CHAPTER-I

INTRODUCTION

1.1 Diabetes

Diabetes mellitus is a complex and a multifarious group of disorders that are characterized by shortage or lack of insulin secretion or action or reduced sensitivity of the tissue to insulin (Prasad *et al*., 2009). Without insulin, body tissue, in particular the liver, muscle and adipose tissue fail to take and utilize glucose from the blood circulation resulting in elevated blood glucose levels, a condition known as hyperglycemia. Persistent hyperglycemia or high glucose levels leads to certain life threatening long term complication causing damages and failure of various organs and cells especially the eyes, kidneys, nerves, heart and blood vessels. Symptoms of marked hyperglycemia include polyurea, polydipsia, weight loss, sometimes with polyphagia and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketonic hyperosmolar syndrome (Hirch, 1995; Brownlee, 2001; Weiss and Sumpio, 2006; Mishra *et al*., 2008; ADS, 2007).

1.2 History of diabetes

The earliest description of diabetes was documented in the writings of Hindu scholars as long as in 1500 BC as "a mysterious disease causing thirst, enormous urine output, and wasting away of the body with flies and ants attracted to the urine of people." The word diabetes was probably coined by Apollonius of Memphis around 250 BC, after the word dia-dainein which literally meant "to go through" or siphon as the disease drained more fluid than a person could consume. Later on, in the year 1675 Thomas Wills incorporated the name "mellitus" which mean sweet tests in latin and because it made the urine sweet (Roussel, 1998; Maccracken, 1997) . In 1776, Matthew Dobson proved that the sweet in urine was due to the production of excess kind of sugar in the urine and blood of people with diabetes (Dobson, 1776). Indians in the ancient time also used ants to test for diabetes (which they referred to as sweet urine disease); they used to observe whether ants were attracted to urine or not. Pathogenesis of diabetes was only understood experimentally in 1900 when Dr Joseph Von Mering and Oskar Minikowski discovered the role of pancreas in diabetes. In 1910, Sir Edward

Albert Sharpey-Schafer discovered that people who lacked a particular single chemical produced by the pancreas developed diabetes and the single compound was named as insulin (Patlak, 2002). The biggest breakthrough of diabetes came in 1921, when Dr Frederick Banting and Charles Best conducted a series of experiment resulting in the isolation of insulin (Banting *et al*., 1992).

1.3 Classification of diabetes

Diabetes mellitus comprises a set of heterogeneous diseases which differ in their etiological, clinical and epidemiological characteristics. In 1979, the National Diabetes Data Group stated the contemporary classification of diabetes and other categories of glucose intolerance with the approval of the expert committee of diabetes, World Health Organization. Later on, during the last decade due to certain uniformity or consistency in the past in defining diabetes and other stages of glucose tolerance, many individuals and groups in the diabetes community expressed the needs for a revision of the nomenclature, diagnostic criteria and classification of diabetes mellitus, seeking an international consensus if possible (Diabetes care, 1997). Assigning a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis, and many diabetic individuals do not easily fit into a single class. Thus, for the clinician and patient, it is less important to label the particular type of diabetes than it is to understand the pathogenesis of the hyperglycemia and to treat it effectively (ADA, 2007).

1.3.1 Type-1 diabetes or Insulin Dependent Diabetes Mellitus (IDDM)

It is an organ specific autoimmune disease which results when the body's system for fighting infection, the immune system turns against a part of the body. In diabetes the immune system attacks and destroys β-cells in the pancreas whose primary function is to produce insulin (Jabon *et al*., 2006). Type 1 is also known as childhood or juvenile diabetes as most people developed it at childhood. Complication associated with this type of diabetes includes congenital abnormalities such as central nervous system, cardiac and skeleton muscle malformations which usually occur during neonatal stages (Wasserfall, 2006). About 10% of all people diagnosed with diabetes have the type I variety. The cause of the disease is not yet fully understood, but it arises from a variety of genetic and environmental factors (Kovats and Johnson , 1997). It has

been shown to be caused as a result of an autoimmune reaction to antigens of β-cells produced by pancreas. Three types of autoimmune exist (Achenbach and Ziegler, 2005). a) Islets cell surface antibodies: These are polyclonal antibodies that react with all the cells of islets i.e (α, β and pancreatic polypeptide cells). About 80% of type 1 diabetics have these auto antibodies. b) Islets cell cytoplasm antibodies: These antibodies are directed against islets cell cytoplasm and about 90% of type 1diabetes has these antibodies. c) Specific antigens targets of islet cells: These antibodies are directed to glutamine acid decarboxylation (GAD) and about 80% of type-1 diabetic have these antibodies.

