Chapter 1

INTRODUCTION

The blue circle, the international symbol for diabetes was introduced by the International Diabetes Federation (IDF) in 2006 to give diabetes a common identity. It supports existing efforts to raise awareness and place the epidemic firmly in the public spotlight.

WHO describes Diabetes mellitus as the metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia with disturbances in the metabolism of carbohydrate, fat and protein resulting from defects in insulin secretion, insulin action or both. It is one among the important noncommunicable diseases (NCD) such as heart disease, stroke, cancer, chronic respiratory disease and it is the leading cause of mortality in the world.

It can cause long term damage, dysfunction and failure of various organs.

1.1 TEN FACTS ABOUT DIABETES (WHO)

Updated in March 2013

1. About 347 million people worldwide have diabetes. There is an emerging global epidemic of diabetes that can be traced back to rapid increase in overweight, obesity and lack of physical activity.

2. Diabetes is predicted to become the seventh leading cause of death in the world by the year 2030. Total deaths from diabetes are projected to rise by more than 50% in the next ten years.
3. There are two major forms of diabetes. T1DM is characterized by a lack of insulin production and T2DM results from the body’s ineffectiveness in the use of available insulin.

4. A third type of diabetes is gestational diabetes. This type is characterized by hyperglycaemia which has first appeared or been recognized during pregnancy.

5. T2DM is much more common than T1DM. T2DM accounts for around 90% of all diabetes worldwide. Reports of T2DM in children were rare in the past; but now it has increased worldwide. In some countries, it accounts for almost half of the newly diagnosed cases in children and adolescents.

6. Cardiovascular diseases are responsible for 50% to 80% of deaths in people with diabetes. Diabetes has become one of the major causes of illness and death in most countries, mainly through the increased risk of cardiovascular disease (CVD).

7. In 2004, an estimated 3.4 million people died from consequences of high blood sugar.

8. Majority of the diabetes deaths (80%) occur in low and middle income countries. In developed countries most people with diabetes are above the age of retirement, whereas in developing countries those most affected are aged between 35 and 64.

9. Lack of awareness about diabetes, combined with insufficient access to health services and essential medicines, can lead to complications such as blindness, amputation and kidney failure.

10. T2DM can be prevented. Thirty minutes of moderate-intensity physical activity on most days and a healthy diet can drastically reduce the risk of developing T2DM. T1DM cannot be prevented.

The world average of diabetic patients was 28.23% in the year 2000. Disability-adjusted life year for diabetes mellitus per 1,00,000 people in 2004 was 1.5% and is also increasing in an uncontrolled way.
According to the data of IDF, approximately 7% of Indian adults are diabetic, and it kills about one million Indians every year.

*Diabetes mellitus* prevalence increases with age, and the number of older persons with diabetes is expected to increase as the population of the elderly increases.

The cause of T1DM or ‘juvenile diabetes’ is not known and it is not preventable with current knowledge.

T2DM is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the role of insulin receptor.

T2DM was found only in adults until recently, but it is now occurring in children also, according to WHO fact sheet 2013 October.

In 2004, an estimated 3.4 million people died as a result of the consequences of high fasting blood glucose. More than 80% of diabetes deaths occur in low and middle income countries.

1.2 GLOBAL PREVALENCE OF DIABETES (Patel P.M. et al., 2006)

Estimates of the year 2000 and projections for 2030

The prevalence of diabetes for all aged groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men. The urban population in developing countries is projected to double between 2000 and 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people above the age of 65.

The above analysis clearly states the dire predicament of the world, if it is not set free from the strangulating tentacles of *Diabetes mellitus*.

Thankfully, WHO and many other health organizations all over the world have taken up multipronged steps to stimulate, support and promote the adoption of
effective measures for the surveillance, prevention and control of diabetes and its complications, particularly in the low and middle-income countries.

1.3 ACTION PLAN OF WHO

- Provide scientific guidelines for diabetes prevention;
- Develop norms and standards for diabetes diagnosis and care;
- Build awareness: WHO and IDF together declared, 14 November to be the world diabetes day. November 14 is the birthday of Frederick Banting, who, along with Charles Best, was instrumental in the discovery of insulin in 1922.
- Conduct surveillance of diabetes and risk factors

The WHO global strategy on diet, physical activity and health complements WHO’s diabetes work by focusing on population-wide approaches to promote healthy diet and physical activity, thereby reducing the growing global problem of overweight and obesity.

By using the existing knowledge, it is possible to overcome the threat of NCD. Modifiable risk factors underlie all the major NCDs. They include the harmful use of tobacco, alcohol, unhealthy diet, insufficient physical activity, overweight/obesity, raised blood pressure, raised blood sugar and raised cholesterol.

According to WHO 63% of death around the world is due to non-communicable diseases (NCD) and 80% of NCD can be prevented.

1.4 CURRENT METHODS OF TREATMENT

In the presently used antidiabetic therapeutic regimen, there are seven different sub-categories. They are

2. Biguanides—orally active. Eg. Metformin
4. Insulin therapies – subcutaneous and i.v infusions are the best even today. Inhalation type was introduced in the year 2006 in US; but it did not
have any superior action than short acting insulin injections; hence it was withdrawn from the market.

5. Meglitinides/ ‘glinides’- orally active. Eg. Mitiglinide, Nateglinide, Repaglinide

   First generation Eg. Acetohexamide, Carbutamide, Chlorpropamide, Metahexamide, Tolbutamide, Tolazamide.
   Second generation Eg. Glibenclamide, Glibomuride, Gliclazide, Glimepiride, Glipizide, Gliquidone, Glyclopyramide.

