2. INTRODUCTION
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Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (ADA, 2008).

Diabetes mellitus, long considered a disease of minor significance to the health, is now taking its place as one of the main threats to human health in the 21st century. Prevalence of diabetes is higher in developed than in developing countries, but the major rise in diabetic population will occur in developing countries. India, China, and the U.S. are currently the countries with the largest number of people with diabetes (King et al., 1998). This increase can be attributed to many factors, including a stressful lifestyle as well as improper dietary habits leading to hyperlipidemia and hypercholesterolemia. This is of economic concern as the disease requires life-long treatment and is also associated with high morbidity from the resulting complications.

Hyperglycemia is defined as an abnormal elevation in blood glucose levels. The American Diabetes Association currently considers a fasting blood glucose of >126 mg dl$^{-1}$ as the cut-off for diabetes (Muoio and Newgard, 2008). Diabetes mellitus has been classified in two forms. Type 1 diabetes mellitus, which accounts for about 10% of all cases of diabetes, is caused by autoimmune destruction of pancreatic $\beta$-cells, producing insulin deficiency. Type 2 diabetes mellitus, the more prevalent form of diabetes, is considered a heterogeneous disease. It results from the combination of insulin resistance and/or a $\beta$-cell secretory defect. An explosive increase in the prevalence of type 2 diabetes is...
predicted for the future. Some 16 million to 17 million people have the condition, and an equal number are thought to be "prediabetic", having early symptoms but not yet the full manifestation of the disease. Even children are no longer exempt to develop type 2 diabetes that until recently rarely affected people before middle age. In the U.S., about 30% of all new cases of diabetes are children with type 2 diabetes (Marx, 2002). While exogenous insulin and other medications can control many aspects of diabetes, assorted complications affecting the vascular system, kidney, and peripheral nerves are common and extremely costly in terms of longevity and quality of life.

Type 1 Diabetes mellitus (IDDM) is characterized by absolute insulin deficiency (insulinopenia); β-cells in the pancreas are gradually destroyed. Type 1 diabetes mellitus (T1DM) is an autoimmune disease governed by multiple genetic and environmental risk factors. T1DM is characterized by a permissive immune system that fails to impose tolerance to arrays of self-antigens. Overt diabetes reflects glucose intolerance due to insulin deficiency. It is the end result of prediabetes, with progressive lymphoid infiltration around and then inside pancreatic islets of Langerhans and subsequent destruction of insulin-producing β-cells by autoreactive T lymphocytes. β-cell stress and death in the course of early islet restructuring are thought to provide sensitizing autoantigens, which expand autoreactive T cell pools in pancreatic lymph node (Mathis et al., 2001; Anderson and Bluestone, 2005).

IDDM is an autoimmune disorder characterized by destruction of the insulin-producing β-cells of the islets of Langerhans in the pancreas and concomitant hyperglycemia (Alberti and Zimmet, 2003). Much of our present understanding about the immunopathology of
IDDM results from studies in the non-obese diabetic (NOD) mouse (Delovitch and Singh, 1997). From these studies several mechanisms have been suggested to contribute to β-cell destruction, including delayed type hypersensitivity reactions mediated by CD4+ Th-1 cells reactive with islet antigens (Haskins and McDuffie, 1990), cytotoxic T-cell (CTL)-mediated lysis of islet cells (Wicker et al., 1994), local production of cytokines (TNF-α and IL-1) that directly damage islet cells (Kagi et al., 1999), and autoantibodies against islet cells (Tisch et al., 1993; Daniel et al., 1995). Recently, it has been reported that Interferon-α initiates type 1 diabetes in nonobese diabetic mice. There is increased expression of several IFN-α-inducible genes in CD4+ T cells and increased production of IFN-α in the PLNs of 3- to 4-week-old NOD mice. Blockade of IFN-α signalling by an anti-IFNAR1mAb during this period significantly delayed the onset and decreased the incidence of T1DM in NOD mice (Li et al., 2008). The pathogenesis of autoimmune diabetes is complex, as expected from the >23 genes found associated with the onset of disease (Wicker et al., 2005), both in humans and the non-obese diabetic (NOD) mouse, the most commonly used animal model. However, IDDM is a multi-factorial autoimmune disease for which susceptibility is determined not only by genetic but also by environmental factors as can be seen in monozygotic twins, where the concordance rate for IDDM is only 50% (Barnett et al., 1981).

Type 2 diabetes mellitus (NIDDM) is characterized by peripheral insulin resistance (reduced uptake of glucose from blood into the skeletal muscle - a progressive decline in insulin action), followed by the inability of beta cells to compensate for insulin resistance (pancreatic beta cell dysfunction), and increased endogenous glucose production (liver).
Insulin resistance is a characteristic metabolic defect that precedes overt beta cell dysfunction and is primarily associated with resistance to insulin-mediated glucose disposal at the periphery and compensatory hyperinsulinemia. The beta cells normally compensate insulin resistance by secreting more amounts of insulin to maintain the glucose homeostasis. In the course of time, however, this beta cell function gets impaired leading to deterioration in glucose homeostasis and subsequent development of impaired glucose tolerance and frank diabetes (Lebovitz and Banerji, 2004).

