Chapter I

Introduction
CHAPTER – 1

1.1. INTRODUCTION

Diabetes mellitus is a disease that has been known to exist for thousands of years and that afflicts ~ 6% of the population in worldwide. Because of its prevalence, chronicity and propensity to cause end-organ damage, diabetes and its complications engenders about 100 billion dollars in healthcare costs each year in the India. Diabetes has emerged as a major healthcare problem in India. According to Diabetes Atlas published by the International Diabetes Federation (IDF), there were an estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people by 2025. The countries with the largest number of diabetic people will be India, China and USA by 2030. WHO estimates that mortality from diabetes, heart disease and stroke costs about $300 billion in India in the year 2025 (Sarah et al., 2004). Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar levels that result from defects in insulin secretion from beta cell of pancreas (Shih and Stoffel, 2002). Numerous clinical studies have focused on the diabetes management and metabolic control to get new insights into the pathogenesis of diabetes in order to discover novel possibilities of preventive and effective treatment. Identification and characterization of integral and Diabetes-Associated Proteins (IDAP) are pivotal in the discovery of novel disease markers and drug targets (Guerreiro et al., 2008). The proteomic component of this portfolio contains grants that have major emphasis in characterizing the proteome or subset of the proteome as it applies to the field of obesity, diabetes, endocrinology and metabolic diseases. For example this above program includes, different organs proteome and plasma/sera proteome studies aimed to identify biomarkers, characterization of the proteome of animal models and cells models, large-scale approaches to study differential protein expression or network. More generally the proteomic program includes all grants that use large-scale approaches for studying proteins (Unlu et al., 1997). It should be noted that a study that uses only one or few antibody for looking at the protein expression during development does not fit within proteomics but a study that uses a set of antibodies (e.g. 20–100) and looks how all these proteins
are changing during development would fit within the proteomic program. Large scale approaches include the use of methodologies like 2D-PAGE, 2D-LC, protein arrays and mass spectrometry by itself or coupled to one of these methodologies for profiling/characterizing the proteome or a subset of the proteome (e.g. phosphoproteome, membrane protein fraction, glycoproteome) (Gorg et al., 2000). Pancreas and other organs are involved in the production of numerous proteins that develop diabetes and diabetes-related complications with the onset of β-cell dysfunction (Kahn, 2003). The broad-range proteomic approaches are applied to investigate the complexity of the mechanisms involved in pancreas and other organs function for identification of new targets of diabetes diagnosis and treatment.

1.2. DIABETES MELLITUS

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both (figure 1.1). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Taylor et al., 1994). Several pathogenic processes are involved in...
the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficient action of insulin on target tissues (Saltiel and Kahn, 2001). Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia (Kahn, 2003).

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome (Arioglu et al., 2002). Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputation and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction (Griffiths et al., 1990). Glycation of tissue proteins and other macromolecules and excess production of polyol compounds from glucose
are among the mechanisms thought to produce tissue damage from chronic hyperglycemia (Diaz-Flores et al., 2004). With diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral vascular and cerebrovascular disease. Hypertension, abnormalities of lipoprotein metabolism and periodontal disease are often found in people with diabetes (figure 1.2) (Gastaldelli et al., 2002). The emotional and social impact of diabetes and the demands of therapy may cause significant psychosocial dysfunction in patients and their families (Le Roith and Zick, 2001).

The vast majority of cases of diabetes fall into two broad etiopathogenetic categories are tabulated in 1.1. In one category (type 1 diabetes), the cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers. In the other, much more prevalent category (type 2 diabetes) (Dineshkumar et al., 2010), the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response (figure 1.3). In the latter category, a degree of hyperglycemia sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before diabetes is detected (Pradhan et al., 2009).
Table 1.1 – Types of diabetes and their aetiology.

