2. REVIEW OF LITERATURE

2.1. Diabetes Definition and Classification

Diabetes is a group of metabolic diseases 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 different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

Modern Classification Systems for Diabetes Mellitus

In 1979, a classification for diabetes mellitus and other categories of glucose intolerance, based on scientific research on this heterogeneous syndrome was developed by an international workgroup sponsored by the National Diabetes Data Group (NDDG) of the National Institutes of Health (NIH) of United States of America (NDDG, 1979). This group recognized diabetes as being a syndrome, a collection of disorders that have hyperglycemia and glucose intolerance as their hallmark characteristics, due either to insulin deficiency or impaired effectiveness of insulin's action or to a combination of these.

The World Health Organization (WHO) expert committee on Diabetes in 1980 endorsed the substantive recommendations of the NDDG (WHO, 1980). These groups distinguished between the two major forms of diabetes, which they termed insulin dependant diabetes mellitus (type 1) and non insulin dependant diabetes mellitus (type 2). The older terms 'juvenile onset', 'maturity-onset' and 'adult-onset' were recommended to be abolished.

American Diabetes Association Classification

In 1996 and 1997, an expert committee of the American Diabetes Association considered the research findings of the last 20 years and proposed some changes to the NDDG/WHO classification scheme (ADAEC, 1997). The new classification is in Table 1. Changes to diagnostic criteria were also proposed. The main features of new classifications are elimination of the terms insulin-dependant diabetes mellitus and non-insulin dependant diabetes mellitus. However the terms type 1 and Type 2 were retained. Forms of diabetes involving pancreatic β-cell destruction, including those cases of autoimmune cause and those cases with unknown etiology were also included.
in type 1 diabetes mellitus. It also included more precise definition of type 2 diabetes with insulin resistance and insulin secretory defects. Each class in Table 1 may be heterogeneous in etiology and pathogenesis, and further research might provide more precise definitions of different types of diabetes mellitus.

The most common form of diabetes is type 2 diabetes that accounts for 85-90% of all diabetes. Globally, a doubling of the prevalence of type 2 diabetes can be expected in the next 10 years, with the largest increase of type 2 diabetes incidence in the developing countries due to changes towards a 'western life-style' (Zimmet, 2000). According to WHO calculations, in the year 2025 there will be 300-350 million adults with diabetes in the world and 80% of them will be found in the developing countries (Zimmet et al., 1997).

<table>
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<th>Table 2.1: ADA classification of diabetes mellitus</th>
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<tr>
<td><strong>Type 1 diabetes mellitus</strong></td>
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<td>Caused by β-cell destruction, often immune mediated that leads to loss of insulin secretion and absolute insulin deficiency. The etiologic agents that cause the autoimmune process and β-cell destruction are not well established. Also includes cases which causes of the β-cell destruction are not understood. Comprises approximately 5% to 10% of cases in diabetes syndrome.</td>
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| **Type 2 diabetes mellitus** |
| Caused by a combination of genetic and non-genetic factors that result in insulin resistance and insulin deficiency. The specific genes are not known but are under intense investigation. Non-genetic factors include increasing age, high caloric intake, overweight, central adiposity, sedentary lifestyle and low birth weight. Comprises of 90% to 95% of the diabetes syndrome. |

| **Other specific types of diabetes** |
| Other types of diabetes comprise a heterogeneous etiologic group that includes those cases of diabetes in which causes are established or at least partially known. The causes include known genetic defects affecting β-cell function or insulin action, diseases of exocrine pancreas, endocrinopathies, drug or chemical induced pancreatic changes. Comprises approximately in 1 % to 2 % cases in the diabetic syndrome. |

| **Gestational diabetes** |
| Caused by insulin resistance and relative insulin deficiency associated with pregnancy. Occurs approximately 3% to 5% of all pregnancies. |
2.2 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus is that form of diabetes caused by the destruction of the insulin producing (β) cells of the pancreas, which are located in islets or islands throughout that organ. The process of destruction can occur over a period of several years, this phase being called the prediabetic phase. Eventually, when sufficient insulin-producing cells are destroyed, production of insulin is inadequate and this results in hyperglycaemia.

Two subclasses are discriminated, an autoimmune class (Type 1A) and idiopathic class (Type 1B). The autoimmune type is a chronic disease with a subclinical prodromal period characterized by cellular-mediated autoimmune destruction of the insulin producing β-cells in the pancreatic islets. The markers of autoimmune disease include antibodies to the islets and insulin, glutamic acid decarboxylase (GAD) and tyrosine phophatases IA-2 and IA2β (Bingley et al., 1997).

Type 1 DM is characterized by an absolute deficiency of pancreatic β-cell function. Most often this is the result of an immune-mediated destruction of pancreatic β cells, but rare unknown or idiopathic processes can contribute. What is evident are four main features: (1) a long preclinical period marked by the presence of immune markers when β-cell destruction is thought to occur; (2) hyperglycemia when 80% to 90% of β cells are destroyed; (3) transient remission (the so-called honeymoon phase); and (4) established disease with associated risks for complications and death. Unknown is whether there is one or more inciting factors (e.g., cow’s milk, or viral, dietary, or other environmental exposure) that initiate the autoimmune process (Daneman, 2006).

2.2.1 Role of autoimmunity in pathogenesis of Type 1 DM

The autoimmune process is mediated by macrophages and T lymphocytes with circulating auto antibodies to various β-cell antigens. The most commonly detected antibody associated with type 1 DM is the islet cell antibody. The test for islet cell antibody, however, is difficult to standardize across laboratories. Other more readily measured circulating antibodies include insulin auto antibodies, antibodies directed against glutamic acid decarboxylase, insulin antibodies against islet tyrosine
phosphatase, and several others. More than 90% of newly diagnosed persons with type 1 DM have one or another of these antibodies, as will 3.5% to 4% of unaffected first-degree relatives. Preclinical β-cell autoimmunity precedes the diagnosis of type 1 DM by up to 9 to 13 years. Autoimmunity can remit in some perhaps less-susceptible persons, or can progress to β-cell failure in others. These antibodies are generally considered markers of disease rather than mediators of β-cell destruction. They have been used to identify individuals at risk for type 1 DM in evaluating disease-prevention strategies. Other nonpancreatic autoimmune disorders are associated with type 1 DM, most commonly Hashimoto thyroiditis, but the extent of organ involvement can range from no other organs to polyglandular failure (Janeway, 1997).

2.2.2 Genetics and Type 1 DM
Type 1 diabetes is not the result of a single gene defect; rather there are at least 17 loci on 10 different chromosomes which are involved in humans. (Todd, 1997) The genes most frequently associated with Type 1 diabetes in western countries are the class II histocompatibility genes DR3 and DR4, which are located on the short arm of chromosome 6. In Japan the association is with DR4 and DRw9 and in China with DR3 and DRw9. Another HLA class II molecule, DQβ, is also involved with lack of aspartate on position 57 conferring susceptibility to Type 1 diabetes. (Aitman, 1995)

2.2.3 Role of viruses in the pathogenesis of Type 1 Diabetes Mellitus
Although genetic predisposition appears to be necessary for the development of type 1 DM, non genetic environmental factors play a critical role in the expression of the disease. Viruses as one environmental factor may directly infect and destroy pancreatic β-cells or trigger β-cells specific autoimmunity.

The etiology of Type 1 DM is believed to have a major genetic component. However, cumulative evidence suggests that environmental factors play an important role in the development of Type 1 DM by influencing the penetrance of diabetes susceptibility genes. The environmental factors thought to play a role this disease include viral infections, certain dietary components and toxins (Yoon, 2002). There appears to be a seasonal variation in the onset of acute type 1 DM, with a peak in autumn. Diseases with seasonal incidents are often caused by viruses. In some cases it is believed that
viruses directly destroy pancreatic β-cells; in other cases, viruses are thought to trigger or somehow contribute to β-cells specific autoimmunity leading to development of type 1 DM (Yoon, 2002). In addition, there is evidence that viruses can also protect against the development of diabetes in the spontaneously diabetic BB rats and NOD mouse (Oldstone, 1988).

**Virus-induced Diabetes in Animals**

Several viruses have been demonstrated to cause diabetes in animals, including encephalomyocarditis (EMC) virus, Coxsackievirus B4 (Toniolo et al., 1982), Kithen rat virus (KRV) (Guberski et al., 1991), rubella virus (Rayfield et al., 1986), retrovirus (Suenaga and Yoon, 1988), bovine viral diarrhea- mucosal virus (Tajima et al., 1992) are suspected of causing DM. Studies using transgenic mice that express novel surface antigens, including viral proteins, have been undertaken to target the antigen involved in the pathogenesis of autoimmune type 1 DM.

**Virus-induced Diabetes mellitus in humans**

Several viruses including mumps virus and coxsackie viruses B3 and B4, were found to infect human β-cells. However, there is as yet no way to prove that these viruses actually infect and destroy human pancreatic β-cells in vivo and cause DM. The in vitro susceptibility of β-cells to certain viruses may not truly reflect in vivo susceptibility because it is known that some viruses grow in cultured cells derived from animals normally resistant to viral infection. Several studies involving the isolation of viruses from pancreas of patients with acute-onset type 1 DM, followed by development of DM in susceptible mice infected with the viral isolates, have provided support for the hypothesis that viruses play a role in pathogenesis of human type 1 DM.

Destruction of pancreatic β-cell function causes hyperglycemia because of an absolute deficiency of both insulin and amylin (Edelman and Weyer, 2002). Insulin lowers blood glucose by a variety of mechanisms including: stimulation of tissue glucose uptake, suppression of glucose production by the liver, and suppression of free fatty acid release from fat cells. The suppression of free fatty acids plays an important role in glucose homeostasis. Increased levels of free fatty acids inhibit the uptake of
glucose by muscle and stimulate hepatic gluconeogenesis (McGarry, 2002). Amylin, a glucoregulatory peptide hormone cosecreted with insulin, plays a role in lowering blood glucose by slowing gastric emptying, suppressing glucagon output from pancreatic α cells, and increasing satiety (Nogid and Pham, 2006). In type 1 DM amylin production, caused by β-cell destruction, is very low.

2.3 Type 2 Diabetes Mellitus
Type 2 DM results from disorders of insulin action and insulin secretion, either of which may be the predominant feature and both of which are usually present when the disease becomes clinically manifest (WHO, 1999). By definition, specific causes are not known and autoimmune destruction of the pancreas does not occur. Type 2 DM is preceded by insulin resistance and impaired glucose tolerance (IGT). Once insulin resistance is pronounced, the likelihood of type 2 DM development depends on the ability of β-cells to adequately compensate by increasing insulin secretion.

Following its discovery by Banting and Best in 1921, insulin rapidly entered the clinical arena and dramatically extended the life spans of individuals with type 1 diabetes mellitus. Chronic treatment with exogenous insulin permitted the recognition of individuals with very high insulin requirements, indicating resistance to the hormone (Rabinowitz, 1970). Many such individuals had secondary causes for insulin resistance, including high titers of antibodies to insulin after chronic treatment with insulin from animal sources (Soeldner and Steinke, 1965), as well as rare genetic defects in insulin signaling (Roth et al., 1975). However, the thriving of patients with type 1 diabetes into their later years and the treatment of individuals with type 2 diabetes with insulin revealed the phenomenon of insulin resistance secondary to obesity and aging, which was widely recognized by the 1970s.

The basic underlying pathogenesis of type 2 diabetes mellitus (DM) results from a failure on the part of the pancreatic β-cell to compensate adequately for the defect in insulin action in insulin-resistant persons. However, the ability to maintain the degree of compensatory hyperinsulinemia necessary to prevent loss of glucose tolerance in insulin-resistant persons does not represent an unqualified victory. In contrast, the combination of insulin resistance and compensatory hyperinsulinemia predisposes to the development of a number of adverse outcomes, including cardiovascular disease.
(CVD), polycystic ovary syndrome (PCOS), nonalcoholic fatty liver disease (NAFLD), and possibly several forms of cancer. In other words, irrespective of the degree of β-cell compensation, the more insulin resistant the person, the more perilous the outlook.

2.3.1 Determinants of Type 2 Diabetes (T2D)

Genetic factors
A genetic susceptibility to T2D is well recognized. Twin studies demonstrate a higher concordance rate for T2D in identical twins ranging from 50 to 90% (Kaprio et al., 1992, Medici et al., 1999, Poulsen et al., 1999) than in dizygotic twins ranging from 25 to 40% (Lo et al., 1991). The lifetime risk for an offspring of a parent with T2D is about 40%, and higher if both parents have diabetes. The relative risk to a sibling of patients with T2D is about 3-fold increased (Poulsen et al., 1999, Weijnen et al., 2002). There are also monogenic forms of diabetes e.g. MODY which account for <5% of all cases with diabetes. Although they show some similarities to classical T2D, they develop at earlier age and show a strong dominant penetrance (Fajans, 1989, Karlsson and Groop, 2007). The new classifications of diabetes call them genetically defined subgroups of diabetes. However, T2D is a paradigm for a complex, multifactorial polygenic disease where multiple genes interact with the environment to cause the disease. The rapid development of large-scale genotyping technologies has led to a breakthrough in the knowledge of genetics of T2D in recent years (Saxena et al., 2007).

Environmental factors
Although there is a strong genetic component to T2D, not all people who are genetically at risk develop the disease. This reflects the important role of the environment in the course of disease development. This fact is supported by the observation of rapid changes in the prevalence of diabetes in certain populations. The most striking changes have been observed in Pima Indians after a change to Western lifestyle. The prevalence of T2D increased by more than 40% over a ten years period in parallel with an increase in obesity (Knowler et al., 1990). T2D was also more common in Japanese migrants to US and their offspring than in their counterparts in Japan (Kawate et al., 1979). The strongest environmental risk factors for developing T2D are obesity (Knowler et al., 1981, Bennett, 1992) and physical inactivity. Studies
in several ethnic groups have shown that the prevalence of diabetes in the physically inactive subjects is about two to three times higher than among physically active people (Kriska et al., 1993). Consequently, several studies have demonstrated the effect of weight loss and physical activity in prevention of T2D (Knowler et al., 2002).

**Metabolic Syndrome**

Recent definition of the metabolic syndrome was established as a consensus by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATPIII) in 2001 (NCEP, 2001). Based on the panel's guidelines, diagnosis of the metabolic syndrome requires the presence of at least three of the following five criteria: elevated fasting plasma glucose levels (>110 mg/dL), visceral obesity (waist circumference >35 inches in women and 40 inches in men), hypertension (>130/85 mm Hg), hypertriglyceridemia (>150 mg/dL), and low high-density lipoprotein (HDL) cholesterol (<40 mg/dL in men and <50 mg/dL in women) (Ford et al., 2002).

Other recognized components of the syndrome include systemic inflammation, a prothrombotic state, and increased oxidant stress (Kereiakes and Willerson, 2003). The increased circulating levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and other proinflammatory cytokines may contribute to some of the metabolic features of the metabolic syndrome, whereas the increased levels of plasminogen activator inhibitor-1 (PAI-1) appear to heighten the risk of atherothrombosis (Sakkinen et al., 2000). Indeed, an increased risk of atherosclerotic disease mortality and morbidity is conferred by the metabolic syndrome.