The causes for the development of Diabetes are environmental (nutrition, physical activity) and genetic. Besides a few percent of all cases with monogenetic disease, several genetic polymorphisms contribute to the development of diabetes. This renders all genes that are involved in the transmission of the cellular insulin response to candidate genes the malfunction of which may be causally involved in the development of diabetes (Saltiel and Kahn, 2001). This disease is often associated with obesity and develops when chronic overnutrition conspires with genetic susceptibility to cause impaired insulin signalling, also known as insulin resistance, as well as a relative insulin deficiency of non-autoimmune aetiology. This contrasts with type 1 diabetes, which is caused by the complete absence of insulin secondary to autoimmune destruction of the pancreatic islet β-cells. Defective insulin secretion and action leads to multiple metabolic abnormalities in type 2 diabetes, including hyperglycemia due to impaired insulin-stimulated glucose uptake and uncontrolled hepatic glucose production, and dyslipidaemia, which includes perturbed homeostasis of fatty acids, triglycerides and lipoproteins. These chronic increases in circulating glucose and lipid levels can further impair insulin secretion and action and
cause other forms of tissue damage by several mechanisms (Muoio and Newgard, 2008). The liver plays a central role in maintaining glucose homeostasis, and the accumulation of hepatic lipids may be an important factor contributing to insulin resistance (Kim et al., 2000). There is increasing evidence that hepatic insulin resistance is associated with an increased production of free fatty acid. The circulating free fatty acid levels are commonly elevated in obese and diabetic subjects, and increased free fatty acid levels lower the ability of insulin to suppress hepatic glucose production by activating gluconeogenesis yet inhibiting glycolysis (Hawkins et al., 2003).

Insulin resistance, in both human and animal models, is commonly associated with several abnormalities in the lipid metabolism, including increased plasma free fatty acid levels, hypertriglyceridemia, hypercholesterolemia and enhanced lipogenesis in the liver. Recent works show that insulin resistance develops as a consequence of the effects of inflammatory and hormonal factors, endoplasmic reticulum (ER) stress, and accumulation of by-products of nutritional ‘overload’ in insulin-sensing tissues (Muoio and Newgard, 2008).

Obesity is associated with a state of chronic, low-grade inflammation (particularly in white adipose tissue). The adipose tissue–derived proinflammatory cytokines such as tumor necrosis factor–alpha (TNF-α) could actually cause insulin resistance in experimental models. Tumor necrosis factor–α is a key molecule in obesity. Its serum concentration increases in obesity. It induces insulin resistance through inhibition of insulin signal transduction, and also modulates secretion of other adipocytokines
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(Hotamisligil et al., 1993). TNF-α, IL-6, resistin, and other pro- or anti-inflammatory cytokines appear to participate in the induction and maintenance of the subacute inflammatory state associated with obesity. Monocyte chemoattractant protein-1 (MCP-1) and other chemokines have essential roles in the recruitment of macrophages to adipose tissue. These cytokines and chemokines activate intracellular pathways that promote the development of insulin resistance and type 2 Diabetes (Shoelson et al., 2006). Recent reports have proposed that TNF-α secreted from macrophages is more important than that from adipocytes itself (Weisberg et al., 2003; Xu et al., 2003). Activated macrophages infiltrate into adipose tissue, and secrete inflammatory cytokines including TNF-α which in turn leads to the dysfunction of adipocytes. TNF-α stimulates adipocytes, and several signal molecules including nuclear factor-κB (NF-κB) are activated in the adipocytes. Among the TNF-α signals, NF-κB pathway is important for regulation of adipocytokine productions (Ruan et al., 2002).

Increased blood levels of cytokines such as TNF-α and IL-6 enhance production of reactive oxygen species (ROS) and reactive nitrogen species (Dobashi et al., 2000). Systemic markers of oxidative stress increase with adiposity, consistent with a role for ROS in the development of obesity-induced insulin resistance (Keaney et al., 2003). Oxidative stress is also known to be an activator of NF-κB (Evans et al., 2002; Ogihara et al., 2004). Shoelson et al. (2006) have reported that advanced glycation end products (AGEs) are non-enzymatic adducts formed between glucose and targeted proteins, particularly those with slow rates of turnover. Prolonged hyperglycemia and the accompanying production of excess quantities of AGEs can activate NF-κB. Activation of NF-κB in turn plays a key role...
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in the gene expression of various cytokines, growth factors, adhesion molecules and inducible nitric oxide synthase (iNOS). These authors further suggest that inflammation is closely linked to the pathogenesis of atherosclerosis and might be a common denominator that links obesity to many of its pathological sequelae. NF-κB regulates many of the proteins that mediate the atherogenic process, in common with the pathogenesis of insulin resistance. Therefore the authors suggest that small increases in obesity-induced inflammation might promote both processes via common mechanisms. This also suggests the corollary that pharmacological decreases in inflammatory activity might coordinately down regulate the production of a number of proteins involved in the pathogenesis of insulin resistance, type 2 diabetes mellitus and cardiovascular disease. Insulin resistance in type 2 diabetes is manifested by decreased insulin stimulated glucose transport and metabolism in adipocytes and skeletal muscle resulting in down-regulation of the major insulin-responsive glucose transporter, Glut4 (Kellerer et al., 1999). Molecular basis of insulin resistance depends on impaired insulin signal transduction with key defects in the glucose transport. The skeletal muscle has a paramount role in energy balance and is the primary tissue for insulin-stimulated glucose uptake and disposal (Smith and Muscat, 2005).

