1. INTRODUCTION

Diabetes mellitus is currently one of the most costly and burdensome chronic diseases and is a condition that is increasing in epidemic proportions throughout the world (King et al., 1998). The diabetes pandemic mostly affects the developing countries like India. India now stands second, next to China with the largest number of diabetic patients in the world.

According to the International Diabetes Federation (IDF) Diabetes Atlas (2011), there were an estimated 61.3 million persons with diabetes in India and this number is predicted to rise to almost 101.2 million persons by 2030. The studies on Indian population (Gupta, 2008) showed that the major risk factor for high prevalence of type 2 diabetes mellitus are, genetic predisposition, insulin resistance, obesity, central obesity (greater abdominal adiposity), urbanisation with change in diet habits and sedentary life style. Rapid urbanisation and industrialisation in developing countries such as India have resulted in dramatic lifestyle changes leading to lifestyle related diseases. Certain unique clinical and biochemical characteristics of this ethnic group collectively called as the “Asian Indian phenotype” is considered to be one of the major factors contributing to the increased predilection towards diabetes and it is estimated that every fifth person with diabetes will be an Indian (Mohan et al., 2007).

Diabetes is one of the world’s leading chronic diseases and has serious social and economic consideration. Billions of dollars are spent every year by healthcare system for treating diabetes and its complications. International Diabetes Federation (IDF) Diabetes atlas (2011) reported that the prevalence of diabetes worldwide has raised and the number would rise from 244.2 million in 2011 to 363 million in 2030.
Introduction

The countries with largest number of Diabetic People will be China, India and USA by 2030. WHO predicts that developing countries will bear the brunt of this epidemic in the 21st century, with 80% of all new cases of diabetes expected to appear in 2020. Diabetes is one of the major causes of premature death worldwide. Every ten second a person dies from diabetes related causes mainly from cardiovascular complications (Das and Rai, 2008). Hence WHO recommends for measures to be taken to control this rapidly spreading epidemic.

Table 1.1  Top ten countries for their projected number of adults with diabetes (millions)

<table>
<thead>
<tr>
<th>Country</th>
<th>Year 2011 (in millions)</th>
<th>Country</th>
<th>Year 2030 (in millions)</th>
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<tbody>
<tr>
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<td>China</td>
<td>129.7</td>
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<tr>
<td>India</td>
<td>61.3</td>
<td>India</td>
<td>101.2</td>
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<tr>
<td>U.S.A.</td>
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<td>U.S.A.</td>
<td>29.6</td>
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<td>12.6</td>
<td>Russian Federation</td>
<td>14.1</td>
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<tr>
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<td>12.6</td>
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<td>19.6</td>
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<td>16.4</td>
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<td>Indonesia</td>
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Courtesy: IDF, Diabetes atlas (2011)

1.1 Diabetes Mellitus

According to the Expert committee on diagnosis and classification of Diabetes Mellitus (1997), Diabetes mellitus is a metabolic and vascular disorder resulting from a defect in Insulin secretion, insulin action or both characterized by
chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism leading to microvascular and macrovascular complications. The word “Diabetes”, a Greek word means “to pass through” and the adjective “Mellitus”, a Latin word means “Honey” was introduced by the English Physician Rollo (1797).

According to the American Diabetes Association (ADA) (1997) for the diagnosis of diabetes, at least one criterion must apply:

- Symptoms of diabetes (polyurea, polydipsia, unexplained weight loss, etc) as well as casual plasma glucose concentration of 11.1 mmol/L (200 mg/dL).
- Fasting plasma glucose of 7.0 mmol/L (126 mg/dL), with no caloric intake for at least 8 h and 2-h plasma glucose of 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (OGTT), with the glucose load containing 75 g anhydrous glucose in water.

Hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis. Insulin deficiency causes wasting through increased breakdown and reduced synthesis of protein. Diabetic ketoacidosis is an acute emergency, which develops in the absence of insulin, because of accelerated fat breakdown to acetyl CoA, in the absence of aerobic carbohydrate metabolism which is converted to acetoacetate and β-hydroxyl butyrate (Rang and Dale, 2007). Hyperglycemia is not only due to deficiency of insulin but also an excess of certain other hormones such as growth hormones, glucocorticoids, and glucagon. Thus other than Pancreas Anterior pituitary and adrenal gland is also involved in glucose homeostasis (Cantrill and Wood, 2003).
Common symptoms of diabetes are lethargy from marked hyperglycemia, polyuria, polydipsia, weight loss, blurred vision and susceptibility to certain infections. This chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs especially the eyes, kidney, nerves, heart and blood vessels (American Diabetes association, 2004).