**2.4 Insulin Signaling and Regulation of Glucose and Lipid Metabolism**

The epidemic of type 2 diabetes and impaired glucose tolerance is one of the main causes of morbidity and mortality worldwide. In both disorders, tissues such as muscle, fat and liver become less responsive or resistant to insulin. This state is also linked to other common health problems, such as obesity, polycystic ovarian disease, hyperlipidaemia, hypertension and atherosclerosis. The pathophysiology of insulin resistance involves a complex network of signaling pathways, activated by the insulin receptor, which regulates intermediary metabolism and its organization in cells.
2.4.1 Proximal insulin-signaling pathways

*The insulin receptor*

The insulin receptor belongs to a subfamily of receptor tyrosine kinases that includes the insulin-like growth factor (IGF)-I receptor and the insulin receptor-related receptor (IRR) (Patti and Kahn, 1998). These receptors are tetrameric proteins consisting of two α- and two β-subunits that function as allosteric enzymes in which the α- subunit inhibits the tyrosine kinase activity of the β-subunit. Insulin binding to the α- subunit leads to derepression of the kinase activity in the β-subunit followed by transphosphorylation of the β-subunits and a conformational change that further increases kinase activity. Insulin, IGF-I and the IRR receptors can form functional hybrids; thus, an inhibitory mutation in one receptor can inhibit the activity of the others (Butler and LeRoith, 2001).

*Insulin receptor substrates*

At least nine intracellular substrates of the insulin/IGF-I receptor kinases have been identified. Four of these belong to the family of insulin-receptor substrate (IRS) proteins (White, 1998). Other substrates include Gab-1, p60\textsuperscript{dok}, Cbl, APS and isoforms of Shc (Pessin and Saltiel, 2000). The phosphorylated tyrosines in these substrates act as ‘docking sites’ for proteins that contain SH2 (Src-homology-2) domains. Many of these SH2 proteins are adaptor molecules, such as the p85 regulatory subunit of phosphatidylinositol-3-OH kinase (PI(3)K) and Grb2, or CrkII, which activate small G proteins by binding to nucleotide exchange factors. Others are themselves enzymes, including the phosphotyrosine phosphatase SHP2 and the cytoplasmic tyrosine kinase Fyn. Substrate binding to these SH2 proteins can regulate their activities, or in some cases their subcellular location. The different IRS proteins seem to serve different functions at the cellular level, probably owing to differences in tissue distribution, subcellular localization and intrinsic activity of the proteins. IRS-1-knockout cells exhibit reduced IGF-I-stimulated DNA synthesis and fail to differentiate into adipocytes in culture. Likewise, the mitogenic response mediated by IRS-2 is weaker than that by produced by IRS-1. IRS-2-knockout cells show a major defect in insulin-stimulated glucose transport. The roles of IRS-3 and 4 are less clear in cultured cells, but some data suggest that these substrates may act as negative regulators of IRS-1 and -2 (Saltiel and Kahn, 2001).
Inhibition of Insulin receptor signaling

In addition to tyrosine phosphorylation, both the insulin receptor and IRS proteins undergo serine phosphorylation, which may attenuate signalling by decreasing insulin-stimulated tyrosine phosphorylation and promote interaction with 14-3-3 proteins (Crapora et al., 1997). These inhibitory phosphorylations provide negative feedback to insulin signalling and serve as a mechanism for cross-talk from other pathways that produce insulin resistance. Several kinases have been implicated in this process, including PI(3)K, Akt, glycogen synthase kinase (GSK)-3 and mammalian target of rapamycin (mTOR). Recent data indicate that obesity-induced attenuation of insulin signalling might arise from the sequential activation of protein kinase C (PKC) and inhibitor of nuclear factor-κB (IκB) kinase (Saltiel and Kahn, 2001).

Insulin action is also attenuated by protein tyrosine phosphatases (PTPases), which catalyse the rapid dephosphorylation of the receptor and its substrates. A number of PTPases have been identified that catalyse dephosphorylation of the insulin receptor in vitro, some of which are expressed in insulin-responsive cells, or upregulated in states of insulin resistance. Most attention has focused on the cytoplasmic phosphatase PTP1B. Knockout of PTP1B leads to increased tyrosine phosphorylation of the insulin receptor and IRS proteins in muscle and improved insulin sensitivity. PTP1B−/− mice are also resistant to diet-induced obesity, suggesting the brain as an important site of action. This combination of effects implicates PTP1B as a potential therapeutic target in diabetes and obesity (Saltiel and Kahn, 2001).

PI(3)K and insulin action

PI(3)K has a pivotal role in the metabolic and mitogenic actions of insulin and IGF-I. Inhibitors of class I PI(3)K, or transfections with dominant negative constructs of the enzyme, block most metabolic actions of insulin, including stimulation of glucose transport, glycogen and lipid synthesis. PI(3)K consists of a p110 catalytic subunit and a p85 regulatory subunit that possesses two SH2 domains that interact with tyrosine-phosphorylated pYMXM and pYXXM motifs in IRS proteins. At least eight isoforms of the regulatory subunits have been identified. These are derived from three genes (p85a, p85b and P55PIK) and alternative splicing of p85a is predominant and thought to be the main response pathway for most stimuli (Fruman et al., 2000). The exact roles of the different regulatory subunits of PI(3)K in insulin action are unclear.
Splice variants possess differences in potencies for enzyme activation, tissue distribution and sensitivity to insulin. Knockout mice with a disruption of all three isoforms derived from the p85a gene die shortly after birth, whereas heterozygous knockout mice or mice lacking only full-length p85a are viable and exhibit improved insulin sensitivity (Saltiel and Kahn, 2001). Cell lines derived from heterozygous knockouts also exhibit increased insulin/IGF-I signalling, which seem to be due to improved stoichiometry of the interaction.

The activation of PI(3)K may transmit multiple signals. PI(3)K catalyses the phosphorylation of phosphoinositides on the 3-position to produce phosphatidylinositol-3-phosphates, especially, phosphatidylinositol-3-phosphates which bind to the pleckstrin homology (PH) domains of a variety of signalling molecules thereby altering their activity or subcellular localization. Moreover, PI(3)K also possesses serine kinase activity, and both the regulatory and catalytic subunits of the enzyme can interact with other signalling proteins. Indeed, recent studies suggest that these proteins may be important in insulin action independent of phosphatidylinositol-3-phosphates generation (Kessler et al. 2001).

Phosphatidylinositol-3-phosphates regulate three main classes of signalling molecules: the AGC family of serine/threonine protein kinases (Peterson and Schreiber, 1999), guanine nucleotide-exchange proteins of the Rho family of GTPases (Mackay and Hall, 1998), and the TEC family of tyrosine kinases (Ziegler et al., 1993). PI(3)K also activates the mTOR/FRAP pathway, and might be involved in regulation of phospholipase D, leading to hydrolysis of phosphatidylcholine and increases in phosphatidic acid and diacylglycerol. The best characterized of the AGC kinases is phosphoinositide-dependent kinase 1 (PDK1), one of the serine kinases that phosphorylates and activates the serine/threonine kinase Akt/PKB. Akt possesses a PH domain that also interacts directly with phosphatidylinositol-3-phosphates, promoting membrane targeting of the protein and catalytic activation. Akt has been suggested to be important in transmission of the insulin signal, by phosphorylation of the enzyme GSK-3, the fork head transcription factors and cAMP response element-binding protein. Although studies using inhibitory or activated forms of Akt have not uniformly inhibited or mimicked insulin actions, deletion of Akt2 produces hepatic insulin resistance in mice. Other AGC kinases that are downstream of PI(3)K include
serum- and glucocorticoid- regulated kinase and the atypical PKCs, PKC-ξ and –λ. Akt and/or the atypical PKCs seem to be required for insulin stimulated glucose transport (Saltiel and Kahn, 2001).

Activity of this pathway is also determined by phosphatidylinositol- 3-phosphates such as phosphatase and tensin homologue (Ogg and Ruvken, 1998) and the SH2 domain-containing inositol-5-phosphatase SHIP. Overexpression of these enzymes leads to decreased levels of phosphatidylinositol-3-phosphates. This might terminate signal transduction and/or change the nature of the phosphoinositides, altering the binding specificity to PH or phox homology domains. Disruption of these genes or reducing expression of these messenger RNAs yields mice with increased insulin sensitivity (Clement et al., 2001).

**The CAP/Cbl pathway and lipid rafts**

In addition to phosphatidylinositol-3-phosphates activity, other signals seem to be required for insulin-stimulated glucose uptake involve tyrosine phosphorylation of the Cbl proto-oncogene (Pessin and Saltiel, 2000). In most insulin-responsive cells, Cbl is associated with the adapter protein CAP, which binds to proline-rich sequences in Cbl through its carboxyl-terminal SH3 domain (Ribon et al., 1998b). CAP is expressed in insulin-sensitive tissues, is markedly induced during adipocyte differentiation and its expression is increased by insulin-sensitizing peroxisome proliferator-activated receptor-γ (PPARγ) agonists (Ribon et al., 1998a).

**Insulin-stimulated phosphorylation cascades**

As is the case for other growth factors, insulin stimulates the mitogen-activated protein (MAP) kinase extracellular signal regulated kinase (ERK). This pathway involves the tyrosine phosphorylation of IRS proteins and/or Shc, which in turn interact with the adapter protein Grb2, recruiting the Son-of-sevenless (SOS) exchange protein to the plasma membrane for activation of Ras. The activation of Ras also requires stimulation of the tyrosine phosphatase SHP2, through its interaction with receptor substrates such as Gab-1 or IRS1/2. Once activated, Ras operates as a molecular switch, stimulating a serine kinase cascade through the stepwise activation of Raf, MEK and ERK. Activated ERK can translocate into the nucleus, where it catalyses the phosphorylation of transcription factors such as p62TCF, initiating a
transcriptional programme that leads to cellular proliferation or differentiation (Boulton et al., 1991). Blockade of the pathway with dominant negative mutants or pharmacological inhibitors prevents the stimulation of cell growth by insulin, but has no effect on the metabolic actions of the hormone (Lazar et al., 1995).

Insulin increases synthesis and blocks the degradation of proteins through activation of mTOR. mTOR is a member of the phosphatidylinositol-3-phosphates family of proteins, but seems to act primarily as a serine, rather than lipid kinase (Raught et al., 2001). The stimulation of this protein kinase involves phosphatidylinositol-3-phosphates kinase activation, although another signal may also be required. mTOR can control the mammalian translation machinery by direct phosphorylation and activation of p70 ribosomal S6 kinase (p70rsk), as well as phosphorylation of the initiation factor 4E for eukaryotic translation (eIF-4E) inhibitor, PHAS1 or 4E-binding protein 1. P70rsk activates ribosome biosynthesis by phosphorylating the ribosomal S6 protein, producing increased translation of mRNAs with a 58-terminal oligopyrimidine tract. P70rsk also requires a second phosphatidylinositol-3-phosphates dependent phosphorylation, presumably catalysed by PDK1. Phosphorylation of PHAS-1 by mTOR results in its dissociation from eIF-2, allowing cap-dependent translation of mRNAs with a highly structured 58-untranslated region. Although the mechanism of activation of mTOR remains unclear, it seems to require the presence of amino acids in the media for full activation by growth factors, and thus may also represent a nutrient sensor (Raught et al., 2001).

### 2.4.2 Glucose and lipid regulation

**Regulation of glycogen synthesis**

Insulin stimulates glycogen accumulation through a coordinated increase in glucose transport and glycogen synthesis. The hormone activates glycogen synthase by promoting its dephosphorylation, through the inhibition of kinases such as PKA or GSK-3 (Cross et al., 1995), and activation of protein phosphatase 1 (PP1) (Brady et al., 1997). Upon its activation downstream of phosphatidylinositol-3-phosphates, Akt phosphorylates and inactivates GSK-3, decreasing the rate of phosphorylation of glycogen synthase, thus increasing its activity state (Cross et al., 1995). Insulin does not activate PP1 globally, but rather specifically targets discrete pools of the phosphatase, primarily increasing PP1 activity localized at the glycogen particle. The
compartmentalized activation of PP1 by insulin is due to glycogen-targeting subunits, which serve as ‘molecular scaffolds’, bringing together the enzyme directly with its substrates glycogen synthase and phosphorylase in a macromolecular complex, and in the process exerting profound effects on PP1 substrate-specific activity. Four different proteins have been reported to target PP1 to the glycogen particle. Despite a proposed common function, no two targeting subunits share more than 50% sequence homology, and this is largely confined to the PP1- and glycogen-binding regions. Over expression of these scaffolding proteins in cells or in vivo results in a marked increase in cellular glycogen levels. Although the mechanism by which insulin activates glycogen-associated PP1 remains unknown, inhibitors of PI(3) K block this effect, suggesting that PI(3)-dependent protein kinases are involved. These scaffolding proteins have a critical permissive role in the hormonal activation of the enzyme, perhaps interacting with additional proteins that regulate the interaction of PP1 with glycogen synthase and phosphorylase (Newgard et al., 2000).

**Regulation of gluconeogenesis**

Insulin inhibits the production and release of glucose by the liver by blocking gluconeogenesis and glycogenolysis. This occurs through a direct effect of insulin on the liver, as well as by indirect effects of insulin on substrate availability (Bergman and Ader, 2000). Insulin can also influence glucose metabolism indirectly by changes in free fatty acids generated from visceral fat, the so called ‘single gateway’ hypothesis. Because visceral fat is less sensitive to insulin than subcutaneous fat, even after a meal there is little suppression of lipolysis by the hormone in this fat depot. The resulting direct flux of fatty acids derived from these fat cells through the portal vein to the liver can stimulate glucose production, thus providing a signal for both insulin action and insulin resistance in the liver.

Insulin directly controls the activities of a set of metabolic enzymes by phosphorylation or dephosphorylation and also regulates the expression of genes encoding hepatic enzymes of gluconeogenesis and glycolysis (Pilkis and Granner, 1992). It inhibits the transcription of the gene encoding phosphoenolpyruvate carboxylase, the rate-limiting step in gluconeogenesis (Sutherland et al., 1996). The hormone also decreases transcription of the genes encoding fructose-1,6-bisphosphatase and glucose-6-phosphatase, and increases transcription of glycolytic
enzymes such as glucokinase and pyruvate kinase, and lipogenic enzymes such as fatty acid synthase and acetyl-CoA carboxylase. Although the transcription factors that control the expression of these genes have remained elusive, new data suggest a potential role for the forkhead family of transcription factors through phosphorylation by Akt-related protein kinases, and the PPARγ co-activator PGC-1.

**Regulation of lipid synthesis and degradation**

Insulin promotes the synthesis of lipids, and inhibits their degradation. Studies suggest that many of these changes require an increase in the transcription factor steroid regulatory element-binding protein (SREBP)-1c. Dominant negative forms of SREBP-1 can block expression of these gluconeogenic and lipogenic genes, whereas overexpression can increase their transcription. Hepatic SREBP levels are increased in some rodent models of lipodystrophy, and this is coordinated with increases in fatty acid synthesis and gluconeogenesis, the exact phenotype observed in genetic models of obesity induced diabetes. Thus, increased expression of SREBP-1c might contribute to the insulin resistance observed in liver of diabetic rodents, with increased rates of both gluconeogenesis and lipogenesis. The pathways that account for the changes in SREBP-1c expression in response to insulin or other metabolic changes are not known, but probably lie downstream of the IRS/PI(3)K pathway (Saltiel and Kahn, 2001).

