Review of Literature

The discovery and development of medicine from galenical to genomical was portrayed with a focus on the potential and role of ayurveda. The natural products, including plants, animals and minerals have been the basis of treatment of human diseases. Ayurveda - as a traditional Indian medicinal system being practised for thousands of years. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on ayurvedic medicinal plants. For ayurveda and other traditional medicines newer guidelines of standardization, manufacture and quality control are required. Employing a unique holistic approach, ayurvedic medicines are usually customized to an individual constitution. Traditional knowledge-driven drug development can follow a reverse pharmacology path and reduce time and cost of development. New approaches to improve and accelerate the joint drug discovery and development process are expected to take place mainly from innovation in drug target elucidation and lead structure discovery (Bhushan Patwardhan et al., 2004).

Combining the strengths of the knowledge base of traditional systems such as ayurveda with the dramatic power of combinatorial sciences and HTS will help in the generation of structure–activity libraries. Ayurvedic knowledge and experiential database can provide new functional leads to reduce time, money and toxicity – the three main hurdles in drug development. These records are particularly valuable, since effectively these medicines have been tested for thousands of years on people (Vaidya and Chorghade, 2004).

The value of traditional medicine is explained as the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. It is stated that throughout the history of mankind, many infectious diseases have been treated with herbals. Among the remedies used, plant drugs constitute an important part. A number of scientific investigations have highlighted the importance and the contribution of many plant families i.e. Asteraceae, Liliaceae, Apocynaceae, Solanaceae, Caesalpinaceae, Rutaceae, Piperaceae,
Sapotaceae used as medicinal plants. Medicinal plants play a vital role for the development of new drugs (export and import diverse parts or bioactive compounds in the current market). The bioactive extract should be standardized on the basis of active compound. The bioactive extract should undergo limited safety studies Tribal healers in most of the countries, where ethnomedical treatment is frequently used to treat cut wounds, skin infection, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snakebite and gastric ulcer. Several authors are currently being undertaken to isolate the active compound(s) by bioassay-guided fractionation from the species that showed high biological activity during screening. Therefore, these scientific investigations may be utilized to develop drugs for these diseases. Further research is deserved to isolate the compounds responsible for the observed biological activity (Perumal samy and Gopalakrishnakone, 2007).

The organic compounds from terrestrial and marine organisms have been used extensively in past and present for the treatment of many diseases and serve as compounds of interest both in their natural form and as templates for synthetic modification. Over 20 new drugs launched on the market between 2000 and 2005, originating from terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates, are described. These approved substances, representative of very wide chemical diversity, together with several other natural products or their analogs undergoing clinical trials, continue to demonstrate the importance of compounds from natural sources in modern drug discovery efforts, he has clearly assessed the values of natural products and its criteria. 23 new drugs derived from natural sources have been launched on the market during 2000 – 2005, even though many pharmaceutical companies have discontinued their programs of drug discovery from natural sources. These new drugs have been approved for the treatment of cancer, neurological diseases, infectious diseases, cardiovascular and metabolic diseases, immunological, inflammatory and related diseases, and genetic disorders, which encompass many of the common human diseases. Besides new drugs launched on the market from 2000 to the present, there are a variety of new chemical entities from natural sources undergoing clinical trials. Further research on
these compounds at industrial, governmental, and academic institutions is seen as vital for the enhancement of human health (Young Won-Chin et al., 2006).

Historically, the majority of new drugs have been generated from natural products (secondary metabolites) and from compounds derived from natural products. During the past 15 years, pharmaceutical industry research into natural products has declined, in part because of an emphasis on high-throughput screening of synthetic libraries. Although the current industry model for drug discovery does not favor natural products, the resource is so vast as to seem unlimited, and these emerging tools will provide exhilarating discoveries leading to new medicines (Jesse Li and John Vederas, 2009).

In the past, medicinal plants were used intensively in folkloric medicine for treatment of various disorders. Today, it is estimated that about 80% of people in developing countries rely on traditional medicines for their primary health care. Traditional medicines are becoming popular, due to high toxicity and adverse effects of orthodox medications. This has led to the sudden increase in the number of herbal industries in the drug market. Several plant species are used by various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy for the treatment of different ailments (Inder Makhija et al., 2011).

