [Diabetes care 1997]. The incidence rate of type II Diabetes mellitus was increasing in Europeans and Polynesians [Valle, 1997] India and China will be the leading countries in having total number of diabetic subjects due to their immense population. In India, currently 30 million people are affected by diabetes mellitus and this figure will double by the year 2025.

CLASSIFICATION

Diabetes mellitus is classified into three types which include Type 1, Type 2 and gestational diabetes.

Type 1 Diabetes mellitus

Type 1 diabetes mellitus formerly known as insulin-dependent diabetes (IDDM), childhood diabetes, or juvenile-onset diabetes. It is characterized by loss of the insulin-producing beta cells of the islets of Langerhans of the pancreas leading to a deficiency of insulin. Type 1 diabetes is partly inherited [Jin, 2008]. Viruses like Cox Sackie Viruses B2, B3, B4, and B5 have been suggested as potential determinants of the disease. Symptoms of type 1 diabetes usually develop over a short period. The principal treatment of type 1 diabetes, even from the earliest
stages, is replacement of insulin. Diet and exercise cannot reverse or prevent type 1 diabetes [Brelot, 2005].

**Type 2 Diabetes mellitus**

Type 2 diabetes is characterized by elevated blood glucose due to target cell resistance to the action of circulating insulin and a qualitative and quantitative deficiency in insulin secretion, pancreatic β cell dysfunction and insulin resistance are the hallmark of type 2 diabetes. Type 2 diabetes accounts for 90-95% of all diagnosed diabetes and usually develops in adults over age 40. Type 2 diabetes is usually associated with family history of diabetes, older age, obesity and lack of exercise, insulin resistance and hyperinsulinemia that eventually lead to impaired glucose tolerance. Diet plays a key role in the management of diabetes. Diet, exercise and blood testing for glucose are also the basis for management of NIDDM. In addition, some people with NIDDM take oral hypoglycemic drugs or insulin to lower their blood glucose levels [Sundaram, 2006].

**Gestational diabetes mellitus**

Gestational diabetes results during pregnancy. It usually ends after delivery, but women with gestational diabetes may develop type-2 later in their life [Schaefer, 2006]. Gestational diabetes results from the body’s resistance to the action of insulin, which is caused by hormones produced by the placenta.
Women with gestational diabetes require treatment to prevent adverse effect to the fetus, usually treated with dietary adjustments or insulin but cannot be treated with oral hypoglycemic because they can harm the fetus [Metzger, 1998].

Other types

Diabetes mellitus may also arise due to the following metabolic and genetic defects or by some chemical drugs.

- Genetic defects in beta cells (autosomal or mitochondrial)

- Genetically-related insulin resistance, with or without lipodystrophy (abnormal body fat deposition)

- Diseases of the pancreas (e.g. chronic pancreatitis, cystic fibrosis)

- Hormonal defects, Chemicals or drugs [Diabetes care, 1998].
Table 1: Symptoms of untreated Insulin dependent diabetes & Non Insulin dependent diabetes.

<table>
<thead>
<tr>
<th>Insulin-dependent diabetes</th>
<th>Non insulin dependent diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequent urination</td>
<td>Frequent urination</td>
</tr>
<tr>
<td>Excessive thirst</td>
<td>Excessive thirst</td>
</tr>
<tr>
<td>Increased appetite</td>
<td>Fatigue</td>
</tr>
<tr>
<td>Weakness</td>
<td>An increase in infections</td>
</tr>
<tr>
<td>Tiredness</td>
<td>Blurred vision</td>
</tr>
<tr>
<td>Urinary tract infections</td>
<td>Tingling in hands or feet</td>
</tr>
<tr>
<td>Recurrent skin infections, such as boils</td>
<td>Impotence in men</td>
</tr>
<tr>
<td>Tingling or numbness in hands or feet.</td>
<td>Absence of menstrual periods in women [Lichiardopol, 2007]</td>
</tr>
</tbody>
</table>
ETIOLOGY AND PATHOGENESIS

Type 1 Diabetes mellitus

In this type of diabetes, there is destruction of β cells of pancreas through an autoimmune or idiopathic process, leading to insulin deficiency and hyperglycemia. It is common in childhood and adolescence but can occur at any age.

Genetic factors

It is well established now that type I diabetes is a polygenic disorder. But about 40% of the familial aggregation of type I diabetes is accounted for the MHC genes (on short arm of chromosome 6) and in particular HLA class II molecules DR and DQ [Gottliab, 1998].

