CHAPTER-I

GENERAL INTRODUCTION
Diabetes mellitus

Diabetes mellitus (DM) is a complex metabolic disorder resulting from an absolute or relative lack of insulin and/or reduced insulin activity, which results in abnormalities in carbohydrate, protein and fat metabolism. Diabetes is characterized by hyperglycemia, glucosuria, hyperlipidemia, polyuria, polyphagia, polydipsia, negative nitrogen balance and sometime ketonemia. According to World Health Organization (WHO) and Indian Council of Medical Research guidelines, criteria for diabetic condition is fasting blood glucose level \( \geq 126 \text{ mg/dL} \). Some of the initial characteristic features of diabetes include increased thirst, hunger, frequent urination, blurred vision and weight loss. Diabetes, a global public health problem is now emerging as a pandemic. Over the past decade, incidence of diabetes is rapidly increasing in developing countries compared to developed countries and it is predicted that this number will almost double in the near future. According to International Diabetes Federation, 415 million (8.8\%) people are living with diabetes globally, which is predicted to rise to 642 million in next 25 years. In India, it is estimated to be about 69.2 million people with diabetes in 2015 and this figure is expected to cross 123.5 million by 2040, next only to China [IDF Diabetes Atlas, 2015]. Diabetes mellitus is ranked 7\textsuperscript{th} among the leading causes of death and is considered 3\textsuperscript{rd} when its fatal complications are considered.

Classification of diabetes mellitus

WHO classification of diabetes:

1. Type 1 diabetes mellitus (IDDM)
2. Type 2 diabetes mellitus (NIDDM): (a) Non-obese, (b) Obese
3. Gestational diabetes mellitus
4. Malnutrition-related diabetes mellitus
5. Other types of diabetes mellitus (associated with specific conditions & syndromes).

1. Insulin dependent diabetes mellitus (IDDM)

It is also referred to as ‘Juvenile-onset diabetes’ or Type I diabetes, occurs due to idiopathic destruction of insulin producing beta-cells in the islets of Langerhans in the pancreases leading to decreased endogenous insulin secretion which is vital for the control of blood sugar and other metabolic functions. The majority of this is of immune-
mediated nature, in which a T-cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin.

2. Non-Insulin-dependent diabetes mellitus (NIDDM)

It is also termed ‘maturity-onset-diabetes’ or Type II diabetes. This tends to occur as a result of obesity and not much exercises, although thin persons may also develop NIDDM. Some people are more genetically at risk than others. Type II DM is much more common than Type I and accounts for about 90% of patients with diabetes. Type II diabetes is a chronic and multifactorial disease characterized by hyperglycemia, a result of impaired insulin secretion from pancreatic beta-cells and insulin resistance in the liver and peripheral tissues.

**Complications of diabetes**

Diabetes is associated with a number of complications.

The complications of diabetes fall into two major categories:

1. **Acute complications:** Acute complications associated with mortality include diabetic ketoacidosis, nonketonic hyperglycemic coma and hypoglycemic reactions. These complications can be readily attributed to alteration in the metabolism and in the level of blood glucose.

2. **Chronic complications:** The major chronic complications include nephropathy, retinopathy, cataract, neuropathy and cardiovascular diseases - due to uncontrolled elevated blood glucose levels account for major increase in morbidity and mortality in diabetic patients.

**Diabetic nephropathy:**

Diabetic nephropathy (DN) is a devastating chronic micro vascular complication of diabetes mellitus, which represents a major cause of end stage renal diseases (ESRD) today. About 30-40% of the diabetic patients suffer from kidney problems after onset of diabetes for 20-25 years. Diabetic nephropathy, a condition characterised by the development of proteinuria with subsequent decline in glomerular filtration rate (GFR) and an increase in arterial blood pressure, which progresses over a period of time. Importantly, kidney disease is also a major risk factor for the development of macro
vascular complications such as heart attack and stroke. Untreated diabetic nephropathy increases the risk of early death due to cardiovascular diseases by 40-fold. The pathological factors influencing the development of diabetic nephropathy is multiple and complex. Hyperglycemia-induced metabolic and hemodynamic stimuli are suggested to be mediators of renal injury, in which reactive oxygen species (ROS) seem to be the common denominator in various pathways and are central to the pathogenesis of hyperglycemic injury. There are several hyperglycemia mediated metabolic mechanisms that lead to DN through oxidative stress, such as polyol pathway, advanced glycosylation end products (AGEs), activation of protein kinase C (PKC), hexosamine pathway, xanthine oxidase activity, mitochondrial respiratory chain deficiency, NAD(P)H oxidase and nitric oxide synthase (NOS) [Brahmachari, 2015]. In addition, hyperglycemia mediated hemodynamic stimuli involving increased systemic and intraglomerular pressure and activation of various vasoactive hormones, which includes the intrarenal renin-angiotensin system (RAS), nitric oxide, vascular endothelial growth factor (VEGF), endothelin, urotensin II, and the kallikrein-kinin system (KKS) [Satirapoj & Adler, 2014; Soetikno, et al., 2014]. These activate inflammatory, pro-oxidant, ischemic, and fibrotic pathways leading to mesangial matrix accumulation; podocyte effacement and loss; glomerular basement membrane (GBM) thickening; endothelial dysfunction; tubular atrophy, fibrosis, and dropout; tubulointerstitial inflammation, and renal arteriolar hyalinosis.