Based on the type, the treatment for Diabetes mellitus involves either administration of exogenous insulin and/or oral hypoglycemic drugs like sulfonylureas (insulin secretagogues), biguanides (reduces hepatic glucose output and increases uptake of glucose by the periphery, including skeletal muscle), thiazolidinediones (insulin sensitizers)
and the newer agents viz. α-glucosidase inhibitors (acarbose and miglitol) and DPP-4 inhibitors (vildagliptin, sitagliptin).

All the approaches mentioned above are not completely satisfactory in a large proportion of patients and there is still a need to look for new drugs as no drug (except strict glycemic control with insulin) has been shown to modify the course of diabetic complications. It is widely agreed that modern polypharmaceutical intervention increases longevity in individuals with diabetes, but finding ways to reduce the excess mortality related to longterm complications remains a priority. To overcome deficiencies in diabetes management, we must become more proactive in minimizing long- and short-term exposure to hyperglycemia. The traditional approach to managing patients with type 2 diabetes includes prescribing a period of lifestyle intervention, followed by introduction of a single oral agent. As glycemic control deteriorates, a second oral agent is added, followed eventually by a third (Nathan, 2002). Newer treatment paradigms suggest that early intervention can profoundly improve prognosis: better glycemic control at the time of initial pharmacologic intervention is associated with lower HbA1c values over time and decreased long-term microvascular and macrovascular complications (Colagiuri et al., 2002). Continuous effort is on for a new hypoglycemic drug with a high potency and little or no side effects. Before the advent of insulin, diabetes was treated with plant medicines. The World Health Organization (WHO) urged researchers to examine whether traditional medicines produced any beneficial clinical results (WHO, 1980). The plant kingdom represents a largely unexplored reservoir of biologically active compounds not only as drugs, but also as unique templates that could serve as a starting point for synthetic...
analogs and an interesting tool that can be applied for a better understanding of biological processes. Folkloric uses are supported by a long history of human experience (Aquino et al., 1995). A multidisciplinary ethnomedical approach by combining aspects of ethnobotany, traditional medicine and modern techniques of natural product chemistry to identify compounds from plant products as potential new therapeutic agents has become necessary (Alarcon-Aguilara et al., 1998).

In the light of the above facts, the main aim of our study was to search new drugs from the traditionally used herbal drugs having some established evidences for the beneficial effects in diabetes and associated disorders. The drug we selected attached for the present investigation is *Helicteres isora* Linn. (Family - Sterculiaceae). Various Ayurvedic formulations contain *Helicteres isora* as one of the constituents and these formulations are used for treatment of various disorders. The root juice of *H. isora* has been used in Indian folklore medicine for the treatment of diabetes for centuries. It has already been reported that ethanol extract of the root and stem-bark aqueous extract produced significant antidiabetic effects in animal models (Chakrabarti et al., 2002; Venkatesh et al., 2004; Kumar et al., 2006a, 2006b).

In preliminary studies in our laboratory, fruits and roots of the plant showed potent antidiabetic activity. So far, a detailed investigation of the antidiabetic and antihyperlipidemic activity of *Helicteres isora* with special emphasis on the mechanism of action at the molecular level and effects on glucose and lipid metabolism regulating genes expression have not been reported.
The objective of the present investigation was

1. To perform phytopharmacological analysis of *Helicteres isora* with special reference to diabetes and its associated conditions like insulin resistance, hyperlipidemia and oxidative stress.

2. To isolate the active constituents from the plant material and characterize them.

3. To study the effects of extracts, fractions and the isolated compounds in STZ-induced type 1 diabetes mellitus and the high fat diet fed and low dose STZ-treated rat model of type 2 diabetes mellitus which simulate the human syndrome.

4. To evaluate the changes in the gene expression of the glucose and lipid metabolism regulating genes in the obese and diabetic C57BL/KsJ-db/db mice in an attempt to explore mechanisms of actions of the drug.

5. To carry out *in-vitro* studies to understand the *in-vivo* activity and thereby understand the mechanism of action of the drug.