Triggering factors

Viral infection:- strong evidence has been obtained only for the possible role of congenital rubella and Enter virus infection especially Coxsackie B virus infection [Hyoty ,2004].

Environmental factors: High intake of nitrates and nitrites and deficiency of vitamin D have been identified as dietary causes of type 1 diabetes [Vaarala; 2004].

Autoimmunity: - The four major auto-antibodies measured clinically are Islet cell antibody (ICA), Insulin auto-antibodies (IAA), antibodies against Glutamic acid decarboxylase-65 and 67 (GAD-65 & 67) and insulinoma associated antibodies (IAS2s). In family studies, individuals
with ICA have greater risk of diabetes. The presence of IAA with ICA further increases the risk.

In neonatal life, auto reactive T cells that are active against host cells are normally destroyed in the thymus (clonal deletion). Cells that escape this process are suppressed in the periphery by regulatory T cells. Failure in either of these processes will result in cells capable of responding to self antigens [Stites, 2001].

Type 2 Diabetes mellitus

Insulin resistance and impaired beta cell function are the major hallmarks of this disease. Though it is not yet clear more studies show that the insulin resistance occurs first followed by beta cell failure [Groop, 2002].

Genetic factors

The most common form of type 2 diabetes is of polygenic inheritance. Several candidate genes have been associated with it on chromosome 1q, 12q and 20q etc. [Florez, 2002]. The less common monogenic form has been named as Maturity onset diabetes of the young (MODY) as named by Fajans et.al. [Elbein, 2002].

Environmental factors

Insulin resistance and beta cell dysfunction are the hallmarks of type 2 diabetes.

Some causes of insulin resistance.

Obesity, aging, reduced physical activity.
Clinical Features

The clinical manifestations of diabetes mellitus vary from patient to patient. Most often the symptoms of hyperglycemia are polydipsia, polyuria and polyphagia. Occasionally they may present diabetic coma or neuropathy, in the absence of symptomatic hyperglycemia.

Table 2: Clinical features of Diabetes mellitus

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Type 1 diabetes</th>
<th>Type 2 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age of onset</td>
<td>Usually &lt;25</td>
<td>&gt;25</td>
</tr>
<tr>
<td>2. Genetic locus</td>
<td>Chromosome 6</td>
<td>Not exactly known</td>
</tr>
<tr>
<td>3. Body habits</td>
<td>Normal to wasted</td>
<td>Obese</td>
</tr>
<tr>
<td>4. Plasma insulin</td>
<td>Low to absent</td>
<td>Normal to high</td>
</tr>
<tr>
<td>5. Plasma glucagon</td>
<td>High, suppressible</td>
<td>High, resistant</td>
</tr>
<tr>
<td>6. Acute complication</td>
<td>Diabetic</td>
<td>Hyperosmolar</td>
</tr>
<tr>
<td></td>
<td>Ketoacidosis</td>
<td>Coma</td>
</tr>
<tr>
<td>7. Response to oral</td>
<td>Unresponsive</td>
<td>Responsive</td>
</tr>
<tr>
<td>hypoglycemic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Response to insulin</td>
<td>Responsive</td>
<td>Responsive to resistant</td>
</tr>
<tr>
<td>therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Auto-antibodies</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>10. Association with</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>autoimmune disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Pancreatic β cell</td>
<td>Destroyed</td>
<td>Decreased number</td>
</tr>
</tbody>
</table>
DIAGNOSIS

The fasting plasma glucose (FPG) and urinary glucose levels are the standard tests for diagnosing diabetes. Repeated fasting plasma glucose levels of 126mg/dl are strongly suggestive of diabetes. Symptoms of diabetes and a random glucose levels above 11.1 m.mol/l (200mg/dl) are strongly suggestive of diabetes. In OGTT, the level of glucose was increased initially and the level remains high, 200 mg/dL (11.1 mmol/L), after 2 hours glucose load. A glycated haemoglobin level of 1% above norm range identifies diabetes in 98% of patients [Kaufmann, 1994].

1. Fasting blood glucose test: After 8 hours fast blood levels are assessed. When the level is greater than 125mg/dl it indicates diabetes.

2. Random blood sugar test (RBS): Blood glucose can be measured in regardless of last ate. This test is useful because glucose levels did not vary widely throughout a day; if they vary widely (200mg/dl) it means diabetes.