1.3.1.1 Pathogenesis of Type-1 diabetes

Destruction of pancreatic β-cells by the autoimmune antibodies leads to a deficiency of insulin secretion causing metabolic disturbances. Extrinsic factors that might affect β cell functioning include damage caused by viruses such as the mumps virus and coxsachie virus B4, by chemical agents, or by destructive cytotoxins and antibodies released from sensitized immunocytes. An underlying genetic defect relating to pancreatic β cell replication or function may predispose a person to the development of β cell failure after viral infections. In addition, specific HLA genes may increase susceptibility to a diabetogenic virus or may be linked to certain immune response genes that predispose patients to a destructive autoimmune response against their own islet cells (auto aggression). Observations that pancreatic β cell damage appears to be lessened when immune suppressive drugs such as cyclosporine or azathioprine are given at the initial manifestation of Type-1 diabetes support the importance of auto aggression by the immune system as a major factor in the pathogenesis of this type of diabetes (Nolte and Karam, 2001).

1.3.2 Type 2 diabetes mellitus

It is commonly known as non-insulin diabetes mellitus. And is a polygenic disorder with obesity related insulin resistance playing a major role in its onset and progression occurs in adult patients aged 40 years and above. It is characterized by excessive hepatic glucose production, decreased insulin secretion from pancreatic beta cells, and insulin resistance in peripheral tissue such as muscle adipose and liver (Ahmed, 2006; Jung *et al.,* 2006). Type-2 diabetes is usually the product of two distinct

abnormalities i.e β cell function and decreased insulin sensitivity (Robertson, 2006). There are convincing data to indicate a genetic component associated with insulin resistance (Kumar *et al*., 1992). Most Type-2 diabetics are obese and these people normally have insulin resistance on liver, muscles and adipose tissues which are the major sites of insulin. Insulin resistance is also caused by acquired factors such as sedentary life style, pregnancy and hormone excess (De-Fronzo, 2004).

1.3.2.1 Pathogenesis of Type-2 diabetes

Type 2 diabetes results in combination of certain environment and genetic factors that may affect insulin production or insulin sensitivity (Hopper and Lips, 2006). Any gene mutation or metabolic disturbance leading to defect in insulin secretion, insulin action, glucose transport or enzyme association with glucose metabolism can theoretically result in hyperglycemia (Tseng, 2004). An example of genetic subtypes of Type-2 diabetes mellitus involves mutations in glucokinase, which phosphorylates glucose to glucose-6-phosphate, leading to impaired glycolysis (Fajans *et al*., 2001). The impaired glucose transport into skeletal muscle and adipose tissues can result from a variety of mechanisms involving insulin receptor defects. Over expression of tumor necrosis factor α (TNF α) in muscle cells has been implicated as an inducer of insulin resistance by increasing the serine phosphorylation of insulin receptor substrate (IRS-1) and (IRS-2), resulting in the reduction in the ability of the IRS molecules to dock with receptor and interact with downstream pathway (Roith and Zick, 2001). Interleukin-6 (IL-6) has also been found to play an important role in the induction of insulin resistance in adipocytes (Lagathu *et al*., 2003). Chronic treatment with IL-6 to adipocyte can diminish expression of β subunit receptor, IRS-1 and GLUT4, resulting in reduced glucose transport (Koistinen *et al*., 2003). Insulin induced activation of β-subunit of insulin receptor, extracellular signal regulated kinases (ERK-1) and (ERK-2) are also inhibited by IL-6. Although the expression of p38 mitogen activated protein kinases (MAPK) phosphorylation is increased in skeletal muscle in patients with T2DM, the insulin stimulated p38 MAPK phosphorylation is only noted in non-diabetic subjects, but not in patients with Type-2 diabetes mellitus (Consoli *et al*., 1990). Another mechanism leading to hyperglycemia in patients with Type-2 diabetes involves the inability to produce hepatic glucose. Enhanced phosphoenolpyruvate carboxykinase (PEPCK), an enzyme catalyzing the rate limiting

step in gluconeogenesis activity leading increased hepatic glucose production in patients with Type-2 diabetes mellitus (Kahn, 2000). Decreased glycogen synthesis has also been reported in patients suffering from T2DM. Pancreatic β-cell dysfunction has also been demonstrated in patients with Type-2 diabetes. Progressive formation of amyloidosis with loss of β cells is always major pathological factor found in patients with Type-2 diabetes mellitus (Marzaban, 2003).

