Introduction
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Glycation is a non-enzymatic reaction of the amino groups of amino acids, peptides, and proteins with carbonyl group of reducing sugars resulting in the formation of complex brown pigments and protein-protein crosslinks. It is occurring slowly but continuously in cells of all living organisms. It was first studied under defined conditions by Louis Camille Maillard in the early 1900s (Maillard and Gautier, 1912). Thus it came to be known as the Maillard reaction (Fig. 1). Exact realization of the importance of Maillard-like reactions in-vivo began in the mid-1970s when studies was done on haemoglobin A$_{1c}$ (HbA$_{1c}$), a naturally occurring minor human haemoglobin that is elevated in diabetics (Bookchin and Gallop, 1968). HbA$_{1c}$ was known to be a post-translational adduct of glucose with the N-terminal valine amino group of the β chain of hemoglobin, in which the glucose was thought to be attached via non-enzymatically formed Schiff base structure. We found that measurement of the elevation of HbA$_{1c}$ in diabetics allowed assessment of the degree of glucose control integrated over several weeks (Koenig et al., 1976). Later on, the significance of the complex, late stage Maillard processes was recognized as mediators of several complications in diabetes (Bunn et al., 1978) and aging (Monnier and Cerami, 1981). The Maillard reaction is actually a complex series of reactions and is sub-divided into three main stages: early, intermediate, and late.

Early Stage:
The nucleophilic addition reaction between a carbonyl group from a reducing sugar (e.g. glucose, fructose, galactose, mannose etc) and a free amino group is initiated with the reversible formation of an adduct known as Schiff base by conversion of the aldehydic carbon–oxygen double bond of the sugar to a carbon–nitrogen double bond with the amine. This reaction occurs over a period of hours. The Schiff base is a thermodynamically unstable form in relation to the equilibrium cycled pyranose or furanose forms. Therefore, the Schiff base gives rise to an enaminol intermediate by rearrangement and, subsequently, to a relatively stable ketoamine compound/Amadori compound and heyns product in case of fructose. Since this reaction does not require the participation of enzymes, the variables which regulate it in vivo are the degree and duration of hyperglycemia, the half-life of the protein, its reactivity in terms of free amino groups, and cellular permeability to glucose. In in vivo conditions, the Amadori
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product reaches equilibrium after approximately 15–20 days and, through irreversible links, accumulates on both short-lived and long-lived proteins (Lapolla et al., 2005).

**Intermediate Stage:**

In the second stage, the Amadori compound further undergoes a series of dehydration and fragmentation reactions generating a variety of carbonyl compounds with some formation of initial cross-linked protein species. Carbonyl compounds are generally more reactive than the original carbohydrate and act as propagators by reactions with free amino groups. Among the most active enhancers of the reaction are \(\alpha\)-dicarbonyls such as methylglyoxal, glyoxal, glucosones, deoxyglucosones and dehydroascorbate (Thornalley et al., 1999).

**Late Stage:**

In the late stage, these propagators again react with free amino groups and, through oxidation, dehydration and cyclization reactions, form yellow-brown, often fluorescent, insoluble, irreversible compounds, usually called Advanced Glycation End-Products (AGEs), sometimes known as “melanoidins”, which accumulate on long-lived proteins and cause damage and extensive protein cross-linking. AGEs are characterized by a wide structural and physicochemical diversity.

Reducing sugars other than glucose can participate in glycation and do so much faster than glucose, which is the least reactive of all sugars. This may explain why glucose has been selected as the major metabolic sugar during evolution (Bunn and Higgins, 1981). Glucose is the major metabolic sugar present in our body. Even in euglycemic normal individual the level of glucose in plasma is between 65 mg/dl to 100 mg/dl. Therefore even in non-diabetic euglycemic individuals’ plasma and other proteins are regularly interacting with glucose and result in formation of glycated end products such as AGEs. Wolffennuttel et al., (1996) suggested that modification of hemoglobin by advanced glycosylation end products would be a better index for long term glycemia in diabetic patients. While glycation can be detected in physiologic conditions like aging, the reactions are considerably faster and more intensive in the pathophysiologic conditions like the uncontrolled diabetes mellitus associated with persistently elevated blood glucose concentration (Thornalley, 2003). In addition to the multiple pathologies mediated by the *in-vivo* generated AGEs, various exogenous sources such as diet and smoking may also
add significantly to the damage caused by those generated in the body (Koschinsky et al., 1997). Recent data showed that, in spite of the fact that sugars are the main precursors of AGE compounds, numerous intermediary metabolites, i.e. alpha-oxoaldehydes, also creatively participate in non-enzymatic glycation reactions. Such intermediary products are generated during glycolysis (methylglyoxal) or along the polyol pathway and can also be formed by autoxidation of carbohydrates (glyoxal). As compared to glycation reactions involving molecules like nucleic acid and lipids, protein glycation has been studied extensively showing numerous structural alterations including exposure of thiols, protein compaction, cross linking, fragmentation and susceptibility to proteolysis (Seidler and Seibel, 2000). Glycation by various sugars of a limited number of amino groups in proteins like hemoglobin, albumin and low density lipoproteins induce number of alterations in proteins and loss of biological activity (Turk, 2001). These include conformational alterations, exposure of hydrophobic residues and thiols, loss in allostERIC sensitivity (Bunn and Briehl, 1970), ligand binding (McDonald et al., 1979) and receptor recognition. In diabetes mellitus, protein glycation and glucose auto-oxidation may generate free radicals, which in turn catalyzes lipid peroxidation (Baynes, 1991).