In adipocytes, glucose is stored primarily as lipid, owing to increased uptake of glucose and activation of lipid synthetic enzymes, including pyruvate dehydrogenase, fatty acid synthase and acetyl-CoA carboxylase. Insulin also profoundly inhibits lipolysis in adipocytes, primarily through inhibition of the enzyme hormone-sensitive lipase (Anthonsen et al., 1998). This enzyme is acutely regulated by control of its phosphorylation state, which is activated by PKA-dependent phosphorylation, and inhibited as a result of a combination of kinase inhibition and phosphatase activation. Insulin inhibits the activity of the lipase primarily through reductions in cAMP levels, owing to the activation of a cAMP-specific phosphodiesterase in fat cells.

**Regulation of insulin sensitivity by fat cell**

Adipose tissue has a special role in insulin resistance. Circulating FFAs derived from adipocytes are elevated in many insulin-resistant states and have been suggested to...
contribute to the insulin resistance of diabetes and obesity by inhibiting glucose uptake, glycogen synthesis and glucose oxidation, and by increasing hepatic glucose output (Bergman and Ader, 2000). Elevated FFAs are also associated with a reduction in insulin-stimulated IRS-1 phosphorylation and IRS-1-associated phosphatidyl inositol 3-phosphate kinase activity. The link between increased circulating FFAs and insulin resistance might involve accumulation of triglycerides and fatty acid-derived metabolites (diacylglycerol, fatty acyl-CoA and ceramides) in muscle and liver. Transgenic mice with muscle- or liver-specific overexpression of lipoprotein lipase exhibit increases in tissue triglyceride content that correlate with decreases in insulin action and activation of IRS-associated phosphatidyl inositol 3-phosphate kinase (Hwang et al., 2001).

Adipokines are substances which are secreted from fat cells have shown to regulate lipid and glucose levels. Leptin, adiponectin and resistin are important paracrine hormones secreted from adipocytes. Expression of TNF-α is also found to be elevated in conditions of diabetes and obesity associated with insulin resistance. The above said hormones appear to play significant role in altering insulin sensitivity and hence glycemic levels in conditions of diabetes (Saltiel and Kahn, 2001).

**Possible causes insulin resistance**

The insulin resistance of obesity and type 2 diabetes is characterized by defects at many levels, with decreases in receptor concentration and kinase activity, the concentration and phosphorylation of IRS-1 and 2, phosphatidyl inositol 3-phosphate kinase activity, glucose transporter translocation, and the activity of intracellular enzymes. Activation of the MAP kinase pathway by insulin is not reduced in type 2 diabetes, perhaps allowing for some of the detrimental effects of chronic hyperinsulinaemia on cellular growth in the vasculature. Genetic and acquired factors can profoundly influence insulin sensitivity. Genetic defects in the insulin receptor are relatively rare, but represent the most severe forms of insulin resistance. Differences in clinical presentation may be due to the severity of the genetic defect, the ability of the mutant receptors to form hybrids with IGF-I or other receptors, and other background genetic or acquired factors that modify the insulin-resistant state.
Type 2 diabetes is polygenic and may involve polymorphisms in multiple genes encoding the proteins involved in insulin signalling, insulin secretion and intermediary metabolism. Targeted deletions of the components of insulin signalling in vivo using homologous recombination have yielded some insight into the complexity of these mechanisms. Although some single defects in the insulin-signalling pathway, such as knockout of the insulin receptor IRS-2 or Akt2, can produce diabetes, knockout of the p85 subunit of PI(3)K, IRS-1 or GLUT4 does not. Conversely, knockout of single genes that are involved in turning off the insulin signal, such as PTP1B and SHIP2, ameliorate diabetes in obese rodents.

Combinatorial knockouts have been produced that mimic polygenic type 2 diabetes with heterozygous deletion of the insulin receptor and IRS-1, of the insulin receptor, IRS-1 and IRS-2, and of IRS-1 and glucokinase. In some of these combinations there has been clear evidence of genetic epistasis. For example, although heterozygous knockout of either the insulin receptor or IRS-1 alone does not produce diabetes, the double-heterozygous knockout produces diabetes in up to 50% of mice. This striking finding gives insight into human type 2 diabetes, where insulin induced down regulation or genetic polymorphisms in the receptor or IRS-1 alone might produce only modest changes in signaling capacity, but when combined can lead to diabetes. One genetic model that produced a surprising phenotype regarding glucose homeostasis emerged from the knockout of the p85a regulatory subunits of PI(3)K. Although, PI(3)K is central to the metabolic actions of insulin, p85a heterozygous knockout mice counter-intuitively exhibit improved insulin sensitivity. Furthermore superimposition of p85a heterozygosity on the insulin receptor/ IRS-1 double-heterozygous knockout protects against diabetes. This surprising protection seems to be due to a unique feature of the insulin-signalling pathway in which the stoichiometric balance between p85a, the catalytic subunit p110 and IRS proteins is critical for optimal signal transduction.

The role of specific tissues in the pathogenesis of diabetes has been explored using the Cre-lox DNA-recombination technology to create tissue-specific knockouts of the insulin receptor and GLUT4. Despite the absence of diabetes in mice with a global knockout of GLUT4, tissue-specific knockouts of GLUT4 in muscle and fat have resulted in severely impaired glucose tolerance. Tissue-specific knockout of the

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insulin receptor has also produced often surprising results. As noted above, despite recognition that insulin stimulates glucose uptake primarily in muscle, mice with a knockout of the muscle insulin receptor have normal glucose tolerance. This occurs, at least in part, as the result of a shift of glucose uptake into fat, with subsequent increases in adipose tissue mass, circulating free fatty acids (FFAs) and triglycerides. Mice with a knockout of the fat-specific insulin receptor also have normal glucose tolerance, whereas the liver-specific insulin-receptor knockout shows both impaired glucose tolerance and decreased insulin clearance with marked hyperinsulinaemia. Perhaps the most surprising results, however, have come from studies of mice with knockouts of the β-cell-specific insulin receptor and neural/brain-specific insulin receptor. The former exhibit a marked defect in glucose-stimulated insulin secretion similar to that observed in type 2 diabetes, whereas the latter exhibit increased food intake, mild adiposity, insulin resistance and hypertriglyceridaemia, as well as reduced fertility due to hypothalamic hypogonadism. Taken together, these findings suggest a unifying hypothesis for type 2 diabetes in which insulin resistance in classic target tissues, such as liver, muscle and fat coupled with insulin resistance in β-cell, brain and other tissues, combine to produce the pathophysiology of type 2 diabetes.

2.5 Biochemistry and Molecular Cell Biology of Diabetic Complications
Diabetes-specific microvascular disease is a leading cause of blindness, renal failure and nerve damage, and diabetes-accelerated atherosclerosis leads to increased risk of myocardial infarction, stroke and limb amputation. Four main molecular mechanisms have been implicated in glucose-mediated vascular damage. All seem to reflect a single hyperglycaemia-induced process of overproduction of superoxide by the mitochondrial electron-transport chain.

Mechanisms of hyperglycaemia-induced damage
Four main hypotheses about how hyperglycaemia causes diabetic complications have generated a large amount of data, as well as several clinical trials based on specific inhibitors of these mechanisms. The four hypotheses are: increased polyol pathway flux; increased advanced glycation end-product (AGE) formation; activation of protein kinase C (PKC) isoforms; and increased hexosamine pathway flux.
A common element linking hyperglycaemia-induced damage

Specific inhibitors of aldose reductase activity, AGE formation, PKC activation and the hexosamine pathway each ameliorate various diabetes-induced abnormalities in cell culture and animal models, there has been no apparent common element linking the four mechanisms of hyperglycaemia-induced damage. When the electrochemical potential difference generated by the proton gradient across the inner mitochondrial membrane is high, the lifetime of superoxide-generating electron-transport intermediates such as ubisemiquinone is prolonged. There seems to be a threshold value above which superoxide production is markedly increased. Hyperglycemia increases the proton gradient above the threshold value as a result of over production of electron donors by the TCA cycle. This, in turn, causes a marked increase in the production of superoxide by endothelial cells. Over expression of manganese superoxide dismutase (MnSOD), the mitochondrial form of superoxide dismutase, abolished the signal generated by reactive oxygen species, and overexpression of uncoupling protein-1 (UCP-1) collapsed the proton electrochemical gradient and prevented hyperglycaemia-induced overproduction of reactive oxygen species.

Inhibition by MnSOD or UCP-1 of hyperglycaemia-induced overproduction of mitochondrial superoxide completely prevented an increase in polyol pathway flux, increased intracellular AGE formation, increased PKC activation and an increase in hexosamine pathway activity in endothelial cells. Overexpression of UCP-1 or MnSOD corrects a variety of hyperglycaemia-induced phenotypes in target cells of diabetic complications. In cultured glomerular mesangial cells, overexpression of MnSOD suppresses the increase in collagen synthesis induced by high glucose. In dorsal root ganglion (DRG) neurons from both wild-type and MnSOD+/− mice, overexpression of MnSOD decreases hyperglycaemia-induced programmed cell death, and in embryonic rat DRG neurons, overexpression of UCP-1 inhibits cleavage of programmed cell death effector caspases. In aortic endothelial cells, overexpression of either UCP-1 or MnSOD completely blocks hyperglycaemia-induced monocyte adhesion, prevents hyperglycaemia-induced inhibition of the anti-atherogenic enzyme prostacyclin synthetase, and prevents hyperglycaemia-induced inhibition of PPAR-γ activation.
The discovery that each of the four main mechanisms implicated in the pathogenesis of diabetic complications reflects a single hyperglycaemia-induced process provides a new conceptual framework for future observation. General areas are of great importance to a more complete understanding of the molecular and cell biology of diabetic complications, hyperglycemic memory, genetic determinants of susceptibility of both microvascular and macrovascular complications and interruption of overproduction of super-oxide by the mitochondrial electron-transport chain would normalize polyol pathway flux, AGE formation, PKC activation, hexosamine pathway flux and NF-κB activation (Brownlee, 2001).

2.5.1 Complications associated with diabetes
Diabetes being a metabolic disorder affect carbohydrate, lipid and protein metabolism. Poor control of glycemic levels as diabetes progresses leads to diabetes induced complications. Diabetes induced complications involve mainly diabetic retinopathy, diabetic cardiomyopathy, diabetic nephropathy and diabetic neuropathy.

2.5.1.1 Diabetic retinopathy
Diabetic retinopathy is one of the most common microvascular complications of diabetes, affecting 80% of patients over 20 years duration of diabetes. Despite, remarkable advances in the diagnosis and treatment of diabetic retinopathy and its associated complications, diabetic retinopathy remains the leading cause of blindness among working age individuals.

Diabetic retinopathy is detected clinically by the presence of visible ophthalmoscopic retinal microvascular lesions in an individual with diabetes mellitus. Retinopathy has been broadly classified as nonproliferative (NPDR) and proliferative diabetic retinopathy (PDR). NPDR is further divided into NPDR with maculopathy, NPDR without maculopathy and pre-proliferative retinopathy. NPDR indicates progressive ischemia in the retinal and an increased risk for the development of PDR and blindness. The prominent clinical features of NPDR include microaneurysms, dot or blot hemorrhages, venous abnormalities and cotton wool spot. Proliferative diabetic retinopathy is the stage before the onset of neovascularization and is characterized by extensive retinal hemorrhages, marked venous bleeding, numerous cotton wool spots or retinal infarcts, intra-retinal vascular abnormalities (IRMA) and marked retinal
ischaemia as evidenced by capillary drop outs in the fundus fluorescein angiogram. Proliferative diabetic retinopathy is characterized by retinal neovascularization, vitreous haemorrhage, vitreoretinal traction and localized retinal detachment.

**Biochemical defects in diabetic retinopathy**

Multiple biochemical pathways have been proposed to explain the pathogenesis of diabetic retinopathy all starting initially from hyperglycemia. These mainly include increased polyol pathway, increased advanced glycation end products formation, activation of protein kinase C (PKC) and increased hexosamine pathway flux (Brownlee, 2001).

**Current treatments for diabetic retinopathy**

Laser photocoagulation is the primary means by which ophthalmologists control the progression of macular edema and neovascularization. The short and long term beneficial effects of photocoagulation have led to its wide clinical acceptance for the treatment of proliferative diabetic retinopathy. Unfortunately, there are many patients in whom laser therapy cannot be done due to an obstructing vitreous hemorrhage or severe fibrous proliferation. Since laser photocoagulation is still an invasive procedure for the destruction of the retinal cells. Future pharmacotherapeutic approaches should be developed to prevent retinal lesions in diabetic subjects.

Research during the past few decades has provided ample evidence that hyperglycemia is one of the main factors driving the onset and progression of diabetic retinopathy. Furthermore, hyperglycemia-induced events regulate a variety of cellular signals including the stimulation growth factors that are implicated in retinopathy. It is possible that in the future, novel therapeutic measures may emerge for the treatment of diabetic retinopathy. In order to discover antipermeability and anti-angiogenic compounds, a more comprehensive understanding of the mechanisms governing the vascularization of the retina is required. Some of the experimental approaches currently under investigation, such as protein kinase C inhibitors, VEGF inhibitors, pigment epithelioid-derived factor, and many other approaches in the treatment of various stages of diabetic retinopathy. Significant efforts have outlined towards the evaluation of the mechanisms underlying diabetic retinopathy in order to achieve newer and better therapies for this potentially preventable cause of blindness.
2.5.1.2 Diabetic cardiomyopathy

Diabetes mellitus is a powerful risk factor for cardiovascular disease associated with high morbidity and mortality rates. Diabetic patients also have an increased incidence of heart failure which has been traditionally attributed to the concurrent presence of ischemic or hypertensive heart disease. Yet, nowadays, according to recent scientific evidence, diabetic myocardial disease (DMD) is more and more being considered as a distinct entity.