Natural products have been the single most productive source of leads for the development of drugs. Over a 100 new products are in clinical development, particularly as anti-cancer agents and anti infectives. Application of molecular biological techniques is increasing the availability of novel compounds that can be conveniently produced in bacteria or yeasts, and combinatorial chemistry approaches are being based on natural product scaffolds to create screening libraries that closely resemble drug-like compounds. Various screening approaches are being developed to improve the ease with which natural products can be used in drug discovery campaigns, and data mining and virtual screening techniques are also being applied to databases of natural products. It is hoped that the more efficient and effective application of natural products will improve the drug discovery process (Alan Harvey, 2008).
During the last 20 years, the considerable and significant advances in tissue culture methodology, the use of chemically-defined cell and tissue culture media, and the availability of mammalian cells have transformed in vitro methods from a new technology to a valuable research tool. In the past, in vitro methodology was used as the last approach in product development. Today, the use of in vitro tests in product development, drug discovery and safety evaluation has become common place, resulting almost exclusively from the evolution of science rather than any fundamental change in philosophy. Yet all in vitro methods are alternatives to animal testing. It is not known how well any in vitro system would recapitulate the in vivo system. Thus, it would be difficult to design an in vitro test battery to replace in vivo test systems. In vitro systems are well suited to the study of biological processes in a more isolated context, therefore, in vitro tests have their greatest potential in providing information on basic mechanistic processes in order to refine specific experimental questions to be addressed in the animal (Sarah wild et al., 2004).

Advantages of in vitro models include the ability to directly manipulate cultivation conditions and isolate the target tissue from the physiological effects of other organs and tissues. Additional unique competitive advantages of in vitro models include: 1) Rapid large-scale screening of drug candidates allows promising substances to be identified at an early stage of the drug development process; 2) The time and costs involved in developing active agents are significantly reduced and 3) Cost-intensive and ethically controversial animal experiments can be reduced to a minimum. In order to determine the best model for antidiabetic screening, it is essential to elucidate and understand the molecular machinery involved in the regulation of blood glucose levels (Roglic et al., 2005).

Diabetes Mellitus

Type 2 diabetes mellitus is a heterogeneous disorder due to a combination of inherited and acquired factors that adversely affect glucose metabolism. It is thought that these factors lead to diabetes mainly by affecting β-cell function and tissue insulin sensitivity. If the amount of insulin produced is too little to allow for glucose
to be used or stored, or if the insulin being produced does not work effectively, glucose accumulates in the blood. Hyperglycaemia develops when rates of glucose release into the circulation exceed rates of tissue glucose uptake. This may occur because release is increased, because uptake is reduced, or due to a combination of factors such as increased release with a lesser increase in uptake (Astrup and Finer, 2000).

In the normal individual, the concentration of glucose in blood is maintained at about 90mg/dl of plasma. However, fasting blood glucose in diabetics may be 300-400 mg/dl and may even reach 1000 mg/dl. Type 2 diabetes is associated with insulin resistance initially and later, as the function of the β-cell decreases, insulin deficiency. Type 2 diabetes is characterized both by abnormalities of insulin secretion progressively leading to secretion failure as well as insulin resistance of all major target tissues (Haiyan Liu, 2008).

Although insulin resistance is important in the early stages of Type 2 diabetes, the failure in adequate β-cell compensation leads to the progression to the diabetic state. Compensation for insulin resistance is through increased secretion per β-cell or by an increase in β-cell mass through neogenesis or replication of the existing β-cells. Beta-cell mass is normally tightly maintained through a balance of β-cell birth (β-cell replication and islet neogenesis) and β-cell death through apoptosis. Most of the increase in β-cell mass with insulin resistance is probably due to increased β-cell number, but β-cell hypertrophy may also contribute (Weir and Bonner-Weir, 2004).

It is suggested that the disease is triggered when the delicate balance between insulin production and insulin responsiveness goes awry. First, cells in muscle, fat and liver lose some of their ability to respond fully to insulin. In response to growing insulin resistance, pancreatic cells temporarily over produce insulin causing hyperinsulinemia. Much of the increase in insulin secretion undoubtedly results from the increase in β cell mass. At some point, β-cells are no longer able to keep glucose levels in the prediabetic range. This failure presumably occurring because of a critical decline of β-cell mass and/or increase in insulin resistance. But the insulin
producing cells eventually die, leading to full-blown diabetes. Therefore Type 2 diabetes results when the body loses the fine-tuned balance between insulin action and insulin secretion. For years up until recently, it was believed that a malfunction in the insulin receptor leads to insulin resistance however researchers are converging on a new hypothesis to explain this metabolic disorder. The shift in thinking occurred as a result of failure in linking insulin receptor malfunction and the disease. It is now believed by some researchers that two related pathways that normally respond to insulin by signalling cells in the tissues to remove glucose, lie at the heart of insulin resistance. They are however unsure as to why the biochemical pathways believed to be involved are not functioning properly. It is believed that defects in the insulin signaling pathway leading to the disease are subtle, as no mutations in the insulin receptor substrate (IRS) genes in diabetics have been uncovered. Due to insulin’s regulation of glucose metabolism being so finely tuned, it is believed that one or two subtle mutations will upset the entire system when combined with the proper environmental insults. Another new insight into Type 2 diabetes is that in order for this disease to develop, insulin resistance must occur in both muscle and liver, leading researchers to conclude that the insulin regulating system must fail at multiple points (Ryosuke Nakano, 2006).