Severity of diabetic nephropathy depends on the duration and level of hyperglycemia. Hyperglycemia condition induces specific cellular effects on resident kidney cells including endothelial cells, smooth muscle cells, mesangial cells, podocytes, cells of the tubular and collecting duct system, and inflammatory cells and myofibroblasts [Forbes & Cooper, 2013]. Glucose transportation in diabetic kidney is upregulated and appears to be part of the response to hyperglycemia [Henry, et al., 1999]. The process of renal glucose reabsorption takes place in the epithelial cells of the proximal tubule, involving Na+-glucose transporters (SGLTs) and facilitative diffusion transporters (GLUTs). Glucose is then transported down its concentration gradient by the facilitative glucose transporter isoforms such as GLUT1 and GLUT2. In diabetic hyperglycemia, higher glucose flux results in the up-regulation of glucose transporter isoforms GLUT1 (mesangial cells) and GLUT2 (S1 tubular cells) and fructose-specific transporter GLUT5 responding to intracellular glucose concentration is involved in diabetic nephropathy [da Silva et al.,
Urinary albumin and transforming growth factor-β1 (TGF-β1) are increased simultaneously with high GLUT1 and GLUT2 protein concentration in the renal cortex of streptozotocin treated diabetic rats and all these changes are magnified by the association with arterial hypertension [Schaan et al., 2005].

**Fig.1.** Interaction between hemodynamic and metabolic mechanisms in the pathophysiology of diabetic nephropathy

The renin-angiotensin system (RAS) plays an integral role in the homeostatic control of arterial pressure, tissue perfusion, and extracellular volume. RAS also influences renal tissue cell infiltration and inflammation in addition to its systemic and local renal hemodynamic effects. Thus, the dysregulation of RAS may lead to hypertension and renal tissue injury. Growing evidence suggested that renin and its receptor play a pivotal role in the development and progression of diabetic nephropathy by stimulating TNF-α and interleukin-1β. In addition, renin itself regulates the expression of TGF-β1 in mesangial cells through a receptor mediated mechanism, which in turn, stimulates plasminogen activator inhibitor-1 (PAI-1), Fibronectin, vascular endothelial growth factor (VEGF), collagen I and IV, confirming its detrimental role in diabetic nephropathy [Huang et al., 2006; Zhang et al., 2008]. Angiotensin II (Ang II) is the most powerful
biologically active product of the RAAS. Over-activation of intrarenal Ang II leads to the development of hypertension and renal injury and results in reduced renal function and structural changes in the kidney [Navar et al., 2003; Navar, 2005]. In addition, Ang II directly induces podocyte injury via the activation of Ang II receptor type 1 (AT1) receptors, independent of hemodynamic changes [Durvasula et al., 2004; Liang et al., 2006; Liebau et al., 2006].

The polyol pathway consists of two enzymes, aldose reductase (AR), the first and rate limiting enzyme, reduces glucose to sorbitol with the aid of its co-factor NADPH, and the second enzyme, sorbitol dehydrogenase (SDH), with its co-factor NAD\(^+\), converts sorbitol to fructose, a process that increases the ratio of NADH/NAD and may result in both oxidative stress and activation of protein kinase C [Sharma & Sharma, 2013]. Consumption of NADPH by AR results in the depletion of the levels of NADPH. NADPH also acts as a cofactor for glutathione reductase, which reduces oxidized glutathione (GSSG) into reduced glutathione (GSH), which is required for glutathione peroxidase (GPx). This ultimately results in decreased antioxidant status of cell. Fructose and its metabolites—fructose-3-phosphate and 3-deoxyglucosone are more potent non-enzymatic glycation agents than glucose. Since sorbitol does not cross cell membranes, its intracellular accumulation results in osmotic stress in renal mesangial and proximal tubular cells; its accumulation has been proposed to interfere with the uptake and metabolism of myo-inositol and altered Na\(^+\)/K\(^+\)-ATPase in the renal tissue [Cohen & Klepser, 1988].

