Introduction and Literature Survey

1. Introduction and literature survey

Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by hyperglycemia with disturbances of carbohydrate and lipid metabolisms resulting from defects in insulin secretion or insulin action or both. Insulin level may be decreased due to the decrease in the beta cell mass and there may also be functional/relative disturbances of beta cells as a result of stress (WHO Expert committee on Diabetes mellitus, 1980). Diabetes mellitus was recognized and distinguished as two types even from 700-200 BC. Earlier it is believed to be a genetically based disorder or resulting due to dietary indiscretions. In the year of 1980, World Health Organization (WHO) classified the diabetes into clinical classes and statistical risk groups. Impaired glucose tolerance (IGT), diabetes mellitus and gestational diabetes are included in clinical classes. Potential abnormality of glucose tolerance is included in statistical risk groups. Degree of insulin deficiency and the etiology were used in combination to classify diabetes. Diabetes may be associated with symptoms or without symptoms depending on the severity of the metabolic abnormality. Some common symptoms are thirst, polyurea and weight loss. It may also progress to ketoacidosis and coma. Broadly diabetes has been divided into two main types, insulin-dependent or type 1 diabetes and non-insulin-dependent or type 2 diabetes (Chandra et al., 2007; Kuzuya et al., 2002).

1.1. Classification of diabetes

1.1.1. Type 1 diabetes or insulin dependent diabetes mellitus (IDDM)

Type 1 diabetes occurs due to autoimmune destruction of insulin-producing beta cells of the pancreas, rendering the pancreas unable to synthesize and secrete insulin. The symptoms of the type 1 diabetes are polyuria (frequent urination), polydipsia (increased

thirst), polyphagia (increased hunger) and loss of body weight. Type 1 diabetes affects 5 to 10% of the people (American Diabetes Association, 2008). In type 1 diabetes, insulin is required for the survival and to prevent the development of ketoacidosis and coma. Type 1 diabetes can occur in any age though common in young individuals (Kyvik et al., 2004).

1.1.2. Type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM)

Type 2 diabetes is most common and is characterized by combination of insulin resistance (physiological condition in which insulin becomes less effective in lowering the blood sugar level) and inadequate insulin secretion. Type 2 diabetes affects 90 to 95% of people. The major symptoms of type 2 diabetes are polydipsia, polyuria, polyphegia, blurry vision, fatigue and irritability (Sharma et al., 2007). Type 2 diabetes occurs mainly due to nurture and nature. The external factors are sedentary habits, inappropriate diet and obesity. In addition, mutation of pancreatic cells may occur in type 2 diabetes (Ramachandran et al., 2002; Scheen, 2003).

1.1.3 Gestational diabetes

Gestational diabetes is form of diabetes which affects pregnant women. It is believed that the hormones produced during pregnancy reduce the woman's receptivity to insulin, leading to high blood sugar level. Gestational diabetes affects 4% of pregnant women. The symptoms of gestational diabetes are fatigue, nausea, vomiting, increase urination, bladder infections and blurred vision. Gestational diabetes may be a temporary phase; it may disappear after pregnancy (American Diabetes Association, 2004).

1.1.4 Genetic defects in beta-cell function

This has been characterized by onset of mild hyperglycemia at an early age presumably before age of 25 years. This type of diabetes typically inherited in an autosomal

dominant pattern. Patients with these form of diabetes referred to as Maturity Onset Diabetes of Young (MODY) (Byrne et al., 1996). Inability in conversion of pro-insulin to insulin due to the genetic abnormalities may also results in diabetes (Clement et al., 1996).

1.1.5. Drug or chemical induced diabetes

Some drugs can produce impairment of insulin secretion. These drugs are not responsible for diabetes but their function may produce insulin resistance. Certain drugs like vacor, pentamidin etc. can destroy the beta cells responsible for insulin secretion leading to diabetes. (Yamagata et al., 1996; Byrne and Feely, 1990).

1.2. Complications of diabetes

Short term complications of diabetes are hypoglycemia, hyperglycemia and ketoacidosis. Long term complications are heart disease, eye disease, kidney disease, neuropathy, peripheral vascular disease etc.

- Cardiac complications- Heart attacks, cardiac arrest and strokes
- Diabetic retinopathy- It shows symptoms of pain in the eyes and may result in loss of vision.
- Renal disease-Body may show swelling and that may pass to all part of the body and subsequently cause increased blood pressure.
- Autonomic neuropathy- Nerves controlling internal organs get damaged.
- Chest pain- Angina may occur. Associated with is shortness of breath, dizziness, headache and stomach or shoulder pain.

1.3 Diabetic prevalence

The number of cases of diabetes mellitus in a particular area at unambiguous time is said to be diabetic prevalence. Diabetes mellitus is widely spreading all over the country due to the population growth, aging, urbanization and physical inactivity. There are some other reasons like improper diet, excess of body weight and lifestyle modifications etc.

1.3.1 Improper diet

Improper diet may contribute in the development of diabetes and may be responsible for low prevalence rate in rural sectors comparing with urban. An analysis of urban and rural diet show the main variant is fat intake (Ghafoarunissa, 1996). The fat intake especially omega-3-fatty acid is low in Indian diet. This low rate of omega-3-fatty acid consumption may be a factor in high diabetic prevalence in India (Mitra et al., 2009). It was being observed that rural Indian diet may not be a significant factor in diabetes. Indians, particularly in rural sectors consume fats and oils mainly through edible oils used in cooking (Mitra, 2008). It was observed that vegetarian diet has a role in reducing the incidence of insulin resistance (Mitra, 2005).

1.3.2 Excess of body weight

Globally a body-mass index (BMI) above 25 increases the chance of getting diabetes though in Indians the figure may be 23 or even below (Drapeau et al., 2003).

1.3.3. Modification in daily life styles

Improper life style may lead to more of visceral obesity and increase in waist diameter leading to insulin resistance. Risk of diabetes is more in urban areas due to lack of physical

activities and alteration in the life style. Proper diet along with some physical exercise will fight against the risk of getting diabetes (Mitra, 2007).

1.3.4. Prevalence of diabetes in India*

Last few years the status of diabetes has been changed from being considered as a mild disorder to one of the major causes of morbidity and mortality. The prevalence of diabetes is swiftly mounting all over the world. High prevalence of diabetes was found among urban Asian Indians (7.3%) when compared with rural prevalence rate (3.1%). In south India, Chennai (6.4%), and Trivandrum (9.2%) showed higher prevalence of diabetes. In India, 12% diabetic prevalence rate was reported in the year of 2009. Due to this rapid increase in the diabetic prevalence, India will have a predicted 80 million diabetic patients in the year of 2030 (Mohan et al., 2008; Dineshkumar et al., 2010a). In 1975, ICMR conducted a study in diabetic prevalence at rural India and results indicated that the diabetic prevalence rate was about 1.5% (Ali et al., 2009). A chronological change in prevalence of diabetes was observed from the period between 1979 and 1995 and resulted in increased level of 40% of diabetic prevalence rate (Singh et al., 1998). India is the home of largest number of diabetic patients as being observed now (PODIS: Prevalence of Diabetes in India Study). This study was focused on diabetic prevalence in urban areas especially the city of Chennai. This study was conducted among 1863 subjects from Chennai and results showed that 4.9% of diabetes prevalence was observed among the subjects. PODIS study also indicated the prevalence of diabetes was about 68.1% in urban areas and 80.8% in rural areas (Sadikot et al., 2004). Diabetes can be found in close relatives of a family due to familial aggregation. It occurred from generation to generation due to Autosomal Dominant (AD) inheritance (Raghupathy et

* Dineshkumar, B., Bhuvaneshwaran, S. P., Kumar, P. V., Mitra, A., and Manjunatha, M. (2010a) A brief description of diabetes in India, *J Pharmacy Res* (Under review).

al., 2007). A study on Maturity Onset Diabetes of Young (MODY) indicated that among the patients who were below the age of 25 years are having diabetes due to AD inheritance. MODY study showed that 10.2% of individual having AD diabetes with 56.9% of family history. 10.6% of individuals who had diabetes showed 36.1% was having one diabetic parent (either father or mother) (Mohan et al., 2009).