Global prevalence of Diabetes mellitus

Diabetes mellitus is one of the commonest diseases affecting the citizens of both developed and poor countries. In South Africa, the number of people suffering from diabetes is believed to be rising steadily. An ethnobotanical study of plants used by the traditional healers, herbalists and rural dwellers for the treatment of diabetes mellitus was conducted in the Eastern Cape Province. The study revealed 14 plant species belonging to six families namely; Asteraceae, Hypoxidaceae, Apocynaceae, Asphodelaceae, Apiaceae and Buddlejaceae. The use of infusions from plant leaves and roots was the common method of herbal preparation. In all cases, the treatment involved drinking the extracts for a long period of time. There was a general belief on the efficacy of the prepared extracts (Erasto et al., 2005).
The Asia-Pacific region is at the forefront of the current epidemic of diabetes. There are currently more than 30 million people with diabetes in the Western Pacific region alone. The World Health Organization predicts that this number will rise dramatically by the year 2025, by which time India and China may each face the problem of dealing with 50 million affected individuals. The problem in the region resulted from a combination of large population size with rapidly rising prevalence rates, particularly of Type 2 diabetes mellitus. Although much heterogeneity exists, rising prevalence rates are being seen throughout the region and appear to be closely associated with westernisation, urbanisation, and mechanisation. The risk for diabetes appears to result from a combination of genetic predisposition and lifestyle change. The most important lifestyle changes relate to changes in dietary habits and physical activity and diabetes risk, particularly in younger individuals, is associated with the development of obesity and particularly central obesity (Cockram, 2000).

Estimated prevalence rates in for urban and rural India are based on national surveys and individual studies. Estimates vary depending on geographical location and year of study conducted in urban India. In the urban population, an Indian Council of Medical Research (ICMR) study in 1972 reported a prevalence of 2.3% which rose to 12.1% in the year 2000. More recently, provided estimates from a nationwide surveillance study of T2DM and found that in urban areas there was a prevalence 7.3% of known T2DM and a prevalence of 3.2% in peri-urban/slum areas Estimates of T2DM prevalence were calculated by applying sampling weights derived from the 2004 census where T2DM was defined by disease history and/or fasting glucose of 7.0 mmol or over. The results indicated that the prevalence for known T2DM was of 6.4%, for undiagnosed T2DM 6.8%, and that 15.5% (Zargar et al., 2000).

Diabetes mellitus – DPP IV inhibitor a novel target

Although being a primary objective in the management of Type 2 diabetes, optimal glycaemic control is difficult to achieve and usually not maintained over time. Type 2 diabetes is a complex pathology, comprising altered insulin sensitivity and impaired insulin secretion. Recent advances in the understanding of the physiological functions of incretins and their degrading enzyme dipeptidyl-peptidase
DPP IV have led to the ‘discovery’ of a new class of oral anti-diabetic drugs. Several DPP IV inhibitors (or gliptins) with different chemical structures are now available. These agents inhibit the degradation of the incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) and hence potentiate glucose-dependent insulin secretion. DPP IV inhibitors inhibit DPP IV activity by almost 100% in vitro, maintaining a >80% inhibition throughout the treatment period in vivo, thus prolonging GLP-1 half-life, and significantly reducing HbA1c generally by 0.7 – 0.8% as well as fasting and postprandial glycaemia. They are well-tolerated with no weight gain and few adverse effects, and, of particular interest, no increase in hypoglycaemic episodes. Although different by their chemical structure and pharmacokinetic properties, the DPP IV inhibitors currently available have proven similar glucose lowering efficacy (HélèneDuez et al., 2012).

The therapeutic potential of inhibitors of post-proline cleaving enzymes like DPP IV has been the focus of recent pharmaceutical research. Three DPP IV inhibitors are in advanced clinical development: saxagliptin (Bristol-Myers Squibb Co.), which is in phase III clinical trials, and vildagliptin (Novartis) and sitagliptin (Merck), both of which have had New Drug Applications filed. Regulatory filings of the first class DPP IV inhibitors are to be expected in 2006. Available data show differences in duration of action and anticipated dosing frequency, whereas data to compare clinical efficacy and safety is not available presently. The DPP IV inhibitors are evaluated being as monotherapy or in combination with other antidiabetic drugs, e.g. metformin, thiazolidinediones, and/or PPARγ agonists. Although human trial results in Type 2 diabetes with DPP IV inhibitors look promising, the lack of selectivity, i.e. inhibition of the structurally related enzymes DPP-8 and DPP-9, has been a potential concern. Based on the crystal structure resolved, it is expected to develop certain therapeutic agents such as small peptide via binding to the catalytic binding site as a ‘“substrate-selective’” DPP IV inhibitor (Yau jan chyan and Lee ming chaung, 2007).