The development of DMD is probably multifactorial. The main pathophysiological mechanisms that are implicated are as follows. (a) Metabolic disorders that are directly triggered by hyperglycemia (Rodrigues et al., 1998). The heart of a diabetic patient shows a primary defect in the stimulation of glycolysis and the oxidation of glucose. There is an increasingly reported evidence supporting that the primary cause of DMD pathogenesis is the deviation in the management of the glucose metabolic substrate by the myocardial cells either due to deficiency in glucose transporters (GLUTs) 1 and 4 of the cellular membrane or to the inhibitory action of the excessive increase in oxidized free fatty acids and the concomitant carnitine deficiency. It has been experimentally shown that this disorder in the use of the metabolic substrate, combined to uncouple oxidative phosphorylation and myocardial oxygen demand, leads to malfunction of the contractile device of the heart. Myocyte bioenergetics is compromised by the concomitant homoeostatic disorders of calcium (reduced sodium–potassium and sodium–calcium ATPase) and myosin deficiency (Belke et al., 2000; Malhotra and Sanghi, 1997; Watts and Marwick, 2003). (b) Insulin resistance, which is expressed as reduced sensitivity to insulin and hyperinsulinemia, is directly associated not only with diabetes, but also with hypertension and coronary artery disease. The linkage of myocardial disease with abnormal metabolism has been identified in patients with diabetes, in obese subjects as well as in those with the metabolic syndrome. Its presence has been linked to left ventricular (LV) early diastolic abnormalities in hypertension (Guida et al., 2001) and obesity (Wong et al., 2004). Experimental and clinical studies have demonstrated that insulin resistance can alter the cardiac contractile function of diabetic patients at the myocyte level (Dutta et al., 2001; Hintz and Ren, 2002) and is associated with LV hypertrophy and diastolic dysfunction (Hirayama et al., 2001). The fasting plasma insulin level was found to be the strongest independent predictor of cardiac mass, both in the normotensive and
hypertensive diabetic subgroups (De Kreutzenberg et al., 2000). (c) Small cardiac vessels’ disease with both structural and functional disorders. The former are manifested as a microvascular remodeling with glassy retrogression, thickening of the intima, formation of capillary microaneurysms and reduction of the transversal intersection of the cardiovascular plexus. Functional changes that occur in the microvasculature are related to reduction of endogenous nitric oxide production and protein kinase C activation, both leading to endothelial dysfunction (Calles-Escandon and Cipolla, 2001). These alterations in capillaries induce microvascular spasm, reduction of capillary density and increased permeability with consequent increases in extracellular volume. The consequent deficiency of the coronary reserve contributes to loss of contractile proteins and necrosis of myocardial cells, with reactive creation of perivascular focal interstitial fibrosis and hypertrophy, increased collagen deposition and development of DMD (Strauer et al., 1997). (d) Disorders of the autonomic nervous system of the heart lead to cardiac sympathetic aponeurosis and counterbalancing increase of the catecholamine levels in the cardiac tissue. This leads to the activation and overenhancement of the apoptosis and oxidative stress processes (Iwai-Kanai et al., 1999; Von Harsdorf et al., 1999). The toxic effects on myocardium are immediate, promoting hypertrophy, interstitial fibrosis, and suppressed contractility to myocytic level, accompanied by increased myocyte necrosis. Cardiac neuropathy is evidenced by attenuation of heart rate and blood pressure responses to breathing, Valsalva and posture, reduction of heart rate variability, and reduction of heart rate recovery after exercise (Marwick, 2006). (e) Myocardial fibrosis and myocyte hypertrophy which are directly associated with the activation of the neurohormonal axis (angiotensin II, endothelin) (Chen et al., 2000; Fiordaliso et al., 2000), the pre-inflammatory cytokines and the presence of oxidative stress (Diamant et al., 2005; Wold et al., 2005) as a reaction to the myocytic necrosis and apoptosis (Frustaci et al., 2000). Experimental studies have shown that diabetes causes defects in cellular calcium transport, defects in myocardial contractile proteins and an increase in collagen formation, which result in anatomic and physiological changes in the myocardium (Ganguly et al., 1983).

There is no standard specific therapeutic strategy to recommend for DMD. However, the aforementioned pathophysiological mechanisms considered to be liable for the development of DMD indicate that different therapeutic approaches may be effective
for the prevention, delay in progress and, eventually, treatment of the emerging heart failure. Since hyperglycemia increases the free fatty acid levels, stimulates the oxidative stress, activates the growth factors and disturbs the calcium homoeostasis and lipid metabolism, it is more than obvious why the accurate regulation of the blood glucose levels is considered to be of major importance. It has been shown that the strict control of DM with intensive treatment with insulin can suspend up to a certain point the progress of DMD (Von Bibra et al., 2004). Insulin resistance and hyperinsulinemia deriving from a combination of environmental and genetic factors can be dealt with a change in lifestyle, physical practice, body weight control and certain medications, which increase insulin sensitivity (metformin, thiazolidinediones (TZDs) and angiotensinconverting enzyme (ACE) inhibitors).

The perspective of modification in the use of the metabolic substrate of the diabetic heart seems to be very attractive. Special metabolically active medications promise the suspension of free fatty acid oxidation and the stimulation of glucose usage by the myocardium as a main metabolic source of energy. Etomoxir, a carnitine palmitoyltransferase inhibitor and dichloroacetate (stimulator of pyruvate dehydrogenase activity), has exhibited conflicting results (Schmidt-Schweda, & Holubarsch, 2000; Lewis et al., 1998). More recently, trimetazidine, a stimulator of the lactate dehydrogenase, according to the first results seems to improve the ventricular function of the diabetic heart, while its action appears to be in favor of the endothelial function by promoting the expression of cGMP and reducing the levels of endothelin-1 (Monti et al., 2006). Regarding sulindac, a second drug in this category (aldose reductase inhibitor), it was announced that it has a cardioprotective activity in experimental models, suspending thus the evolution of DMD (Krishna et al., 2005). Another study reported that diabetic patients with autonomous neuropathy and confirmed cardiac dysfunction may be stabilized and partially suspend the evolution of DMD, when treated with another reductase inhibitor (zopolrestat) (Johnson et al., 2004).

Currently in experimental stages also are glycation end product cross-link breakers which reduce the collagen storages of diabetic myocardium. Besides, they promise to have a therapeutic role in diabetic patients by improving cardiac function while reducing aortic stiffness, amending LV elasticity and, finally, inhibiting DM
progression to clinically obvious heart failure (Liu et al., 2003). Regulation of the lipidemic profile with a change in lifestyle but mainly with the use of statins is a useful therapeutic tool at the physician's disposal. The cardioprotective activity of statins is attributed to both the reduction of lipid levels in blood and their well-studied pleiotropic activities (endothelium protective activity, stabilization of atheromatic plaque, anti-inflammatory activity).

Treatment of hypertension represents an essential aspect in the management of diabetic patients, as it has been proved that for every 10 mmHg lowering of blood pressure there is a 15% reduction in the total mortality. Apart from the aforementioned regulation of metabolic homoeostasis (glucose, lipids, hypertension), physical exercise seems to have additional beneficial results: it improves the endothelial dysfunction and the function of cardiac and peripheral skeletal musculature; it reduces the activity of the excessively stimulated neurohormonal systems; it inhibits the prethrombotic environment and promotes fibrinolysis.

As already mentioned, oxidative stress, i.e., the imbalance amongst the inherently derived products of oxidation and their neutralization by inherent anti-oxidatives, may trigger the initiation and promote the evolution of DMD. Therefore, it is normal for the scientific interest to intensively turn to the research and implication of an anti-oxidative therapy on the primary stages of the disease. So far, the research underlines the fact that anti-oxidatives (vitamin E, carnitine, α-lipoic acid) are very promising, mainly regarding the delay in progress of the autonomous cardiac neuropathy and also the occurrence of complications in the diabetic heart (Lo et al., 2002; Wold et al, 2005).

The administration of ACE inhibitors to diabetic patients with CHF has proven that, it does not only delay the progress to more severe stages and reduce mortality, but it may also be used as a primary prevention of imminent CHF development. This fact is attributed to the competence of ACE inhibitors to facilitate blood flow through microcirculation into both skeletal and cardiac muscles; to improve insulin activity and use of the glucose metabolic substrate in myocytic level; to inhibit the neurohormonal hyperactivation, and eventually to delay or to prevent the development of interstitial myocardial fibrosis and cardiovascular remodeling. An
alternative choice and equally reliable solution are angiotensin II receptor antagonists, which also block the renin–angiotensin–aldosterone axis effectively. Therefore, they should probably take part in the early treatment of DMD taking into consideration the status of renal function which is often seriously affected in diabetic subjects. Despite the unquestionable evidence of the benefit of beta-adrenergic blockers when administered for CHF, according to great contemporary studies, they are still insufficiently prescribed to diabetic patients with CHF (probably due to the fact that they weaken the response to potential incidents of hypoglycaemia, affect the lipidemic profile and inhibit insulin expression in Type 2 DM). Perhaps, carvedilol must be the beta-blocker of choice for diabetic patients, due to the fact that it has beneficial effect on insulin tolerance and neutral activity on lipidemic profile, causes peripheral vasodilatation and, furthermore, has an anti-oxidative and anti-apoptotic activity, promoting the reverse cardiovascular remodelling. Other beta-blockers that are indicated in CHF are bisoprolol, metoprolol and nebivolol. The latter with extra beneficial activity on endothelial dysfunction (which is obviously observed in DMD) potentially has its own special role in the treatment of the disease. According to the published reports and the fact that diabetic patients obviously need a more aggressive management, it is easy to come to the conclusion that the use of beta-blockers must by all means be recommended in DMD, except, of course, in cases where it is contraindicated (Mytas et al., 2008).

2.5.1.3 Diabetic nephropathy

With the global epidemic of type 2 diabetes mellitus, diabetes has become the leading cause of end stage renal failure (ESRF) in developed countries. Approximately 20–30% of all diabetic subjects will develop evidence of diabetic nephropathy, which represents a continuum from microalbuminuria, to overt nephropathy or macroalbuminuria, and finally ESRF.

Diabetic nephropathy occurs as a result of an interaction between haemodynamic and metabolic factors (Cooper, 2001). Haemodynamic factors that contribute to the development of diabetic nephropathy include increased systemic and intraglomerular pressure, as well as activation of vasoactive hormone pathways including the renin angiotensin system (Zatz et al, 1986) and endothelin (Hargrove et al, 2000). These haemodynamic pathways activate intracellular second messengers such as protein
kinase C (PKC) (Xia et al., 1994), MAP kinase (Haneda et al., 1997), nuclear transcription factors such as NF-κB (Barnes and Karin, 1997) and various growth factors such as the prosclerotic cytokine, TGF-β and the permeability enhancing growth factor, vascular endothelial growth factor, VEGF (Cooper et al., 1999). Glucose dependent pathways are also activated within the diabetic kidney and result in enhanced oxidative stress, renal polyol formation (Dunlop, 2000) and the accumulation of advanced glycation end products (AGEs) (Soulis-Liparota et al., 1991). In combination, these pathways ultimately lead to increased renal albumin permeability and extracellular matrix accumulation, resulting in increasing proteinuria, glomerulosclerosis and ultimately tubulointerstitial fibrosis. There appears to be a complex interplay between hemodynamic and metabolic pathways which contribute to the development of diabetic nephropathy. While proven therapies against the development of the disease include strict glycaemic control and the use of inhibitors of the rennin angiotensin system, more novel strategies to influence vasoactive hormone action or inhibit various metabolic pathways such as advanced glycation and specific protein kinase C isoforms provide important promise for the future. It is predicted that a combination of therapies that target different steps in the pathophysiology of the disease will be required to further reduce the rate of progression and ultimately prevent the development of diabetic nephropathy in the future (DCCT, 1993).

2.5.1.4 Diabetic neuropathy

Diabetic distal symmetric sensorimotor polyneuropathy affects at least 50% of diabetic patients, and is the leading cause of foot amputation. Two largest clinical trials in subjects with Type 1 and Type 2 diabetes i.e., Diabetes Control and Complication Trial (DCCT) and UK Prospective Diabetes Study (UKPDS) indicate that intensive therapy and improved blood glucose control reduce incidence and slow progression of peripheral diabetic neuropathy (PDN) thus implicating hyperglycemia as a leading causative factor (Stratton et al., 2000; Boulton, 1998). A number of mechanisms have been proposed to link chronic hyperglycemia to diabetes-induced deficits in motor and sensory nerve conduction velocities (MNCV and SNCV) and small fiber sensory neuropathy. The vascular concept of PDN implies that diabetes-induced endothelial dysfunction with resulting decrease in nerve blood flow (NBF),
vascular reactivity, and endoneurial hypoxia has a key role in functional and morphological changes in the diabetic nerve.

Endothelial changes in *vasa nervorum* have been attributed to multiple mechanisms including increased aldose reductase (AR) activity, non-enzymatic glycation and glycoxidation, activation of protein kinase C, oxidative-nitrosative stress, changes in arachidonic acid and prostaglandin metabolism (Cameron et al., 2001), and, recently, decreased expression of the vanilloid receptor 1 in *vasa nervorum*, increased production of angiotensin (AT) II and activation of the AT1-receptor, activation of poly(ADP-ribose) polymerase-1 (PARP), nuclear factor-κB (NF-κB), cyclooxygenase-2 (COX-2), and others. The neurochemical concept of PDN suggests the importance of similar mechanisms in the neural elements of PNS i.e., Schwann cells, spinal cord oligodendrocytes, and dorsal root ganglion neurons. Other pathobiochemical mechanisms such as 1) metabolic abnormalities i.e., downregulation of Na⁺/K⁺ ATP-ase activity, "pseudohypoxia" - an increase in free cytosolic NADH/NAD⁺ ratio putatively linked to increased conversion of sorbitol to fructose by sorbitol dehydrogenase, changes in fatty acid and phospholipid metabolism, 2) impaired neurotrophic support, 3) changes in signal transduction, and 4) dorsal root ganglion (DRG) and Schwann cell mitochondrial dysfunction and premature apoptosis have also been invoked (Obrosova et al., 2009).

### 2.6 Oxidative Stress and Antioxidant Treatment in Diabetes

Diabetes and insulin resistance can greatly increase the risk of cardiovascular disease in the many people displaying these increasingly prevalent disorders. Oxidative stress has also been postulated to be the main metabolic abnormality causing microvascular complications including retinopathy, nephropathy, and neuropathy as a result of hyperglycemia and diabetes (Giugliano et al., 1996). Oxidative stress occurs when the amount of reactive oxygen species (ROS) exceeds the levels of neutralizing agents called antioxidants. The increase of oxidative stress in patients who have diabetes and poor glycemic control or insulin resistance is most likely a result of abnormal metabolism such as hyperglycemia, dyslipidemia, and elevated levels of free fatty acids (FFAs) (Brownlee, 2001). Although it has been shown that oxidant production is increased in vascular cells exposed to high glucose concentrations and in various cardiovascular tissues originating from diabetic and insulin-resistant states, it is still
relatively unknown how significant a role oxidative stress plays in the pathogenesis of diabetic microvascular and cardiovascular complications.

**Induction of oxidants by Diabetic or Insulin-resistant states**

There is a wealth of evidence showing that oxidant production is increased when microvascular or cardiovascular cells are exposed to hyperglycemia or elevated levels of FFAs. Cultured vascular cells exposed to high concentrations of glucose and vascular tissues from animals and patients with diabetes have increased levels of oxidative stress markers such as gluco-oxidants, glycated compounds, oxidized low density lipoprotein, superoxidents, and nitrotyrosine. (Brownlee, 2001) Likewise, diabetic animals and patients have been shown to have elevated levels of isoprostanes, 8-hydroxydeoxyguanosine, and lipid peroxides in the plasma or urine. Despite the growing evidence that the production of oxidants is increased in diabetic and insulin resistant states, it has not been clearly shown that oxidative stress is responsible for the microvascular or cardiovascular pathologies in diabetes and insulin resistance. A lack of clear and definitive evidence of tissue damage caused by oxidants could be due to the large reserve of antioxidants in both plasma and cells acting to diminish the effects of increased production of oxidants. This is corroborated by the findings that various antioxidants in plasma and cells have not been shown to be decreased consistently in the diabetic state. For example, there are as many studies that have reported a decrease in plasma vitamin C or E levels as studies that have found no change in diabetic states. (Campoy, 2003; Will, 1999) However, it is also likely that antioxidant levels in the plasma are not reflective of the levels in the tissues. Nonetheless, ample evidence showing that oxidative stress markers are increased suggests that antioxidant defense in the tissues is not adequate to neutralize the oxidants produced by diabetes or insulin resistance.