Madras Diabetic Research Foundation (MDRF) utilized the analytical findings from the observational studies of Chennai Urban Population Study (CUPS) and Chennai Urban Rural Epidemiology Study (CURES) to devise and to stimulate interventions with greater utilitarian benefits. CUPS showed alarming diabetes prevalence rate of 12.0% among the total number of 1399 subjects belongs to urban area of Chennai. CURES predicted diabetes prevalence rate of 15.5% among the total number of 26,001subjects belongs to rural area of Chennai (Mohan et al., 2001). In addition, not only the diabetes prevalence but also prevalence's of Coronary Artery Disease (CAD) and hypertension were compared with urban and in rural areas. More prevalence rate was found in urban areas. They are more frequent with patients associated with diabetes than with the non-diabetic patients. Excess of body weight and obesity, sedentary lifestyle, higher visible fat intake was significantly associated with diabetes (Boddula et al., 2008). Type 2 diabetic prevalence will be high among Indian adults (7.7%, 439 million adults) in the year of 2030 due to the demographic changes such as ageing and risk factors (obesity and sedentary life style). Suitable remedy has to be identified to control the alacrity of the increasing prevalence (Ahuja, 1991).

1.3.5. Prevalence of diabetes in Bengal [*]

Diabetic prevalence in rural Bengal is in between 3.5% - 5.7%. Three districts of West Bengal have high prevalence of diabetes-Howrah (13.2%), Kolkata (12%) and Burdwan (8.7%). Prevalence is comparatively low in Purulia (2.7%), Bankura (3.0%), Dinajpur South (3.6%) and Dinajpur North (3.5%). Rural Bengal shows that Muslims have lowest prevalence (4.8%) and it is highest in Hindus (5.4%) while Christians (5.1%) in between. The prevalence of diabetes is more in meat eaters (7.2%). It is most in pork eaters (7.6%), intermediate in chicken eaters (6.4%) and lowest in those who take goat/sheep (6.1%). In vegetarians it is 5.8% and in fish eaters it is 5.2%. In the vegetarians those who consume ghee it is least (3.8%) while it is highest in those who take coconut oil or groundnut oil (6.8%), in mustard oil consumers it is 5.6%. This study indicated that changes in socio-economic pattern and life styles may be important to prevent the prevalence of diabetes in West Bengal (Pradhan et al., 2009).

1.3.6. Tests used for diagnosis of diabetes

- **Fasting plasma glucose (FPG) test:**

  The fasting plasma glucose (FPG) test measures blood sugar levels and is used to diagnose diabetes. Relatively simple and inexpensive, the test exposes problems with insulin functioning. It is most reliable test when done in the morning. People with a fasting glucose level of 100-125 milligrams per deciliter (mg/dL) have a form of pre-diabetes called Impaired Fasting Glucose (IFG).

  If a person has IFG then he has an increased risk of developing type 2 diabetes. A level of 126 mg/dL or above is confirmed by repeating the test on another day to

[*] Pradhan, R., Dineshkumar, B., and Mitra, A. (2009) Some salient points in Type 2 diabetes prevalence in rural Bengal, *Studies on Ethno-Medicine 3*, 127–31.

confirm the diabetes (Diabetes diagnosis, Fasting plasma glucose test, 2009). The results are predicted as:

Plasma glucose level is 99mg/dl or below → diagnosed as normal.

Plasma glucose level is 100-125 mg/dl → diagnosed as pre-diabetes.

Plasma glucose level is 126 mg/dl → diagnosed as diabetes.

- **Oral glucose tolerance test (OGTT)**

The test had been done two times. First test is done to the patient fasting for at least 8 hours and the second test is done to the patient 2 hours after drinking a glucose-containing beverage containing 75 grams of glucose dissolved in water. It is used to diagnose both pre-diabetes and diabetes. (The oral glucose tolerance test, 2008).

Results are predicted as:

Plasma glucose level is 139 mg/dl and below → diagnosed as normal.

Plasma glucose level is 140-199 mg/dl → diagnosed as pre-diabetes.

- **Random plasma glucose test**

Random plasma glucose test also called as casual plasma glucose test. It is used to diagnose diabetes along with an assessment of symptoms. But pre-diabetes diagnosis is not done. The results have to be confirmed by performing the second test on a different day (Random plasma glucose test, 2010). A random, or casual, blood glucose level of 200 mg/dl or higher, plus the presence of the following symptoms, can mean a person has diabetes,

  - increased urination
  - increased thirst and unexplained weight loss

- **Gestational diabetes detection**

Diagnosis has been done based on plasma glucose values. The test is done during pregnancy period, preferably by using 100 grams of glucose in liquid. Blood glucose levels are tested four times during the test. If the test result showed glucose level as above the normal level for two times while performing the test then the women has gestational diabetes (American Diabetes Association 2003).

1.3.7. Drugs used for the treatment of Diabetes

Sulphonylureas

It primarily stimulates the pancreatic insulin secretion that in turn reduces hepatic glucose output and increases peripheral glucose disposal. They are classified into first generation and second generation sulphonylureas.

First generation sulphonylureas	*Second generation sulphonylureas*
a) Acetohexamide	a) Glibenclamide
b) Chlorpromide	b) Glyburide
c) Tolazamide	c) Glipizide
d) Tolbutamide	d) Glicazide

Mode of action:

It is also called as insulin secret gouges. They initially stimulate the pancreatic insulin secretion that in turn reduces hepatic glucose output and increases peripheral glucose disposal. They block the potassium channel and allow the influx of calcium to the pancreatic beta-islets of langerhans cells. As a result an increased amount of insulin is released. After several months, insulin level in the blood will return to premedication level but the glucose level in the blood will remain in a reduced state. The drug is also having some other functions like, the rate of release of glucose in the blood stream is slowed, they increase the number of

insulin receptors on the cell membrane. The second generation sulphonylureas have some advantages than first generation sulphonylureas. Even a small milligram of the drug is more efficient and they have low side effects. Single dose for a day is more effective than second generation that enables the compliance.

Biguanidines

The biguanidine drugs were introduced in 1957. Phenformin and metformin are the two groups of biguanidine. They suppress excessive hepatic glucose production and increased utilization of glucose in the peripheral blood. When they are used alone, they are called as anti-hyperglycemic agents rather than hypoglycemic agents because they do not stimulate endogenous insulin secretion and thus hypoglycemia does not occur.