1.1.1 Types of Diabetes Mellitus

Type 1 Diabetes Mellitus

On the basis of etiology, patients with type 1 diabetes have little or no endogenous insulin secretory capacity and who therefore require insulin therapy for survival. The two main forms of clinical type 1 diabetes are

- Type 1a (about 90% of type 1 diabetic patients) which is thought to be due to immunological destruction of pancreatic $\beta$ cells resulting in insulin deficiency, and
- Type 1b (idiopathic, about 10% of type 1 diabetic patients), in which there is no evidence of autoimmunity (Bastaki, 2005).

Type 2 Diabetes Mellitus

Type 2 or adult onset is the commonest form of diabetes and is characterized by disorders of insulin secretion and insulin resistance (Defronso et al., 1997). Defective beta cells become exhausted, further fuelling the cycle of glucose intolerance and hyperglycemia. The etiology of Type 2 Diabetes Mellitus is multifactorial and probably genetically based, but it also has strong behavioral components.
Introduction

Gestational Diabetes mellitus (GDM)

GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Gestational diabetes occurs in about 2% of pregnancies and usually appears in second or third trimester at the time when pregnancy associated insulin antagonistic hormone peaks. After delivery, glucose tolerance usually reverts to normal (American Diabetes association, 2004).

Other specific types

a) Genetic Defects in insulin action,
   b) Disease of exocrine pancreas includes fibrocalculus,
   c) Pancreatopathy,
   d) Endocrinopathies,
   e) Drugs or chemical induced Diabetes,
   f) Infection,
   g) Uncommon forms of immune mediated, and
   h) Other genetic syndromes associated with diabetes (Bastaki, 2005).

1.1.2 Complications of diabetes

Diabetes related complications can be broadly classified as

1. Microvascular complications include neuropathy (nerve damage), nephropathy (kidney disease) and vision disorders (eg. retinopathy, glaucoma, cataract and corneal disease).
2. Macrovascular complications include heart disease, stroke and peripheral vascular disease (which can lead to ulcers, gangrene and amputation).
3. Other complications of diabetes include infections, metabolic difficulties, impotence, autonomic neuropathy and pregnancy problems.
Oxidative stress may play an important role in the pathogenesis of diabetic neuropathy, a condition characterized by pain and numbness of the extremities (Aruoma et al., 2006). Much of the effects of oxidative stress may be mediated indirectly via a reduction in nerve blood flow. Diabetic nephropathy is one of the major causes of end stage renal failure (ESRF). In diabetic nephropathy, there is thickening of glomerular basement membrane and accumulation of matrix material in the mesangium with increasing rates of urinary excretion of albumin. Diabetic retinopathy is the leading cause of new onset blindness in adults. In retinopathy, hyperglycemia increases retinal blood flow causing increased production of vasoactive substance and endothelial cell proliferation resulting in capillary closure (Abdulrazzaq, 2008).

1.1.3 Current pharmacological treatment for diabetes

The major goal in treating diabetes is to minimize any elevation of blood sugar (glucose) without causing abnormally low levels of blood sugar. Type 1 diabetes is treated with insulin, exercise, and a diabetic diet. Type 2 diabetes is treated first with weight reduction, a diabetic diet and exercise. When these measures fail to control the elevated blood sugars, oral medications are used. If oral medications are still insufficient, treatment with insulin is considered.

Insulin

In 1977, the gene for human insulin was cloned, and through modern recombinant DNA technology, manufactured human insulin was made available.

The widely used Human insulin includes
Introduction

- Rapid-acting insulin- such as insulin lispro (Eli Lilly), insulin aspart (Novo Nordisk), or insulin glulisine (Sanofi-Aventis), begin to work about 5 minutes after injection, peak in about 1 h, and continue to work for 2 to 4 h.
- Regular or Short-acting insulin- reaches the bloodstream within 30 minutes after injection, peaks anywhere from 2 to 3 h after injection, and is effective for approximately 3 to 6 h.
- Intermediate-acting insulin- generally reaches the bloodstream about 2 to 4 h after injection, peaks 4 to 12 h later and is effective for about 12 to 18 h.
- Long-acting insulin (ultra lente)- Reaches the bloodstream 6 to 10 h after injection and is usually effective for 20 to 24 h. There are also two long-acting insulin analogues, glargine and detemir. They both tend to lower glucose levels fairly evenly over a 24 h period with less of a peak of action than ultra lente (Bastaki, 2005).

The adverse effects of Insulin include IgE-mediated local cutaneous allergic reactions and hypoglycemia resulting from an inappropriately large dose.