3. Glucose tolerance test: Glucose tolerance is a test in which glucose is given and blood samples taken afterwards to determine how quickly it is cleared from the blood. In the most commonly performed version of the test, an oral glucose tolerance test (OGTT), a standard dose of glucose is ingested by mouth and blood levels are checked two hours later [Kasper, 2005].

4. HbA1c test: RBC was made up of hemoglobin. Glucose sticks to the hemoglobin to make a glycosylated hemoglobin molecule called A1C or
HbA1C red cells live for 8 – 12 weeks before they are replaced. By measuring the HbA1C it can tell how high the blood glucose has been on average over the last 8 -12 weeks.

>6.5% = diabetes  
<6.0% = normal

Table 3: Diagnosis of Diabetes mellitus

<table>
<thead>
<tr>
<th>Fasting plasma Glucose Test (mg/dl)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 99 and below</td>
<td>Normal</td>
</tr>
<tr>
<td>2 100 to 125</td>
<td>Pre-diabetes (impaired fasting glucose)</td>
</tr>
<tr>
<td>3 126 and above</td>
<td>Diabetes</td>
</tr>
<tr>
<td>O GTT</td>
<td></td>
</tr>
<tr>
<td>1 139 and below</td>
<td>Normal</td>
</tr>
<tr>
<td>2 140 to 199</td>
<td>Pre-diabetes (impaired glucose tolerance)</td>
</tr>
<tr>
<td>3 200 and above</td>
<td>Diabetes</td>
</tr>
</tbody>
</table>
Complications of Diabetes mellitus

Diabetes mellitus if not properly managed, controlled or treated leads to several acute and long term complications. Acute complications of diabetes include hypoglycemia, diabetic ketoacidosis and hyperosmolar non-ketonic coma. Late complications of diabetes include diabetic retinopathy, neuropathy and nephropathy.

Diabetic ketoacidosis

Diabetic ketoacidosis is clinically defined as absolute insulin deficiency aggravated by hyperglycemia, dehydration and acidosis, thus producing derangements in intermediary metabolism. [Umpierrez, 1995] It is mostly diagnosed in type 1 diabetic patients and than in type 2 diabetes. Ketoacidosis is caused by insulin deficiency and an increase in catabolic hormone, leading to hepatic overproduction of glucose and ketone bodies. A fruity odour is often detected in the breath, is due to the respiratory system’s efforts to rid the body’s acetone. Diabetic ketoacidosis, if not promptly treated with insulin leads to coma and death [Keller, 1986].

Hyperglycemic hyperosmolar non ketotic coma

Hyperglycemic hyperosmolar nonketotic coma is commonly seen in type II diabetic patients due to relative insulin deficiency and hyperglycemia, usually > 1,000 mg/dl with associated elevated serum osmolality (>300 mosm/kg), dehydration, progressing to coma if uncorrected. Ketonuria is mild in such patients but plasma bicarbonate
and pH are reduced in them [Yares, 2003]. There is high concentration of lactic acid in blood. Prevention accomplished through education, self monitoring of blood glucose, self-care, avoidance of dehydration, awareness and avoidance of medications that may precipitate the disorder [Arieff, 1972].

**Hypoglycemia**

Hypoglycemia, also called as low blood sugar, occurs when the blood glucose level drops too low to provide enough energy for body's activities. The causes of hypoglycemia include low intake of meals or snacks, excessive doses of insulin or oral hypoglycemic agents, severe exercise and excessive drinking of alcohol. The symptoms of hypoglycemia include hunger, nervousness and shakiness, perspiration, dizziness or light headedness, sleepiness, confusion, difficulty in speaking and feeling anxious or weak. Usually hypoglycemia can easily be treated by eating or drinking something with carbohydrate. But left untreated, hypoglycemia can lead to loss of consciousness and even sometime fatal [Brownlee, 1981].

**Diabetic retinopathy**

Diabetic retinopathy is the serious eye related complication of diabetes and is the leading cause of new cases of blindness in adults due to poor management and control of blood glucose. The major signs of diabetic retinal damage include blurry or double vision, rings or black spots, dark spots and pain or pressure in one or both eyes. Diabetic retinopathy can be controlled by maintaining the blood glucose level and proper management of diabetes mellitus [Harvey, 2006].
Neuropathy

Neuropathy is a common complication of IDDM and NIDDM. This complication affects more than 60% of people with type I diabetes and leads to loss of feeling and weakness in the feet, legs, hands, and arms. Diabetic neuropathy causes problems to the digestive tract, heart, and sex organs. The best way to prevent neuropathy is to keep the blood glucose levels as close to the normal range as possible [Greena, 1992].