Dipeptidyl peptidase IV/CD26 is a Type 2 integral membrane protein consisting of a hydrophobic N-terminal domain, a transmembrane region, and a C-terminal domain.
containing the catalytic triad that acts on oligopeptides by selectively removing N terminal dipeptides. In addition to its catalytic function, DPP IV/CD26 plays important structural and signaling roles in cells involving binding sites capable of associating with diverse proteins, DPP IV exhibits a strong preference for peptides with proline (Pro) or alanine (Ala) as the penultimate (P1) amino acid, but it is now established that it can also act efficiently on peptides with N-termini consisting of Xaa-Serine (Ser). There are also differences of opinion as to whether it is therapeutically beneficial to administer inhibitors that induce partial or complete, irreversible or reversible, inhibition and whether single or multiple drug doses are preferable (Christopher McInstosh et al., 2006).

In the last years, synthetic inhibitors of DPP IV (DP IV; EC 3.4.14.5, CD26) were found to be promising drugs for the treatment of different disorders including autoimmune diseases. A selection of these inhibitors is already in clinical trials for the treatment of Type 2 diabetes and under in vivo investigation in psoriasis, rheumatoid arthritis (RA), colitis, and multiple sclerosis (MS). DPP IV is an ectoenzyme releasing N-terminal dipeptides from peptides with proline in the penultimate position. It exists in a membrane-bound and a secreted form and has access to extracellularly localized substrates. In the immune system, DPP IV is expressed on the surface of T cells, B cells, and natural killer cells. Some chemokines, including CC chemokine ligand 5 (regulated on activation, normal T cell expressed and secreted) and CXC chemokine ligand 12 (stromal cell-derived factor-1), were identified to be hydrolyzed by DPP IV (Sabine Wrenger et al., 2006).

Apart from DPP IV, Glucagon-like peptide-1 (GLP-1) is one of the important insulin-releasing hormones (incretins) and in postprandial released by the entero glucagon-producing L cells in the lower gut, i.e., the ileum. GLP-1 regulates not only blood glucose via stimulation of glucose-dependent insulin secretion, but also the inhibition of gastric emptying and glucagon secretion. In addition, GLP-1 may regulate food intake in the central nervous system and glycogen synthesis in adipose tissue and muscle. Recent studies have shown that disruption of the GLP-1 receptor gene results in fasting hyperglycaemia and abnormal glycemic excursions after glucose challenge together with reduced levels of glucose stimulated insulin,
emphasizing the essential role of GLP-1 in the control of blood glucose (Hironobu mitani et al., 2002).

Streptozotocin induced diabetes is a well documented model of experimental diabetes. Previous reported literature indicates that the type of diabetes and characteristics differ with the employed dose of STZ and animal and species used. Streptozotocin induced diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia. STZ is a pancreatic β cell toxin that induces rapid and irreversible necrosis of β cells, whereas a single diabetogenic dose of STZ (70-250mg/kg, body weight) has been demonstrated to induce complete destruction of β cells in most species within 24 hour, multiple sub-diabetogenic doses of STZ partially damage islets, thereby triggering an inflammatory process leading to macrophage and subsequent lymphocyte infiltration, which is followed by the onset of insulin deficiency (Sachin Arora et al., 2009).

The new insights into Type 2 diabetes are mainly from studies in knockout mice, however, human genetics does not necessarily correlate with animal data, therefore not everyone is convinced and the old dogma is still being followed. The major causes of insulin resistance of the skeletal muscle in the prediabetic state may be discussed as genetic background, obesity related insulin resistance and of physical inactivity. Type 2 diabetes is known to have both genetic and environmental determinants and is strongly associated with age and obesity (Philip wenzel, 2008).