<table>
<thead>
<tr>
<th>S. no</th>
<th>Former Name</th>
<th>Preferred Names</th>
<th>Aetiology of Diabetes</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>Type I</td>
<td></td>
<td>Genetics, immunological factors, Viral Infections, Other Environmental Factors and drugs.</td>
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<tr>
<td></td>
<td>juvenile diabetes</td>
<td>Type 1 diabetes</td>
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<td></td>
<td>insulin-dependent diabetes</td>
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<tr>
<td></td>
<td>mellitus IDDM.</td>
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<td>2.</td>
<td>Type II</td>
<td></td>
<td>Impaired insulin release, Insulin resistance, Beta Cell Dysfunction and Inflammation</td>
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<td></td>
<td>adult-onset</td>
<td>Type 2 diabetes</td>
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<tr>
<td></td>
<td>diabetes noninsulin-dep.</td>
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<tr>
<td></td>
<td>dependent diabetes mellitus</td>
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<tr>
<td></td>
<td>NIDDM.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Diabetes Insipidus</td>
<td>Diabetes Insipidus</td>
<td>Diabetes insipidus is caused by the inability of the kidneys and imbalance of antidiuretic hormone.</td>
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Diabetes is a polygenic disease, meaning many different genes contribute to its expression. Depending on locus or combination of loci, it can be dominant, recessive or somewhere in between. The strongest gene, IDDM1, is located in the MHC Class II region on chromosome 6, at staining region 6p21. Certain variants of this gene increases the risk for decreased histocompatibility characteristic of diabetes such as DRB1 0401, DRB1 0402, DRB1 0405, DQA 0301, DQB1 0302 and DQB1 0201, which are common in North Americans of European ancestry and in Europeans (Bluestone et al., 2010). Many genetic syndromes are accompanied by an increased
incidence of diabetes mellitus. These include the chromosomal abnormalities of Down’s syndrome, Kline-felter’s syndrome and Turner’s syndrome (Rimoin, 1976).

Diet, is also a major factor responsible for causing diabetes. Eating too much of Carbohydrates, fats and proteins are all harmful to the body. Our body in general needs a balanced diet to produce energy for performing vital functions. Too much of food, hampers the pancreas from performing its function of insulin secretion. Hence, with insufficient insulin secretion, the blood sugar level rises, leading to diabetes mellitus (Seshiah et al., 2004). Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With the exception of cancer, damage to the pancreas must be extensive for diabetes to occur. However, adenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes (Cersosimo et al., 1991). In type 1 autoimmune diabetes there is a selective destruction of insulin-secreting beta cells. Around the time of clinical presentation, insulitis, a chronic inflammatory infiltrate of the islets affecting primarily insulin containing islets. The inflammatory infiltrate consists primarily of T lymphocytes; CD8 cells and CD4 cells, there are fewer B lymphocytes and macrophages are relatively scarce. Beta cell death may involve the Fas apoptotic pathway since they have been shown to express Fas, infiltrating T lymphocytes express Fas-L and apoptotic beta cells have been described (Foulis, 2008). Obesity is also one of the major factors causing diabetes. Excessive body weight as compared to the height of an individual, serves as a predisposing factor for diabetes mellitus. It is commonly seen in patients at 40 years of age suffering from type 2 non-Insulin dependent diabetes mellitus. Due to extra amount of fat in the body, the insulin does not function properly in the body (Parvez et al., 2007). Of hypothesized mediators of insulin resistance, recent findings have profiled potential roles for inflammation and proinflammatory cytokines, other fat cell-derived cytokines, free fatty acids and inhibitory serine/threonine (Ser/Thr) phosphorylation of upstream elements of insulin signalling (Werner et al., 2004). Certain viruses have been associated with β-cell destruction. Diabetes occurs in patients with congenital rubella, although most of these patients have HLA and immune markers characteristic of type 1 diabetes. In addition, coxsackievirus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the diabetes (Pak et al., 1988). Several hormones (e.g., growth
hormone, cortisol, glucagon and epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing’s syndrome, glucagonoma and pheochromocytoma) can cause diabetes (Berelowitz and Eugene, 1996). This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is removed. Zanosar is the trade name for streptozotocin, an antibiotic and antineoplastic agent used in chemotherapy for pancreatic cancer; it kills beta cells, resulting in loss of insulin production. Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetron) is an oxygenated pyrimidine derivative. It also kills beta cells, resulting in loss of insulin production (figure 1.4). The cytotoxic action of both these diabetogenic agents is mediated by reactive oxygen species. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β cells (Szkudelski, 2001; Wilson et al., 1984).