Diabetic nephropathy

This devastating complication of diabetes causes slow deterioration of the kidneys and their function, which can eventually lead to kidney failure. About one third of people with type I diabetes develop nephropathy. Microalbuminuria is an important marker for kidney damage. The major signs include decreased kidney function, nausea and vomiting, itchy skin, a metallic taste in the mouth, hard bone swelling in the limbs and eyelids. People with kidney failure must either have dialysis treatment or receive a kidney transplant [Grenffel, 1986].
Mechanism of Complications [Jaleel, 2005].

Four main theories have been put forth to explain the complications of diabetes.

1. Advanced Glycation end products (AGEs):- The serum levels of AGEs correlate with the level of glycemia [Leonardi O, 2003].

2. Sorbitol pathway: - Increased sorbitol concentration in cells alters redox potential, increases cellular osmolality, generates reactive oxygen species and causes cellular dysfunction [Stirbling, 1986].

3. Through protein kinase C (PKC):- Hyperglycemia increases the formation of diacylglycerol leading to activation of PKC. Along with other actions, it alters the transcription of genes for fibronectin, type IV collagen etc., in endothelial cells and neurons.

4. Through Hexosamine pathway: - Hyperglycemia increases the flux through hexosamine pathway which generates fructose-6-phosphate, which alters the function of proteins like nitric oxide synthase or gene transcription for TGF-β.

A unifying mechanism is that hyperglycemia leads to increased production of ROS in mitochondria which in turn activates all the above cited pathways.
It is well known that several plant extracts decrease blood glucose levels, due to the presence of hyperglycemic agents in their extracts.

Table 4: List of Medicinal plants with antidiabetic effects.

<table>
<thead>
<tr>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia arabica</em> (Babul)</td>
</tr>
<tr>
<td><em>Aegle marmelos</em> (Bengal Quince, Bel or Bilva)</td>
</tr>
<tr>
<td><em>Allium cepa</em> (onion)</td>
</tr>
<tr>
<td><em>Allium sativum</em> (garlic)</td>
</tr>
<tr>
<td><em>Aloe vera</em></td>
</tr>
<tr>
<td><em>Eugenia jambolana</em> (Indian gooseberry, jamun)</td>
</tr>
<tr>
<td><em>Mangifera indica</em> (Mango)</td>
</tr>
<tr>
<td><em>Momordica charantia</em> Aloe barbadensis</td>
</tr>
<tr>
<td><em>Azadirachta indica</em> (Neem)</td>
</tr>
<tr>
<td><em>Coccinia indica</em> (bitter gourd)</td>
</tr>
<tr>
<td><em>Ocimum sanctum</em> (holy basil)</td>
</tr>
<tr>
<td><em>Phyllanthus amarus</em> (bhuwwala)</td>
</tr>
<tr>
<td><em>Pterocarpus marsupium</em></td>
</tr>
<tr>
<td><em>Trigonella foenum graecum</em> [Grover, 2002; Scartezzini, 2000; Seth, 2004]</td>
</tr>
</tbody>
</table>
ANTIDIABETIC DRUGS

Insulin Preparations

1. Rapid acting insulin: Insulin Lapsro, Insulin aspart

2. Short acting insulin: Regular, Regular Humulin

3. Intermediate acting insulin: Lente humulin, Lente, NPH Humulin, NPH.

4. Premixedinsulins: Novolin70/30, Humulin 70/30, 50/50, etc


Oral hypoglycemics:

Following the observation in 1918 that guanidine had hypoglycemic effect; guanides were tried for diabetes treatment in 1962, but were abandoned a few years later for fear of hepatotoxicity. In 1930 it was hypoglycemic effect of sulfonamides was noted which later led to the discovery of sulfonylureas in 1954 [Bennet, 2003].