7. Thiazolidinediones- orally active- also known as ‘glitazones’. Eg. Darglitazone, Pioglitazone, Rosiglitazone.

   In addition to this, antidiabetic medicines such as exenatide, liraglutide and pramlintide are given as injections.

   In the use of all the above antidiabetic agents, there are one or more side effects or other limitations. It is clear that the world urgently requires a better antidiabetic agent with minimum toxicity and side effects. It should be readily available, easy to store and cost effective. In an attempt to find a better alternative, I began my search for a plant product, that would be ever renewable and environment friendly.

1.5 TRADITION OF MEICINAL PLANTS

Ayurveda which originated in India is one of the oldest still extant, health traditions in the world. It is based on Sankhya philosophy, which means ‘rational enquiry into the nature of the truth’. The meaning of ‘Ayur’ is life and Veda is knowledge or science. i.e. Ayurveda means the knowledge of life.

   Concepts of Ayurveda are adopted from Atharvaveda. Since the period of Bharadwja (pre-historic), Ayurveda has been practiced in India. Under the guidance of the physician Atreya, Agnivesh wrote encyclopedic treaties (8th century BC). Charaka revised it around 300 BC and wrote the Charakasamhita. Sushruta, the first known surgeon wrote Sushruthasamhita (500-300 BC). Nagarjuna 150-250 AD father of ‘bhasmas’ (‘iatrochemistry’) is also considered as a great contributor.
Vagbhata, son of the renowned Ayurvedic physician Simhagupta (7th century AD) wrote Ashtangahridaya and Ashtanga samgraha. In the above classics, the use of many herbs, mineral preparations and animal parts are explained (Patwardhan B and Mashelkar RA, 2009). In and around the world now, people use more natural/plant medicines through Ayurveda, Traditional Chinese Medicine (TCM) and new products of modern medicine derived from natural products.

Plant derived medicines initially were dispensed in the crude herbal formulations/concoctions such as tincture, tea, decoction, powder, poultice etc. (Samuelson G, 2004). In Atharvaveda, use of the extract of opium poppy as an analgesic is mentioned. Galenic is the crude extract formulation used in the old days and is named after the Greek physician Galan (AD 129-200). Serturner, first isolated morphine from opium in the early 19th century; it opened the new era of identification of chemical constituents which are responsible for the pharmacological action (Butler MS, 2004). Since then, the isolation and characterization of active pharmaceutical ingredients (API) has been continuing. The recent developments in technology are being utilized for the standardization of herbal medicines by the identification, characterization and quantification of specific marker components present in them.

1.6 IMPORTANCE OF PLANT PRODUCTS

Despite very stringent and demanding regulatory processes, post approval or postmarketing withdrawal of new drugs continues. It is a deplorable fact that the safety of new agents cannot be known with certainty until a drug has been in the market for many years (Lasser KE et al., 2002). This has led to a re-examination of the process of drug regulation and increased concern that the current process is inadequate for the protection of public health (Ray WA and Stein CM, 2006). (Remember about the withdrawal of the new anticoagulant Ximelagatran of Astra Zeneca, the Cox II inhibitor, Vioxx of Merck, the cancer vaccine Provenge, of Dendron etc…). At this moment, the combined use of the basic principles, experimental wisdom, holistic approach and systematic use of the database of Ayurveda may offer useful bioprospecting tools as an efficient discovery engine (Patwardhan B et al., 2004).
Important shortcomings of the New Chemical Entity (NCE) derived by synthesis are (a) toxicity (b) side effects including adverse effects (c) emergence of drug resistant microorganisms (Dhanukar et al., 2000). In addition to that, there are no available medicines to treat the emerging/recently identified diseases (Harvey AL, 2008).

Plant drugs are expected to have normal biochemical pathways for their metabolism and are expected to have minimum toxicity and side effects. The traditional knowledge based bioprospecting offers better leads for the treatment of AIDS and cancer. It is estimated that over 100 new natural product-based leads are in clinical development (Gupta R et al., 2005). About 60% of anticancer and 75% of anti-infective drugs approved from 1981 to 2002 could be traced to natural origins (Patwardhan B et al., 2008). Since most of these compounds are part of routinely used traditional medicines, their tolerance and safety are relatively better known than other synthetic chemical entities entering first in human studies (Cordell and Colvard, 2005).

Here we need to consider about the large plant biodiversity available. In India, over 7500 plant species have been reported to be used in traditional and ethnomedicines (Sanjappa M, 2005). Globally, around 55,000 species of plants have been used for traditional medicines; but only a small portion has been investigated scientifically to establish their activity (Cordell and Colvard, 2005).

Knowledge of the precious traditional medicines is confined more or less to the individuals, families or tribes and is not documented. As there was only verbal communication, effective transfer of knowledge and documentation did not take place. As per the data of NAPRALERT, systematic investigation leading to the isolation of API has not taken place in 55% of the ethnomedically used plants (Evans et al., 1998).

Several secondary metabolites of plants are accredited with antibacterial, antifungal, antiviral actions, which contribute to their defence mechanisms. The biological relevance of the natural products is evidenced by its ability to interact with multiple proteins and is thus regarded as privileged structures. Privileged structures are compounds, which are able to bind with various proteinaceous
receptors (Patwardhan B and Mashelkar R A, 2009). Due to the above reason they often display multiple biological activities.