**Pathways of Oxidant Production**

Increased oxidant production is a result of abnormal metabolism of glucose, FFAs, and other reactive metabolites in insulin-deficient and insulin-resistant states. (Evans et al, 2003) In hyperglycemia, increases of oxidants can arise from several processes. For example, gluco-oxidants and advanced glycated endproducts are created by non-enzymatic reactions thereby adding to the increased levels of oxidants. (Bayens & Thorpe, 1999) These processes can also form oxidized low-density lipoprotein and
nitrotyrosine both extracellularly and intracellularly. It has been suggested that the production of superoxide due to hyperglycemia can also be derived from glycolysis and mitochondrial oxidative phosphorylation. Elevated intracellular glucose can alter the redox balance by increasing flux through the aldose reductase pathway, inhibition of the electron transport chain in the mitochondria, activation of oxidases, and alteration of NADPH/NADP ratios. (Dunlop, 2000) Furthermore, byproducts of these processes can cause activation of certain signaling cascades such as protein kinase C (PKC), which can activate oxidases to increase superoxide production. Thus, metabolism of high concentrations of glucose can activate NADPH oxidase in vascular cells independent of mitochondrial metabolism to increase oxidants. (Inoguchi et al., 2000) For example, elevation of glucose can increase NADPH oxidase activity by activating or increasing the expression of the phox47 subunit of the enzyme in endothelial cells and monocytes by activation of PKC through the \textit{de novo} synthesis of diacylglycerol (DAG).

![Diagram](attachment:diagram.png)

\textbf{FIGURE 2.1.} The toxins and signaling pathways contributing to hyperglycemias’s adverse effects for complications.
In summary, oxidant production by hyperglycemia stems not just from a single dominant route but through multiple pathways that present several targets for therapeutic intervention (FIG. 1). Elevated FFA levels, also a consequence of diabetes and insulin resistance, can increase oxidant production by \( \beta \) oxidative phosphorylation via mitochondrial metabolism. It has been shown that malondialdehyde levels and NF-\( \kappa \)B expression are increased in insulin-resistant states without hyperglycemia in vascular tissues as well as in muscle and adipose tissues. Furthermore, it was demonstrated that glutathione levels in the plasma can be decreased by FFA infusion. Therefore, increased oxidant production in diabetic and insulin-resistant states can originate from the metabolism of both glucose and FFAs through multiple pathways.

**The Role of Oxidative Stress in Diabetic Microvascular and Cardiovascular Complications**

Clearly, there is ample evidence to support an increase of oxidative stress in diabetes and insulin resistance; however, it is far from proven that oxidative stress can cause the vascular abnormalities of diabetes. For cardiovascular disease, increases in oxidative stress have also been reported in aging and in non-diabetic patients. Experimental findings indicate that increases in oxidative stress may be responsible for accelerating the risk of cardiovascular disease in diabetic or insulin-resistant states because of elevated levels of glucose or FFAs. The sharing of common risk factors for cardiovascular disease amongst non-diabetic, insulin-resistant, and diabetic patients are reasonable because pathologies for cardiovascular disease amongst these three groups of patients are very similar, although the progression and severity of the pathologies are different. However, it is not clear at all whether oxidative stress is causing microvascular pathologies because microvascular pathologies are observed only in the diabetic state and are directly associated with hyperglycemia. In contrast, microvascular pathologies such as pericyte loss and mesangial expansion, which contribute to diabetic retinopathy and nephropathy, respectively, are not reported in the insulin-resistant state or aging populations even though they experience oxidative stress to a similar level as diabetes. Thus, an increase in oxidative stress induced by FFAs, lipids, or other processes are not adequate to cause microvascular pathologies of diabetes. (Brownlee, 2001) It is still possible that oxidative stress may accelerate the microvascular pathologies even though it is unlikely that microvessel pathologies of diabetes are initiated by oxidative stress. Lastly, the damage caused by oxidative stress...
stress is likely to be tissue specific because hyperglycemia appears to increase oxidant production in many cell types and tissues that do not manifest significant pathologies.

**Antioxidants as Therapeutics**

Because oxidative stress may contribute to diabetic cardiovascular complications, it leaves the question as to whether antioxidants as therapeutics can prevent or delay the onset of complications in diabetic states. Many different types of antioxidants such as vitamin C, vitamin E, β-carotene, lipoic acids, and others have been studied in cultured vascular cells, in animal models of diabetes, and in diabetic patients. (Bursell et al., 1999)

It has been reported that some antioxidants such as vitamins C and E, lipoic acid, antioxidative enzymes, taurine, acetylcysteine, and others prevented hyperglycemia-induced biological changes including cytokine expression, matrix synthesis, and cellular growth and turnover. (Koya et al., 1997) There is mounting evidence to support the notion that antioxidants such as those listed above can prevent, and even reverse, many early changes in the vascular and neurological tissues from diabetic animals. In the following, we will provide a brief review of the evidence available on the effects of antioxidant treatment to prevent or halt diabetic vascular complications.

**Animal Studies**

Similar to cell culture experiments, many antioxidant studies have used diabetic and insulin-resistant rodents. In the antioxidant studies that have used these rodents, antioxidants have been evaluated for their effectiveness in preventing or delaying the onset of vascular and neurological complications. (Giugliano et al., 1996; Bursell et al., 1999; Ruhe & Mcdonald, 2001) These studies have yielded mostly positive effects of antioxidant treatment on preventing or stopping the early changes of diabetic nephropathy, neuropathy, retinopathy, endothelial dysfunction, and surrogate markers of atherosclerosis. Vitamins C and E and α-lipoic acid (a superoxide scavenger) are required to regenerate glutathione and oxidized vitamins C and E. Administered alone or in combination, they have been shown to be effective in ameliorating diabetic complications by demonstrating improvement of nerve conduction velocity and blood flow to the peripheral nerves, leukocyte adhesion in the retina, cataract formation, and
Surprisingly, α-lipoic acid alone has been reported to normalize other diabetic abnormalities besides oxidative stress, such as improving glycemic control, and may even prevent the onset of diabetes and insulin resistance. (Packer et al, 2001) Therefore, this leaves the question as to whether the effectiveness of α-lipoic acid in treating diabetic complications is due to antioxidative actions or an ability to mediate through another mechanism to improve glycemic control and preserve pancreatic islet function.41 However, α-lipoic acid was not able to reverse several other vascular changes, such as the decrease in retinal blood flow corrected by high doses of vitamin E. (Abiko et al, 2003) Although both α-lipoic acid and vitamin E are potent antioxidants, vitamin E was able to reverse many more neural and vascular changes of diabetes than was α-lipoic acid, indicating that vitamin E at high doses has a greater variety of effects than those typically associated with an antioxidant. Many studies using a variety of diabetic animal models have characterized the effectiveness of vitamins C and E. When administered alone or in combination to diabetic animals, vitamins C and E normalized such parameters as levels of lipid peroxidation, isoprostanes, plasma malondialdehyde, and other cellular markers of oxidative stress such as NF-κB. (Bursell et al, 1999; Studer et al, 1997) Furthermore, not only have vitamins C and E been shown to improve biochemical changes, they also have been shown to improve early functional markers of complications including diabetic retinopathy, nephropathy, and neuropathy. These vitamins can ameliorate or reverse cardiovascular abnormalities such as forearm blood flow, nerve conduction velocity, vascular permeability and contractility, endothelial dysfunction, and albuminuria.44 Some studies have even reported that in diabetic animal models, late pathological changes in the retina and the peripheral nerves can be prevented by vitamins C and E.33,46 In other studies, high doses of vitamin E normalized parameters of oxidative stress and inhibited vascular abnormalities caused by hyperglycemia-induced production of DAG and PKC activation in the retina and glomerulus. (Koya et al, 1997) The mechanism by which vitamin E acts to prevent diabetic complications is still relatively unclear. However, doses of d-α-tocopherol at 50 μM or higher have been shown to decrease PKC activity by activating DAG kinase, thereby decreasing the levels of the DAG pool.46 The action of d-α-tocopherol to reduce DAG levels and decrease glucose-induced PKC activation has been shown to occur not only in the retina, glomerulus, and
macrophages of diabetic animals (Koya et al, 1997) but also in cell types such as aortic smooth muscle cells in response to various growth factors, suggesting it may play a therapeutic role in preventing or delaying atherosclerosis in non-diabetic states (Ozer & Azzi, 2003). Although these data generally suggest a positive effect for diabetic complications in animals without serious adverse side-effects of high- or lowdose antioxidant treatment, it has been shown to improve only early changes and potential surrogate markers of vascular or neurological complications. Besides the studies on early nonproliferative microvascular changes in the retina, very few studies have indicated that antioxidant treatment in animals will prevent or delay the onset of late pathologic changes of diabetic microvascular or cardiovascular diseases. (Kowluru & Kennedy, 2001)

**Clinical Studies**

In clinical studies, antioxidant treatments such as vitamins C and E and α-lipoic acid provided positive results to show that they can prevent or stop only early surrogate markers of diabetic retinopathy, nephropathy, neuropathy, and cardiovascular disease, in parallel with neutralizing oxidative markers in the plasma or circulatory monocytes. For example, studies have shown that α-lipoic acid improved symptoms of diabetic polyneuropathy and increased glucose transport in skeletal muscle cells.(Packer et al, 2001, Van dam, 2002) Studies involving vitamins C and E, individually or in combination, been reported to improve early oxidative stress markers in the plasma, urine, and circulating cells, as well as endothelial dysfunction and microalbuminuria.47,57,58 We have shown that retinal blood flow and renal hyperfiltration can be normalized with high doses of vitamin E (1800 IU/day) in type 1 diabetic patients in a placebo-controlled trial.37 Although there is evidence that vitamin E can ameliorate early neuronal and vascular changes caused by oxidative stress, larger studies, such as the Heart Outcomes Prevention Study using a dose of 400 IU/day of vitamin E, d-α-tocopherol, did not show improvement of microvascular or cardiovascular damage in greater than 3,000 individuals who have had diabetes for several years.59 However, smaller studies using a dose of 600 mg/day or higher of vitamin E, the Cambridge Heart Antioxidant Study and the Secondary Prevention with Antioxidants of Cardiovascular Disease in End-Stage Renal Disease (SPACE), provided suggestive evidence that vitamin E may improve cardiovascular
function.60,61 However, most large studies using vitamin E alone or in combination with other antioxidants have not yielded positive benefits for decreasing the development or progression of diabetic microvascular and cardiovascular pathologies or mortality.

In summary, there are differences in response to antioxidants in experimental diabetes in the prevention of associated complications. Studies in experimental models provide a foundation for the clinical studies but results should be interpreted cautiously since the experimental models of diabetes, duration and type of antioxidant treatment and markers of oxidative stress investigated in these studies exhibit a wide range.

2.7 Indian Medicinal Plants with Hypoglycemic Activity

Since time immemorial, various plants and plant derived compounds have been used in the treatment of diabetes to control the blood sugar of the patients. The use of herbs in the management of diabetes mellitus has been prevalent in Indian society from a long time. Several medicinal plants have reported to possess potential hypoglycemic activity in Indian system of medicines. Various plant species from Indian biosphere, having potent hypoglycemic activity are described in the following section (Table 2.2).

Table 2.2: Selected Indian medicinal plants with blood sugar lowering activity

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>Activity with route of administration/dosage</th>
<th>Reported mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia arabica</em> (Lam.) Muhl. ex Willd. Common name: Indian Gum Arabic tree (Family: Leguminosae)</td>
<td>Hypoglycaemic activity of 94% seed diet in normal rats orally with no blood sugar lowering activity in alloxanized rats at the same dose level (Singh et al., 1975)</td>
<td>Acts through release of insulin from pancreatic beta cells, which accounts for the hypoglycemic activity (Singh et al., 1975; Wadood et al., 1989) Hypoglycemic effect of powdered seeds in normal rabbits (2, 3 and 4 mg/kg) administered orally.</td>
</tr>
<tr>
<td><em>Aegle marmelos</em> (L.) Correa ex Roxb. Common name: Holy Fruit Tree (Family: Rutaceae)</td>
<td>Hypoglycemic effect of aqueous decoction of the plant root bark (1 ml/100 mg) in normal fasted rats (Karunanayake et al., 1984) Increases utilization of glucose; either by direct stimulation of glucose uptake or via the mediation of enhanced insulin secretion (Sachdewa et al., 2001a)</td>
<td></td>
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</tbody>
</table>
direct stimulation of glucose uptake or via the mediation of enhanced insulin secretion (Sachdewa et al., 2001a) and also decreases the elevated glucose and glycosylated hemoglobin levels (Kamalakkanan et al., 2003)

Antihyperglycemic activity of aqueous leaf extract in alloxanized rats (Ponnachan et al., 1993)

Antihyperglycemic activity of aqueous leaf extract in streptozotocin induced diabetic rats (Das et al., 1996)

Hypoglycemic and antioxidant activity of leaves in diabetic male albino rats (Upadhya et al., 2004)

Antihyperglycemic and antioxidant activity of the plant in alloxanized rats (Sabu and Kuttan, 2004)

Antihyperglycemic activity of the leaves in glucose induced hyperglycemic rat at an oral dose equivalent to 250 mg/kg (Sachdewa et al., 2001a)

Antihyperglycemic activity of aqueous fruit extract (250 mg/kg, twice daily for 1 month) in streptozotocin induced female albino Wistar diabetic rats (Kamalakkanan et al., 2003)

Hypoglycaemic activity of water extract of fruits in streptozotocin-induced diabetic Wistar rats (125 and 250 mg/kg) twice a day for 4 weeks, orally (Kamalakkanan and Prince, 2003)

Antioxidant activity of the aqueous extract of fruits in streptozotocin diabetic rats (125 and 250 mg/kg), orally for 30 days (Kamalakkanan and Stanely, 2003)

| Allium cepa L. | Hypoglycemic activity of ether soluble fraction of onion (0.25 mg/kg p.o.) in normal rabbits (Augusti, 1973) | Lowers blood glucose level and has potent antioxidant activity, which may account for the hypoglycemic potential (Augusti, |
potent antioxidant activity, which may account for the hypoglycemic potential. Hypoglycemic activity of the bulb in rabbits in an oral glucose tolerance test at 2 g/kg (Gupta et al., 1977)
Antihyperglycemic, antioxidant and hypolipidemic activity of a diet containing 3% freeze dried onion powder upon prolonged administration in STZ diabetic rats (Babu and Srinivasan, 1997)

<table>
<thead>
<tr>
<th>Allium sativum L.</th>
<th>Antihyperglycemic activity of ethanol, petroleum ether and ethyl acetate extract in alloxanized rabbits at a dose of 0.25 mg/kg, orally (Jain and Vyas, 1975) Antioxidant activity of allicin, isolated from garlic (Rabinkov et al., 1998)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common name:</td>
<td>garlic (Family: Alliaceae)</td>
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<tr>
<td>Has strong antioxidant activity and rapid reactivity with thiol containing proteins responsible for the hypoglycemic property (Rabinkov et al., 1998)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Aloe vera (L.) Burm.f.</th>
<th>Hypoglycemic activity of the plant (200 and 300 mg/kg p.o.) on normal fasted rats, oral glucose-loaded rats and streptozotocin-induced diabetic rats (Rajasekaran et al., 2004) Hypoglycemic effect of aloe and its bitter principle in alloxanized mice (Ajabnoor, 1990) Antihyperglycemic activity of dried sap in five non-insulin-dependent diabetic patients and in alloxanized Swiss albino mice (Ghannam et al., 1986)</th>
</tr>
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<tbody>
<tr>
<td>Common name:</td>
<td>Aloe (Family: Aloaceae)</td>
</tr>
<tr>
<td>Maintains glucose homeostasis by controlling the carbohydrate metabolizing enzymes (Rajasekaran et al., 2004) and stimulates insulin release from pancreatic beta cells (Ajabnoor, 1990)</td>
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</table>