During prolonged high glucose, reducing sugars react with amino group of proteins non-enzymatically leading to a class of irreversibly cross-linked moieties termed Advance Glycation End Products (AGEs) [Grandhee & Monnier, 1991]. The flux of glucose through the polyol pathway would also increase AGEs formation. AGEs, as well as binding of AGE to their receptors (RAGE), elicit oxidative stress and evoke vascular inflammation and thrombogenesis, thus participating in diabetic nephropathy. AGE receptors are present on various renal cell types including proximal tubular cells, mesangial cells, and podocytes. The AGEs-RAGE-mediated ROS generation stimulates
Fig. 2. Polyol pathway

production of prosclerotic growth factors such as TGF-β1 and connective tissue growth factor (CTGF) via Mitogen-activated protein kinase (MAPK), Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and/or PKC pathways in both mesangial and renal tubulointerstitial cells [Rohilla, et al., 2011]. Several lines of evidence indicated that TGF-β1 induction is also mediated by the hexosamine pathway [Schleicher & Weigert, 2000]. These changes may mainly contribute to glomerular hypertrophy, basement membrane thickening, and mesangial extracellular matrix expansion, which is associated with significant proteinuria and albuminuria and glomerulosclerosis.

Nitric oxide (NO) is a key gaseous regulatory molecule secreted by the endothelium and a major modulator of endothelial function with extensive metabolic, vascular, and cellular effects. A low level of NO is beneficial for several physiological and cellular functions, while higher levels may cause detrimental effects. Increased NO levels in cells may react with superoxide anion to generate peroxynitrite radical, which binds to proteins and thus affects their function [Adela et al., 2015].

Hyperglycemia-mediated oxidative stress has been shown to be related to the increased peroxidation and low antioxidant status that provokes the oxidative attack on cellular macromolecules such as lipids, proteins and DNA. Among the oxidative damages, base modifications such as oxidation of deoxy guanosine to 8-hydroxy-2′-deoxyguanosine (8-OHdG) and subsequent mutations of mitochondrial DNA (mtDNA), have received increasing attention in recent years. It is widely accepted that mtDNA is 10–20 times more vulnerable to oxidative damage and subsequent mutations than nuclear DNA.
DNA. Oxidative stress has also been implicated in the development of several glomerular or tubulointerstitial diseases, because ROS exert numerous toxic effects on the kidney.

**Diabetic retinopathy**

Retinopathy is one of the common micro vascular and potential blinding complications of diabetes mellitus. Among approx. 415 million people living with diabetes worldwide, it is expected that one third of them will be affected by diabetic retinopathy during their lifetime. Today, more than 93 million people suffer from diabetic eye diseases, with diabetic retinopathy being the leading cause of blindness among the working age population [Nentwich & Ulbig, 2015]. Retinopathy can be broadly categorised into three different stages: back-ground diabetic retinopathy, pre-proliferative retinopathy and proliferative retinopathy (characterised by growth of new blood vessels). In the first stage of retinopathy, hyperglycemia initiates thickening of capillaries basement membrane and causes death of pericytes that supports the vessel wall. Following this, micro aneurysms and vascular leakage take place leading to the blockage of retinal capillaries and induction of local hypoxia. Subsequently, endothelial cells die resulting in closure of capillaries and increased area of non-perfusion. Pre-proliferative diabetic nephropathy is identifiable by the areas of increased retinal hypoxia and multiple haemorrhages because of the loss of vascular patency. Increased areas of non-perfusion stimulates the generation of angiogenic factors leading to the formation of new blood vessels, a characteristic feature of proliferative diabetic retinopathy. Subsequently, retinal detachment may take place, causing vision loss or blindness [Majumdar & Srirangam, 2010]. Hyperglycemia and hypoxia are the two major factors involved in the development and progression of diabetic retinopathy. Among the several pathogenic mechanisms that may contribute to diabetic retinopathy, namely, increased polyol pathway, activation of protein kinase C (PKC), increased expression of growth factors such as vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1), hemodynamic changes, accelerated formation of advanced glycation end products (AGEs), oxidative stress, activation of the renin-angiotensin-aldosterone system (RAAS), prostaglandins, cyclooxygenase-2 (COX-2) and nitric oxide (NO) are indicated in the process, all of which contribute to vascular permeability and angiogenesis.
Diabetic cataract

Cataract is considered as one of the most common and secondary complication of diabetes which causes visual impairment. Cataract termed cloudiness or opacification of eye’s natural lens is the leading cause of blindness worldwide, affecting approximately 18 million people. Cataracts occur at an early age and 2–5 times more frequently in diabetic patients. Eye is more susceptible to hyperglycemia induced toxicity. Eye lens contains structural proteins called crystallins which accounts for 90% of total lens protein (33%) content, made up of α-crystallin, β-crystallin, and γ-crystallins. The transparency and refractive index property of the lens is mainly due to the high content of these crystallines and are long lived and specific to the lens. The lens fibre arranged in order, the sparseness of the cellular organelles, the peripheral location of the fibre nuclei away from optic axis, the small extracellular space [Yorio & Bentley, 1976] and the narrowing of the fibre membranes also impacts the transparency of the lens. The loss of transparency or opacification and refractive index of lens in diabetes may be due to the swelling and breakdown of fibres, expansion of extracellular space or aggregation of proteins, mainly crystallins.