Oral hypoglycemic Agents

Alpha-glucosidase inhibitors are oral anti-diabetic drugs used for type 2 diabetes mellitus that acts by preventing the digestion of carbohydrates (such as starch). Examples of alpha-glucosidase inhibitors include: acarbose, miglitol, voglibose etc. The delay in carbohydrate digestion increase the amount of fermentable carbohydrate reaching the colon resulting in dose-related flatulence, diarrhoea and abdominal bloating (Bastaki, 2005).

Sulfonylureas primarily lower blood glucose levels by increasing the release of insulin from the pancreas. It also further increases insulin levels by reducing hepatic clearance of the hormone (Davis and Granner, 2001), but run the risk of causing hypoglycemia, including coma. Other side effects of sulfonylureas include nausea, vomiting, cholestatic jaundice, agranulocytosis, aplastic and hemolytic anemias, generalized hypersensitivity reactions, and dermatological reactions.
Meglitinides such as repaglinide and nateglinide work on the pancreas to promote insulin secretion. Unlike sulfonylureas that bind to receptors on the insulin producing cells, meglitinides act through a separate potassium based channel on the cell surface and nateglinide has major therapeutic effect by reducing postprandial glycemic elevations in type 2 diabetes mellitus patients (Rang and Dale, 2007). Meglitinides has been associated with hypoglycemia, headaches, muscle and joint aches and sinus infections.

Biguanides like Metformin and phenformin increase glucose uptake and utilisation in skeletal muscle reducing insulin resistance and also inhibit gluconeogenesis. The commonest unwanted effects of metformin are dose-related gastrointestinal disturbances e.g. anorexia, diarrhoea, nausea. Lactic acidosis is a rare but potential fatal toxic effect, and metformin should not be given to patients with renal diseases, hepatic diseases, hypoxic pulmonary disease, heart failure or shock.

Thiazolidinediones like Pioglitazone lowers blood glucose by improving target cell response to insulin i.e., increasing the sensitivity of the cells to insulin. Thiazolidinediones increase glucose transport into muscle and adipose tissue by enhancing the synthesis and translocation of specific forms of the glucose transporters. Contraindicated in liver disease, heart failure, pregnancy and in breast-feeding women (Bastaki, 2005).

1.2 Role of herbs in diabetes management

Plants have been the major source of drugs in Indian Medicine (Shukla et al., 2000). Earliest description of curative properties of medicinal plants is found in Rig Veda (2500-1800 BC). Charaka Samhita and Sushruta Samhita give extensive description on various medicinal herbs (Kirtikar and Basu, 1975).
The World Health Organisation (WHO) has listed nearly 21,000 plants, which are used for medicinal purposes around the world. Among these, 2,500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest grower of medicinal herbs and is called as the Botanical Garden of the World (Seth and Sharma, 2004). Over the last two decades hundreds of plants used in folk medicine for diabetes have been screened for their biological activity in both in vitro and in vivo assays (Bailey and Day, 1989; Ivorra et al., 1989).

1.2.1 Clinical trials on Herbal antidiabetics

Extensive studies were carried out in animals and human by Baldwa et al. (1977) and Khanna et al. (1981) to study the antidiabetic activity of *Momordica charantia* and Shanmugasundaram et al. (1981; 1983) studied *Gymnema sylvestre*. Recently in 2009, Soumya et al. showed that *Allium sativum, Aloe vera, Artocarpus heterophyllus, Asteracanthus longifolia, Bauhinia forclita, Coccinia indica, Ficus carica, Panax quinquefolius, Myrcia uniflora, Ocimum sanctum, opuntia streptacantha, Silymarin, Trigonella foenum, Hordeum vulgare, Ginkgo biloba, Withania somnifera* etc. were some of the other medicinal plants that are tested on human subjects.

1.2.2 New trends in herbal therapy

Amylin, a polypeptide that is cosecreted with insulin have demonstrated to inhibit insulin release and muscle glycogenesis. Amylin is thought to play a major role in the disturbed metabolism associated with Diabetes Mellitus. Hence in the 32nd Congress of the International Union of Physiological Sciences (IUPS, 1993) it was reported that medicinal plants with amylin antagonism may be considered as a frontier in the search for novel antidiabetic agents for metabolic control in diabetic patients. Randle et al., (1994) has reviewed the role of disturbed free fatty acids.
metabolism as a major factor in the development of diabetes mellitus and plants showing effect on lipid metabolism can be tested for anti diabetic activity (Soumya et al., 2009).