Mode of action

Metformin and phenformin are widely used. Eventhough it may increase the glucose utilization in peripheral cells to a lesser degree by decreasing insulin resistance in muscle cells, they mainly suppress the excessive glucose production. When it is taken alone hypoglycemia will not occur because it does not stimulate endogenous insulin secretion. Hypoglycemia may occur if it is taken along with insulin or sulfonylurea. Metformin have some additional functions like lowering triglyceride and low density lipoprotein (LDL) cholesterol levels while increasing high density lipoprotein cholesterol (HDL). This drug particularly helps type 2 diabetes patients.

Alpha-glucosidase inhibitors

Acarbose is an example for alpha-glucosidase inhibitor. It is used in management of post-prandial hyperglycemia. The breakdown of disaccharides, polysaccharides and other carbohydrates to monosaccharide is slowed down by acarbose. The enzymatic generation and subsequent glucose absorption is reduced. Alpha-glucosidase inhibitor does not prevent the absorption of carbohydrates but it delays the process. Lowering of total insulin output of the

pancreas, increased insulin sensitivity, mild decrease in triglycerides, no side effects to patients are some of the functions of alpha-glucosidase inhibitors.

Meglitinide

It is ultra short acting drug that acts directly on the beta cells of pancreas and increase the secretion of insulin. The problem of pulsatile release of insulin in type 2 diabetes patients is corrected by this drug. It blocks the potassium channels on the pancreatic islet-beta cells. As a result, the secretion of insulin is increased by the influx of calcium into the cells.

Thiazolidinediones (TZD)

Increased insulin sensitivity and increased glucose uptake are the primary functions of TZD. The TZD have some effects on hepatic glucose uptake. They will not stimulate pancreas to secrete more insulin. The group has been hepatically metabolized and has minimal side effects. It can be dosed once a day. They have notable effects on lipids. They have minimal effects on LDL, triglyceride and favorable effects on HDL. Rosiglitazone and pioglitazone are approved for monotherapy and also being used along with metformin and insulin (Prato and Pulizzi, 2006; Sweet success: Diabetes and pregnancy news letter, 2008; Lebowitz, 2005).

Pramlintide

By augmenting endogenous amylin, pramlintide aids in the absorption of glucose by slowing of gastric emptying, promoting satiety via hypothalamic receptors (different receptors than for GLP-1), and inhibiting inappropriate secretion of glucagon, a catabolic hormone that opposes the effects of insulin and amylin. Pramlintide is an analogue of amylin, a small peptide hormone that is released into the bloodstream by the β-cells of the pancreas along with insulin usually after a meal. Like insulin, amylin is deficient in individuals with diabetes.

Exenatide

Exenatide displays biological properties similar to human glucagon-like peptide-1 (GLP-1), a regulator of glucose metabolism and insulin secretion. According to the package insert, exenatide enhances glucose-dependent insulin secretion by the pancreatic beta-cell, suppresses inappropriately elevated glucagon secretion, and slows gastric emptying, although the mechanism of action is still under study.

1.4. Chronological developments in diabetes management research

Diabetes has been with human race from long back. It is not a newly born disease. We came to knew about it in 1552 B.C. Since this period, many of Greek as well French physicians had worked on it and made us aware of the nature of disease and the organs responsible for it. In 1870s, a French physician had discovered link between diabetes and diet intake. Due to this reason, formulation of individual diet plan came into picture. Diabetes was recognized as a devastating and deadly disease for nearly 2000 years. Aretaeus described the destructive nature of the affliction which he named diabetes from the Greek word for "siphon" in the first century A.D. Ancient time physicians like Aretaeus recognized the symptoms of diabetes but were powerless to effectively treat it. Aretaeus recommended oil of roses, dates, raw quinces and gruel. In 1900-1915, diabetic diet was formulated with inclusion of milk, oats and other fiber containing foods. Function of insulin**,** its nature, along with its use started from 1920 -1923. Effective management strategy of diabetes got momentum after the discovery of insulin at University of Toronto in 1921 – 1922 by Dr. Banting, Prof. Macleod and Dr .Collip. In the decade of 1940, it has been observed that different organs like kidney and skin are also affected if diabetes is creeping for a long term. Oral hypoglycemic drugs have been manufactured in the year of 1955. Scientists are

continuously working to relieve patients from diabetes, by discovering the relevant drugs and making new researches (Tables 1.1).

1.4.1. Non-communicable diseases (NCD) in India

Prevalence of type 2 diabetes, commonly known as maturity onset diabetes or adult diabetes is in explosive rise at the Indian subcontinent. The exact etio-pathological reasons being unknown and varied factors both nature and nurture are being blamed. Niles thrifty hypothesis and genetic variations are under closed study by a group of workers. Other groups laid emphasis in nurture and focus lifestyle, diet and environment. Dilman (1989) through a series of articles published in Lancet had claimed hypothalamus to be a failing rheostat due to constant stress (ontogenetic and adaptational model). Spectrum of disease is a graphic representation of variations in the manifestation of disease. Figure 1.1 describes disease in a community, which can be compared with an iceberg. The floating tip of the iceberg represents the clinical cases. The vast submerged portion of the iceberg represents the latent and in-apparent, pre-symptomatic, undiagnosed cases and carriers in the community. The water line represents demarcation between apparent and in-apparent diseases. In some diseases (hypertension, diabetes, anemia, malnutrition, mental illness etc.) the unknown morbidity, i.e., the submerged portion of the iceberg far exceeds the known morbidity. The hidden part of the iceberg thus constitutes an important, undiagnosed reservoir of infection or diseases in the community and its detection as well as control is a challenge to modern world. India and other developing countries are in the process of epidemiologic transition. Hence, there is a need to observe the current status of Non-Communicable Diseases (NCD) in India. NCD will account for 73% of deaths and 60% of the global disease burden by 2020, and will account for a major proportion of disease and deaths in India. Deaths are mostly due to heart disease, strokes, diabetes mellitus, cancers and lung diseases. It is estimated that India

accounts for 17% of global cardiovascular mortality, and this is projected to rise to 50% in the future (Reddy and Yusuf, 1998). NCD patterns over several decades suggest that there is a steady increase in NCD in India. Diabetes prevalence is increasing at an alarming rate and there is a prominent rural-urban divide for most cases, although CURE studies in Chennai indicates that there is a progressive flattening of the rate of increase of diabetes and impaired glucose tolerance over the last 15 years, particularly for the latter (Ramachandran, 2005; Gupta and Gupta, 1997; Mohan et al., 2006). Commonly associated with in persons with insulin resistance (precursor of type 2 diabetes) are obesity, hypertension, dyslipidaemia and atherosclerosis, coronary arterial and cerebro-vascular disorders (CAD and CVD). The phenotypic expression of the genetically predisposed individual depends on the degree of gene expression, insulin resistance and hyper-insulinemia. Consequentially, both genetic tendencies as well as hyperinsulinemia/ insulin resistance have a role in the management of these disorders. A person with insulin resistance may not be resistant to all actions of insulin and increase in insulin values may lead to more anabolic effects particularly on arterial smooth muscles, leading to proliferation of these cells and atherosclerosis. There is a positive correlation between systolic blood pressures and 2-hour post-prandial insulin concentrations in hypertensive obese individuals. The defect in insulin resistance may be at post receptor level in impaired glucose tolerance stage and at the receptor and post-receptor level in frank type 2 diabetes.