The diagnostic criteria for diabetes mellitus have been modified from those previously recommended by the WHO. The revised criteria for the diagnosis of diabetes are shown in table 1.2. Three ways to diagnose diabetes are possible, and each must be confirmed, on a subsequent day, by any one of the three methods given in table 1.2. For example, one instance of symptoms with casual plasma glucose ≥ 200 mg/dl (11.1 mmol/l), confirmed on a subsequent day by 1) FPG ≥ 126 mg/dl (7.0 mmol/l), 2) an OGTT with the 2-h postload value ≥ 200 mg/dl (11.1 mmol/l), or 3) symptoms with a casual plasma glucose ≥ 200 mg/dl (11.1 mmol/l), warrants the diagnosis of diabetes (WHO, 1985).
Table 1.2 – Criteria for the diagnosis of diabetes mellitus

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<tr>
<td>1</td>
<td>Symptoms of diabetes plus casual plasma glucose concentration ≥ 200 mg/dl (11.1 mmol/l). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weightloss.</td>
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<tr>
<td>2</td>
<td>FPG ≥ 126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.</td>
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<tr>
<td>3</td>
<td>2 h PG ≥ 200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by WHO (2), using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.</td>
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The early detection and consequently early treatment might well reduce the burden of diabetes and its complications. Modifying eating habits and increasing physical activity are typically the first steps toward reducing blood sugar levels (Langtry et al., 1998). Sometimes blood sugar levels remain high in people with diabetes even though they eat in a healthy manner and exercise. When this happens, medications taken in pill form may be prescribed (Langtry et al., 1998). The medications work in several different ways. These include improve the effectiveness of the body’s natural insulin, reduce blood sugar production, increase insulin production and inhibit blood sugar absorption. Oral diabetes medications are sometimes taken in combination with insulin (Melander et al., 1989). The Diabetes Control and Complications Trial (DCCT) Research Group (1993) stated, “Control of blood glucose is an effective strategy in clinical complications of diabetes mellitus and even optimal control of blood glucose cannot prevent complications in diabetes, which suggests an alternative therapeutic approach is needed”. In recent years, there has been renewed interest in plant medicine (Prince et al., 1998) as an alternative treatment against different diseases and herbal drugs are generally nontoxic as reported from research work conducted on experimental animal. However, in most cases the efficacy of this traditional treatment regimen is unproven. Thus, the study of herbal medicine against
1.3. PLANT MEDICINE

Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago (Solecki, 1975). From that point the development of traditional medical systems incorporating plants as a means of therapy can be traced back only as far as recorded documents of their likeness. However, the value of these systems is much more than a significant anthropologic or archeologic fact. Their value is as a methodology of medicinal agents, which, according to the WHO, almost 65% of the world’s population have incorporated into their primary modality of health care. According to the WHO, 80% of the world’s people depend on

Figure 1.5 – Flow chart of sequence for the study of plants used in traditional medicine (Farnsworth et al., 1985).
traditional medicine and 25% of the medical drugs are based on plants for their primary health care needs (Farnsworth et al., 1985). The main goals of using plants as sources of therapeutic agents are (figure 1.5): i) to isolate bioactive compounds for direct use as drugs, e.g. digoxin; ii) to produce bioactive compounds of novel or known structures as lead compounds for semi synthesis to produce highly activity and/or lower toxicity, e.g. metformin; iii) to use agents as pharmacologic tools, e.g. mescaline; and iv) to use the whole plant or part of it as a herbal remedy, e.g. Cranberry and Echinacea (Fabricant and Farnsworth, 1996).

The number of higher plant species (angiosperms and gymnosperms) on this planet is estimated at 250,000 (Ayensu and DeFilipps, 1978), with a lower level at 215,000 (Cronquist, 1988) and an upper level as high as 500,000 (Schultes, 1972). Of these, only about 6% have been screened for biologic activity and a reported 15% have been evaluated phytochemically (Verpoorte, 2000). With high throughput screening methods becoming more advanced and available, these numbers will change, but the primary discriminator in evaluating one plant species versus another is the matter of approach to finding leads. There are some broad starting points to selecting and obtaining plant material of potential therapeutic interest. However, the goals of such an endeavor are straightforward. Plants have an advantage in this area based on their long-term use by humans (often hundreds or thousands of years). One might expect any bioactive compounds obtained from such plants to have low human toxicity (Farnsworth, 1990).