In 1997, the first thiazolidindione, troglitazone was introduced in to the market but was withdrawn in 2000 because of its hepatotoxic effect [Jarvinen, 2004].
Classification of oral hypoglycemic agents

I. Insulin secretagogues:

[A]. Sulfonylurea’s:
   a. First generation
      Ex: Tolbutamide, Chloropropamide, Tolazamide
   b. Second generation
      Ex: Glibenclamide, Glipizide, Glyburide

[B]. Meglitinide : Repaglinide
[C]. D-Phenylalanine derivative : Nateglinide

II. Biguanide : Metformin, Phenformin

III. Thiazolidindione : Pioglitazone, Rosiglitazone,

IV. $\alpha$-Glucosidase inhibitors : Acarbose, Miglitol
Treatment:

In case of type 1 diabetes mellitus insulin therapy is carried out while in case of type 2 diabetes mellitus oral hypoglycemic agents like sulfonylurea or biguanide can be administrated.

Laboratory models

Laboratory animals of various species have been used for study of hypoglycemic activity, including mouse, rat, guinea pig, rabbit, dog, cat etc. but rat, being omnivorous, resembles man nutritionally. Before any test is done rat should be fasted for at least 18 hours to attain stable glycemia [Laurence, 1964].

Plant extracts

*Phyllantus amarus* whole plant aqueous extract was purchased from Laila Gangaraju group, Vijayawada, and *Piper nigrum* seeds were bought from kochi. A combination of *Phyllantus amarus* and *Piper nigrum* was prepared in 8:2 ratio.
Table 5: Figures of *Phyllanthus amarus* and *Piper nigrum*

<table>
<thead>
<tr>
<th>Fig 1</th>
<th>Fig 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phyllanthus amarus</em></td>
<td><em>Piper nigrum</em></td>
</tr>
</tbody>
</table>
Table 6: Classification of *Phyllantus amarus* & *Piper nigrum*

<table>
<thead>
<tr>
<th></th>
<th><em>Phyllantus amarus</em></th>
<th><em>Piper nigrum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kingdom</strong></td>
<td>Plantae</td>
<td>Plantae</td>
</tr>
<tr>
<td><strong>Division</strong></td>
<td>Angiospermae</td>
<td>Angiospermae</td>
</tr>
<tr>
<td><strong>Class</strong></td>
<td>Dicotyledoneae</td>
<td>Dicotyledoneae</td>
</tr>
<tr>
<td><strong>Order</strong></td>
<td>Tubiflorae</td>
<td>Piperales</td>
</tr>
<tr>
<td><strong>Family</strong></td>
<td>Euphorbiaceae</td>
<td>Piperaceae</td>
</tr>
<tr>
<td><strong>Genus</strong></td>
<td>Phyllanthus</td>
<td>Piper</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>amarus Schum. &amp; Thonn.</td>
<td>nigrum Linn</td>
</tr>
<tr>
<td><strong>Part used</strong></td>
<td>Whole plant</td>
<td>Seeds</td>
</tr>
</tbody>
</table>
Phyllanthus amarus

Description:

A herb that grows up to 10-60 cm tall, widespread throughout the tropics and subtropics in sandy regions as a weed in cultivated and wastelands [Bagchi, 1992].

The plant is bitter, astringent, cooling, diuretic, stomachic, febrifuge and antiseptic. It is useful in dropsy, jaundice, diarrhoea, dysentery, intermittent fevers, and diseases of urino-genital system, scabies ulcers and wounds1-3. Alkaloids, flavonoids, geraniin, tannin which is an in vitro anti-viral agent, lignans such as hypophyllanthin, and phyllanthin were the major plant constituents. It has anti-viral, hepatoprotective, hypoglycemic activities. [Ross, 1999]

Anti hyper glycemic activity:

An aqueous extract of the leaves of Phyllanthus amarus was administered at a dosage of 5 mg/kg by oral route [Moshi, 1997]. The observation showed blood glucose lowering effect in normal and alloxan diabetic rabbits, the extract lowered blood sugar level even after the administration of glucose.
**Piper nigrum**

In Ayurveda, black paper (*Piper nigrum*), long pepper (*Piper longum*) and ginger (*Zingiber officinalis*) are termed as 'trikatu'. Ayurvedic Material Medica mentioned these three compounds as essential ingredients of many prescriptions, *Piper nigrum* also known to have hypoglycemic activity [Kaleem et al, 2005].

**Description:**

*Piper nigrum* is a climbing perennial shrub. Fruits are globose and bright red when ripe. Fruits botanically described as drupe. The plant is widely cultivated in India, Ceylon and other tropical countries [Dalby, 2002]. This climbing perennial shrub grows in hot and moist places. Black Pepper (or perhaps long pepper) was believed to cure illness such as constipation, diarrhea, earache, gangrene, heart disease, hernia, hoarseness, indigestion, insect bites, insomnia, joint pain, liver problems, lung disease, oral abscesses, sunburn, tooth decay, and toothaches [Turner, 2004].