1.7 MEDICINES DERIVED FROM THE PLANTS AND THE NEW VISION

Some of the popular plant derived API and medicines are arteether, betulinic acid, camptothecin, delta-9-tetrahydrocannabinol, etoposide, galantamine, nitisinone, paclitaxel, pilocarpine, reserpine, scopolamine, tiotropium, tubocurarine, vincristine etc. (Philipson JD and Anderson LA, 1989).

At present the pharmaceutical research is moving away from single molecule or single target approach to combinations and multitarget approaches (Wermuth and Camille G, 2004) i.e. drug discovery and development need not always be confined to new molecular entities. Rationally designed, carefully standardized, synergistic traditional herbal formulations and botanical drug products with robust scientific evidence can also be alternatives (Zimmerman G R etal., 2007). Thus drug discovery strategies based on natural products and traditional medicines are re-emerging as attractive options.

Single drugs, however, may not be an optimal way to treat a patient, with many characteristics that are highly individual, associated, of course with the challenges of genetic diversity. Herbal preparations from the crude extracts may offer greater chances for success where conventional single drugs have been disappointing; the reason could be the presence of more than one API which act through different receptors and mechanisms. Thus it can be suggested that drug discovery need not always be confined to the discovery of a single molecule. The current ‘one drug fits all’ approach may be unsustainable in the future. The growing interest in the polypill concept is indicative of the need to collectively address multiple targets, risk factors or symptoms (Kumar V et al., 2008).

‘Life’ in Ayurveda is conceived as the union of body, senses, mind and soul. The concept of ‘Prakriti’ or human constitution plays a central role in understanding health and disease in Ayurveda. This is similar to modern pharmacogenomics. Genome-wide functional screening against disease targets may be a practical approach.
The combined use of Ayurveda and functional genomics in biological screening may reveal the pathway analysis of crude and active components (Deocaris CC et al., 2008). Pharmacogenomics is now significantly influencing drug discovery and genotyping is recommended for drugs that are metabolized by enzymes whose genes have inactivating polymorphisms (Relling MV and Hoffman JM, 2007). Efforts to correlate genotype and phenotype-based traditional methodology of classifying humans into three major ‘Prakriti’ types or constitutions described in Ayurveda have opened an exciting scientific chapter and will help the progress of individualized medicine approaches (Patwardhan B et al., 2005). Whole genome expression and biochemical correlates of extreme constitutional types are defined in Ayurveda (Prasher B et al., 2008).

In the management and treatment of polygenic diseases there is renewed interest in multi-ingredient synergistic formulations (Hong-Fang Ji et al., 2009). Thus, the traditionally used polyherbal formulations (Traditional Indian medicine-TIM) also could be explored as an option for multitarget therapeutic and prophylactic applications. Over thousands of years Ayurveda, TIM, TCM and other global indigenous knowledge (GIK) have developed various practical theories to create polyherbal formulations, in which multiple agents contained in one formula, act synergistically (Vaidya RA et al., 2003).

The FDA however maintains the same standards for safety and efficacy for marketing approval of a product, whether it is a botanical-sourced product or a synthesized purified chemical. Thus, to get FDA approval for the traditional herbal formulations, scientific evidence with the support of chemistry, reproducible analytical and manufacturing data are to be created with control/standard values.

A holistic approach based on biological evaluations seems to be more appropriate to evaluate therapeutic efficacy and pharmacodynamics of traditional medicines for drug development (Veerporte R, 2005). It is also suggested that, instead of randomized controlled trials, strategies of pragmatic clinical trials are better suited for traditional medicine-inspired reverse pharmacology approaches (Fonnebo V et al., 2007). Sufficient pharmacoepidemiological evidence, based on actual clinical use, can thus be generated to support their safety and efficacy (Vaidya
R A et al., 2003). It also considers a large volume of mostly anecdotal human data in the place of well-controlled animal studies and human trials in an IND (US FDA manual of policies and procedures, 2012). Systematic data collection from the use of existing formulations can certainly help the drug discovery processes to identify safe medicines and synergistic formulations. In such a situation, traditional medicine data remains very important.

Issues related to the appropriateness of conventional biomedical and clinical models for evaluating efficacy of traditional medicine are yet to be resolved.

1.7.1 REVERSE PHARMACOLOGY: A PRAGMATIC APPROACH

In this process ‘safety’ remains the most important starting point and the efficacy becomes a matter of validation. The novelty in this approach is the combination of living traditional knowledge such as Ayurveda and the application of modern technology and processes to provide better and safer leads. By utilizing the traditional knowledge, in reverse pharmacology the routine process of ‘laboratory-to-clinic’ is reversed as ‘clinics-to-laboratories’ (Vaidya ADB, 2006). Since most of the active components are part of routinely used traditional medicines, their tolerance and safety are relatively better known than other synthetic chemical entities entering for the first time in human studies (Patwardhan B et al., 2010).

1.7.2 INDIAN INVOLVEMENT

The department of Ayurveda, Yoga, Unani, Siddha, and Homoeopathy (AYUSH) in India has recently established a research center at the University of Mississippi USA to facilitate scientific investigations on Indian herbal drugs. Such efforts are expected to improve quality assurance, enhancing the chance of regulatory approvals and improving the acceptance of traditional drug products and formulations.