Most large pharmaceutical manufacturers and some small biotechnology firms have the ability to screen 1,000 or more substances per week using high throughput in vitro assays. It was estimated that in 1991 in the United States, for every 10,000 pure compounds (most likely those based on synthesis) that are biologically evaluated (primarily in vitro), 20 would be tested in animal models and 10 of these would be clinically evaluated and only one would reach U.S. Food and Drug Administration approval for marketing. The time required for this process was estimated as 10 years at a cost of $231 million (U.S.) (Vagelos, 1991).
Ethnobotany is not new to India because of its rich ethnic diversity. Jain printed out that there are over 400 different tribal and other ethnic groups in India (Jain, 1991). The tribals constitute about 7.5% of India’s population. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of India and there are many reports on the use of plants in traditional healing by either tribal people or indigenous communities of India (Saikia et al., 2006). Apart from the tribal groups, many other forest dwellers and rural people also possess unique knowledge about plants (Jain, 1991).

With this above literature review, we have planned to investigate the effects of *Cynodon dactylon* (L.) Pers. in diabetic rats and also we have analysed identification and characterization of integral and Diabetes-Associated Proteins (IDAP.) Many ethnobotanical surveys on medicinal plants used by the local population have been performed in different parts of the world including India, Morroco, Saudi Arabia, Taiwan, Trinidad and Tobogo (Bnouham et al., 2006). They are many plants having antidiabetogenic effect (*Opuntia streptacantha* Lem, *Trigonella foenum graecum* L, *Momordica charantia* L, *Ficus bengalensis* L, *Polygala senega* L., *Gymnema sylvestre* R., *Allium sativum* and *Citrullus colocynthis*). *Cynodon dactylon* is one of them (Santosh et al., 2008). *Cynodon dactylon* (L) Pers. (Family: Poaceae) is an herbal plant commonly known as ‘Arugampul’ in Tamilnadu in India, which is treated as a blessed plant (figure 1.6). This grass grows almost in all parts of the world. It is a short C4 grass, which is rhizomatous, stoloniferous and water-stress tolerant (Bethel et al., 2006). Traditionally, juice of this plant is commonly consumed as health drink during the early morning in south India. *C. dactylon* is used by traditional healers for purifying the blood, anuria, conjunctivitis, diarrhoea, gonorrhea, itches and stomachache (Muthu et al., 2006). *C. dactylon* is claimed to have anti-microbial (Ahmed et al., 1994) properties. The plant is diuretic and traditionally known as a remedy for urinary infections, kidney stones and congestion (Atmani et al., 2009). The root and rhizomes are also used in the treatment of depression, vomiting, cough, epilepsy and hemorrhage (Miraldi et al., 2001). It has been reported recently that the plant possesses protective effect against streptozotocin induced hepatic injury in rats (Singh et al., 2008).
1.3.1. Plant Description

**Cynodon dactylon (L.) Pers.**

- **Kingdom**: Plantae (Plants)
- **Subkingdom**: Tracheobionta (Vascular plants)
- **Super division**: Spermatophyta (Seed plants)
- **Division**: Magnoliophyta (Flowering plants)
- **Class**: Liliopsida (Monocotyledonss)
- **Sub-class**: Commelinidae

*Figure 1.6 – Illustration of plant Cynodon dactylon (L.) Pers. The plant was taxonomically identified and authenticated by Rev Dr S John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St Joseph’s College (Autonomous), Tiruchirappalli, Tamil Nadu, India.

Voucher specimen No.: RHCD02*
Order : Cyperales  
Family : Poaceae (Grass family)  
Genus : Cynodon Rich. (Bermuda grass)  
Species : dactylon (L.) Pers. (Bermuda grass)  
Botanical name : Cynodon dactylon (L.) Pers.  
Description : Perennial grass, very variable, with long rapid growing, creeping runner or stolons  
Distribution : Throughout Asia  
Parts used : Whole plant  
Uses : antiseptic, aperient, astringent, cyanogenetic, demulcent, depurative, purifying the blood, anuria, biliousness, conjunctivitis, diarrhoea, gonorrhoea, itches and Stomachache.