The impact of NCD is very high particularly considering their chronic nature and impact on individuals, families and the society. The framework for the development of NCD, while including genetic causes, links lifestyle factors such as an increased energy intake, and a decreased physical activity, to the development of obesity, which in turn is related to the development of NCD. There is every reason to believe that the dual burden of under and over-nutrition exists in communities and families of every countries which have undergone a

nutritional and epidemiologic transition. An unexplored area of research is the role of the nutrition transition in the genesis of NCD in the individuals who were previously chronic energy deficient. The fetal programming theory is a relevant explanation for the onset of NCD, lifecycle interventions relevant to the high prevalence of low-birth weight in India and its link to NCD need to be assessed (Rich-Edwards et al., 1999; Bhargava et al., 2004). In particular, diet, physical activity and 'social' variables including social support systems and psychological variables such as depression have been inadequately documented, along with biochemical and genetic markers of diabetes. Identification of candidate genes for diabetes and atherosclerosis are particularly exciting as prospective rural urban comparisons will allow for the study of gene-environment interactions in two groups which are presumed to be genetically similar, but with very divergent lifestyles (Sharma, 2008).

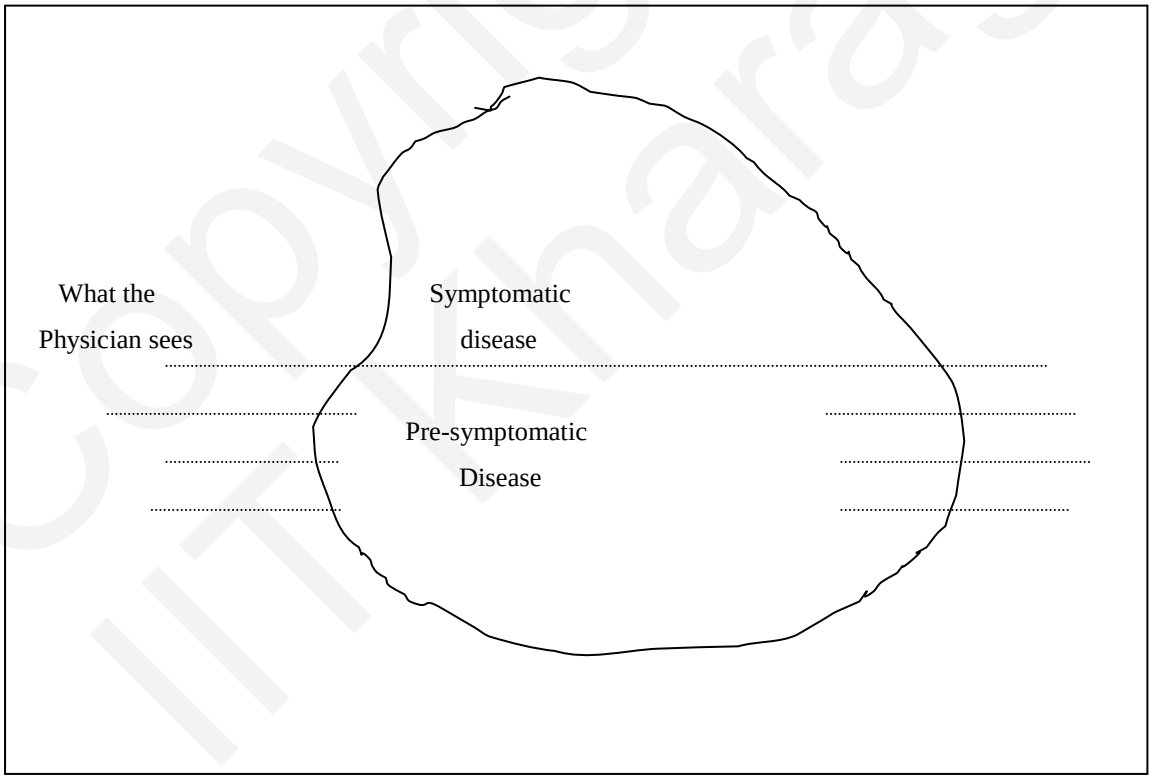

Figure 1.1. **Disease in a community and its different phases (Park, 2000)**

1.4.2. Management Policies for Chronic Diseases

- Clinic management of specific chronic condition,
- Care of patient with multiple chronic condition and frail elderly people,
- Practice base interventions that address chronic disease care, for example, informatics, strategies, patient education (Davis et al, 1999).

Table 1. 1. Post Insulin Era

Year of the research	Description of the work
1921	Pancreatic extracts demonstrated to lower blood sugar levels in experimental diabetic dogs (Banting and Best)
1922	Insulin first used in human (Leonard Thompson)
1923	"Iso-electric point" produced larger quantities of higher potency insulin from animal sources - enough to satisfy commercial need (Lilly)
1925	First international insulin unit defined (one unit = 0.125 mg of standard material) U-40/80 insulin became available
1926	Crystallized amorphous insulin adds to insulin stability (Abel)
1936	Addition of zinc to protamine insulin (P 21) to create a prolonged duration of action of the hormone (Scoott, Fisher, Hagedorn)
1939	Globin insulin with a shorter duration of action than P 21 developed
1950	N P H (Neutral Protamine Hagedorn) insulin developed with controlled amounts of protamine (Nordisk)
1951	Lente insulin developed by acetate buffering of zinc insulin (Novo, Hollas-Mollar)
1955	Structure of insulin delineated (Stanger and Co-workers)
1960	Radio-immuno assay of insulin became available (Berson and Yalow)
1967	Pro-insulin discovered (Steiner and Oyez)
1971	Insulin receptor defined (Roth, Cuatrecasus and co-workers)
1972	U-hundred insulin introduced to promote better accuracy in administration
1973	Small dose intravenous insulin treatment for acidosis emerges as alternative to large dose subcutaneous treatment
1976	C-peptide became clinical tool
1977	Insulin gene cloned (Ullrich, Rutter, Goodman and co-workers)
1978	Purified "Single-peak" Pork insulin introduced (Lilly)
1979	Open loop insulin delivery system clinically available (Timberline)
1981	Insulin receptor kinase activity described (Kasaga, Kahn and co-workers)
1982	Re-combinant DNA insulin available (Lilly) and enzymatic conversion of pork insulin sequence to human insulin sequence developed (Novo)
1990-2001	Re-combinant DNA insulin from fungus Lispro Insulin Re-combinant DNA insulin from plants (Gene gun technique and others) Oral insulin available as a pill in an acrylic-based gel like coating-tested in diabetic rats and dogs at Nicholas University by Aron Fross Insulin glargine, a recombinant analogue of human insulin with a shift in isoelectric point producing an action similar to normal basal insulin secretion.
2001-2010	Newer oral anti-diabetic drugs Grade IV trial in progress of oral insulin

	Plant cultured insulin Insulin analogues Automated synchronized MEMS based insulin delivery system Use of Nano-technology

Sources: Kahn, 1996; Naik, 2001; Rosenstock, 2001; Hammilton-Wescler et al. 1999; Shah, 2001

1.4.3. Management of Diabetes

A variety of technologies have been developed to support diabetes patient's efforts at self care and provide an alternative to traditional education occurring within outpatient clinics. Many tips had been followed by patients for the management of diabetes mellitus; to keep blood glucose in control for the diabetes patients throughout the day one may need: diet modifications, regular exercises, medicines (tablets/insulin injections/alternative medicines). An emotional stress (a death in family, displeasure at work or at home) may increase and disturb the control of diabetes. Patients need to discuss the problem with their doctors for suitable adjustments in dosage of medications and stress control exercises. The main aim of Diabetes Self Management Education (DSME) is to help patients to take care of their health by educating them by improving their knowledge and skills for self directed behavioral changes that will enable them to integrate self management in their daily life and ultimately reduce the risk of complications. By keeping a good control of diabetes at all times, patients will be able to prevent the complications of diabetes affecting the nerves, eyes, kidneys, heart and blood vessels (Wong et al., 2009).