Among the environmental factors causing insulin resistance, obesity is of predominant importance. Obesity is defined as an excess of body weight that is mainly attributable to an increased body fat accumulation. Obesity is the most important modifiable risk factor for Type 2 diabetes. About 90 percent of people with this form of the disease are overweight. An excess of body fat is associated with a deterioration of glucose utilisation and promotes development of Type 2 diabetes, particularly in those with a genetic predisposition for the disease (www.geocities.com/jqjacobs).
Diabetes and its comorbidities - Hyperlipidemia

Treatment of Type 2 diabetes is not limited to just glycaemic control. Rather, the proper management of hyperglycaemia, weight, blood pressure, and lipids can have benefits in terms of slowing the progression of Type 2 diabetes, decreasing the risk of CV disease, and improving overall health. Current antihyperglycaemic treatments are predominantly insulin-dependent. These treatments can effectively manage HbA1c, and treatment may be influenced by the patient’s comorbidities and any potential treatment-related adverse events (Jens Juul Holst and Carolyn, 2004).

The metabolic syndrome is a cluster of various cardiovascular disease risk factors: diabetes and pre-diabetes, abdominal obesity, hyperlipidaemia and high blood pressure. People with metabolic syndrome are twice as likely to die from and three times as likely to have a heart attack or stroke compared with people without the syndrome. It was observed the frequency of metabolic syndrome (MetS) in patients with Type 2 diabetes mellitus and study the relationship of insulin resistance with the metabolic syndrome and its different clinical parameters (Shahid Ahmed et al., 2010).

There are a number of different hypotheses explaining the mechanism of insulin resistance in obesity. It was proposed that tumor necrosis factor alpha (TNF-α) is released by adipose tissue and is able to impair insulin signaling through serine kinase and tyrosine phosphatase dependent modulation of the insulin signaling chain at the level of the insulin receptor and substrates (IRS). Another proposed mechanism of insulin resistance in obesity is related to a protein molecule which interferes with insulin action. Membrane glycoprotein PC-1 (plasma cell antigen-1) has been shown to reduce the uptake of glucose by cells through inhibiting insulin receptor tyrosine kinase activity. Obesity’s contribution to the onset of diabetes may be by increasing the levels of PC-1 (www.pslgroup.com). The increase in obesity prevalence has led the World Health Organization (WHO) to refer to a ‘global epidemic’ to describe the obesity issue. Body weight and fat losses are essential if the health burden which obesity imposes on a great proportion of individuals throughout the world is to be alleviated (Shinji Susa et al., 2008).
Insulin

Despite significant advances in past years on the chemistry and biology of insulin and its receptor, the signaling mechanisms involved in the various biologic responses to insulin remain somewhat elusive. Progress in this area has been complicated by the pleiotropic nature of the actions of insulin. The relative activation and coordination of these distinct cellular processes by insulin varies with cell type, state of differentiation of the cell, presence of other hormones, and insulin dose response and time course, suggesting that insulin action involves a network of inter-related and independent pathways with differing levels of divergence regarding mechanisms of regulation (Saltiel, 1990).

Insulin is the most potent anabolic hormone known and it is essential for appropriate tissue development, growth, and maintenance of whole-body glucose homeostasis. It regulates glucose homeostasis at many sites, reducing hepatic glucose output (via decreased gluco-neogenesis and glycogenolysis) and increasing the rate of glucose uptake, primarily into striated muscle and adipose tissue (in Fig-1). It also profoundly affects lipid metabolism, increasing lipid synthesis in liver and fat cells, and attenuating fatty acid release from triglycerides in fat and muscle (Pessin and Saltiel, 2000).
As mentioned in the above Figure-1 Insulin is the principal regulator of energy metabolism. When glucose or other nutrients are absorbed from the gastrointestinal tract, this elicits insulin secretion. Insulin regulates the metabolism of multiple fuels. Selected actions of insulin are indicated (+, activation; -, inhibition). Insulin activates transport of glucose into muscle and adipose tissue, and promotes synthesis of glycogen and triglycerides. Insulin also inhibits hepatic glucose production by
inhibiting both glycogenolysis and glucogenesis. Insulin does not directly regulate the metabolism of red blood cells, which uses glycolysis to provide energy. Although the brain uses glucose in the fed state, it can also use ketone bodies when levels raise high enough (e.g., during fasting) (Taylor, 2000).

Importance of Antioxidant Activity

The antioxidant defense system represents a complex network with interactions, synergy and specific tasks for a given antioxidant. Recent studies showed that majority of the plasma antioxidants are depleted in Type 2 diabetes patients. The oxidative stress and resultant tissue damage are hallmark of chronic diseases and cell death, and diabetes is not the exception. It was recently discussed some of the paradigms of oxidative stress in diabetes. Whether oxidative stress occurs at an early stage in diabetes, preceding the appearance of complications, or whether it is merely a common consequence of the tissue damage reflecting the presence of complication, is a matter of scientific debate. But, it is true that oxidative stress plays an important role in diabetic complications. Argument was made that treatment of diabetes with antioxidant therapy is like applying water to a burning house and is certainly helpful in limiting the conflagration. Obviously, finding out the real cause and understanding becomes the secondary consideration to be explained and addressed later. Nonetheless, the rationale for the therapeutic use of antioxidants in cases of diabetes and other critical disease conditions is emerging fast (Ashok Tiwari and Madhusudhana Rao, 2002).