In an attempt to pull industry and academia together to explore the potential of herbal drug development, CSIR, initiated the national network project known as New Millennium Indian Technology Leadership Initiative (NMITLI) (Padma TVet al., 2005).
In the management of osteoarthritis, a herbal drug development project was initiated under the NMITLI scheme, a network of sixteen different institutions which included national research institutions, modern medicine hospitals and pharmaceutical industries of India. After detailed literature studies and discussions with Ayurvedic physicians at various levels including national level consultations, they judiciously short-listed botanical drugs, conducted animal pharmacology studies and then open label observations by clinicians (Chopra A et al., 2010). This led to the design of a few variants of synergistic polyherbal formulations that were found to be safe and devoid of any genotoxicity or mutagenic activity (Chopra A et al., 2006). This project was completed in five years and all the formulations were manufactured under Good Manufacturing Practices in accordance to US FDA guidance to industry for botanical drugs. Finally the best formulation was selected that led to one Indian and one Patent Cooperation Treaty (PCT) application (Patwardhan B et al., 2008).

1.7.3 INVOLVEMENT OF OTHER NATIONS AND WORLD BANK FUNDING

Herbalome is an ambitious project from China that is expected to undertake high-throughput screening (HTS), toxicity testing and clinical trials to identify active compounds and toxic contaminants in popular recipes to identify scores of drug candidates (Stone R, 2008). The chemical compound dehydrocorybulbine was isolated from the underground tubers of the corydalis plant and identified as a part of Herbalome project (YZhang et al., 2014). It can effectively alleviate three different types of pain.

In Tanzania, the Tanga AIDS Working Group (TAWG) has used indigenous knowledge (IK) in an attempt to alleviate suffering from HIV/AIDS (Kayombo EJ, 2007). During the recent launch of the Global Indigenous Knowledge and Innovation Partnership (GIKIP) in New York, the Tanga project was showcased prominently.
1.7.4 PRESENT SCENARIO AND THE FUTURE

Drug discovery and development is an extremely complex, time consuming (not less than 20 years) and capital involved process. Another major challenge in drug discovery is due to toxicity in human trials and it is known that drugs with novel mechanisms have higher toxicity/failure rates. It is estimated that only one in five thousand lead compounds will advance through clinical trials and will get FDA approval (Dickson M and Gagnon JP, 2009). Better validated preclinical targets with proof-of-concept of better efficacy and safety of drugs can, however, minimize such failures. Thus, the reverse pharmacology approach can be useful to reduce failure rates (Kola I and Landis J, 2004).

There is growing evidence to show that old molecules find new applications through a better understanding of traditional knowledge and clinical observations. Eg., Forskolin isolated from *Plectranthus barbatus* (synonym *Coleus forskohlii*) has been studied, to treat conditions of asthma (Gonzalez Sanchez Ret al., 2006). Similarly, antimicrobial berberine alkaloids (originally isolated from *Berberis aristata*) are being investigated in dyslipidemia with a mechanism different from statins (Xian Shang et al., 2013).

Polyherbal formulation Artrex®, containing four herbals is patented in US. A recent review on national pharmacopoeias from several countries reveals that, at least 120 distinct chemical substances from different plants have utility as life saving drugs. Recently NIH started extensive research for anti-inflammatory compounds from turmeric, ginger and boswellia with the aid of Ayurveda knowledge.

A large number of promising lead molecules that have come out of Ayurveda include rauwolfia alkaloids for hypertension, psoralsens for vitiligo, holarrhena alkaloids for amoebiasis, guggulsterons as hypolipidemic agents, *Mucuna pruriens* for Parkinson’s disease, piperidines as bioavailability enhancers, baccosides for memory retention, picrosides for hepatic protection, phyllanthins as antivirals, curcumins for inflammation, withanolides, many other steroidal lactones and glycosides as immunomodulators (Patwardhan B, 2005).

Interestingly, a few natural products are found useful in diseases unrelated to the traditional use of the product. E.g. *Catharanthus roseus* was initially used as a
hypoglycaemic agent in the folk medicine, but is now known to contain useful antineoplastic compounds (vinca alkaloids) (Williamson T et al., 1996). Therefore, the screening should not be limited to the application of traditional use, but should also include other possible activities.

1.7.5 FUTURE PERSPECTIVES

Seeking new synergistic combinations and improvements in bioavailability are innovative strategies, which can play a significant role in drug development. Below explained are some of the advances in the use of API derived from plants in combination therapy. In animal studies, a combination of artemisinin derivative and curcumin has been reported to show a synergistic interaction in killing \textit{Plasmodium falciparum} leading effectively to the total survival of test animals (mice) used (Nandakumar DN et al., 2006). There have been several studies on piperine showing that its combination improved bioavailability of synthetic drugs such as propranolol, theophylline and rifampicin. The clue for piperine as a bioenhancer that came from Ayurveda (Navin Atal and Bedi KL, 2010). Such bioavailability enhancing activity may have numerous advantages in drug development including reduction in dose, thus toxicity and treatment costs.

Many countries including India, China, Korea, Malaysia, Brazil, South Africa, Australia and the like are becoming increasingly aware of the value of their traditional knowledge and they are working to protect the intellectual property rights of their natural resources. The Traditional Knowledge Digital Library (TKDL) developed by CSIR and AYUSH offer unique technologies (http://www.tkdl.res.in/). AyuSoft(http://ayusoft.cdac.in/) has converted classical Ayurvedic textbook knowledge into comprehensive, authentic, intelligent and interactive repositories with complex analytical tools that can be used as a powerful discovery resource(Patwardhan B and Bodekar G, 2008).The online Encyclopedia of Indian Medicinal Plants developed by the Foundation for Revitalization of Local Health Traditions (FRLHT) now known as Indian Institute of Ayurveda and Integrative Medicine (IIAIM), Bangalore, India (http://www.frlht.org.in/) are the two most available valuable resources. The World Health Organization’s Commission on Intellectual Property and Innovation in Public Health has also recognized the
promise and role of traditional medicine in drug development with the qualities, (a) low cost (b) non toxic(Patwardhan B, 2005). The Government of India’s golden triangle project integrating biomedicine, modern sciences and traditional medicine is indicative of a trend where traditional sciences like ‘Ayurveda’ are increasingly embracing the scientific evidence-base and the spirit of robust research(Mashelkar RA, 2005, Cooper E L, 2008, Sharma RK and Prahlad S Patki, 2010).