<table>
<thead>
<tr>
<th>Artemisia pallens Wall. Ex DC.</th>
<th>Antihyperglycemic activity of aerial parts (100 mg/kg, orally) in glucose-fed hyperglycaemic and alloxan-induced diabetic rats. Moderate hypoglycaemic effect (1000 mg/kg) in fasted normal rats (Subramoniam et al., 1996)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common Name:</td>
<td>Davana (Family: Compositae)</td>
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<tr>
<td>Inhibits glucose re-absorption or increase in peripheral glucose utilization (Subramoniam et al., 1996)</td>
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<thead>
<tr>
<th>Annona squamosa L.</th>
<th>Hypoglycemic activity of aqueous leaf extracts in streptozotocin-nicotinamide induced diabetic rats (Shirwaikar et al., 2004) Hypoglycemic and antihyperglycemic activities of ethanolic leaf-extract (350</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common name:</td>
<td>Sugar apple (Family: Annonaceae)</td>
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<tr>
<td>Lowers blood glucose level (Shirwaikar et al., 2004)</td>
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<tr>
<td><strong>Andrographis paniculata</strong> Nees Common name: King of Bitter. (Family: Acanthaceae)</td>
<td>mg/kg, orally) in normal, streptozotocin (STZ)-diabetic rats and alloxanized rabbits (Gupta et al., 2005)</td>
</tr>
<tr>
<td><strong>Azadirachta indica</strong> A.Juss. Common name: Neem (Family: Meliaceae)</td>
<td>Hypoglycemic activity of hydro alcoholic plant extract in normal rats and hypoglycemic activity in glucose fed and streptozotocin induced diabetic rats (Chattopadhyay et al., 1987a; Chattopadhyay, 1996)</td>
</tr>
<tr>
<td><strong>Beta vulgaris</strong> L. Common name: Garden beet (Family: Chenopodiaceae)</td>
<td>Hypoglycemic activity of Betavulgarosides II–IV, isolated from the root of Beta vulgaris L. in an oral glucose tolerance test in rats (Yoshikawa et al., 1996)</td>
</tr>
<tr>
<td><strong>Boerhavia diffusa</strong> L. Common name: Tar vine (Family: Nyctaginaceae)</td>
<td>Hypoglycemic activity of aqueous leaf extract at a dose of 100, 200 and 400 mg/kg in alloxan induced diabetic rats (Chude et al., 2001)</td>
</tr>
<tr>
<td><strong>Cassia auriculata</strong> L.</td>
<td>Hypoglycemic and antihyperglycemic activity of aqueous leaf extract (200 mg/kg p.o., daily for 4 weeks) in normal and alloxan induced diabetic rats (Satheesh and Pari, 2004)</td>
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<tr>
<td><strong>Common name:</strong> Tanner’s Cassia</td>
<td>Flower extract in streptozotocin-induced diabetic rats upon oral administration at different doses for 30 days (Pari and Latha, 2002; Latha and Pari, 2003a)</td>
</tr>
<tr>
<td><strong>Family:</strong> Leguminosae</td>
<td>Antihyperglycemic activity of the seed extracts in type II diabetic Long Evans rat (Chakrabarti et al., 2005)</td>
</tr>
<tr>
<td><strong>Caesalpinia bonducella</strong> (L.) Roxb.</td>
<td>Hypoglycemic and antihyperglycemic activities of the aqueous and 50% ethanolic seed extracts in normal and streptozotocin-diabetic rats (Sharma et al., 1997)</td>
</tr>
<tr>
<td><strong>Common name:</strong> Chinese Cinnamon</td>
<td>Antihyperglycemic activity of the seed extracts in type II diabetic Long Evans rat (Chakrabarti et al., 2005)</td>
</tr>
<tr>
<td><strong>Family:</strong> Caesalpiniaceae</td>
<td>Hypoglycemic activity of aqueous and ethanolic extracts in chronic type II diabetic model with an increase in secretion of insulin from isolated islets (Chakrabarti et al., 2005)</td>
</tr>
<tr>
<td><strong>Cajanus cajan</strong> (L.) Millsp.</td>
<td>Glucose tolerance enhancing activity of aqueous leaf and stem extract in oral glucose tolerance test (Esposito Avella et al., 1991)</td>
</tr>
<tr>
<td><strong>Common name:</strong> Pigeon pea (Family: Fabaceae)</td>
<td>Hypoglycemic activity of cooked diet in healthy human volunteers (Panlasigui et al., 1995)</td>
</tr>
<tr>
<td><strong>Citrullus colocynthis</strong> (L.) Schrad.</td>
<td>Hypoglycemic activity of aqueous extract (300 mg/kg), glycosidic and saponin extract (50 mg/kg), orally in normal rabbits (Nmila et al., 2000)</td>
</tr>
<tr>
<td>Cucurbitaceae</td>
<td>Blood glucose lowering activity of aqueous seed extract in normal and streptozotocin (STZ)-induced diabetic rats upon daily oral administration for 2 weeks (Al-Ghaithi et al., 2004)</td>
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<tr>
<td><strong>Coccinia indica</strong>&lt;br&gt;Wight &amp; Arn.&lt;br&gt;Common name: Ivy gourd&lt;br&gt;(Family: Cucurbitaceae)</td>
<td>Hypoglycemic activity of alcoholic leaf extract in normoglycemic guinea pig (Mukherjee et al., 1972)&lt;br&gt;Hypoglycemic activity of the leaves in alloxanized dogs upon oral administration (Singh et al., 1985)&lt;br&gt;Hypoglycemic and antihyperglycemic activity of the ethanolic root extract in fasted and glucose-loaded animal models (Chandrasekar et al., 1989)&lt;br&gt;Hypoglycemic effect of 95% ethanol extract of the leaves in normal fed and 48 h starved rats (Hossain et al., 1992)&lt;br&gt;Blood glucose lowering activity of 60% ethanol leaf extract (200 mg/kg, orally) (Shibib et al., 1993)&lt;br&gt;Hypoglycemic activity of the leaf extract in a double blind control trial in human subjects (Platel and Srinivasan, 1997)&lt;br&gt;Antihyperglycemic activity of dried extract (500 mg/kg p.o., for 6 weeks) in 30 diabetic patients (Kamble et al., 1998)</td>
</tr>
<tr>
<td><strong>Casearia esculenta</strong>&lt;br&gt;Roxb.&lt;br&gt;Common name: Carilla Fruit&lt;br&gt;(Family: Flacourtiaceae)</td>
<td>Antihyperglycaemic activity of root extract (300 mg/kg p.o. for 45 days) in normal and streptozotocin-induced diabetic rats (Prakasam et al., 2002)&lt;br&gt;Blood glucose lowering activity of aqueous extract in normal and glucose loaded rats. Antihyperglycemic activity of the extract in streptozotocin-diabetic rats along with reduction in the increased plasma thiobarbituric acid reactive substance and blood urea (Prakasam et al., 2003a)&lt;br&gt;Antioxidant activity of aqueous extract in</td>
</tr>
</tbody>
</table>
### Review of Literature

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Activity and Description</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Catharanthus roseus</strong> (L.) G. Don</td>
<td>Hypoglycemic activity of ethanolic leaf extract in normal rats upon oral administration at graded dose. Hypoglycemic activity of the extract (500 mg/kg) in streptozotocin rats and in oral glucose tolerance test (Chattopadhyay et al., 1991)</td>
<td>Increases metabolism of glucose (Singh et al., 2001) and enhances secretion of insulin either from the beta cells of Langerhans or through extrapancreatic mechanism (Nammi et al., 2003)</td>
</tr>
<tr>
<td><strong>Enicostemma littorale</strong> Blume (Family: Gentiaceae)</td>
<td>Antihyperglycemic activity of whole plant aqueous extract in alloxan induced diabetic rats along with reduction of glycosylated haemoglobin and glucose-6-phosphatase activity in liver (Vijayvargia et al., 2000) Insulin enhancing activity of a single dose of aqueous extract of plant (15 g dry plant equivalent extract per kg) in alloxan-induced diabetic rats (Maroo et al., 2002) Glucose lowering activity of aqueous extract (2 g/kg p.o.) daily for 6 weeks in neonatal non-insulin-dependent diabetes mellitus (NIDDM) rats along with a</td>
<td>Enhances glucose-induced insulin release from isolated rat pancreatic islets, mediated through K (+)-ATP channel-dependent pathway (Maroo et al., 2002)</td>
</tr>
</tbody>
</table>
### Eugenia jambolana Lam. (syn. Syzygium cumini L.)

**Common name:** Indian black berry  
(Family: Myrtaceae)

**Hypoglycemic activity of pulp extract of the fruits in normal as well as STZ diabetic rats upon oral administration (Achrekar et al., 1991)**

Blood glucose lowering activity of aqueous seed extract (2.5 and 5.0 g/kg body weight p.o. for 6 weeks) along with an increase in total haemoglobin and antioxidant activity in diabetic rats (Prince et al., 1998)

The blood glucose lowering activity of alcoholic extract (100 mg/kg p.o.) in alloxan diabetic rats along with reduction in urine sugar and lipids in serum and tissues (Prince et al., 2004a)

Hypoglycemic effect of aqueous, alcoholic extracts and lyophilized powder (200 mg/kg per day) of the plant in hyperglycemic animals (Grover et al., 2000)

Antihyperglycemic and antihyperinsulinemic activity of aqueous extracts (400 mg per day) in fructose fed rats (Vikrant et al., 2001)

Reduction in plasma glucose concentration by the extract (200 mg/kg) upon administration for 50 days in STZ induced diabetic mice (Grover et al., 2000a)

May be mediated through an insulin release mechanism (Achrekar et al., 1991) or due to alteration in hepatic and skeletal muscle glycogen content and hepatic glucokinase, hexokinase, glucose-6-phosphate and phosphofructokinase levels in diabetic mice (Grover et al., 2000). It also enhances serum insulin activity (Sharma et al., 2003) and exhibits normoglycemia and better glucose tolerance (Ravi et al., 2004a)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Description</th>
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<tbody>
<tr>
<td>Decrease in elevated cholesterol, triglyceride and creatinine levels</td>
<td>(Murali et al., 2002)</td>
</tr>
<tr>
<td>Reduction in glycosylated haemoglobin, liver glucose-6-phosphatase activity and significant increase in serum insulin levels of the diabetic rats by aqueous extract</td>
<td>(Maroo et al., 2003)</td>
</tr>
<tr>
<td>Antioxidant activity of the whole plant aqueous extract (1 and 2 g/kg) in alloxanized rats upon oral administration for 45 days</td>
<td>(Srinivasan et al., 2005)</td>
</tr>
<tr>
<td>Blood glucose lowering activity of alcoholic extract (100 mg/kg p.o.) in alloxan diabetic rats</td>
<td>(Achrekar et al., 1991)</td>
</tr>
<tr>
<td>Antihyperglycemic and antihyperinsulinemic activity of aqueous extracts</td>
<td>(Vikrant et al., 2001)</td>
</tr>
<tr>
<td>Reduction in plasma glucose concentration</td>
<td>(Grover et al., 2000a)</td>
</tr>
<tr>
<td><strong>Ficus bengalensis L.</strong></td>
<td>Hypoglycemic activity of ethanolic seed extract in alloxan-induced diabetic rabbits along with hypolipidemic effect (Sharma et al., 2003)</td>
</tr>
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</tr>
<tr>
<td>Common name:</td>
<td>Hypoglycemic activity of ethanolic whole seeds, kernel (100 mg/kg of body weight) and seed coat extracts in streptozotocin-induced diabetic rats (Ravi et al., 2004a)</td>
</tr>
<tr>
<td>Banyan tree</td>
<td>Hypoglycemic activity of inorganic trace elements, obtained from the seeds in streptozotocin-induced diabetic rats (Ravi et al., 2004b)</td>
</tr>
<tr>
<td>Family: Moraceae</td>
<td>Antioxidant activity of ethanolic seed kernel extract in streptozotocin-induced diabetic rats upon oral administration (Ravi et al., 2004c)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Ficus bengalensis L.</strong></th>
<th>Hypoglycemic activity of ethanolic bark extract and a glucoside isolated from the plant in normal and alloxan diabetic rabbits (Augusti, 1975)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose lowering activity of bark extract in streptozotocin-induced diabetic animals upon oral administration and enhancement of serum insulin levels in normoglycemic and diabetic rats (Achrekar et al., 1991)</td>
<td>Blood sugar lowering activity of a dimethoxy derivative of leucocyanidin 3-O-beta- d-galactosyl celllobioside isolated from the bark in normal and moderately diabetic rats along with an increase in serum insulin in the diabetic rats at a dosage of 250 mg/kg for a 2 h period upon oral administration (Kumar and Augusti, 1989)</td>
</tr>
<tr>
<td>Antihyperglycemic activity of dimethoxy derivative of perlargonidin 3-O-alpha-1 rhamnoside (250 mg/kg, single dose study and 100 mg/kg/day long term</td>
<td>Stimulates insulin secretion from beta cells of islets of langerhans (Achrekar et al., 1991; Augusti et al., 1994) and inhibits insulin degradative processes (Kumar and Augusti, 1989)</td>
</tr>
<tr>
<td><strong>Hibiscus rosa sinensis</strong> L.</td>
<td>Hypoglycemic activity of single dose of ethanol extract of the plant in glucose-loaded rats at 120 min and blood glucose lowering effect after repeated administration for seven consecutive days at 30, 90 and 120 min after glucose loading (Sachdewa and Khemani, 1999). Hypoglycemic activity of alcoholic leaf extract (250 mg/kg p.o. for seven consecutive days) in glucose induced hyperglycemia model in rats (Sachdewa et al., 2001b). Blood glucose lowering activity of ethanol flower extract in streptozotocin induced diabetic rats along with a reduction in total cholesterol and serum triglycerides (Sachdewa and Khemani, 2003).</td>
</tr>
<tr>
<td><strong>Helicteres isora</strong> L.</td>
<td>Plasma glucose lowering activity of ethanolic root extract (300 mg/kg, after 9 days of administration) in insulin resistant and diabetic C57BL/KsJdb/db mice associated with a reduction in plasma triglyceride level (Chakrabarti et al., 2002). Antihyperglycemic activity of butanol root extracts (250 mg/kg) in glucose loaded rats (Venkatesh et al., 2004).</td>
</tr>
<tr>
<td><strong>Ipomoea batatas</strong> (L.) Lam.</td>
<td>Hypoglycemic effect of the plant against diabetic Zucker fatty rats and inhibition</td>
</tr>
<tr>
<td>Common name: Sweet potato (Family: Convolvulaceae)</td>
<td>of the increased blood glucose level in a glucose tolerance test in rats (Kusano and Abe, 2000) Postprandial glucose suppression effect (reduced blood glucose level by 16.5% at 30 min) of Peonidin 3-O-[2-O-(6-O-E-feruloyl-beta-d-glucopyranosyl)-6-O-Ecaffeoyl-beta-d-glucopyranoside]-5-O-beta-d-glucopyranoside, a diacylated anthocyanin, isolated from storage roots in male 8-week-old Sprague-Dawley rats upon single oral administration (Matsui et al., 2002)</td>
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<tr>
<td>Common name: Mango (Family: Anacardiaceae)</td>
<td>Hypoglycemic activity of aqueous leaf extract (1 g/kg p.o.), given along with as well as 60 min before glucose administration in streptozotocin-induced diabetic rats (Aderibigbe et al., 1999) Hypoglycemic activity of Mangiferin (10 and 20 mg/kg, i.p. once daily for 28 days) in STZ induced diabetic rats and improvement in oral glucose tolerance in glucose-loaded normal rats upon chronic administration (10 and 20 mg/kg, i.p.) for 14 days (Muruganandan et al., 2005)</td>
</tr>
<tr>
<td>Common name: Momordica cymbalaria Fenzl ex Naudin (Family: Cucurbitaceae)</td>
<td>Blood glucose level reducing activity of fruit powder in fasted alloxan-induced diabetic rats after a treatment for 15 days (Rao et al., 1999) Blood glucose lowering effect of aqueous fruit extract in alloxan diabetic rats (Rao et al., 2001) Antihyperglycemic activity of aqueous fruit extract (0.5 g/kg dose for 6 weeks) in alloxan-induced diabetic rats upon oral administration (Kameswara Rao et al., 2003a)</td>
</tr>
<tr>
<td>Common name: Mucuna pruriens (L.) DC.</td>
<td>Blood glucose lowering activity of powdered seeds (0.5, 1 and 2 g/kg) in normal rabbits and hypoglycemic activity</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Description</td>
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<tr>
<td>Velvet bean (Family: Leguminosae)</td>
<td>of the seed (1 and 2 g/kg body weight) in alloxan-diabetic rabbits (Akhtar et al., 1990)</td>
</tr>
<tr>
<td>Morus alba L. (Family: Moraceae)</td>
<td>Hypoglycemic activity of hot water extract of leaves in fasted and non-fasted streptozotocin induced diabetic mice at a dose of 200 mg/kg, i.p. (Chen et al., 1995)</td>
</tr>
<tr>
<td>Murraya koenigii (L.) Spreng. (Family: Rutaceae)</td>
<td>Fasting as well as post-prandial blood sugar lowering effect of leaf-powder in Type II diabetic patients upon administration for a period of 1 month (Iyer and Mani, 1990)</td>
</tr>
<tr>
<td>Ocimum sanctum L. (Family: Lamiaceae)</td>
<td>Hypoglycemic activity of 70% ethanolic leaf extract in normal, glucose fed and STZ diabetic rats, orally. The extract also potentiated the action of exogenous insulin in normal rats (Chattopadhyay, 1993)</td>
</tr>
<tr>
<td><strong>Plant</strong></td>
<td><strong>Common Name</strong></td>
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<tr>
<td><em>Punica granatum</em> L.</td>
<td>Pomegranate</td>
</tr>
<tr>
<td><em>Salacia reticulata</em> Wight.</td>
<td>Salacia</td>
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</tbody>
</table>
| **Swertia chirayita** (Roxb. ex Fleming) H. Karst. | Blood glucose lowering activity of hexane fraction of 95% ethanol extract (250 mg/kg) in fed, glucose loaded and tolbutamide pretreated animals (Sekar et al., 1987)  
Insulin releasing effect of the hexane fraction of the plant (250 mg/kg body weight p.o. per day for 28 days) in albino rats along with a significant rise in liver glycogen (Chandrasekar et al., 1990)  
Blood sugar lowering activity of swerchirin, (1,8-dihydroxy-3,5-dimethoxyxanthone), isolated from hexane fraction of the plant in fasted, fed, glucose loaded and tolbutamide pretreated albino rats (Bajpai et al., 1991)  
Blood sugar lowering effect of Swerchirin (50 mg/kg p.o.) in healthy and streptozotocin treated (35 mg/kg i.v.) Charles Foster strain albino rats (Saxena et al., 1991) | Stimulates insulin release from islets of Langerhans by depleting aldehyde-fuchsin stained beta-granules and immunostained insulin (Saxena et al., 1993) |
| **Scoparia dulcis** L. | Hypoglycemic activity of aqueous leaf extract (0.15, 0.30 and 0.45 g/kg body | Suppresses glucose influx into the polyol pathway leading to |
name: Sweet Broomweed  
Family: Scrophulariaceae