Both increased aldose reductase (AR) activity and oxidative stress have been implicated in the pathogenesis of diabetic complications. The important role of the two mechanisms in DR (diabetic retinopathy) is supported by findings in animal models of diabetes and galactose feeding that manifest virtually identical alterations in gene expression. AR is also responsible for retinal VEGF protein expression in early diabetes (Irina Obrosova et al., 2003).

Increasing evidence in both experimental and clinical studies suggested that oxidative stress plays a major role in the development and progression of both
Type 1 and Type 2 diabetes mellitus. There has been considerable recent debate regarding the extent to which increased oxidative stress contributes towards the development of diabetic complication. The facts that the role of antioxidant compound in both protection and therapy of diabetes mellitus were also emphasized in previous scientific studies. There is an evidence that glycosylation of various protein may itself induce the generation of oxygen-derived free radicals in diabetic condition. Hyperglycemia results in the generation of free radical which can exhaust antioxidant defense thereby leading to the disruption of cellular function, oxidative damage to membrane and enhance the susceptibility to lipid peroxidation and diffuse from the site of tissue damage which is measured by malondialdehyde level. Five fold increases in plasma malondialdehyde level was observed in diabetic rats compared to normal rats which is similar to earlier report (Suresh kumar et al., 2012).

NAFLD (Non Alcoholic Fatty Liver Disease)

The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes. By definition, diabetes mellitus is categorized as a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. In the other hand, much more prevalent category, Type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. Despite the great interest in the development of new drugs to prevent the burden of complications associated with this disease and the raised interest in the scientific community to evaluate either raw or isolated natural products in experimental studies (Frode and Medeiros, 2008).

The typical characteristic of T2DM is insulin resistance (IR). A major risk factor for IR is obesity, which is generally caused by western-style high-fat diet and physical inactivity. Excess circulating lipids resulting from high-fat diet induce liver and muscle steatosis, which is tightly associated with the development of IR in these two tissues. In addition, IR may further increase the severity of tissue steatosis and
hyperlipidemia that contribute to the pathogenesis of cardiovascular disease and therefore, in addition to controlling hyper glycemia, effective correction of dyslipidemia and tissue steatosis should be considered for ideal treatment of T2DM. However, hypolipidemic activity is not included in most of the antidiabetic medicines presently in use. Searching for new reagents that are able to control both hyperglycemia and abnormal lipid profile is necessary to manage this disease (Tao zheng et al., 2012).

Non-alcoholic steatohepatitis is a pathological condition characterized by accumulation of lipids in the liver of non-alcoholic individuals and consequent oxidative stress leading to cirrhosis of liver in the long run. It has received clinical importance only recently after a long lag phase of ignorance mainly due to its asymptotic nature, lack of relevant diagnostic tests and erroneous misinterpretation with hepatitis. Incidence of NASH in USA is 30% of the adult population (Bellentani and Marino, 2009). Even, in Asian countries, number of individuals being diagnosed with NASH is on the rise. Obese individuals are at maximum risk of developing NASH and a strong positive correlation exists between insulin resistance (IR) and development of NASH (Thounaojam et al., 2009a).

Once hepatic steatosis is established, other factors, including oxidative stress, mitochondrial dysfunction, gut-derived lipopolysaccharide and adipocytokines, may promote further hepatocellular damage. In many previously published studies of non-alcoholic fatty liver disease, either genetic rodent models, such as the leptin deficient(ob/ob) or the leptin resistant (db/db) mouse, or diet induced rodent models, such as the dietary methionine/ choline deficient model have been utilized. Recently, it has been demonstrated that rodents fed a diet with either high cholesterol/cholate content or a high fat content can develop hepatic steatosis. This have been recently reported that high cholesterol (1% cholesterol and 0.3% cholate) diet can induce hepatic necrosis and macrophage infiltration in addition to steatosis. Since C57BL/6 mice are susceptible to high fat diet-induced obesity and develop insulin resistance and hepatic steatosis, in the present studies C57BL/6J mice were fed a high fat and cholesterol containing diet (with 45% Kcal fat and 0.12% cholesterol) chronically to study the progression of non-alcoholic fatty liver disease. The livers of these mice
enlarged, had evidence of steatosis, and displayed varying degrees of fibrosis and necroinflammation, all of which are hallmarks of human non-alcoholic fatty liver disease (Shuqin zheng et al., 2008).