A strong mutually respecting relation and partnership between Ayurveda, academia and industry coupled with robust evidence-based research is possibly the right way forward.

1.8 DIABETES: THE PRESENT DAY CHALLENGE

The high blood sugar in T2DM can occur, either because the pancreas does not produce enough insulin or cells do not respond to the insulin that is produced(David G Gardner and Dolores M Shoback, 2011). This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).

The glucose concentration in the blood is raised beyond its renal threshold when (about 10 mmol/L (180mg/dl), although this may be altered in certain conditions, such as pregnancy), reabsorption of glucose in the proximal renal tubule is incomplete, and part of the glucose remains in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst.

Pre-diabetes is an important condition that occurs when a person’s blood glucose levels are higher than normal, but not high enough for a diagnosis of T2DM.

Diabetes without proper treatments can cause many complications. Acute complications include hyperglycemia, diabetic ketoacidosis, and nonketotic hyperosmolar coma. Adequate treatment of diabetes is thus important, as well as blood pressure control and lifestyle factors such as smoking cessation and the maintenance of a healthy body weight.
Introduction

*Diabetes insipidus* (DI) is a condition characterized by frequent and heavy urination, excessive thirst and an overall feeling of weakness (Diabetes fact sheet, ADA, 2011). This condition may be caused by a defect in the pituitary gland (a deficiency of arginine vasopressin (AVP), also known as antidiuretic hormone (ADH)) or in the kidney (an insensitivity of the kidneys to ADH). In DI, blood glucose levels are normal.

Humans are capable of digesting starch and some disaccharides such as sucrose into simpler forms, most notably the monosaccharide glucose, the principal carbohydrate energy source used by the body.

Insulin regulates cells in the liver, muscle, and fat tissue to take up glucose from the blood (except central nervous system cells). Insulin is also the principal control signal for conversion of glucose to glycogen (which is a starch-like polymer chain made up of glucose molecules) for internal storage in liver and muscle cells. Lowered glucose levels result in the reduced release of insulin from the β-cells, in the reverse conversion of glycogen to glucose (glycogenolysis) and then glucose generation by gluconeogenesis.

Insulin and its related proteins have been shown to be produced inside the brain, and reduced levels of these proteins are linked to Alzheimer's disease (Susannae M De la Monte and Wands JR, 2008, Wen Fu et al., 2013).

In β-cells, insulin is synthesized from the proinsulin precursor molecule by the action of proteolytic enzymes, known as prohormone convertases (PC1 and PC2), as well as the exoprotease carboxypeptidase E (Steiner D F et al., 1967). These modifications of proinsulin remove the center portion of the molecule (i.e., C-peptide), from the C- and N-terminal ends of proinsulin.

The middle segment of proinsulin is between the N-terminal B-chain and the C-terminal A-chain. It is a pancreatic peptide of about 31 residues, depending on the species. Upon proteolytic cleavage of proinsulin, equimolar insulin and C-peptide are released. C-peptide immunoassay has been used to assess pancreatic β-cell function in diabetic patients with circulating insulin antibodies or exogenous insulin. Half-life of C-peptide is 30 minutes, almost 8 times that of insulin.
Insulin is produced and stored in the body as a hexamer (a unit of six insulin molecules), while the active form is the monomer. The hexamer is an inactive form with long-term stability, which serves as a way to keep the highly reactive insulin protected, yet readily available. A fast-reacting drug means, insulin injections do not have to precede mealtimes by hours, which in turn gives diabetics more flexibility in their daily schedules (Dunn M F, 2005).

The first phase release is rapidly triggered in response to increased blood glucose levels. Increasing the amount of glucose, researchers then monitored the additional production of insulin and found that insulin production did increase to higher levels when insulin was already present. Members of Dr. Goldfine’s research laboratory in Boston, Massachusetts have confirmed in humans that when blood sugar levels rise in healthy people, insulin signals β-cells to increase production of insulin (Proceedings of the National Academy of Sciences, USA, March 2010).

Dr. Rohit Kulkarni, proved in animal models, that insulin is important in regulating its own production, led the pilot experiments in mice a decade ago. Several different studies in mice formed the basis for the above experiments in people.

The second phase is a sustained, slow release of newly formed vesicles triggered independently of sugar.

Other substances known to stimulate insulin release include the amino acids arginine and leucine, parasympathetic release of acetylcholine (via phospholipase C), sulphonylurea, cholecystokinin (CCK, via phospholipase C) (Cawston Erin E and Miller Laurence J, 2010) and the gastrointestinal-derived incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP).

Although glucose levels are a primary regulator of insulin secretion, signaling through different GPCRs can have positive or negative effects on insulin secretion through their regulation of intracellular signaling pathways. One group of GPCRs present in islets is the adrenergic family of receptors. These receptors are named for their agonists, adrenalin (epinephrine) and noradrenalin (norepinehrine), examples of catecholamines. While these receptors play a key role in the regulation of blood pressure, scientists now know that members of the adrenergic family of
GPCRs, including both the $\alpha_2$ and $\beta_2$ receptors, also regulate islet function. (For example, $\alpha_2$-adrenergic receptors inhibit insulin secretion and stimulate glucagon secretion, while $\beta_2$-adrenergic receptors stimulate both insulin and glucagon secretion).