| weight for 45 days p.o.) in experimental diabetic rats along with a reduction in glycosylated haemoglobin and an increase in total haemoglobin (Pari and Venkateswaran, 2002)  
Blood glucose, sorbitol dehydrogenase, glycosylated hemoglobin, thiobarbituric acid reactive substances, hydroperoxides reducing and plasma insulin, glutathion peroxidase, glutathion S-transferase enhancing activities of aqueous plant extract (200 mg/kg) in the liver of streptozotocin adult diabetic male albino Wistar rats (Latha and Pari, 2004)  
Plasma insulin and plasma antioxidants enhancing activity of aqueous extract for 6 weeks at a dose of 200 mg/kg p.o. in diabetic rats (Pari and Latha, 2004)  
The insulin secretagogue activity of the plant extracts in isolated mice pancreatic islets at a dose of 10 mg/ml (Latha et al., 2004a)  
In vitro insulin secretagogue activity of the extract of this plant in rat insulinoma cell lines (RINm5F cells) treated with streptozotocin (Latha et al., 2004b)  
increased activities of antioxidant enzymes and plasma insulin and decreases activity of sorbitol dehydrogenase (Latha and Pari, 2004). Also potentiates insulin release from pancreatic islets (Latha et al., 2004b) |

2.8 Plant Description

2.8.1. *Solanum nigrum* Linn.

2.8.1.1 Characteristics of Family: Solanaceae

The family solanaceae is represented by 90 genera and between 2000 and 3000 species which are widely distributed throughout tropical and temperate regions of the world. (Edmonds 1978a; D’Arcy 1991).

The Solanaceae, to which the genus Solanum L. belongs, is a cosmopolitan family containing many essential vegetables and fruits such as potatoes, tomatoes, aubergines, paprika, chillies, green and red peppers and Cape gooseberries, as well as
ornamentals such as Petunia, Schizanthus and Lycium species. It also contains tobacco (Nicotiana spp.) one of the most harmful yet economically important plants in the world together with many other plants of both poisonous and medicinal value such as belladonna or deadly nightshade (Atropa belladona L.), stramonium (Datura stramonium L.) and black henbane (Hyoscyamus niger L.).

The following are the genera included in it:


2.8.1.2 Solanum nigrum Linn.

Distribution:
The generic name Solanum is generally considered to be derived from the latin solamen, and to refer to the quieting or sedative effects associated with many of the species. The plant commonly grows throughout India in dry parts up to an elevation of 2100 m. (Anonymous, 2002).
The Taxonomy is as follows:

Kingdom : Plantae
Subkingdom : Tracheobionta
Superdivision : Spermatophyta
Division : Magnoliophyta
Class : Dicotyledons
Subclass : Asteridae
Order : Solanales
Family : Solanaceae
Genus : Solanum L.
Species : Solanum nigrum L.
The presence of the plant in various parts of India has resulted in different names for the different regions. Some of them are as follows: (Anonymous, 2002,).

Hindi : Makoi
Telugu : Kachchipandu
Tamil : Munatakali
Gujarati : Piludi
Marathi : Kamuni
Sanskrit : Kakamachi
Bengali : Gurkamai, Kakamachi, Tulidun
English : Black nightshade

2.8.3 Morphological characteristics
The species of this genus are unarmed herbs, sometimes suffrutescent, occasionally shrubs or epiphytes.

Plants are subglabrous to villous annuals up to 70 cm high, covered with simple multicellular hairs with glandular or eglandular heads.

Stems decumbent to erect, terete or angled, the angles with smooth or dentate ridges; sparsely to densely pubescent with simple, uniseriate, multicellular, patent or appressed hairs with eglandular or glandular heads, interspersed with spherical four-celled glands.

Leaves are ovate, ovate-lanceolate, and ovate-rhombic to lanceolate, 2.5-7.0 cm long and 2.0 to 4.5 cm broad, margins entire to sinuate-dentate.

Inflorescences are simple, lax and often extended cymes, 5 to 10-flowered; peduncles 14 to 28 mm fruiting when usually erecto-patent; pedicels much shorter, recurved in fruit. Calyces 1.2-2.5 mm long, slightly accrescent, deflexed or adhering to base of mature berry, sepals usually ovate. Corollas stellate, white with translucent basal star, 5 to 7 mm radius, usually 1.5-3 times as long as calyx. Anthers yellow, 1.5 to 2.5 mm long. Pollen 29.5 to 33.9 μm diameter. Styles 2.8 to 3.5 mm long, not exserted beyond anthers.
Berries usually broadly ovoid, dull purple to blackish or yellowish-green, 6-10 mm broad, remaining on plants or falling from calyces when ripe.

Seeds 1.7-2.4 mm long, 26 to 60 per berry. Sclerotic granules absent.


**Therapeutic uses:**
In ayurvedic system of medicine, the entire plant is recommended for its medicinal properties. Leaves, fruits stem and roots of *Solanum nigrum* have been used in ethnomedicine for several medicinal properties: diseases of heart and eye, in pains, piles, inflammation, leucoderma, itch, worms in the ear, dysentery, vomiting, cough, asthma, bronchitis, fever, urinary discharges, favor conception, facilitate delivery laxative, liver disease, psoriasis and ringworm. *Solanum nigrum* plant is also known as herbal medicine for the treatment of diabetes (Mishra 1997; Khare 2007; Anonymous 1935).

The juice of this plant he mentioned inflamed throats, eye inflammations, shingles, ringworm, running ulcers, testicular swelling, gout and ear pains. In Europe, ‘S. nigrum’ has been used as a remedy for convulsions, and has been administered as a soporific in Germany especially for children, with leaves being placed in babies’ cradles to promote sleep in “Bohemia” (Czech Republic) (Grieve 1931). The bruised fresh leaves used externally are reputed to ease pain and reduce inflammation; they are applied to burns and ulcers by the Arabs. Leaf juice has also been used for ringworm, gout and earache, while it is also reputed to be a good gargle and mouthwash when mixed with vinegar (Grieve 1931).

In North America, the Houmas Indians use an infusion made from boiled roots of this ‘species’ to administer to babies with worms, and crushed green leaves mixed with a grease to make poultices for sores, while the Rappahannocks used a weak infusion to cure insomnia. There are relatively few reports of these species being used medicinally in South America, an exception being the moderate narcotic action
attributed to flowers and leaves resulting in their use to calm fever and combat the effects of alcoholic excesses in southern Ecuador (Heiser 1963).

In India, the ‘plant’ is noted for its antiseptic and antidysenteric properties and is given internally for cardalgia and gripe. An infusion of the plant is used as an enema for infants with abdominal upsets. The plant is also a household treatment for anthrax pustules when it is applied locally. It is further reported to have emollient, diuretic and laxative properties and its decoction is regarded as both antispasmodic and narcotic. Freshly prepared extracts of the plant are apparently effective in the treatment of cirrhosis of the liver and also serve as an antidote to opium poisoning. An alcoholic extract of leaves is active against Staphylococcus aureas and Escherichia coli. Infusions or decoctions of the plant, after transient stimulation, are reported to depress the central nervous system and the reflexes of the spinal cord. Small doses increase cardiac activity while large doses decrease it. Extracts also reduce blood pressure.

Berries apparently possess tonic, diuretic and cathartic properties and are also useful in heart diseases and as a domestic treatment for fevers, diarrhea, ulcers and eye troubles (Anonymous 1965a, 1965b). The seeds are reportedly used to treat gonorrhea and dysuria (Jain and Borthakur 1986). In Pakistan Akhtar and Muhammad (1989) showed that a powder from the aerial parts of the plant could be “antiulcerogenic”. In China leaves are used as a febrifugal or detoxicant drug. Medicinally used preparations consist of dried aerial parts of plants which are used as a diuretic, antihypertensive and anticancer agent for infections of the urinary system, hypertension and cancer of the digestive system (Schilling et al. 1992). Fresh leaves are also used to treat wounds. In the Orient Tandon and Rao (1974) reported that the fruits and juices of ‘S. nigrum’ are used to cure stomach ailments, fevers and blood impurities and young shoots to cure skin diseases. In the Philippines, leaf extracts are apparently used to restore body skin pigment. In East Africa the raw fruit is chewed and swallowed for treatment of stomach ulcers or for general abdominal upsets which lead to continued stomach-ache. Infusions of leaves and seeds are rubbed onto the gums of children who have developed crooked teeth. Pounded leaves are soaked in water, fermented and used for the treatment of boils, ulcers and swollen glands. Unripe berries are used to treat ring worms. Various parts of the plant are also believed to cure malaria, black fever, dysentery and urinary infection (Kokwaro
1976). The Zulus use an infusion as an enema for abdominal upsets in children; the southern Sotho rub burnt and powdered root in to scarifications on the back for the relief of lumbago; a paste made from unripe berries is used among African tribes as an application for ringworm; the Xhosa also use the plant for disinfecting anthrax-infected meat; in Zimbabwe the plant is used as a remedy for malaria, black water fever and dysenteries, while the juice or decoctions of the herb were formerly made into an ointment for foul ulcers (Watt and Breyer-Brandwijk 1962). In Kenya unripe fruits are applied to aching teeth and squeezed onto babies’ gums to ease pain during teething. Leaves and fruits are pounded and the infusion used against tonsillitis. Furthermore, the same mixture is believed to increase the strength of weak people and prevent skin eruptions. Raw roots of S. nigrum were also found to be eaten for stomach-ache in Tanzania.

**Phytochemistry:**

The occurrence of the steroidal alkaloid solasodine and of solasodine-like alkaloids in most species belonging to the genus Solanum has resulted in a number of phytochemical surveys of various taxa from different geographical regions throughout the world. The toxicity and/or medicinal effects of these plants are generally attributed to these glycoalkaloids. Those so far identified in the black nightshade include solanine, solasonine, solamargine and chaconine (Everist 1974; Cooper and Johnson 1984).

The most comprehensive study dealing with species belonging to the section Solanum was carried out by Carle (1981) who analyzed plant material from 55 strains representing 32 so-called species for both steroidal alkaloids and the biogenetically closely related steroidal sapogenins. He demonstrated that the characteristic steroidal alkaloid solasodine was absent from plant material of all these samples, though it was present in their unripe fruits. However, the steroidal sapogenins, identified as diosgenin and tigogenin, were universally present though not always together, in both the vegetative parts and the unripe berries. The importance of these substances ties in their potential use as raw materials for the industrial production of hormonal steroids.

Pushpa Khanna and Rathore (1977) had earlier reported significant amounts of diosgenin (1.2%) and solasodine (0.65%) in green berries of the Indian diploid ‘S.
nigrum’ (=S. americanum) with lesser amounts of both chemicals in the Indian tetraploid (=S. villosum) and hexaploid (=S. nigrum) species.

So far, few attempts have been made to isolate and identify the various chemicals responsible for the medicinal effects observed in species belonging to Solanum section Solanum. The little work done on glycoalkoloids, which are said to be responsible for anticancer activity, indicated that solasonine and solamargine, from leaves and unripe fruits, are the two most important.

Solasonine concentrations are reported highest during fruiting white solamargine is highest during flowering (Aslanov and Novruzov 1978).