1.3.2. Advantages of Herbal Medicine (Muthu et al., 2006)

- Allopathic medicines are very costly. In contrast, herbal medicines are very cheap. This cost effectiveness makes them even more alluring.

- Herbal medicines can be brought without prescription and they are available in almost all health stores. Some herbs can even be grown at home. Ailments, herbal medicines are considered more effective than allopathic medicines.

- Herbal medicines do not have any side effects, as they are free from chemicals. They are also milder than allopathic medicines.

- The natural detoxification process of the body is effectively enhanced by herbal medicines. They can be used to cleanse the colon, improve digestion and food absorption. Herbal medicines are also very good in boosting the immune system.

- Herbal medicines are very effective in curing various digestive disorders like colitis, indigestion, peptic ulcers and irregular bowel movements. These types of medicines are best for people who are allergic to various types of drugs.

- Herbal medicines are also effective in boosting the mental health. Some herbal medicines are very good in reducing the cholesterol level in the blood stream. They are also used to treat coronary artery diseases. Herbal medicine can be used to reduce weight by regulating appetite.
1.4. PROTEOMICS AND BIOMEDICINE

Proteomics emerged in the beginning of the 1990s with the appearance of new methods for protein analysis. The proteome encompasses an important concept whose counterpart for man was introduced more than 25 years ago (Anderson and Anderson, 1982), during the initial development phase of 2-DE (O’Farrell, 1975). The last 15 years has witnessed a revolution in molecular biology. The result has been an era of holistic biology, where it is now accepted that biology must be studied on a global scale (Williams, 1999). The field of proteomics involves the study of proteomes and represents an exciting new way to pursue biological and biomedical science. It takes a broad, comprehensive and systematic approach to understand biology that is generally unbiased and not dependent upon existing knowledge. Proteomics is primarily discovery based rather than hypothesis limited.

However, it is now accepted that the proteome of an organism or tissues is more than simply a catalogue of all the proteins encoded by the genome as it also includes the dynamic changes. Proteomic research includes the determination of the structure and function of the complete set of proteins produced by the genome of an organism, including co-translational modification and PTMs, the interactions of these proteins with small and large molecules of all types, the determination of the expression of these proteins as a function of time and physiological condition and finally, the coordination of this information into a unified and consistent description of the organism (Pandey and Mann, 2000; Norin and Sundstrom, 2002). The last part of the definition represents the ultimate goal of proteomics, which is to understand not only the nature of the components and how they change with time and condition but also how they integrate to produce a living entity. The sequencing of the human genome and of numerous pathogens has provided the field of proteomics a sequence based framework to understand disease. This opens the door to the discovery of new biomarkers for early diagnosis and eventually find more efficient and safer therapeutics for a wide range of pathologies (Williams, 1999).
1.5. PROTEOMICS IN DRUG DISCOVERY

The drug discovery and development processes include steps such as target identification, selection of candidates, drug toxicity studies, validation of potential drugs and clinical testing. Furthermore, in some of the cases, drug design is necessary. It is obvious that all these steps are quite costly (hundred million US$) and time consuming (years). As proteins are the principal targets of drug discovery, proteomic tools have taken relevance in the drug discovery/development process (Banks et al., 2000). Proteomics provide new strategies for potential target identifications and validation process. Proteomics can reveal molecular information of the mechanistic basis of drug action, their toxicity and information about why some cells become drug resistant. Proteomics can accelerate target identification, because many targets can be identified at the same time by global protein profiling comparison of normal and disease tissue or diseased tissue versus drug-treated tissue. However, the use of proteomics in drug discovery is limited by many challenges inherent of proteomics tools. One important challenge is that 70% of drug targets are membrane proteins (membrane receptors and ion channel) that generally appear in low abundance (Wu and Yates, 2003)]. Inclusively with these limitations, in recent years, many proteomic researchers have focused their efforts on drug discovery.