1.3.3 Type-3 diabetes or Gestational diabetes

Some women develop gestational or Type-3 diabetes late in pregnancy, caused by the hormones of pregnancy or a shortage of insulin. Although this form of diabetes usually disappears after the birth of the baby, women who have had gestational diabetes have a 40-60 percent chance of developing Type-2 diabetes later in life (Mayfield, 1998). Gestational diabetes occurs more often in some ethnic groups and among women with a family history of diabetes. Women with gestational diabetes may not experience any symptoms.

1.3.3.1 Pathogenesis of Type-3 diabetes

Pathogenesis of gestational diabetes milltus (GDM) is mainly due to the resistance of insulin to stimulate glucose disposal and to suppress both glucose production and fatty acid levels (Catalano *et al*., 1999; Xiang *et al*., 1999). Also a large defect in pancreatic β cell function is consistently found in women with prior GDM (Buchanan, 2001). Recently, it has also been proposed that events leading to the development of GDM are triggered by an antigenic load which is the fetus itself. Human leukocyte antigen-G (HLA-G) expression, which functions to protect the fetus from immune attack by down-regulating cytotoxic T cell responses to fetal trophoblast antigens, is postulated to protect pancreatic islet cells as well. The interaction between HLA-G and nuclear factor-κB (NF-κB) has been suggested to be central in the events leading to GDM development. Also it has been postulated that the development of DM in patients who have undergone organ transplantation is analogous to GDM development in a proportion of pregnancies. In both cases, an antigenic load triggers the diabetogenic process (Oztekin, 2007).

1.4 Complications of diabetes mellitus

Complications of diabetes acquire the greatest costs of diabetes to the patient and the health service. Uncontrolled hyperglycemia in both type of diabetes leads to the development of both acute and chronic complications; however complications are less common and severe in people who control blood glucose levels (Weiss and Sumpio, 2006). Acute complications of diabetes mellitus include diabetic ketoacidosis, abdominal pain, dehydration, accelerated breath where the patient requires medical emergency. Hypoglycemia or abnormally low blood glucose is also a complication that is normally cause by drugs used for diabetes. In this case the patient may become agitated, sweaty, and have many symptoms of sympathetic activation of the autonomic nervous system resulting in feelings similar to dread and immobilized panic (Kovats and Johnson, 1997). Long term or chronic complications arise only after many years of exposure to high blood glucose, high blood pressure and high cholesterol compounded by age, inactivity, obesity and smoking (NDA, 2011). It includes cardiovascular diseases, hypertension, chronic renal failure, retinal damage, nerve damage, erectile dysfunction and macro vascular damage which may cause poor healing of wounds particularly of the feet and can lead to gangrene which may require amputation and eventually premature death (WHO, 1999; Ortiz *et al*., 2007). Hyperglycemia lead to increase production of mitochondrial reactive oxygen species (ROS), which activate a number of metabolic pathways viz, the polyol pathway, formation of advanced glycation end product, hexosamine pathway and the protein kinase C pathway whose end products contribute to the development of long term complication of diabetes (Weiss and Sumpio, 2006).

1.5 Medicinal plants and herbs for diabetes

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity till date due to its certain escalating factors while comparing with synthetic oral hypoglycemic drugs, which are currently the main form of treatment for diabetes. These limitations, of currently available antidiabetic pharmacological agents have prompted researchers all over the world to investigate alternative antidiabetic remedies. In particular, consideration is given to plants and herbs used by traditional healers and herbalists as antidiabetic remedies with the hope

of discovering new natural products that can be used or developed into safe, inexpensive and effective antidiabetic remedies. It is estimated that 80% of the world's population, mostly from developing countries, depends on traditional medicine for primary health care. Furthermore, while largely unrecognized, it is estimated that 25% of all prescribed medicines contain some ingredient(s) derived from plants. India is the largest producer of medicinal herbs and is called as botanical garden of the world (Seth and Sharma, 2004). In India more than 70% of the population uses herbal drugs for their health. There is a vast experience-based evidence for many of these drugs. There are also a number of Institutes/Universities in India carrying our research on herbal drugs and medicinal plants.