The sympathetic nervous system via $\alpha_2$-adrenergic stimulation inhibits the release of insulin. However, it is worth noting that epinephrine activates $\beta_2$-receptors on the $\beta$-cells in the pancreatic islets to promote insulin release (Layden BT et al., 2010). This is important, since muscle cannot benefit from the raised blood sugar resulting from adrenergic stimulation, unless insulin is present to allow for GLUT-4 translocation in the tissue. Therefore, beginning with direct innervation, norepinephrine inhibits insulin release via $\alpha_2$-receptors, and then subsequently, circulating epinephrine from the adrenal medulla. Epinephrine will then stimulate $\beta_2$-receptors, thereby promoting insulin release.

Insulin release from the $\beta$-cells slows or stops when the glucose level comes down to the usual physiologic value. If blood glucose levels drop lower than this, especially to dangerously low levels, release of hyperglycemic hormones (most prominently glucagon from islet of Langerhans $\alpha$-cells) forces release of glucose into the blood from cellular stores, primarily liver cell stores of glycogen. By increasing blood glucose, the hyperglycemic hormones prevent or correct life-threatening hypoglycemia. Release of insulin is strongly inhibited by the stress hormone norepinephrine (noradrenalin), which leads to increased blood glucose levels during stress.

Evidence of impaired first-phase insulin release can be seen in the glucose tolerance test, demonstrated by a substantially elevated blood glucose level at 30 minutes, a marked drop by 60 minutes, and a steady climb back to baseline levels over the following hourly time points.

*Special transporter proteins in cell membranes allow glucose from the blood to enter a cell.* These transporters are indirectly under blood insulin's control in certain body cell types (e.g., muscle cells). Low levels of circulating insulin, or its absence, will prevent glucose from entering those cells (e.g., in T1DM). More commonly, however, there is a decrease in the sensitivity of cells to insulin (e.g., the
reduced insulin sensitivity characteristic of T2DM), resulting in decreased glucose absorption. In either case, there is ‘cell starvation’ and weight loss, sometimes extreme. In a few cases, there is a defect in the release of insulin from the pancreas. Either way, the effect is the same: elevated blood glucose levels.

Activation of insulin receptors leads to internal cellular mechanisms that directly affect glucose uptake by regulating the number and operation of protein molecules in the cell membrane that transport glucose into the cell.

Two types of tissues are most strongly influenced by insulin, as far as the stimulation of glucose uptake is concerned: muscle cells (myocytes) and fat cells (adipocytes). The former are important because of their central role in movement, breathing, circulation, etc., and the latter because they accumulate excess food energy for future needs. Together, they account for about two-thirds of all cells in a typical human body.

*Insulin binds to the extracellular portion of the alpha subunits of the insulin receptor.* This, in turn, causes a conformational change in the insulin receptor that activates the kinase domain residing on the intracellular portion of the beta subunits. The activated kinase domain autophosphorylates tyrosine residues on the C-terminus of the receptor as well as in the IRS-1 protein.

*After the signal has been produced, termination of signaling is then needed.* As mentioned below in the section on degradation, endocytosis and degradation of the receptor bound to insulin is a main mechanism to end signaling. In addition, signaling can be terminated by dephosphorylation of the tyrosine residues by tyrosine phosphatases. Serine/Threonine kinases are also known to reduce the activity of insulin. Finally, with insulin action being associated with the number of receptors on the plasma membrane, a decrease in the amount of receptors also leads to termination of insulin signaling.

Once an insulin molecule has docked onto the receptor and effected its action, it may be released back into the extracellular environment, or it may be degraded by the cell. The two primary sites for insulin clearance are the liver and the kidney. The liver clears most insulin during first-pass transit, whereas the kidney clears most of the insulin in systemic circulation. Degradation normally involves
endocytosis of the insulin-receptor complex, followed by the action of insulin-degrading enzyme. An insulin molecule produced endogenously by the pancreatic β-cells is estimated to be degraded within about one hour after its initial release into circulation (insulin half-life ~ 4–6 minutes)(William C Duckworth et al., 1998, Palmer B F et al., 2010).

Lack of insulin appears to be the chief factor involved in diabetes; 90% of diabetics seem to suffer from T2DM (Wilson P W and Kannel W B, 2002). Thus, antihyperglycaemic drugs are necessary for the treatment of T2DM (Ratner R E 2007, Roglic G et al., 2005). In T2DM patients, several key pathogenic abnormalities contribute to increased blood glucose levels, including abnormal insulin secretion caused by impaired β-cell function and insulin resistance in target tissues (Taylor S I, 1999). In the long run, a T2DM patient may become T1DM.

Scientists generally think that reduced insulin production by the pancreas, a hallmark of T2DM, is due to the death of the β-cells. However, a new study by Columbia University Medical Centre (CUMC) researchers shows that β-cells do not die, instead revert to a more fundamental, undifferentiated cell type. Pancreatic β-cell dedifferentiation leads to decline in insulin production in T2DM. The findings suggest that strategies to prevent β-cells from de-differentiating, or to coax them to re-differentiate, might improve glucose balance in patients with T2DM. The study was conducted in mice (Columbia University medical room, Sep., 2012).

The **insulin receptor (IR)** is a transmembrane receptor that is activated by insulin, IGF-I, IGF-II and belongs to the large class of tyrosine kinase receptors (Ward C W and Lawrence M C, 2009).