1.5. Dietary management of diabetes considering rural India (role of vegetable oils)*

The prevalence of diabetes varies throughout India. Nearly 12% of the adult population in Delhi, 10% in Mumbai, 12.5% in Bangalore, 13.5% in Chennai, and 16% in

* Dineshkumar, B., Bhuvaneshwaran, S. P., Vigneshkumar, P., Mitra, A. (2010b) Management of diabetes with some edible oils in India, International Journal of Biosciences, Healthcare Technology and Management (Communicated).

Hyderabad showed the prevalence of diabetes (Mukherjee et al., 2009). Pradhan et al. (2009) have found that the overall prevalence of diabetes in different districts of rural Bengal is between 5% and 10% when both rural and urban populations are considered. Many authors have reported on the basis of short-term studies that the rural Indian diet may be diabetogenic in nature; therefore, diet could play a significant role in controlling the disease. An increase in the protein content of the diet not only reduces the prevalence of diabetes and is easier to comply with and is more satiating (Mitra and Bhattacharya, 2005; Raheja et al., 1970; Sanders et al., 1985; Ghafoarunissa, 1996). In south Asian populations, it has been reported that the diet has a significant role in the development of insulin resistance syndrome, dyslipidemia, and subclinical inflammation (Mitra and Bhattacharya, 2006; Luscombe et al., 2002).

Several dietary imbalances, such as a low intake of monounsaturated fatty acids (MUFA), omega-3 polyunsaturated fatty acids (PUFAs) and fibre and a high intake of fats, saturated fats, carbohydrates and *trans*-fatty acids, increase insulin resistance. A high intake of omega-6 PUFAs has been correlated with fasting hyperinsulinemia, specifically in children and young individuals (Misra et al., 2009). It has been advocated that a low-fat diet is good for health, but it has the disadvantage of lowering high-density lipoprotein–cholesterol (HDL-C) levels in the blood (Ornish, 1996). Low intake of omega-6 PUFAs in the diet helps maintain normal BMI and reduce insulin resistance in Asian adolescents and young adults. Dyslipidemia in Asian Indians is usually characterized by hypertriglyceridemia, low levels of HDL-C, and high levels of small, dense LDL-C, which frequently occur with postprandial hyperlipidemia. Excess postprandial lipid levels are considered a component of insulin resistance syndrome (Garg et al., 1994; Dineshkumar et al., 2010b). Boden et al. (2005) showed that, in a small group of obese patients with type 2 diabetes, a low-carbohydrate diet, along with reduced caloric intake, for 2 weeks resulted in a spontaneous reduction in energy

intake to a level appropriate for patient height and weight loss, as well as significant improvements in 24-h blood glucose profiles, insulin sensitivity, and hemoglobin A_{1c}, with decreased plasma TG and bad cholesterol levels. Garg et al., (1988) reported that partial replacement of complex carbohydrates with MUFA in the diet of patients with type 2 diabetes do not increase LDL-C concentrations and may improve glycemic control, as well as plasma TG and HDL-C levels. Dietary recommendations to prevent type 2 diabetes should focus more on the quality of the fats and carbohydrates in the diet rather than the quantity alone, as well as taking into consideration a balance between total energy intake and expenditure to prevent overweight and obesity (Hu et al., 2001).

Teuscher et al. (1987) observed that high carbohydrate/cassava intake combined with low protein consumption (8% caloric supply) does not cause diabetes. Dietary modifications were relied more than exercises among the rural Indians. Most of the lacunae in knowledge prevailed in drug therapy of diabetes (Shah et al., 2009). In rural India changes in diet is principally in fats intake. In India an average human being consume 13-20 gram of fat through his daily diet. This consumption helps to derive 30-40% of total energy. The recommended daily dietary allowance of fat by ICMR (Indian Council for Medical Research) for a normal human being is 34g. A wide variation in dietary fat consumption levels in different areas of India has been observed to be 9.5 g in Karnataka, 10.5 g in Tamilnadu, 1.0 g in Maharashtra, 18.0 g in Gujarat, 21.7 g in Kerala and 25.7 g in Kolkata (Punekar, 1985). In India, the fat intake in rural diet is low. Especially omega-3-fatty acid and omega-6- fatty acid intake is very low in the rural diet with a high amount of carbohydrate content (Mitra et al., 2007). In India, edible oil such as sesame oil, sunflower oil, coconut oil, ghee, mustard oil, palm oil, peanut oil and soya bean oil are being used widely in the preparation of food. Edible oil acts as a source of essential fatty acids which cannot be synthesized by our body. Edible oil has high nutritional value due to presence of saturated and unsaturated fatty acids

(mainly Omeag-3-fatty acid and Omega-6-fatty acid). Every year 10 million tons of edible oil has been consumed by Indian people (Ramesh and Murughan, 2008). Optimum consumption of edible oils may help in control of diabetes in rural Indian people. Consumption of proper edible oil with diet may also be low cost treatment for diabetes**.**

1.6 Health management strategies in India using alternative medicine particularly plants

Indian rural society is a dynamic conglomeration of traditional values and modernization. While traditional values are welcomed in rural sectors, in urban sector modernization is sweeping and it infiltrates to the villages. As a result Western medicine is getting wide acceptance but affordability is of concern. Lacunae of Western medicine are inability to provide selective remedies in chronic, degenerative, auto-immune, viral, neo-plastic and other diseases. Indian society believes more in results than scientific validations. Ayurveda and plant based medicine is being practiced from Vedic civilizations in India and is flowing through generations. Patanjali, the famous Yogi, pioneers the idea of Yogic Cure Therapy. Unani gained momentum from 6^{th} century BC when Muslims invaded India (Mitra, 2008).

The Indian Systems of Medicine and Homoeopathy were given an independent identity in the Ministry of Health and Family Welfare in 1995 by creating a separate Department, which was renamed as Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) in November 2003 and is entrusted with the responsibility of developing and propagating these ancient and holistic systems towards the health care of the people. These systems have marked superiority in addressing chronic conditions and offer a package of promotive and preventive interventions. An outlay of Rs.775 crore has been allocated for the Department during the Tenth Five-year Plan. The Plan allocation for 2006-07 was Rs. 381.60 crore (AYUSH, 2010). It is actively engaged in

conducting clinical researches in health care, drug research, survey and cultivation of medicinal plants, pharmacognosy, phyto-chemistry, pharmacology, toxicology, drug standardization and literary research for revival of the ancient classical literature.