Several of these proteomic approaches compare drug treated versus -untreated cancerous cell lines. Cecconi et al. (Cecconi et al., 2003). compared the protein expression profile of pancreatic ductal carcinoma cell lines (PaCa44) before and after treatment with 50-aza-20-deoxycytidine (DAC), a DNA methylation inhibitor. Using 2-DE, they found that of 700 spots detected, 45 polypeptide chains were differentially expressed between treated and untreated samples. After treatment 32 proteins were downregulated and 13 upregulated. Nearly all 45 proteins were identified by MALDI-TOF. Proteomics has addressed cell drug-resistance mechanisms, too and could be a contributor to the drug-resistance of these cell lines, so this protein could be an important target to prevent resistance (Liu et al., 2006).
Another topic of proteomic application is the measurement of drug toxicity. These studies have identified many toxin-associated proteins which may be used as biomarker of toxicity in drug screening (Kennedy, 2001). In addition, detected proteins can help understand the mechanism of toxic damage. Drug side effects are common problems but the mechanisms of action of drug toxicity in human organs are little known. Kidney, liver and heart are the organs where proteomics of drug toxicity have been largely applied. These approaches studied the mechanisms of various drugs such as the derivatives of puromycin, pyrimidine, gentamicin, 4-aminophenol, D-serine, cisplatin and cycloheximide (Newsholme et al., 2000).

1.6. PROTEOMIC TECHNOLOGIES: TWO-DIMENSIONAL ELECTROPHORESIS AND MASS SPECTROMETRY

In this system, complex mixtures of proteins are separated by gel electrophoresis. In the first dimension, separation is based on relative charge according to isoelectric point (isoelectric focusing [IEF]); in the second dimension, separation is based on molecular mass (O’Farrell, 1975). Once separated, the proteins are visualised as an array of apparent ‘spots’ by treating the gel with one of a variety of protein stains, including Coomasie Blue, silver and Sypro Ruby or by pre-staining with Cy dyes (100s to 1,000s of spots can be resolved on a single gel). The stained protein spots are imaged by specialised equipment and differences in protein expression between samples can be determined using specialised software (Sivakumar, 2002). A variety of analysis programs are available which allow for comparison of multiple samples by aligning gel images. Assessment can then be made of changed protein patterns (often characteristic of PTMs) or levels (indicative of variant expression). For protein identification, protein spots of interest are excised from the
gel, fragmented by proteases (most often trypsin) and the resulting mixture of peptides is then spotted onto a MALDI-MS plate. The samples are then dried and coated with MALDI matrix to promote peptide ionisation for MS analysis. This involves separation of peptides according to their time of flight (TOF), which is dependent upon mass to charge (m/z) ratio. The resultant peptide m/z ‘fingerprint’ is then compared against databases (such as Mascot, www.matrixscience.com) of theoretical peptide fingerprints of all proteins to identify the protein(s) of interest (figure 1.7) (Henzel et al., 2003).

The field of diabetes research has expanded as the laboratory and bioinformatics tools for unravelling complex phenotypes have evolved. Proteomics is the latest research tool to be employed in this context and it perhaps holds more promise than the genetic analyses that have been prevalent for the past decade or so. The aim of this research is to Identification and characterization of ‘Integral and Diabetes-Associated Proteins-Differential Protein Expression Analysis’ (IDAP-DPEA) by using advance proteomic techniques and their potential application to the study of diabetes.

1.7. AIM AND OBJECTIVES:

To study the anti-diabetic activity of plant extract and analysis of differential protein expression in diabetic rats to elucidate the pathological mechanism of diabetes and associated complications in vital organs and also to find out potential therapeutic target for diabetes mellitus.

- To analyze the anti-diabetic, hypolipidemic and antioxidant activity of C. dactylon.
- To find out the phyto-chemical constituents in C. dactylon, which is responsible for anti-diabetic activity and control the diabetes complication?
- To find out the optimum dosage of C. dactylon for anti-diabetic activity.
- To elucidate differential protein expression in different organs and its role in diabetes associated complication.
- To discover a novel disease markers and drug targets for diabetes mellitus and diabetes associated complication.