Multiple mechanisms have been proposed with respect to the development of cataractous lens in diabetic condition through oxidative stress namely, polyol pathway and non-enzymatic glycation reactions. Hyperglycemia induced free radicals take a major role in the development of cataract. Healthy lens is rich in antioxidants such as ascorbic acid, Vitamin-E, reduced glutathione (GSH) and antioxidant enzymes- superoxide reductase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase. ROS produced during normal oxidative metabolism are eliminated by these efficient scavenging systems, but an imbalance between production and scavenging of ROS can result in excessive levels of either molecular oxygen or ROS, thus resulting in increased oxidative stress.

The polyol pathway has been described as the primary mediator of diabetes-induced oxidative stress in the lens. In hyperglycemia condition, polyol pathway of glucose metabolism becomes active, aldose reductase (AR), the first and rate-limiting enzyme in the pathway, reduces glucose to sorbitol using NADPH as a cofactor; sorbitol is then metabolized to fructose by sorbitol dehydrogenase that uses NAD⁺ as a cofactor. This process, increases the ratio of NADH/NAD and depletion of NADPH that may also act as
a cofactor for glutathione reductase and nitric oxide synthase. This results in a depletion in cofactors led imbalance in cellular defence mechanism causing oxidative stress and activation of protein kinase C [Sharma & Sharma, 2013]. In the eye lens, sorbitol is produced faster than it is converted to fructose by the enzyme sorbitol dehydrogenase. The increased accumulation of sorbitol in the lens creates a hyperosmotic effect that results in an infusion of fluid to counter the osmotic gradient. These osmotic changes further result in degeneration of hydropic lens fibers, collapse and liquefaction of lens fibers, ultimately resulting in the formation of lens opacities and sugar cataracts. Furthermore, studies have shown that osmotic stress in the lens caused by sorbitol accumulation induces apoptosis in lens epithelial cells (LEC) leading to the development of cataract [Pollreisz & Schmidt-Erfurth, 2010].

Fructose produced by the polyol pathway can become phosphorylated to fructose-3-phosphate, which is broken down to 3-deoxyglucosone; both compounds are powerful glycosylating agents that enter in the formation of advanced glycation end products (AGEs) [Pollreisz & Schmidt-Erfurth, 2010]. Since it is believed that fructose is more reactive than glucose, because it has an ability to adopt to the open chain form, it can react with the free amino groups of various proteins (crystallins) leading to the non-enzymatic glycation via the formation of Schiff bases and further rearrangements and conformational changes by the Maillard reactions, leading to the formation of AGEs.

**Diabetic neuropathy**

Neuropathy is a common complication of diabetes and is associated with wide range of clinical manifestations. It is widely accepted that the toxic effects of hyperglycemia play an important role in the development of this complication. Over 90% of patients with >25 year of long lasting diabetes may develop neuropathy. Although pain is one of the main symptoms of diabetic neuropathy, its pathophysiological mechanisms are not yet fully known. Diabetic neuropathic pain (DNP) is characterized by tingling, burning, sharp, shooting, and lancinating or even as electric shock sensations. It is usually considered moderate to severe and often worse at night, causing sleeping disturbs [Schreiber et al., 2015]. Diabetic neuropathy is characterized as clinical or subclinical based on sign and symptoms as distal symmetric polyneuropathy, focal or multifocal neuropathy and autonomic neuropathy depending on its distribution [Becker, 2001]. Neuropathy affects all peripheral nerves including pain fibre, motor neurons and the
autonomic nervous system. The distal symmetrical polyneuropathy (DSPN) is the commonest clinical form of diabetic neuropathy, affecting more than 90% of the patients, generally affecting the toes and distal foot, and slowly progresses to feet and legs over a period of time. Occasionally patients with diabetes can develop focal or multifocal neuropathies that include cranial nerve involvement and limb and truncal neuropathies. Therefore diabetic neuropathy affects all organ system that may be associated with other autonomic dysfunction also. Hyperglycemia is a key factor underlying diabetic neuropathy, but other changes may also involve including genetic predisposition, osmolyte accumulation, oxidative stress, ischemia neurotropic factor deficiency and immunologic molecular interplay.