Three important events connected with the current status of herbal medicine include: the Alma-ata Declaration (WHO, 1978); the manual on quality control methods for medicinal plant materials and the general guidelines for methodologies on research and evaluation of traditional medicine (WHO, 1998). Currently, medicinal plant research is a rapid growing area. This is illustrated by the fact that the number of citations in PubMed from 1990-2007 containing the word "phytotherapy" was less than 100 in 1990, but rose to over 1000 in 1998, then to about 12000 in 2005, and to over 15000 in 2007 (Wikipedia-phytotherapy, 2008). In 1999, the world market for herbal remedies in different countries were in US $19.4 billion, with Europe in the lead (US$6.7 billion), followed by Asia (US$5.1 billion), North America (US$4.0 billion), Japan (US$2.2 billion) and the rest of the world (US$1.4 billion). Currently over 50, 000 plants are in use for medicinal purposes worldwide (Current status of medicinal plants, 2003). The World Health Organization had over time produced a large volume of data on how medicinal plant materials and their products should be handled, starting from the collection of materials, through manufacturing and then to clinical trials. These guidelines are intended to facilitate the work of regulatory authorities, scientific academies, industries and inter-regional agencies concerned with the regulation and trade of herbal medicines. It is purposive that assessment by these agencies would reflect current scientific knowledge and that such assessments would form the basis for classifying herbal medicines for the purpose of regulation, trade and other purposes. The issues raised (WHO, 2000) in assessing the efficacies of herbal medicines are:

1. Whether the ingredients and their pharmacological actions are known and whether these have any relations with observed clinical results.

2. Whether the indications for use of the medicine are specified. Evidence that such indications are evidence-based must be rigorously sought, unless they relate to minor disorders, unspecific complaints or prophylactic use.
3. If long-term traditional use has not been established, it is needful to seek fresh clinical evidence.

Considering the WHO guidelines AYUSH has following guidelines:

1. Increase in area under cultivation of medicinal plants of AYUSH systems – The government has approved a National Mission of Medicinal Plants at a total outlay of Rs. 640 crores for the 11th Plan. This envisages among other things cultivation of medicinal plants for which the best indicator of success in the short term is the area brought under cultivation, which would lead to assured availability of crude resources.
2. Increase in area under conservation of rare & endangered species of medicinal plants – Endangered species of medicinal plants are in deep crisis considering the availability of medicinal plants on the whole. Conservation of such species is imperative and has additional area covered under conservation has an important industry. Average number of incidents of biotic /abiotic interference per area annually would provide a good measure of the success or otherwise of the conservation efforts.
3. Adoption of organic and good agriculture & collection practices – This would lead to better resource management by sensitizing farmers, growers and other stakeholders.
4. Species coverage under conservation & cultivation – there is a need for authentic data especially on trade and commerce of medicinal plants.

5. For standardization and testing of drugs, various agencies have been put in plan by the Government of India. Four different pharmacopoeia committees are working for preparing official formularies / pharmacopoeias to evolve uniform standards in preparation of drugs of Ayurveda, Siddha, Unani and Homeopathy and to prescribe working standards for single drugs as well as compound formulations. A drug quality control cell is working in the department to deal with the matters pertaining to licensing, regulation and control of drugs and the spurious manufacture of Ayurvedic, Unani and Siddha Drugs and other matters. Two apex Laboratories, namely, Pharmacopoeial Laboratory for Indian Medicine (PLIM) and Homoeopathic Pharmacopoeial Laboratory (HPL) are functioning as Standard Setting-Cum-Drug-testing Laboratories for Indian Medicines and Homoeopathy respectively. Indian Medicines Pharmaceutical Corporation Ltd. (IMPCL), a Public Sector Undertaking, manufactures classical Ayurveda and Unani drugs. Every Herbal Formulation must be standardized as per WHO guidelines. The objective of WHO guidelines is to define basic criteria for the evaluation of quality, safety and efficacy of drugs herbal medicines (Phillipsion, 1989). The Siddha system of medicine uses around 600, Ayurveda 700, Unani 700 and modern medicine about 30 plants species. In India, 60% of registered physicians are involved in non-allopathic systems of medicine. In addition to the nearly 400,000 Ayurvedic practitioners, there are over 170,000 homeopathic physicians; India has about 500,000 medical doctors. Reliance on Ayurvedic medicine is heavy in certain regions of India, such as Kerala in the Southwest (Kottakkal Arya Vaidya Sala, 2010). Many Ayurvedic practitioners in small villages are not registered. Projection is being made that after information technology, herbal technology will be India's biggest revenue earner (Chaudhri, 1996).

An estimate of WHO demonstrates about 80% of world population depends on natural products for their health care, because of side effects and high cost of modern medicine (Satakopan, 1994). World Health Organization currently recommends and encourages traditional herbal remedies in natural health care programs because these drugs are easily available at low cost and are comparatively safe. Due to the contribution of numerous significant factors, the market of herbal medicines has grown at an expressive rate worldwide. Some of them are: preference of consumers for natural therapies; concern regarding undesirable side effects of modern medicines and the belief that herbal drugs are free from side effects since millions of people all over the world have been using herbal medicines for thousands of years; great interest in alternative medicines due to traditional acceptance; preference of populations for preventive medicine due to increasing population age; the belief that herbal medicines might be of effective benefit in the treatment of certain diseases where conventional therapies and medicines have proven to be inadequate; tendency towards self-medication; improvement in quality, proof of efficacy and safety of herbal medicines and high cost of synthetic medicines (Calixto, 2000). Indian Ayurveda medicine has used herbs such as turmeric as early as 1900 B.C (Aggarwal et al, 2007). Many other herbs and minerals used in Ayurveda were later described by ancient Indian Vedic researchers such as Charaka and Sushruta during the 1st millenium BC. The Sushruta Samhita attributed to Sushruta in the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources (Girish and Shridhar, 2007). The second millennium, however, also saw the beginning of a slow erosion of the leading position held by plants as sources of therapeutic effects. This began with the Black Death, which the then dominant Four Element medical system. A century later, Paracelsus introduced the use of active chemicals as drugs (like arsenic, copper sulfate, iron, mercury, and sulphur). These were accepted even though they had toxic effects as they are

effective to treat Syphilis considered the most dreaded disease. The rapid development of chemistry and the other physical sciences, led increasingly to the dominance of chemotherapy – chemical based medicines - as the orthodox system based on evidence of the twentieth century. Present day herblite must use proper technology to free the medicine from toxic contaminants and it must be evidence based. The knowledge about and use of alternative medicine is widespread among future health care professionals and the students also expressed a strong interest in the topic. In-depth studies on the knowledge and use of alternative medicine among other health care professionals and among ethnic minorities are urgently needed and may help to better manage the treatment of minor disorders as well as chronic diseases (Heike et al., 2006).