Ethnobotanical surveys indicate that more than 1200 plants are used in traditional medical systems for their suspected hypoglycemic activity (Marles and Farnsworth, 1995).The hypoglycemic activity of a large number of these plants/plant products has been evaluated and confirmed in animal models as well as in human beings (Gupta *et al*., 2005). Earlier the plants have been used as crude extracts which consisted of numerous active compounds. Some of these compounds may act synergistically, while at times they can have antagonist effects. Lately it has been focused on ethnobotany and ethnopharmacognosy in which many bioactive compounds were isolated and characterized. Among these are alkaloids, flavanoids, glycolipids, glycosides, polysaccharides, peptidoglycans, hypoglycans, galactomannans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids, saponins, dietary fibers and inorganic ions. Even the discovery of widely used hypoglycemic drug, metformin came from the traditional approach of using *Galega officinalis* (Gurub-Fakim, 2006). Thus, plants are a potential source of anti-diabetic drugs however there is a major hindrance in amalgamation of herbal medicine in modern medical practices due to lack of scientific and clinical data proving their efficacy and safety.

1.5.1 Mechanism of action of antidiabetic medicinal plants

There are several possible mechanisms through which medicinal plants can act to control the blood glucose level by interfering with one or more of the processes involved in glucose homeostasis. Moreover, during the past decade and especially in last few years some of the new bioactive drugs isolated from hypoglycemic plants

showed antidiabetic activity with more efficacy than synthetic oral hypoglycemic agents (Chauhan *et al*., 2010). Therefore, plants, as folk remedies, are widely used to treat diabetes mellitus. The reported mechanisms whereby herbal antidiabetic remedies reduce blood glucose levels and are summarized as follows: stimulation of insulin synthesis and/or secretion from pancreatic beta-cells, regeneration of damaged pancreatic beta cells, increased of insulin sensitivity (enhancement of glucose uptake by fat and muscle cells), protective/inhibitory effect against insulinase, mimicking the action of insulin (acting like insulin), alteration of the activity of some enzymes that are involved in glucose metabolism, improved glucose homeostasis: increase of peripheral utilization of glucose, increase of synthesis of hepatic glycogen and/or decrease of glycogenolysis acting on enzymes, inhibition of intestinal glucose absorption, reduction of glycogenic index of carbohydrates, reduction of the effect of glutathione etc (Tanira, 1994; Bnouham *et al*., 2006; Chauhan *et al*., 2010).

It can be concluded from previous reports that the majority of antidiabetic medicinal plants exert their blood glucose lowering effect through stimulation of insulin release from pancreatic beta cells or through alteration of some hepatic enzymes involved in glucose metabolism. Another point of note in the above mentioned reviews is that a given plant and/or its product may exert its blood glucose lowering effect through a combination of more than one mechanism (Grover, *et al*., 2002; Bnouham *et al*., 2006).

1.6 Prevalence of Diabetes mellitus

Diabetes mellitus is one of the most common metabolic disorders across the world and number of diabetic patients is on rise. 371 million people were reported globally with diabetes in 2012 comparing to 366 million in 2011 and this is expected to rise to 552 million by 2030. This equals to approximately three new cases every ten seconds or almost ten million per year. Diabetes caused 4.8 million death worldwide in 2012 with more than 471 billion USD were spent on health care for diabetes.

Most people with diabetes live in low and middle income countries like India, and these countries will also see the greatest increase over the next few years. It is clear that in the last two decades, there has been a marked increase in the prevalence of diabetes among both urban as well as the rural Indians. Although in rural India the

prevalence of diabetes is much lower than in the urban population, even here the prevalence rates are rapidly rising, though clearly more studies are needed (Gupta *et al*., 2006). India is presently home to 62 million diabetics, an increase of nearly 2 million in just one year. By 2030, India's diabetes numbers are expected to cross the 100 million mark. The recently published ICMR-INDIAB national study reported that there are 62.4 million people with type-2 diabetes and 77 million people with pre diabetes in India. So diabetes burden are getting worse in India and is also ways ahead then its immediate neighbors.