Prolonged hyperglycemia can lead to cellular damage in several ways, first high glucose flux and excess glycolysis can lead to overload of the mitochondrial electron transport chain and generation of ROS. Second, high glucose led polyol pathway can increase cellular osmolarity; reduce NADPH levels, and leads to oxidative stress. Finally, increased flux through the hexosamine pathway is associated with inflammatory injury. Another consequence of hyperglycaemia is the generation of advanced glycation end products (AGEs). These extracellular AGEs also bind to the receptor for AGE (RAGE), initiating inflammatory signalling cascades, activating NADPH oxidases, generating oxidative stress and Long-term inflammatory responses including the upregulation of RAGE and activation of NF-κB [Callaghan et al., 2012].

**Diabetes and Cardiovascular diseases**

Diabetes is a prime risk factor for cardiovascular diseases (CVD). Patient with diabetes have a 2-4 fold higher risk than do non-diabetic individual of developing atherosclerosis and its complications, which includes stroke, myocardial infarction (MI) and peripheral vascular disease. The relative risk of the incidence of stroke or MI was increased 2-3 fold in those with type-2-DM and risk of death increased by 2-fold independent of other risk factors [Unachukwu & Ofori, 2012]. Diabetic condition also leads to heart failure, which may arise from myocardial damage resulting from an ischemic, thrombotic event. In this case, endothelial dysfunction, oxidation and glycation of atherogenic lipids, and the hypercoagulability of the blood are major contributors to the patient’s heart failure [Dokken, 2008]. Several conditions have been proposed to explain the acceleration of vascular alterations in diabetes, including hyperglycemia,
accelerated formation of AGEs, increased oxidative stress, hypertriglyceridemia, a high low-density lipoprotein/high-density lipoprotein (LDL/HDL) ratio, hypoinsulinemia and genetic variables.

Atherosclerosis is a complex process involving numerous cell types and important cell-to-cell interactions that ultimately lead to progression from the “fatty streak” to formation of more complex atherosclerotic plaques. These complex atherosclerotic plaques may then destabilize and rupture, resulting in myocardial infarction, unstable angina, or strokes [Forbes & Cooper, 2013]. There are a number of mechanisms by which hyperglycemia can contribute to the development and progression of diabetic heart failure. The extent and frequency of diastolic dysfunction is directly proportional to HbA1c level and in diabetic cardiomyopathy is thought to be the result of myocellular hypertrophy and myocardial fibrosis. The increase in oxidative stress in diabetic hearts has been found to decrease NO levels, worsen endothelial function, and stimulate the inflammatory mediators to induce myocardial injury by a variety of mechanisms including enhanced vascular permeability, apoptosis, recruitment of invasive leukocytes and promotion of ROS production [Dokken, 2008]. Further, numerous studies have demonstrated increased generation of free radicals, activated leukocytes and hypercoagulability states play a crucial role in the pathogenesis of cardiovascular complications in diabetes.

The relationship between diabetes and hyperlipidemia is a well-recognised phenomenon. Hypercholesterolemia is a feature frequently observed in diabetes, contributing to the high prevalence of accelerated coronary heart diseases. Hypercholesterolemia is an independent risk factor for diabetic nephropathy. Diabetic patients with low blood cholesterol concentration are reported to exhibit better renal function than those having high blood cholesterol. Dyslipidemia condition is highly correlated with pathogenesis of atherosclerosis and up to 97% of patients with diabetes are dyslipidemic, being a cardiovascular risk factor characterised by increased triglycerides and decreased HDL-cholesterol found in the plasma of diabetes. In diabetes, the predominant form of lipid is LDL, which is more atherogenic because they are small, dense, more easily penetrable and form stronger attachments to the arterial wall, and they are more susceptible to oxidation. Oxidized LDL is pro-atherogenic; because once they are oxidized, they acquire new properties that are recognized by the immune system as
“foreign” particle. Thus, oxidized LDL produces several abnormal biological responses such as attracting leukocytes to the intima of the vessel, improving the ability of the leukocytes to ingested lipids and differentiate into foam cells and stimulating the proliferation of leukocytes, endothelial cells and smooth muscle cells, all of which are steps in the formation of atherosclerotic plaque. In diabetic condition, LDL particles are also glycated similar to a process of glycation of protein haemoglobin; this lengthens the half-life of LDL particles and therefore increases the ability of LDL in promoting atherogenesis [John et al., 2016].

**Dietary management of diabetes**

In the management of any disease condition, dietary factors play a very important role. The role of food constituents in the etiology of chronic diseases such as diabetes and in their prevention/management has been increasingly understood in recent years. Before the introduction of therapeutic use of insulin, diet was the main form of treatment of the disease, and dietary measures included the use of traditional medicines mainly derived from plant sources. These traditional medicines are invariably from plant sources that do not form the constituents of our normal diet. However, several common constituents of the diet are also traditionally recommended for regular consumption, and some are additionally taken as infusions and decoctions. Among these are herbs, spices, and vegetables.