Various other chemicals have been identified in species associated with the section Solanum. Fresh leaves of S. nigrum are said to contain 1 mg/100 g of ascorbic acid (Watt and Breyer-Brandwijk 1962).

The flavonols have been widely analyzed for their use as indicators of phylogenetic relationships in the Solanaceae, both within and between various generic sections. Schilling (1984) isolated 10 flavonoids from leaf extracts of 11 species belonging to the section Solanum; they were all flavols with the predominant glycosidic moiety being glucose. Other chemicals identified include coumarins (such as scopoletin), flavonols and anthocyanidins for S. scabrum in Nigeria (Gbile and Adesina 1984) and the anthocyanin pigments found in European samples of this species (Francis and Harborne 1966).

The latter authors found that the major pigment (93%) in the berries of this species was petanin (petunidin-3-(p-coumaroyl rutinoside)-5-glucoside) with minor pigments constituting the remainder.

There are also various reports of the anthocyanins responsible for the purple/black berry colouration and the carotenoids responsible for the red/orange/yellow berry colouration, but these are mostly concerned with the inheritance of these characters rather than their biochemical analyses. Rao (1978), however, identified the
anthocyanin pigment present in the Indian forms of both S. nigrum and S. americanum as “petunidin3-(p-coumoryl) rhamnosyl glucoside”.

**Pharmacological review**

Literature review has shown that the pharmacological activity of the different extracts has been studied in various disorders.

Sultana et al (1995) have reported that ethanolic extract of leaves of hepatoprotective plant; *Solanum nigrum* and *Cinchium intybus* have free radical mediated DNA damage inhibiting properties which produce dose dependent action.

Perez et al.(1998) reported that on intraperitoneal injection, the ethanolic extract significantly prolonged pentobarbital inducing sleeping time, produced alteration in general behaviour pattern, reduced exploratory behaviour pattern, suppressed the aggressive behavior, affected locomotor activity and spontaneous motility. The observations suggest that the fruit of S.Nigrum possesses potential CNS depressant action which may be correlated with increased parasympathetic tone.

Prashanth kumar et al (2001) reported cytoprotective role of *Solanum nigrum* against gentamicin-induced kidney cell damage in vitro. The 50% ethanol extract of the whole plant of *Solanum nigrum* was tested in vitro for its cytoprotection against gentamicin-induced toxicity on Vero cells. Cytotoxicity was significantly inhibited as assessed by the Trypan blue exclusion assay and mitochondrial dehydrogenase activity (MTT) assay. The test extract also exhibited significant hydroxyl radical scavenging potential, thus suggesting its probable mechanism of cytoprotection.

Ikeda et al (2003) explored cytotoxic activity of steroidal glycosides from *Solanum* plants. Here the said activity was measured on human lung cancer (PC-12) and human colon cancer (HCT116) cells. The potency of activity was observed to be decreased in the order of spirostane, furostane, spirosolane, and pregnane type steroid glycoside. It was also suggested that the activity depend on the kind of oligosaccharide moiety and aglycon moiety.
Raju *et al* (2003) reported the protective effect of dried fruits of *Solanum nigrum* L against carbon tetrachloride induced hepatic damage in rats. The ethanolic extract of the fruit showed remarkable hepatoprotective activity. The activity was evaluated using biochemical parameters such as serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and total bilirubin.

Jainu *et al* (2004) reported antioxidant effect of methanolic extract of *solanum nigrum* berries (SBE) on aspirin induced gastric mucosal injury. In this study SBE inhibited the increase in area of gastric mucosal lesions in aspirin induced ulceration in rats. These protective effects were observed at oral dose of SBE 500 mg/kg body wt and a recovery of 70.12% was observed with in 7 days.

Huseini *et al* (2005) reported the efficacy of Liv-52 on liver cirrhotic patients in a randomized double blind placebo controlled first approach in which Solanum nigrum is one of the ingredient. The result demonstrated that the patients treated with Liv-52 for 6 months had significantly better child-plug score, decreased serum ALT and AST. The protective effect of Liv-52 can be attributed to the diuretic, anti-oxidative, and immunomodulating properties of the component herbs.

Lee *et al* (2005) investigated apoptotic effect of glycoprotein isolated from *Solanum nigrum* L. SNL glycoprotein has obvious cytotoxic and apoptotic effects (>50 % cell death) at 40μg/ml SNL glycoprotein for 2 hr in human colon carcinoma cells (HT-29). The study indicated that the SNL glycoprotein has a stimulatory effect on mitochondrial cytochrome c, cleavage of pro-caspase-9, pro-caspase-3, and poly (ADP-ribose) polymerase (PARP) proteins in HT-29 cells.

Lee *et al* (2006) reported that glycoprotein (150-kDa ) isolated from *Solanum nigrum* L induces apoptosis through the mitochondrial apoptotic signal pathway in HCT-116 cells rather than through intracellular reactive oxygen species.

Zakaria *et al* (2006) reported that chloroform extract of leaves *Solanum nigrum* L. was found to exhibit remarkable antinociceptive, anti-inflammatory and antipyretic activities in a dose-independent manner.
Jainu et al (2006) reported that Solanum nigrum L fruits extract (SNE) possesses antiulcer and ulcer healing effects on experimental ulcer models. SNE showed concomitant attenuation of gastric secretory volume, acidity, and pepsin secretion in ulcerated rats. In addition, it accelerated the healing of acetic acid induced ulcers. Further, it inhibits H⁺K⁺ ATPase activity and decreases the gastrin secretion in ethanol induced ulcer model.

Fatimi et al (2007) reported antioxidant, antimicrobial and cytotoxic toxic activities of selected plants including Solanum nigrum. The observed antibacterial and antifungal activities of Solanum species are well known and probably caused by the alkaloids.

Heo et al (2004) reported glycoprotein isolated from Solanum nigrum L. inhibit the DNA-binding activities of NF-kB and AP-1, and increases the production of nitric oxide in TPA-stimulated MCF-7 cells.

An et al (2005) reported Solanum nigrum produces nitric oxide via nuclear factor-[kappa] B activation in mouse peritoneal macrophages. It was found that there was a marked cooperative induction of NO production. Nitric oxide (NO) is an anti-tumor molecule produced in activated macrophages.

2.9.1 *Acacia catechu* Willd.

2.9.1.2 Characteristics of Family: Mimosaceae

The Mimosaceae are mostly tropical and subtropical trees and shrubs comprising about 40 genera and 2,000 species. The leaves are nearly always alternate, stipulate, and bipinnately compound (rarely once-pinnate) they usually have swollen petiole bases called pulvini that commonly function in orientation of the leaf. The inflorescence is commonly a capitulum (also called a head). The flowers are hypogynous to slightly perigynous, have radial symmetry, petals that are valvate in bud, and commonly a 5-parted calyx and corolla. The stamens are distinct to strongly monadelphous, numerous (rarely as few as 10), and are generally more showy than the perianth. The pistil is simple, comprising a single style and stigma, and a superior ovary with 2-many marginal ovules in a solitary locule. The fruit is usually a legume.
Distribution:

*Acacia catechu* Willd. is a moderately sized deciduous tree, up to 3m high. The leaves are pinnate with a pair of recurved prickles at the base of the rachis. Flowers are pale yellow in cylindrical spikes. Pods are glabrous oblong. The plant is distributed throughout the Sub–Himalayan tract of Punjab to Assam ascending to 1200m., Peninsular region - particularly in drier parts, Madhya Pradesh, Maharashtra, Gujarat, Bihar, Rajasthan and Tamil Nadu.

The taxonomy is as follows:

Kingdom : Plantae  
Division : Magnoliophyta  
Class : Magnoliopsida  
Order : Fabales  
Family : Fabaceae  
Subfamily : Mimosoideae  
Genus : Acacia  
Species : *Acacia catechu*

The presence of the plant in various parts of India has resulted in different names for the different regions. Some of them are as follows: (Anonymous, 2002.)

Hindi : Katha, Khair.  
Telugu : Khadiramu, Kachu.  
Tamil : Kadiram, Karangalli, Karungali.  
Gujarati : Kher, Kherio-baval, Katho.  
Marathi : Khair, Khaira.  
Sanskrit : Balapatra, Gayatri, Khadira, Tiktasara.  
Bengali : Khayera  
English : Cutch tree, Catechu.
2.9.1.3 Morphological characteristics

A moderate sized tree, not reaching more than 30 to 40 feet high and often smaller, with a short somewhat crooked trunk, and numerous irregular stragglng branches; bark brown or dark grey, rough, red and fibrous within; young branches are smooth or pubescent, with a pair of sharp, hooked, brown prickles, just below the position of the stipules of each leaf.

Leaves are numerous, alternate, stalked; petiole with a few prickles, bipinnate, 5 to 8 inches long; pinnae in 10 to 20 pairs, 1-2 inches long, narrow; leaflets opposites, 20-
30 pairs in each pinna, sessile, overlapping, linear, blunt, 1/8-1/4 inch long, glabrous or pubescent, entire.

Flowers pale yellow, sessile in peduncled axillary spikes. Calyx glabrous or downy; corolla- segments rather broader; stamens about twice as long as corolla, the filaments not combined into a tube at the base; a small disk surrounds the stalk of the ovary.

Fruits flat brown pods, shiny and with a triangular brak at the apex and narrow at the base; seeds 3-10 per pod.

**Phytochemistry**
The important chemical constituents reported in the heartwood are catechin, catechutannic acid, epicatechin, catechin tetramer, dicatechin, gallocatechin, kaempferol, taxifolin, isorhamnetin, (+) afzelechin, L-arabinose, D-galactose, D-rhamnose and aldobiuronic acid (Ismail and Asad, 2009).

The important chemical constituents reported in the heartwood are catechin, catechutannic acid, epicatechin, catechin tetramer, dicatechin, galallocatechin, kaempferol, taxifolin, isorhamnetin, (+) afzelechin, L-arabinose, D-galactose, D-rhamnose and aldobiuronic acid (Ismail and Asad, 2009). The medicinal properties of *Acacia catechu* may be due to the antioxidant properties of these constituents (Devi et al., 2011).

**Pharmacological review**
Literature review has shown that the pharmacological activity of the different extracts has been studied in various disorders.

Jayasekhar et al (1997) reported the ethyl acetate extract of *Acacia catechu* decreased the levels of CCl₄ induced elevated enzyme levels in acute and experimental models and there is marked reduction of increased serum bilirubin content of CCl₄ induced acute and chronic liver damage experiment possesses fairly good hepatoprotective activity.
Ray *et al* (2006) reported that seven days treatment of ethyl acetate extract of *Acacia catechu* at dose of 250 and 500 mg/kg, p.o showed significant antipyretic activity (P< 0.01), antidiarrhoeal activity was significant in single dose of 250 mg/kg (P< 0.001) in respect of latent period of onset of diarrhoea, average number of stool passed and purging index. Significant reduction of blood glucose level was observed in normal rats following single dose treatment with the test drug at dose of 500 mg/kg, p.o. significant hypoglycaemic activity was also evident in diabetic rats at the dose of 250 and 500 mg/kg, p.o.(P< 0.01) and significant hepatoprotective activities of in albino rats in the same mentioned dose.

Singh *et al* (2005) reported antimicrobial activity of some natural dyes including *Acacia catechu*. The wool samples dyed with *Acacia catechu* dye shows 10-15 % reduction in bacterial growth.

Naik *et al* (2003) reported antioxidant activity of individual herbal components used in ayurvedic medicines containing *Acacia catechu*. The polyphenolic content of this plant shows significant antioxidant potential by measuring its ability to inhibit lipid peroxidation, SOD, DPPH assay and ABTS \(^{-}\) radical decay.

Zhang *et al* (2008) reported low concentration of condensed tannins from catechu significantly inhibits fatty acid synthase and grow of MCF 7 cells. Condensed tannins from catechu inhibited the growth of breast cancer cells, while they had no effect on normal cells, and the inhibition was related to their ability to suppress FAS. More importantly, the inhibition of both FAS activity and the growth of breast cancer cells by the tannins from catechu were exhibited without proteins being precipitated.

Kamble *et al* (2008) reported hepatoprotective activity studies of herbal formulatilons containing *Acacia catechu* against CCL\(_4\) induced and paracetamol induced hepatotoxicity.

Surveswaran *et al* (2007) reported systemic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants including *Acacia catechu*. 
Maisuthiasakul et al (2007) reported assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants including Acacia catechu. In chewing plants the EC$_{50}$ value of bark extract from Acacia catechu wild. Was lowest (0.05µg/µg DPPH) and give highest antiradical activity among all studied plant parts. The highest antiradical activity of this plant reflects the highest phenolic content and flavanoid content.

Pingale (2010) reported hepatoprotection by acacia catechu in CCL4 induced liver dysfunction. The heartwood powder of Acacia catechu has been treated in the form of aqueous slurry. From the findings of blood biochemical parameters it could be inferred that the treatment with this plant material is effective against CCL4 induced dysfunction of the liver, justifying the use this plant by folk healers. The decreased levels of serum bilirubin after treatment with heartwood powder of Acacia catechu restores the normal functional status of the liver. This hepatoprotective effect was supported by light microscope studies.

Devi et al (2011) investigated preliminary study on the antioxidant activity of the alcohol extract was carried out by evaluating the free radical scavenging activity by the method of DPPH radical scavenging assay. The extract gave very good radical scavenging activity comparable with that of ascorbic acid which was used as the standard.

Karwani et al (2011) reported antisecretory and antiulcer activity of acacia catechu against indomethacin plus pyloric ligation induced gastric ulcers in rats. Results of the study suggest that the extract can be used for the prevention and treatment of gastric ulcers.

**Traditional uses**

Cutch from wood— powerful astringent (in urinary and vaginal discharge), antidiarrhoeal, haemostatic; used for treating excessive mucous discharges, haemorrhages, relaxed conditions of gums, throat and mouth, stomatitis, irritable bowel; also used as an antileprotic drug. Along with other therapeutic applications, The Ayurvedic pharmacopoeia of India indicates the use of dried pieces of heartwood in inflammations, skin diseases and urinary disorders, recommends its use as a blood purifier, in diseases caused by lipid disorders. (Anonymous 2007)
The decoction of bark mixed with milk is taken to cure cold and cough. The bark decoction is either alone or used in combination with opium to cure severe diarrhea. *Katha* after drying is applied on lemon slice and taken regularly with empty stomach to cure piles. Heartwood of khair is boiled with other ingredients to prepare the decoction. It is taken as tea by the pregnant ladies to keep warm their body. It is also given to cure fever due to cold during the pregnancy. A decoction is served to women after 2-3 days of child delivery, prepared by boiling katha along with Ellachi (cardamom). It is believed that it provides strength to the body and also helps in secretion of milk. The water boiled with the heartwood chips of *Khair*, is used to take bath by women after delivery. It is considered beneficial to cure the body pains. *Katha* or decoction of heartwood is applied in mouth and on tongue to cure mouth ulcer. It is also applied externally on ulcers, boils, skin eruptions and on gums as disinfectant.

This plant material is used as anodyne, astringent, bactericide, refrigerant, stimulant, styptic, masticatory expectorant and antiphlogistic. It is also used in asthma, cough, bronchitis, colic, diarrhoea, dysentery, boils, in skin affections and sores and for somatitis. The bark is used as an anthelmentic, antipyretic, antiinflamatory, in bronchitis, ulcers, anaemia and gum troubles.

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