Pepper gets its spiciness mostly from the piperine compound, which is found both in the outer fruit and in the seed. There is an increasing interest and medical need for the improvement of bioavailability of a large number of drugs which can be performed by piperine. Pepper contains volatile oil, the crystalline alkaloids, piperine, piperidine, piperettine and a resin. The minor alkaloids present are piperitine, piperolein A, piperolein B, pipermane, trichostachine. The pungency is ascribed to piperine and the resin. Its medicinal activities depend mainly on its pungent resin and volatile oil [Shoba, 1998].
**Metformin**

Metformin is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes. Evidence is also mounting for its efficacy in gestational diabetes, although safety concerns still preclude its widespread use in this setting.

**Medical uses**

Metformin is primarily used for type 2 diabetes however is increasingly being used in polycystic ovary syndrome, [Lord, 2003] non-alcoholic fatty liver disease (NAFLD) and premature puberty.

**Mechanism of action**

Metformin improves hyperglycemia primarily through its suppression of hepatic gluconeogenesis. Metformin activates AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats; activation of AMPK is required for Metformin's inhibitory effect on the production of glucose by liver cells.

Activation of AMPK is required for inhibiting the expression of the hepatic gluconeogenic genes PEPCK and Glc-6-Pase. The mechanism by which biguanides increase the activity of AMPK remains uncertain; however, research suggests that Metformin increases the amount of cytosolic AMP (as opposed to a change in total AMP or total AMP/ATP)[ Zhang, 2007].
In addition to suppressing hepatic glucose production, Metformin increases insulin sensitivity, enhances peripheral glucose uptake (by phosphorylating GLUT-4 enhancer factor), increases fatty acid oxidation, [Collier, 2006] and decreases absorption of glucose from the gastrointestinal tract. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors [Bailey, 1996].

AMPK probably also plays a role, as Metformin administration increases AMPK activity in skeletal muscle. AMPK is known to cause GLUT4 deployment to the plasma membrane, resulting in insulin-independent glucose uptake.

Some metabolic actions of Metformin do appear to occur by AMPK-independent mechanisms; a 2008 study found "the metabolic actions of Metformin in the heart muscle can occur independent of changes in AMPK activity and may be mediated by p38 MAPK- and PKC-dependent mechanisms [Saeedi et al, 2008]."
Diabetes mellitus, the nation’s third or fourth leading cause of
death, is characterized by hyperglycemia due to abnormality in
carbohydrate metabolism resulting from defect in insulin action and
secretion.

Alloxan is a glucose analogue taken through GLUT 2 transporter
causes diabetes when it is administered intraperitonially induces chemical
diabetes. Alloxan is commonly employed to induce diabetes mellitus in
experimental animals as well as to study the antidiabetic effect of
medicinal plants and their constituents. Increased oxidative stress
induced by alloxan is the possible mechanism of its diabetogenic action.

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**Molecular formula:** C₄H₂N₂O₄

Free radical induced lipid peroxidation play an important role in
the causation and complications of diabetes mellitus. Alloxan causes
diabetes through excessive generation of oxygen free radicals which
leads to damage of pancreatic beta cells.

Metformin is an oral hypoglycemic drug classified under,
biguanaides. The drug is also used in combination with a strict diet and
exercising regime for controlling high blood sugar in non-insulin
dependent diabetic patients.
An aqueous extract of the whole plant of *Phyllanthus amarus* was administered at a dosage of 10 mg/kg by oral route [Moshi et al, 1997]. The observation showed a lowering blood glucose level in alloxan diabetic rabbits.

An aqueous extract of *Piper nigrum* is known to have hypoglycemic activity which increases gradually and was observed to be maximum at the end of the study period in alloxan diabetic rats [Kaleem et al 2005].

So far certain studies were conducted for evaluating the hypoglycemic activity of Phyllantus amarus and Piper nigrum separately. But in the present study a herbal recipe mentioned in the Ayurvedic therapeutics of 17th century namely “Yogaratnakaram” was taken up for scientific evaluation, which suggests that *Phyllanthus amarus* when combined with *Piper nigrum* will give effective hypoglycemic activity[edited by Nishiteswar.k,2007].

The main objective of present study is to,

1. Evaluate the hypoglycemic activity of *Phyllanthus amarus* and *Piper nigrum*. 