**Spices as useful antidiabetic adjuncts**

Spices are consumed as food adjuncts to impart flavour and colour to our food (Srinivasan, 2005). Several health beneficial physiological effects of commonly consumed spices have been experimentally or clinically documented in recent decades. A few commonly consumed spices are now understood to possess significant hypoglycemic and hypolipidemic influence in different experimental situations in both animals and humans. Spices such as fenugreek (*Trigonella foenum-graecum*) seeds, garlic (*Allium sativum*), onion (*Allium cepa*), and black cumin (*Nigella sativum*) are recognized to possess hypoglycemic influence in diabetic situation. Limited animal studies have also documented beneficial antidiabetic influences of curcumin of turmeric (*Curcuma longa*), cumin seeds (*Cuminum cymminum*) and curry leaves (*Murraya koenigii*).
Fig. 3. Possible mechanism of antidiabetic action of spices
The high content of mucilage galactomannan in fenugreek is responsible for a lower dietary glucose uptake and hence believed to facilitate glycemic control in diabetics. The two Allium spices – onion and garlic largely owe their antidiabetic property to sulfur compounds allyl propyl disulfide and diallyl sulfide, which have insulinotropic activity.

Additionally, these sulfur compounds have insulin sparing action by protecting from sulhydryl inactivation [Srinivasan 2005]. The hypoglycemic effect of black cumin might be due to its volatile oil and thymoquinone. Mechanism of hypoglycemic effect of black cumin is multifactorial including, elevation of insulin, attenuation of insulin resistance, hepatic gluconeogenesis and oxidative stress in diabetic animals [Bamosa 2015]. Curry leaves shows protective effects in experimental diabetes, possibly by decreasing oxidative stress and preservation pancreatic β-cell integrity [Vijayanand S. 2015]. Curcumin yellow principle of turmeric - have been found to be effective in improving the glycemic status and glucose tolerance in diabetic animals/type-2 diabetic patients. Several common spices have been evaluated for cholesterol lowering effect in various experimental conditions both in animals and humans. Dietary fenugreek, garlic, onion curcumin and red pepper are found to be effective as hypocholesteromic under conditions of diabetes induced hyperlipidemia.

**Fenugreek (Trigonella foenum-graecum L.) seeds**

Fenugreek (Trigonella foenum-graecum) is an annual leguminous herb belonging to the family Fabaceae cultivated in India and North African countries. It is variously called in different languages, viz., Fenugrec (French), Methi (Hindi), Bockshorklee (German), Fieno greco (Italian), Pazhitnik (Russian), Alholva (Spanish), Koroha (Japanese), Hulba (Arabian), Halba (Malaya), and K’u-Tou (China). The seeds are used as spices in many countries, whereas the leaves are used as herb (dried leaves) and vegetables (fresh leaves) in the diet. Fenugreek seeds have been in use for over 2500 years. The seeds are frequently employed as a spice for seasoning, a flavoring agent in the cuisines of the Indian subcontinent, used both in whole and powdered form in the preparation of pickles, vegetable dishes, pancakes and spice mixes. India is the major producer of fenugreek and its main consumer for culinary and medicinal uses. During 2011–12, estimated production was 1,16,000 tonnes of seeds from an area of 93,605 hectares [Vidyashankar 2014]. Largest fenugreek production state in India is Rajasthan and other growing states are Tamil Nadu, Gujarat, Madhya Pradesh, Punjab and Uttar Pradesh.
Fenugreek seeds are aromatic, bitter, carminative, galactogogue, antibacterial and may be eaten raw or cooked. The bulk of the seed (50%) constitutes unavailable carbohydrates consisting of gel-forming soluble fibre (30%), mostly galactomannan and an insoluble fibre (20%). The RDA for dietary fibre is 20–30 g per day. Dietary fibre are indigestible complex carbohydrates found in plant foods, and are an essential ingredient in a healthy diet. Fibre in the diet improves health by replacing calories, increasing chewing time, suppressing appetite, controlling overeating, and arresting weight-gain. Dietary fibre also induces satiety (feeling of fullness), delays gastric emptying and increases mouth-to-cecum food transit time [Srinivasan, 2006]. Fenugreek also contains approximately 4 to 8% saponins and about 1% alkaloids, contributing to bitterness, gastric stimulation, increased acidity, and increased appetite. Steroidal saponin diosgenin present in seeds is used in manufacturing of many pharmaceuticals, such as progesterone. Trigonelline, an alkaloid, is thought to reduce glycosuria in diabetes [Ambasta, 2000].