The present study, the black tea major component theaflavins were assessed for their hepatic lipid-lowering potential when administered in fatty acid overload conditions both in cell culture and in an animal experimental model. It has been suggested that increased free FAs supplied to the liver play a major role in the early stage of this disease, supporting the idea that high circulating FAs are the major risk factor of fatty liver. Although this common syndrome is usually considered benign and without crucial clinical significance, it may progress to fibrosis, cirrhosis, and even hepatocellular carcinoma. To date, some FAS inhibitors, such as cerulenin and C75, are being investigated to reduce hepatic fat content, but applications are limited by some side effects. Recent data collected in several laboratories indicate that AMP-activated protein kinase (AMPK) plays a key role in regulating carbo-hydrate and fat metabolism, serving as a metabolic master switch in response to alterations in cellular energy charge. AMPK is known to play a major role in energy homeostasis by coordinating adaptive responses in low-energy metabolic states. Based on this, AMPK cascades have emerged as novel targets for the treatment of obesity and fatty liver (Chi-li-lin et al., 2007).

Almost one-quarter of the adults in the world population have excessive hepatic fat accumulation, and non-alcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease in many industrialized countries. Although the pathogenesis of NAFLD was complex, recent research confirmed that the disorder of gastrointestinal hormone played an important role in this disease. In terms of Traditional Chinese medicine theory, the etiology of NAFLD is poor diet or emotional disorders, and the pathogenesis for the disease is the combination of phlegm and blood stasis. The Yiqihuoxue formula has been used in clinical practice to treat NAFLD. However, the effective components in the formula and the underlying molecular mechanism needs to be further investigated (Shaodong Chen et al., 2013).
The liver is the most important organ for the intermediary metabolism of lipids, and also manufactures cholesterol for normal body functions. Over the last few years, a growing body of evidence suggests that AMP-activated protein kinase (AMPK), a serine threonine kinase comprising of a catalytic a subunit and regulatory β and γ subunits, represents a point of convergence of regulatory signals monitoring systemic and cellular energy status. AMPK has been implicated as a key regulator of physiological energy dynamics by limiting anabolic pathways, which prevent further ATP consumption, and facilitating catabolic pathways, which increase ATP generation. Activation of hepatic AMPK leads to increases in fatty acid oxidation and simultaneously inhibition of hepatic lipogenesis, cholesterol synthesis, and glucose production. In addition to a short-term effect on specific enzymes, AMPK also modulates the transcription of genes involved in lipogenesis and mitochondrial biogenesis. Among the large number of AMPK downstream targets, acetyl-CoA carboxylase (ACC) is well identified. ACC is an important rate-controlling enzyme in the synthesis of malonyl-CoA, which inhibits carnitine palmitoyl transferase 1 (CPT-1), and regulates acyl-CoA inflow and β-oxidation in the mitochondrial outer membrane. This is a rate-limiting step for fatty acid oxidation. Consequently, AMPK cascades have emerged as novel targets in the treatment of fatty liver (Thing Fong Tzeng et al., 2013).

Insulin resistance, oxidative stress and cytokine imbalance are key pathophysiological mechanisms in non-alcoholic fatty liver disease (NAFLD). A two-hit hypothesis has been proposed since 1998, in which a first hit is able to induce liver fat accumulation and a second hit prompts steatosis progression to non-alcoholic steatohepatitis. The presumed factors initiating second hits are oxidative stress and subsequent lipid peroxidation, proinflammatory cytokines (TNF-α) and hormones derived from adipose tissue (Sawsan Zaiton et al., 2011).

Although exercise and diet improvement may reduce the overall magnanimity of this disease, development of novel dietary supplements or drugs that successfully prevent the onset of NASH is desired. Recently, consumption of natural antioxidants and hepatoprotective plant products has gained popularity in last few decades
mainly due to their cost effectiveness and minimal side effects (Yayalac et al., 2013).

Recent studies have also shown effectiveness of herbal drugs with multifactorial therapeutic potential as a possible therapy against NASH. The effect of herbal supplementation on lipid accumulation, lipid peroxidation, cytotoxicity and cell viability were evaluated in oleic acid treated HepG2 cells. Supplementation of NASH mice with extract prevented high fat diet induced elevation in plasma marker enzymes of liver damage, plasma and hepatic lipids. Further, addition of the extract to in vitro HepG2 cells minimized oleic acid induced lipid accumulation, higher lipid peroxidation, cytotoxicity and reduced cell viability. These in vivo and in vitro studies suggest that extract has the potential of preventing high fat/fatty acid induced NASH mainly due to its hypolipidemic and antioxidant activities (Thounaojam et al., 2010).