Metabolically, the insulin receptor plays a key role in the regulation of glucose homeostasis, a functional process that under degenerate conditions may result in a range of clinical manifestations including diabetes and cancer (Ebina Y and Ellis L, 1985).

The function of an insulin receptor is to control the movement of the hormone insulin from the blood stream into certain types of cells. Insulin receptors are not present in all the cells. An insulin receptor responds to a cell’s need for insulin by moving back and forth from the surface to the interior of the cell.
Upregulation is when the insulin receptors move to the surface of the cell and down-regulation is when the receptors move back.

**The actions of insulin in removing glucose from the blood, which are enabled by insulin receptors, also assist in maintaining stable blood glucose levels. This is important because some types of cells, such as nerve cells, do not have insulin receptors and do not use insulin to regulate the intake of glucose. These cells uptake glucose through diffusion, and they are very much affected by blood glucose levels.**

Insulin resistance (impaired insulin sensitivity) is a physiological condition where cells are no longer able to respond to the normal actions of the hormone insulin. Cells are not able to take in glucose, amino acids and fatty acids. Thus, glucose, fatty acids and amino acids ‘leak’ out of the cells. A decrease in insulin/glucagon ratio inhibits glycolysis which in turn decreases energy production.

In such cases, the pancreas secretes more insulin to compensate for the insulin resistance. People with this syndrome have high levels of insulin in the blood as a marker of the disease rather than a cause.

**Poorly functioning insulin receptors can lead to insulin resistance, which occurs when too few insulin receptors are at the cells surfaces to respond to insulin, allowing glucose to enter. The cells are in effect starving, but they don’t have the means to allow glucose to enter.**

Insulin resistance can lead to T2DM. In this disorder, the body produces sufficient insulin but the insulin and the receptor are not able to bind together, leaving high levels of glucose in the blood. This disease can lead to blindness and cardiovascular disease.

The insulin receptor on binding with insulin changes shape so that the kinase regions inside the cell become activated. The activated insulin receptor then activates a number of different targets within the cell. The targets are often enzymes, leading to the increase or decrease in the different chemical reactions involving glucose, as described earlier. The effect of insulin on liver cells is called a tyrosine kinase second messenger system.
Introduction

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<tr>
<th>Transporter</th>
<th>Tissue distribution</th>
<th>Special properties</th>
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</thead>
<tbody>
<tr>
<td>GLUT-1</td>
<td>Most cells.</td>
<td>High capacity, relatively low $K_m$ (1-2mM).</td>
</tr>
<tr>
<td>GLUT-2</td>
<td>Liver, beta cells, hypothalamus, basolateral membrane small intestine.</td>
<td>High capacity but low affinity (high $K_m$, 15-20mM) part of “the glucose sensor” in β-cells. Carrier for glucose and fructose in liver and intestine</td>
</tr>
<tr>
<td>GLUT-3</td>
<td>Neurons, placenta, testes.</td>
<td>Low $K_m$ (1mM) and high capacity.</td>
</tr>
<tr>
<td>GLUT-4</td>
<td>Skeletal and cardiac muscle, fat.</td>
<td>Activated by insulin. $K_m$ 5mM.</td>
</tr>
<tr>
<td>GLUT-5</td>
<td>Mucosal surface in small intestine, sperm.</td>
<td>Primarily fructose carrier in intestine.</td>
</tr>
</tbody>
</table>

Table 1.1 Glucose transporter proteins, tissue distribution and special properties

The outer membrane of eukaryotic cells has a lipid bi-layer structure. Most metabolically active water-soluble materials are effectively hindered from crossing these membranes. Small channels are found in these membranes and these do allow low-molecular weight compounds (MW < 80) to diffuse into and out of cells. However, “simple” compounds such as sugars and amino acids are much larger. Carriers are necessary if these materials are to gain access to cells. These are usually large proteins that span the cell membrane. They are specific; transporting only the molecules they recognize and are listed in Table 1.1.

Five proteins with a high degree of homology are involved in concentration-driven transport of glucose over cellular membranes. Each of these has special physiological functions and tissue distribution.
These transport proteins mediate facilitated transport, i.e., they can only transport glucose (or fructose) from areas of high concentration to areas of lower concentration. The sugar is bound by the protein, a flip-flop mechanism reverses the membrane direction of the sugar-protein complex, the sugar is released and the protein flips around once more to initiate a new cycle. In principle the GLUT family can transport glucose both into and out of cells. In most tissues the internal glucose concentration is quite low; transport can only proceed from the extracellular area into the cell. In gluconeogenetic tissues (liver and kidney), intracellular glucose concentration can exceed blood glucose concentration in the post-absorptive or fasting states. Export of glucose from liver and kidney occurs through GLUT-2.

The glucose transporter present in pancreatic beta-cells is GLUT-2. The combination of the high $K_m$ glucose carrier and glucokinase with its high $K_m$ (5 mM) insure that the presence of increasing levels of circulating glucose is noted only when this exceeds around 5 m moles/liter. Initiation of insulin secretion by glucose occurs only when glucose levels increase from basal levels after intake of a carbohydrate meal.