1.7. Screening of plants having anti-diabetic effects at close vicinity of IIT Kharagpur Campus*

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethno botanical information reports about 800 plants that may possess anti-diabetic potential (Grover at al., 2002). Apart from these there are plenty of plants with anti-diabetic activities. Some commonly available plants having anti-diabetic effects present in the Indian Institute of Technology (IIT) Kharagpur campus are: *Acalypha indica*, *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Musa sapientum*, *Mangifera indica*, *Murraya koenigii*, *Ocimum sanctum*, *Phyllanthus amarus*, and *Tinospora cordifolia*. They are selected, after being authenticated by herbalist, for the purpose of screening the bio-active components. It is being observed that the active principles from the plant sources have various mechanisms of actions to elucidate the anti-diabetic effects (Dineshkumar et al., 2010c). These may be:

* Dineshkumar B., Mitra, A., Manjunatha M. (2010c) A Comparative Study of Alpha Amylase Inhibitory Activities of Common Anti-diabetic Plants at Kharagpur 1 Block. *International Journal of Green Pharmacy 4*, 115-121.

1. Act on the β-cells of the pancreas and to stimulate the secretion of insulin,
2. Inhibit α-cells for the release of hyperglycemic factors like glucagon,
3. Enhance the effect of insulin,
4. Defer the process of digestion and consequent glucose release thereon,
5. Assist in inhibiting the synthesis of various enzymes like glucose-6-phosphatase, fructose diphosphatase and pyruvate carboxylase

1.8. Isolation and characterization of bio-active components from plants*

Large numbers of phyto-compounds are biologically active in nature. The biological active compounds may be polyphenols, terpenoids, alkaloids, enzymes, proteins, aminoacids and sugars. The quantitative estimation and qualitative effects of active plant products depends on various factors particularly the geographic location, surrounding environment, plant cycles etc. Hence, the effects of bio-active components need to be validated based on geographic locations for predicting the possible outcome (Hoareau and Dasilva, 1999). These compounds may present in the crude form and can be isolated and purified after extraction followed by chromatographic techniques. Bio-active compounds from medicinal plants can be extracted by various types of extraction methods like soxhlation, maceration, percolation, infusion and decoction. Separation and purification of biological active compounds can be done by chromatographic techniques like column chromatography, paper chromatography, Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) and High Performance Thin Layer Chromatography (HPTLC). High Performance Thin Layer Chromatography (HPTLC) is used

* Dineshkumar, B., Kumar P. V., Bhuvaneshwaran, S. P., and Mitra, A. (2010d) Isolation and characterization of Natural products from Medicinal plants-A Review, *Journal of Natural Remedies* (Under review).

to detect and quantifying the compounds present in the extracts. High Performance Liquid Chromatography (HPLC) involves applying high pressure for the more separation of compounds. Gas Chromatography (GC) is used in the separation of volatile compounds. The isolated biological active compounds are characterized by analyzing the functional groups present in them, determining the molecular weight and identifying the structure of the unknown compounds. Structure elucidation tools such as Fourier Transform Infra Red spectroscopy (FTIR) is used to determine the functional group of the compounds. Mass Spectrometry (MS) is used in the determination of the molecular weight of a compound. Nuclear Magnetic Resonance spectroscopy (NMR) is used to determine the structure of the unknown compounds with aid of electrons. (Dineshkumar et al., 2010d).

1.8. 1.Quantitative structure activity relationship (QSAR)*

The evaluation of therapeutic potentials of compounds can be assessed by non-biologically by QSAR. The biological activity of the compounds is mainly determined based on physiochemical properties, structural and molecular information. The physiochemical properties of the compounds are analyzed based on molecular parameters like solubility, hydrophobicity, lipophilicity, steric property, electronic property, chemical topology and functional groups present in them. The Computer Aided Molecular Design (CAMD) is used to detect the molecular properties and structure-function relationships of the chemical compounds (Dineshkumar et al., 2010e).

1.9. Detection of active drug metabolites in the body*

* Dineshkumar, B., Kumar P. V., Bhuvaneshwaran, S. P., and Mitra, A. (2010e) Structure activity relationship of anti-diabetic and anti-obesity agents, *Journal of Pharmaceutical Sciences and Research* (Accepted).

* Bhuvaneshwaran, S. P., Dineshkumar, B., Kumar P. V., and Mitra, A. (2010) Non-Conventional Approaches to *In-vivo* Drug Tracking and Targeting - A Review, *International Journal of Biological Sciences and Technology 2*, 1-10.

The drug molecule produces specific action on targeted tissues or organs to normalize their biological functions. The drug molecules in specific tissues or organs has important role in diagnosis or prevention of disease in human. The drug molecules can be designed with structure based or ligand based drug designing methods. In addition, the drug molecules in targeted tissues or organs can be detected by non-conventional techniques like imaging techniques such as Single Photon Emission Computed Tomography (SPECT), Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI), Near Infra Red (NIR) spectroscopy along with magnetic based technology, nanotechnology and laser technology (Bhuvaneshwaran et al., 2010).

1.10. *In vitro* studies using alpha amylase and alpha glucosidase inhibitory assay*

One of the therapeutic approaches for type 2 diabetes is to decrease the post-prandial hyperglycemia. Inhibition of alpha amylase and alpha glucosidase limits postprandial glucose levels by delaying the process of carbohydrate hydrolysis and absorption (Bell, 2004). Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha-linked polysaccharides such as starch and glycogen, the end product is glucose and maltose. It is the major form of amylase found in humans and other mammals. Although found in many tissues, amylase is most prominent in pancreatic juice and saliva in which each have their own isoform of human alpha amylase. Alpha glucosidase enzyme present in the brush border of small intestine and helps in hydrolysis of oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharide in the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of carbohydrates. Less glucose is absorbed because the carbohydrates are not broken down into glucose molecules. In diabetic patients, the short-term effects of these

* Dineshkumar, B., Mitra, A., Manjunatha, M. (2009) *In vitro* and *in vivo* studies of anti-diabetic Indian medicinal plants-A Review, Journal of Herbal Medicine and Toxicology 3, 9-14.

inhibitors are decrease in blood glucose levels: the long term effect is a small reduction in glycosylated hemoglobin (A1C) level. The plant based alpha amylase and alpha glucosidase inhibitors offer a prospective therapeutic approach for the management of post-prandial hyperglycemia (MeCue et al., 2004). Hexane extract from *Achillea ligutica* exhibited higher alpha amylase inhibitory activity than its methanolic extract. *In vitro* α-amylase inhibitory activities of seven indigenous medicinal plants of Mauritius (namely *coxi lacryma-jobi* (Poaceae), *Aegle mamelos* (Rutaceae), *Artocarpus heterophyllus* (Moraceae), *Vangueria madagascariensis* (Rubiaceae), *Azadirachta indica* (Meliaceae), *Eriobotrya japonica* (Rosaceae) and *syzigium cumini* (Myrtaceae) showed that only *Artocarpus heterophyllus* can be useful in lowering of blood glucose levels by inhibiting alpha amylase enzyme activity. Ethanol extracts of *Azadirachta indica* leaves, *Mangifera indica* stem bark as well as petroleum ether extract of *Murraya koenigii* leaves exhibited alpha amylase inhibitory activities (Dineshkumar et al., 2010c). Azadirachtolide isolated from chloroform extract of *Azadirachta indica* leaves and characterized by HPTLC, FTIR, ESI-MS and NMR and it decreased FBS, TG, TC, LDL, VLDL level as well as increased HDL level in streptozotocin-induced diabetic rats. The bodyweight also restored by azadirachtolide (at a dose of 50 and 100mg/kg) when compared with diabetic control rats (Dineshkumar et al., 2010f). Mangiferin isolated from ethanloic extract of *Mangifera indica* stem bark and characterized by HPTLC, FTIR, ESI-MS and NMR. Mangiferin (at a dose 10 and 20mg/kg) exhibited significant anti-diabetic as well as hypolipidemic effects in type 2 diabetic model rats. The bodyweight also restored by mangiferin (at a dose 10 and 20mg/kg) when compared with in type 2 diabetic rats (Dineshkumar et al., 2010g). Mahanimbine was isolated by petroleum ether extract of *Murraya koenigii* leaves and characterized by HPTLC, FTIR, ESI-MS and NMR. Mahanimbine (at a dose 50 and 100mg/kg) were administrated as a single dose per week to the diabetic rats for 30 days. Elevated FBS, TG, TC, LDL, VLDL levels were reduced and