1.7 Significance of the study

In India, indigenous remedies have been used in the treatment of diabetes mellitus since the time of Charaka and Sushruta (6th century BC) (Grover and Vats, 2001). The World Health Organization (WHO) has listed 21,000 plants which are used for medicinal purposes around the world. Among these, 2500 species are in India. India is the largest producer of medicinal herbs endowed with a wide diversity of agro-climatic conditions and is called as botanical garden of the world (Seth and Sharma, 2004).

A wide and diverge range of plant have been reported in the Indian literature that have beneficial effects in the treatment of diabetes. Most of these plants have been claimed to possess hypoglycemic properties but most claims are anecdotal and few have received adequate medical or scientific evaluation. Those that have been evaluated may be grouped into three categories: a) plants from which a reputedly hypoglycemic compound or partially characterized hypoglycemic fraction has been prepared; b) plants reported to exert a hypoglycemic effect, but the nature of the active principle is unestablished; and c) plants that reputedly exert a hypoglycemic effect, but the scientific evidence is unclear (Noor *et al*., 2013; Bailey, 1989). So far several phytochemicals, including alkaloids, flavonoids, glycosides, glycolipid, galactomannan, polysaccharides, peptidoglycan, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids, saponins, dietary fibers and inorganic ions has been isolated that affects various metabolic cascades, which directly or indirectly affect the level of glucose in the human body (Grover and Vats, 2001). However, only a few have been subjected to detailed scientific investigation therefore,

still there is a need for modern research in the identification of phytochemical compound(s), their target(s) and their modes of action and combination therapy of plant products with synthetic drugs that will provide treatment for all and justify the role of novel traditional medicinal plants having anti-diabetic potentials.

Eugenia operculata Roxb. also known as *Cleistocalyx operculata* Roxb. is a small or medium sized evergreen tree widely distributed within Sub-Himalayan forests and tract. The leaves and buds of this plant have been used as an ingredient in various beverages, common tea for gastrointestinal disorders and as an antiseptic for dermatophytic disorders for many years (Loi, 1986). The fruit is eaten for rheumatism. A concentrated of the root infusion is used against painful joints. The bark is acrid, bitter and astringent and is given in dysentery, bronchitis and biliousness. Previous reports also revealed that the *E. operculata* buds had various biological activities in vitro and in vivo such as anticancer, antitumor, antihyperglycemic and cardio tonic action (Loi, 1986; Ye *et al*., 2005a,b; Mai *et al*., 2007; Anthony *et al*., 2002). High contents of polyphenols and flavonoids were known to have antioxidant and anticarcinogenic properties. Also it stimulated human lymphocyte proliferative responses and significantly enhanced NK cells activity (Sriwanthana *et al*., 2007). The stem bark is used for ritual and religious in North-Eastern parts of India. Also in folk medicine, the ash of dried bark is given in a dose of 1.25g with water on empty stomach or 1hour before lunch and dinner for 40 days to diabetic patients. Aqueous extract of *E.operculata* flower buds inhibited the rat intestinal α glucosidase, maltase and sucrase activities, with IC_{50} values of 0.70 and 0.47mg/ml respectively, inhibition of the activity of carbohydrates hydrolyzing enzymes plays an important role in the prevention and treatment of diabetes. Again prolonged administration of *E.operculata* extract at a dosage of 500mg/kg b.w for 8 weeks to STZ induced diabetic rats clarified more antihyperglycemic effects viz restoration of the weight gain, reduction of blood glucose and urine volume (Mai and Chuyen, 2007).

However, despite the various bioactive phytochemical constituents and diverse medicinal properties attributed to this plant, no detailed biochemical studies have been carried out to shed light on the role of *E.operculata.* leaves in diabetes. Hence, the present study was carried out in an attempt to emphasis the antidiabetic properties of

E.operculata in STZ induced diabetic mice and also to characterize the responsible compounds.

1.8 Aim and objectives of the work

In the present study emphasis is made on the isolation and characterization of antidiabetic compound(s) from *Eugenia operculata* Roxb. This study has the following objectives.

i) Screening for anti-diabetic activity.
ii) Isolation/fractionation of the active compounds from the selected plant.
iii) Characterization of isolated/fractionated compounds.