The insulin-sensitive glucose transporter, GLUT-4, is found bound to internal cellular membranes where it is inactive. Most researchers agree that GLUT-4 is bound to the Golgi apparatus. GLUT-4 is brought to the plasma membrane by an ATP requiring process. The transport protein molecules that arrive at the surface membrane contribute to glucose transport. Another ATP-dependent mechanism transports GLUT-4 back to the Golgi apparatus where these molecules are once more inactive. Insulin shifts the balance between exocytosis and endocytosis such that the number of functional GLUT-4 molecules in the plasma membrane increases, thereby activating glucose uptake. Note that muscle activity can increase the number of GLUT-4 molecules in the plasma membrane through the same mechanism. Muscle activity and depletion of intracellular glucose alone (without increased insulin levels) activates glucose uptake.
1.9 DIAGNOSIS

Diabetes mellitus is characterized by recurrent or persistent hyperglycaemia, and is diagnosed by demonstrating any one of the following: (Alberti K G and Zimmel P Z, 1998).

- Fasting plasma glucose level $\geq 7.0$ mmol/l (126 mg/dl)
- Plasma glucose $\geq 11.1$ mmol/l (200 mg/dl) two hours after a 75 g oral glucose load as in a glucose tolerance test.
- Symptoms of hyperglycaemia and casual plasma glucose $\geq 11.1$ mmol/l (200 mg/dl)
- Glycated haemoglobin (Hb A\textsubscript{1C}) $\geq 6.5\%$ (Diabetes Care, January 2010, ADA).

T1DM can be distinguished from T2DM by C-peptide assay, which measures endogenous insulin production.

Newly diagnosed diabetes patients often get their C-peptide levels measured as a means of distinguishing T1DM and T2DM. C-peptide levels are measured instead of insulin levels because insulin concentration in the portal vein is two to ten times higher than in the peripheral circulation. The liver extracts about half the insulin reaching it in the plasma, but this varies with the nutritional state. The pancreas of patients with T1DM is unable to produce insulin, and, therefore, they will usually have a decreased level of C-peptide, whereas C-peptide levels in T2DM patients are normal or higher than normal. Measuring C-peptide in patients injecting synthetic insulin can help to determine how much of their own natural insulin these patients are still producing, or if they produce any at all.

Insulin is used medically to treat T1DM and some forms of T2DM. Patients with T2DM are often insulin resistant and, because of such resistance, may suffer from a “relative” insulin deficiency. Some patients with T2DM may eventually require insulin if other medications fail to control blood glucose levels adequately. Over 40\% of those with T2DM require insulin as part of their diabetes management plan.
1.10 CLASSIFICATION OF HERBAL ANTIDIABETICS (Patel P M et al., 2006)

The wide range of chemical constituents present in the plants acts on different sites in the body and some demonstrate hypoglycemic / antidiabetic properties. Experimental evidence suggests that, all these agents act through different mechanisms of action. On the basis of mechanism of action, antidiabetic plants are classified as below.

1. Plants mimicking the action of insulin. *Momordica charantia*

2. Plants that increase insulin secretion from β-cells. *Panax ginseng* (release of insulin from cells; *Pterocarpus marsupium*, *Gymnema sylvestra*, and *Morus bombysis* (regeneration of β-cells); *Lythrum salicaria*, *Trigonella foenum-graecum*, *Cassia tamala* and *Swertia chirayata* (increase circulating insulin levels)

3. Plants that inhibit glucagon secretion. Ke-Tang-Ling (an oriental antidiabetic drug)

4. Plants that reduce glucose absorption from gastrointestinal tract. *Cyamopsis tetragonolobus*, *Cuminum nigrum*, *Andrographis paniculata*, *Pterocarpus marsupium*, *Ocimum sanctum*, *Saccharum officinarum*.

5. Plants that increase glucose uptake by muscle. *Swertia chirayata*


8. Plants that increase glucose uptake by lipocytes. *Ocimum sanctum*, *Swertia japonica*.
9. Plants that increase glucose uptake by GLUT-4 in skeletal muscles. Sangbackpitang, a preparation containing Morus bombycis, Panax ginseng, Liriope muscari, Pueraria thunbergiana, Poria cocos, Dioscorea batatas, Cinnamomum cassia, Glycyrrhiza uralensis.


12. Plants increasing glyoxalase 1 activity in liver and creatinine kinase levels in tissue. Trigonella foenum-graecum.

Studies conducted in different countries and published in 34 reliable medical journals have been effectively reviewed in an article (Chan C H, Ngoh G C, Yusoff R, 2012). The study showed that Asian and African continents have 56% and 17% share of the worldwide distribution of therapeutic herbal plants, respectively. Remarkable improvements have been recorded in the control of diabetes using plant extracts from the Asian countries India and China since 1995.

The information collected shows that plant leaves are about 20% more favourable for storing active ingredients, as compared to other parts of herbal plants. A brief review on the extraction techniques for the mentioned parts is also included. Furthermore, the acting mechanisms for the antidiabetic activity were described, and the related active ingredients were identified. The findings reveal that most of the antidiabetic research is focused on the alteration of glucose metabolism to prevent diabetes.

Mechanism of action of quercetin (stimulation of insulin release and regeneration of pancreatic islets), kaempferol (promotes hypoglycaemic effect), apigenin (improves glucose tolerance and increase plasma insulin level), catechins (exhibit hypoglycaemic, glucose oxidizing and insulin mimetic activities), 3-hydroxy benzoic acid (enhances serum insulin level and liver glycogen content), are reported therewith.
1.11 SUMMARY

In the management of the disease discussed in this chapter, current methods of treatment, medicines used and its limitations are presented. Due to the limitations of the currently used medicines, search for newer agents are in progress. A brief list of currently used natural products with the possible mechanism through which they act is also included here.

The chemistry of insulin including biogenesis is then discussed. Pathophysiology of Diabetes mellitus and after that the role of insulin in the homoeostasis of glucose and its related mechanisms are also elaborated.

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