It has been a well-recognized fact that dietary fibre offers substantial benefits to persons with diabetes mellitus. Epidemiological studies have shown that prevalence of diabetes is lower in a population with high fibre intake than in a Western population with low fibre intake. In regions such as Japan, India and the West Indies, where intake of dietary fibre is high, the specific complications of diabetes are less frequent than in Western countries where the fibre intake is low. Cardiac complications and diabetic gangrene among diabetics are less common in India and Japan than in the USA. Fenugreek seeds commonly used in India and other countries as a condiment, are an excellent source of dietary fibre and hence advantageous in the context of diabetes [Srinivasan, 2006].

The probable hypoglycaemic action of fenugreek seeds might be due to delaying gastric emptying by direct interference with glucose absorption. In addition, gel-forming dietary fibre reduces the release of insulinotropic hormones and gastric inhibitory polypeptides. Lipid lowering effect might be attributed to dietary fibre which enhances the conversion of hepatic cholesterol to bile acids or increase the catabolism of LDL via the apo-B, -E receptor (Srinivasan, 2006).
Table-1. Composition of mature fenugreek seeds

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Component</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Protein</td>
<td>30 g</td>
<td>S</td>
<td>16 mg</td>
</tr>
<tr>
<td>Fat</td>
<td>7.5 g</td>
<td>Cl</td>
<td>165 mg</td>
</tr>
<tr>
<td>Fiber</td>
<td>50 g</td>
<td>Mn</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Sapogenins</td>
<td>2 g</td>
<td>Zn</td>
<td>7.0 mg</td>
</tr>
<tr>
<td>Trigonelline</td>
<td>380 mg</td>
<td>Cr</td>
<td>0.1 mg</td>
</tr>
<tr>
<td>Ca</td>
<td>160 mg</td>
<td>Choline</td>
<td>50 mg</td>
</tr>
<tr>
<td>Mg</td>
<td>160 mg</td>
<td>Vitamin C</td>
<td>43 mg</td>
</tr>
<tr>
<td>P</td>
<td>370 mg</td>
<td>β-Carotene</td>
<td>96 μg</td>
</tr>
<tr>
<td>Fe</td>
<td>14 mg</td>
<td>Thiamine</td>
<td>340 μg</td>
</tr>
<tr>
<td>Na</td>
<td>19 mg</td>
<td>Riboflavin</td>
<td>290 μg</td>
</tr>
<tr>
<td>K</td>
<td>530 mg</td>
<td>Nicotinic acid</td>
<td>1.1 mg</td>
</tr>
<tr>
<td>Cu</td>
<td>33 mg</td>
<td>Folic acid</td>
<td>84 μg</td>
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Fig.4. Fenugreek seeds

Additionally, 4-hydroxy isoleucine present in fenugreek seeds increases the glucose induced insulin secretion in pancreatic beta cells, and also inhibits the activities of α-amylase and sucrase [Sauvaire et al., 1998]. Trigonelline of fenugreek seeds acts by affecting β-cell regeneration, insulin secretion, and activities of enzymes related to glucose metabolism [Yoshinari & Igarashi, 2010].
**Onion (Allium cepa L.)**

Onion (Allium cepa) is a bulbous vegetable that belongs to the family Liliaceae. Onions are believed to have originated from Afghanistan/ Iran/ USSR region and are now grown in almost every country of the world. The onion plant has been grown and selectively bred in cultivation for at least 7,000 years. It is a biennial plant, but is usually grown as an annual, which may be red, white, or yellow in colour, and consumed in its tender state, raw, ripe. India is the second largest onion growing country in the world, after China. The Major Onion producing states in India are Maharashtra, Karnataka, Madhya Pradesh, Gujarat, Bihar, Andhra Pradesh, Rajasthan, Haryana and Tamil Nadu. World onion production has been increased by at least 25%, in the past 10 years, to the current output of about 44 million tons, making it the second most important horticultural crop after tomatoes [FAO Statistics, 2011]. Onions are versatile; can be baked, boiled, braised, grilled, fried, roasted, sautéed, or eaten raw in salads, used as a main ingredient in their own right. Onions are a staple in Indian cuisine, used as a thickening agent for curries and gravies.

![Fig.5. Onion bulb](image)