1.2.3 Mechanisms of action of the hypoglycemic plants

Herbal drugs exhibit their hypoglycemic activity through various mechanisms similar to those of allopathic drugs as follows

- **Herbal drugs acting like insulin and those acting on insulin secreting β cells**

  Khanna et al. (1981) isolated a hypoglycemic peptide (polypeptide –p) from seeds of *Momordica charantia* which is reported as a very effective hypoglycemic agent when administered subcutaneously to langurs and humans. The aqueous extract of several culinary and medicinal plants effectively mimics *in vitro* insulin action by enhancing adipose glucose uptake (Broadhurst et al., 2000). *Canavalia brasiliensis, Diocela virgata, Diacelea rostrata* and *Cratylia floribunda* stimulated autophosphorylation of the insulin receptor *in vitro* showing “insulin like activity” (Cavada et al., 2003). *Momordica charantia* has demonstrated a beta cell regeneration capacity in streptozotocin-diabetic rats (Ahmed et al., 1998; Rotshteyn and Zito, 2004). *Gymnema sylvestre* (Shanmugasundaram et al., 1990), *Vinca rosea* (Chattopadhyay et al., 1992), and *Urtica pilulifera* (Kavalali et al., 2003) also demonstrated beta cell regeneration activities.

- **Herbal drugs decreasing intestinal glucose absorption**

  Complex carbohydrates and high molecular weight galactomannans slow intestinal absorption of glucose by decreasing gastric emptying. Leguminous plants in diet reduce blood sugar levels and cholesterol levels because of their dietary fiber content. Polyphenolic compounds reduce the dietary glucose uptake in the intestinal
epithelium and control the blood glucose levels (Shimizu, 1999). Prashanth et al. (2001) and Yamamoto et al. (2004) reported that plants inhibit alpha-glucosidase enzyme in vitro with best efficacy than acarbose without any side effect even after prolonged use of these plants.

➢ **Effect of medicinal plants on renal glucose transport**

The aqueous extract of the seeds of *Fraxinus excelsior* and leaves of *Retam reatam* exhibit potent increase of glucosuria with a parallel decrease in glycemia (Eddouks and Maghrani, 2004; Maghrani et al., 2003) similar to Phlorizin-like mechanism which inhibit renal glucose reabsorption when administered orally or intravenously.

➢ **Effect of medicinal plants on the hepatic glucose metabolism**

Pushparaj et al. (2001) reported that the activity of glucose-6-phosphatase, a key enzyme of gluconeogenesis, was significantly decreased after oral administration of aqueous extract of *Averrhoa bilimbi* in streptozotocin-induced diabetic rats. In another study, Shibib et al. (1993) showed that pretreatment of diabetic rats with *Momordica charantia* resulted in depression of hepatic gluconeogenic enzymes and regulates the blood glucose level.

➢ **Herbal drugs improving insulin resistance**

Thiazolidinediones are used to improve insulin resistance via activation of a nuclear factor regulating the transcription of genes involved in lipid and glucose metabolism known as PPARα. But failed to control insulin resistance complications such as diabetic dyslipidemia, hypercoagulation, fibrinolysis and hypertension (Yki-Jarvinen, 2004). Mae et al. (2003) reported that the ethanolic extract of *Glyrrhiza uralensis* (Licorice) exerted PPARγ ligand-binding activity leading to the amelioration
of diabetic state in KK-Ay diabetic mice (an animal model of genetically type 2 diabetes with hyperinsulinemia) and prevented hypertension in spontaneously hypertensive rats.

Herbal remedies have quite often given potential leads for developing new biologically active molecules for treatment of various diseases or disorders. Indian systems of medicine viz., Ayurveda, Siddha, Unani besides the traditional medical practices of the tribes are presently being globally reviewed in search of new drug substances, particularly for treatment of diabetes or disorders where modern medicine is not able to provide complete cure or prove safety.

**Figure I. Major site of action of hypoglycemic herbs**

(Hongxiang et al., 2009)
1.3 Selection of the plants for the study

The following herbal drugs are selected for the study as they have the reputation of being used in folk/traditional medicine to treat diabetes or their antidiabetic activity has been scientifically reported. These herbal drugs along with blood glucose lowering effect also possess hypolipidemic and antioxidant effects. The major objective of selecting these plants is that these are commonly available with minimal or no cost of procuration and hence very cost effective.

The plants used in the study are:

- Ripe fruits and seeds of *Momordica charantia*,
- Leaves of *Aloe vera*,
- Leaves of *Annona squamosa*,
- Leaves of *Gymnema sylvestre* and
- Whole plant of *Scoparia dulcis*

Bio-guided combinations of these plant extracts were analysed for antidiabetic and antioxidant activity through *in vitro* methods and based on the statistical evaluation two efficient combinations were selected. Antidiabetic and antioxidant potentials of the selected two combinations were further evaluated in STZ-induced diabetic rats. Finally, the molecular mechanism of antidiabetic activity of the selected efficient combination was analysed by determining the expression of PPARγ and GLUT4 in 3T3-L1 adipocytes.