Insulin is released from the pancreatic β-cell directly from the granules by exocytosis and the movement of the granules to the cell membrane in response to stimulation probably involves microfilaments and microtubules. Insulin is released from pancreatic β -cells at a low basal rate and at a much higher stimulated rate in response to a stimulus. The most important stimulus for insulin secretion is an increase in the extracellular concentration of glucose. Within one minute of the addition of glucose to the tissue an increased rate of secretion occurs. The response of insulin secretion to the change in glucose concentration is sigmoidal so that there is little response below 5mM, and a 50% response at about 8mM (Newsholme and Leech, 1992).

Pancreatic β cells secrete insulin in a pulsatile fashion and, in response to a square-wave increase in interstitial glucose concentration, release insulin in a biphasic manner characterized by a “spike” lasting approximately 10 minutes (first-phase release) and followed by gradually increasing release (second-phase release). It has been suggested that these different phases of insulin released represent two different intra-islet pools: one – a rapidly releasable pool accounting for about 5% of islet insulin – represents granules close to the cell membrane and is thought to be
responsible for first-phase insulin release. The second is a reserve pool, the release of which requires adenosine triphosphate dependent mobilization of insulin-containing granules into the rapidly releasable pool for subsequent exocytosis. Both phases of insulin release are important for maintaining normal glucose homeostasis. However, considerably more emphasis has been placed on the importance of first-phase insulin, assuming that this is the major determinant of “early” insulin release, that is, the increase in plasma insulin levels observed during the initial 30 minutes following glucose or meal ingestion (Gerich, 2000).

The proposed hypothesis of insulin secretion: An increase in the extracellular glucose concentration above 5mM increases proportionally the rate of glycolysis (through operation of the glucose/glucose 6-phosphate cycle) and this raises the concentration of phosphoenol pyruvate which, increases the rate of uptake of calcium ions and probably increases the rate of release from intracellular calcium stores. An increased cytosolic concentration of calcium ions, via calmodulin, causes contraction of the microfilaments or microtubules and hence results in an increased rate of exocytosis and insulin secretion. The hypothesis attempts to link the rate of glycolysis, calcium ions and rate of insulin secretion (Newsholme and Leech, 1992).

Potassium channels are sensitive to ATP and function in coupling cell metabolism to membrane potential in many tissues. K\(^+\)ATP channels have been found in a variety of tissues including heart, pancreatic \(\beta\)-cells, skeletal muscle, smooth muscle and the central nervous system. K\(^+\)ATP channels comprise an octameric complex of pore-forming Kir6.x subunits and regulatory sulfonylurea receptors (SURs). There are three isoforms of the sulfonylurea receptor, SUR1 and two spliced variants of SUR 2, SUR 2A and SUR 2B. The SUR1-Kir 6.2 and SUR2B-Kir 6.2 or Kir 6.1, constitute the cardiac and vascular smooth muscle-type channels, respectively (Gribble and Ashcroft, 2000).

SUR is a member of the family of ATP-binding cassette (ABC) transporter proteins and appears to be the major determinant of the pharmacological properties of K\(^+\)ATP channels. K\(^+\)ATP channels which consist of SUR1 and Kir 6.2 do not only
occur in β cells but are also present in the alpha, delta and pancreatic polypeptide cells of the pancreatic islets. The K+ATP (SUR1-Kir 6.2) channels mediate glucose-induced insulin secretion in pancreatic cells. K+ATP channels are modulated by intracellular ATP/ADP ratios: ATP closes the K+ATP dependent channels, while ADP opens them. When the level of blood glucose increases, glucose enters β-cells via GLUT 1, 2 and 3 transporters, which are insulin independent. Following glucose metabolism, the intracellular ATP/ADP ratio increases thereby inactivating the K+ATP channels. This increase in the ATP concentration causes closure of the K+ATP sensitive channels and the efflux of K+ through the channel is decreased causing depolarization of the membrane. This in turn results in the opening of the voltage-dependent calcium channels with a subsequent increase in the intracellular Ca2+, which initiates insulin secretion. The entry of glucose into cells is a crucial step in life-supporting processes since glucose is the main monosaccharide in nature that provides carbon and energy for almost all cells. The passage of glucose into cells depends on different parameters, including expression of the appropriate glucose transporters in the target tissues and hormonal regulation of their function (Gorovits and Charron, 2003).

Glucose is a hydrophilic compound; it cannot pass through the lipid bilayer by simple diffusion, and therefore requires specific carrier proteins to mediate its specific transport into the cytosol. Cells take up glucose by facilitated diffusion, via glucose transporters (GLUTs) associated with the plasma membrane (Medina and Owen, 2002).