Onion has approximately 90% water with high content of dietary fibre, sugar and various chemicals of distinctive biological activity. Onions are richest source of dietary flavonoids including anthocyanins, which impart a red to purple color to some varieties and the flavonols such as quercetin and its derivatives which are responsible for the yellow and brown skins of different varieties [Griffiths et al., 2002]. Quercetin 3,4-
diglucoside and quercetin 4-glucoside are in most cases reported as the main onion flavonols in literature [Caridi et al., 2007]. Onions also contain high concentrations of sulfur compounds such as dimethyl trisulfide, propenyl propyl disulfide, dipropyl disulfide, propenyl methyl disulfide and methyl propyl trisulfide dipropyle trisulfide [Mathur et al., 2011]. Therefore, the hypoglycemic property of onion may be due to the presence of sulfur compounds as well as flavonoids such as quercetin. The blood glucose lowering effect is probably due to the presence of bioactive compounds that may enhance glucose uptake in peripheral tissues by improving insulin sensitivity and / or secretion and by increasing the level of NADP$^+$/NADPH ratio. It is also suggested that these disulfide compounds have the effect of sparing insulin from -SH inactivation by reacting with endogenous thiol-containing molecules such as cysteine, glutathione, and serum albumins. As quercetin, a major flavonoid found in onion, has a strong antioxidant activity, it promotes normoglycemia and decreases oxidative stress. The hypolipidemic property of onion might be due to the presence of S-methyl cysteine sulfoxide (SMCS), which contains a cysteine moiety, and has been reported to raise the level of hepatic cholesterol 7α-hydroxylase activity, a key enzyme in the synthesis of bile acids from cholesterol. The increase in the faecal bile acids and sterols excretion from the body might be one of the mechanisms for lowering cholesterol [Haleagrahara et al., 2009].
Scope of present intervention

The role of food constituents in the etiology of chronic diseases and in their prevention/management has been increasingly understood in recent years. A few commonly consumed spices are now understood to possess significant hypoglycemic and hypolipidemic influence in both animals and humans. Spices such as fenugreek (*Trigonella foenum-graecum*), garlic (*Allium sativum*), onion (*Allium cepa*), and black cumin (*Nigella sativa*) are recognized to possess hypoglycemic influence. Limited animal studies have also documented beneficial antidiabetic influences of curcumin of turmeric (*Curcuma longa*), cumin seeds (*Cuminum cymminum*) and curry leaves (*Murraya koenigii*). The hypoglycemic potential of fenugreek seeds has been documented even in human subjects. The high content of mucilage galactomannan in fenugreek is responsible for a lower dietary glucose uptake and hence believed to facilitate glycemic control in diabetics. It is reported that dietary fenugreek powder (5%) significantly improved glucose homeostasis and renal function in alloxan diabetic rats by delaying carbohydrate absorption, attenuating inflammation and improving kidney antioxidant status.

The two Allium spices – onion and garlic largely owe their antidiabetic property to sulfur compounds allyl propyl disulfide and diallyl sulfide, which have insulinotropic activity. Additionally, these sulfur compounds have insulin sparing action. About 65 to 80% of onion’s dry matter consists of non-structural carbohydrates such as low molecular-weight fructans. These fructooligosaccharides (FOS) of varying molecular size constitute the main carbohydrate reserve of onion. Two intervention studies done on healthy humans have indicated no clear effects of fructooligosaccharides on blood glucose and serum lipids. A study has showed that chronic consumption of 20 g fructooligosaccharides per day increases basal hepatic glucose production without any effect on insulin-stimulated glucose metabolism or serum lipids.

Dietary fenugreek, garlic, onion and curcumin are also documented to have health beneficial hypocholesterolemic and hypotriglyceridemic influences. The relationship between diabetes and hyperlipidemia is a well-recognised phenomenon. Hypercholesterolemia is a feature frequently observed in diabetes, contributing to the high prevalence of accelerated coronary heart diseases. Hypercholesterolemia is an independent risk factor for diabetic nephropathy. Diabetic patients with low blood
cholesterol concentration are reported to exhibit better renal function than those having high blood cholesterol.

It has been demonstrated that dietary fenugreek and onion are independently effective in the management of diabetes. The fibre-rich fenugreek seeds and Allium spice - onion are now understood to possess significant hypoglycemic as well as hypolipidemic action, and may also possess beneficial antioxidant potential in diabetic situation. The hypolipidemic and antioxidant potential of spices are likely to have far-reaching implication in alleviating secondary complications associated with diabetes. This needs to be verified with appropriate investigations. The mechanism of action of these two is dissimilar with respect to beneficial modulation of glucose homeostasis. Hence the combined use of these two food ingredients may have an additive health beneficial influence.

Hence, it was proposed to make exhaustive studies to explore synergy between dietary fenugreek seeds (as a provider of soluble fibre) and onion in deriving health benefits to the maximum with respect to: (a) hyperglycemia and attendant abnormalities in experimental diabetes, (b) beneficial modulation of compromised antioxidant status, and (c) renal lesions, cataract and cardiac disease. The basic information generated in this investigation is likely to lead to an effective dietary strategy in the management of the complications of diabetes. In this context, the following objectives were envisaged in the research programme:

**Objectives**

1. To evaluate the additive effect of a combination of dietary fenugreek seeds and onion on glucose homeostasis and diabetes related abnormalities in lipid metabolism in experimentally induced diabetic rats.

2. To assess the potential of dietary fenugreek seeds and onion – both independently and in combination for the beneficial modulation of secondary complications – renal disease, cataract and CVD in streptozotocin induced diabetic rats.