increased level of HDL level was observed by intra-peritoneal administration of mahanimbine (at a dose of 50 and 100mg/kg) in diabetic rats. The bodyweight also restored by mahanimbine (50 and 100mg/kg) when compared with diabetic control rats (Dineshkumar et al., 2010h). Ethanolic extract of *Andrographis paniculata* exhibited appreciable alpha glucosidase and weak alpha amylase inhibitory effects. *Andrographis paniculata* can be considered as a potential candidate for the management of type 2 diabetes mellitus (Subramanian et al., 2008). Isolated compounds such as (-)-3-O-galloylepicatechcin and (-)-3-O-galloylcatechcin from *Bergenia ciliata* showed anti-diabetic activity by inhibiting intestinal alpha glucosidase and porcine pancreatic alpha amylase (Kawabata et al., 2008). Methanolic extract of *Tournefortia haetwegiana* exhibited anti-diabetic activity by inhibiting the intestinal alpha glucosidase enzyme in diabetic rats (Estrada-Soto et al., 2007). Aqueous extract of *Commelina communis* L showed alpha glucosidase inhibitory activity (Kyung-Hea Cho et al., 2004).

1.11. *In vitro* studies on insulin secretion

Anti-diabetic agents usually affect different pathways of glucose metabolism such as insulin secretion, glucose uptake by target organs etc. Therapeutic approaches directed towards incretins (Hansotia and Drucker, 2005) and transcription factors such as peroxisome proliferator-activated receptors —PPAR (Rosenson, 2007) are gaining momentum though insulin receptor, glucose transporters based therapies are yet to be explored. Studies using natural products have been reported by researchers to explore futuristic and deterministic predictions *in vivo* models (Iwashima et al., 2001, Storling et al., 2005).

1.12. In *vitro* studies using insulin-secreting cell lines

The most widely used insulin-secreting cell lines are RIN, HIT, beta-TC, MIN6 and INS-1 cells. These cell lines release mainly insulin and small amounts of glucagon and somatostatin. These cell lines play important role in the study of molecular events of the function of β-cell. Utilization of glucose by pancreatic beta cells glucose leads to increase in cytoplasmic ATP/ADP ratio, closure of ATP-sensitive potassium channels, activation of voltage-dependent Ca^{2+} channels and elevation of the intracellular Ca^{2+} concentration triggers insulin secretion. In type 2 diabetes ion channel activity of pancreatic β-cells shows abnormality and inappropriate pattern of release of insulin (Affourtit and Brand, 2006). These pathways can be studied with isolated pancreatic β-cells from either normal (control) or diabetic rat/mouse. Pathways related to insulin resistance may be observed in cell lines of adipocytes such as murine 3T3-L1 cells and rat L6 muscle (Maddux et al., 2001). Aqueous extracts of *Momordica charantia* showed cell repairing effects and stimulation of insulin secretion in HIT-T15 Hamster pancreatic β-cells (Xiang et al., 2007). Methanol extract of *Pterocarpus marsupium* exhibited glucose transport activity in a PPARγ mediated PI3 kinase dependent fashion as well as isoflavone from *Pterocarpus marsupium* showed glucose transport activity in a PPARγ mediated but PI3 kinase independent fashion (Anandharajan et al., 2005).

1.13. *In vivo* animal models of diabetes mellitus

Most experiments in diabetes are carried out on rodents, although some studies are being performed in larger experimental animals diabetes can be induced by pharmacologic, surgical or genetic manipulations in several animal species though the murine model is most commonly selected due to the availability of over 200 well-characterized inbred strains (Thakur et al., 2009).

1.14. Pharmacological induction of diabetes

Streptozotocin (STZ) and alloxan treated mice/rat models have been usually documented in ethnopharmacology in assessing the therapeutic potentialities. Both drugs exert their diabetogenic action when being administered parenterally, intravenously, intra-peritoneally or subcutaneously. The dose of these agents required for inducing diabetes depends on various factors like the animal species, route of administration and anthropometric evaluations etc. According to the administered dose syndromes similar to either type 1 or type 2 diabetes mellitus or glucose intolerance can be achieved experimentaly (Thakur et al., 2009). The destruction of pancreatic β-cells by both drugs is associated with a huge release of insulin which makes animals more susceptible to severe hypoglycemia which may be lethal. Thus, following treatment with either STZ or alloxan, animals are fed with glucose solution (5%) for 12–24 hours. This results in an increase of glucose levels in comparison to control animals due to insulin deficiency. Therefore, the administration of either STZ or alloxan must be done in the fasting period (8–12 h), followed by addition of glucose solution to avoid the hypoglycemic complications. Besides rats, dogs and mice, other animal species such as rabbits and monkeys have been employed to induce diabetes by these protocols though rabbits and pigs are more resistant to STZ (Rees and Alcolado, 2005). In diabetes induced by chemical drugs the majority of studies report the amount of reduction of blood glucose and that is always evaluated after a period of fasting following acute or chronic treatment with the specific natural product.

1.15. Surgical models of diabetes

Another technique used to induce diabetes is the complete removal of the pancreas with animal species such as rats, pigs, dogs and primates (Masiello, 2006). Choi et al., (2004) systematically evaluated the action or relative glucose uptake in various tissues of 90%

pancreatectomized rats by using either hyperglycemic or euglycemic hyperinsulinemic clamp methodologies. This experimental design permits to evaluate if the compound has some effect upon both resistance to and secretion of insulin. However the process requires technical expertise, adequate surgical room environment, adequate post-operative analgesia and antibiotic administration to avoid the high risk of animal infection.

1.16. Transgenic mice

Significant advancements have also been noticed, especially with the advent of transgenic mice, there have been no studies carried out involving natural products and these models. Certainly, the high costs restrict their study in sophisticated protocols which explore mechanisms of potential therapeutic agents (Phytochemical) that either stimulate pancreatic β-cell growth or inhibit pancreatic β-cell death (Meiton, 2006).

1.17. Human Trials and Marketing

After successful animal trial the active metabolite needs to undergo human trialing following the guidelines given by Director-General Indian Council of Medical Research (2006). After successful trial (phase 1, phase 2 and phase 3) the metabolite will undergo phase 4 trial (post market) and will be inducted in the market following the specific drug rules of the country.