**Fig. 1:** Glycation of a protein by glucose and subsequent formation of AGEs (Ahmed, 2005).
ADVANCED GLYCATION END PRODUCTS (AGEs):
AGEs are complex, heterogenous molecules that cause protein cross-linking, exhibit browning and generate fluorescence. The formation of AGEs in-vitro and in-vivo is non-enzymatic and dependent on the turnover rate of the chemically modified target, time and sugar concentration. Persistent hyperglycemia induces abnormal changes such as increase of advanced glycation end products (AGEs) formation, increase of polyol pathway flux, and activation of protein kinase C isoforms (Brownlee, 2001; Evans et al., 2002). Protein modification with AGE is irreversible, as there are no enzymes in the body that would be able to hydrolyze AGE compounds. These structures then accumulate during the lifespan of the protein on which they have been formed. For example, lens crystalline proteins (Stevens et al., 1978), insulin (Dolhofer and Wieland, 1979), proteins of erythrocyte membrane (Miller et al., 1980), bovine serum albumin (Arakawa and Timasheff, 1982), human serum albumin (Shaklai et al., 1984), enzymes (Coradello et al., 1982), high and low density lipoproteins (Kirstein et al., 1990), peripheral nerve myelin (Greene, 1983), elastin (Baydanoff et al., 1987) and immunoglobulin G (Newkirk et al., 2003).

CLASSIFICATION OF AGES:
AGEs are usually grouped into fluorescent, non-fluorescent compounds and cross linking AGEs. Some authors also grouped them into toxic and non-toxic AGEs. Toxic AGEs seem to derive from glycolaldehyde or glyceraldehyde and their structures remain to be elucidated (Sato et al., 2006). Many AGEs fluoresce under UV light and are capable of intra and inter-molecular cross-linking, but not all share these properties (Wautier and Schmidt, 2004). On the basis of these two properties AGEs can be classified into three categories (Fig. 2).

1. **Fluorescent AGE crosslinks:**— Protein-protein crosslinks by these structures in-vivo are thought to be responsible for a major share of the deleterious effects of AGEs in diabetes and aging. Along with brown colour, fluorescence is one of the qualitative properties classically used to estimate these AGEs. For example, pentosidine, crossline, pentodilysine, vesperlysine A, B and C. Pentosidine was first isolated and identified from dura mater collagen and has since been identified in many tissues (Sell et al., 1991).
2. Non-fluorescent AGE crosslinks:- Although their ease of detection makes them useful markers of AGE formation, the fluorescent AGE cross-links are thought to account for only one percent or less of the total cross-linking structures formed under physiological conditions (Dyer et al., 1991). Thus, the major AGES responsible for protein-protein cross-linking in-vivo are non-fluorescent structures that have not yet been conclusively identified. The structure of three common examples of this class are imidazolium dilysines, alkyl formyl glycosyl pyrrole and arginine-lysine imidazole.

3. Non-crosslinking AGES:- Besides the cross-linking AGES, a number of non-crosslinking AGES have been reported under physiological conditions. They may have deleterious effects as precursors of cross-links or as biological receptor ligands causing a variety of adverse cellular and tissue changes. Pyrraline, carboxymethyllysine and imidazolones are examples of non-crosslinking AGES.

AUTOXIDATIVE GLYCATION AND GLYCOXIDATION:
Oxidation processes are important in the formation of many AGES (Lapolla et al., 2005). Two routes have been proposed for AGES formation. The first involves auto-oxidation of free sugar. Monosaccharides, like glucose, exist in equilibrium with their enediol, which can undergo autoxidation in the presence of transition metals to form an enediol radical (Wolff and Dean, 1987). This radical reduces molecular oxygen to generate the superoxide radical (O$_2^-$) and becomes oxidised itself to a dicarbonyl ketoaldehyde that reacts with protein amino groups forming a ketoimine. This is referred to as antioxidative glycation and is outlined in Fig. 3(a). Ketoimines are similar to, although more reactive, than Amadori products and participate in AGE formation.
Fig. 2: Chemical structure of (a) Fluorescent cross-linking AGEs, (b) Non-fluorescent cross-linking AGEs, (c) Non-cross-linking AGEs (Ahmed, 2005).
The second mechanism involves autoxidation of Amadori products to AGEs as shown in Fig. 3(b). Protein-bound products of the Amadori pattern, in the presence of molecular oxygen and transition metals, are oxidized and give origin to highly reactive protein-enediols generating protein-dicarbonyls and superoxide radical. The protein dicarbonyl compounds can participate in AGE formation and referred to as glycoxidation products. Once formed, the superoxide radicals can be converted to highly reactive hydroxyl radical via the fenton reaction. ROS such as \( \text{O}_2^{*-} \), \( \text{H}_2\text{O}_2 \), and \( \cdot\text{OH} \) may contribute to these reactions, which require trace levels of catalytic redox-active transition metal ions. The process also includes oxidative steps and is therefore called glycoxidation (Bousova et al., 2005; Elgawish et al., 1996; Yim et al., 1995).

**RECEPTORS FOR AGES (RAGE)**

A number of AGE receptors (RAGE) have been identified in macrophages, endothelial and several other types of cells (Skolnik et al., 1991). Phagocytic cells expressing RAGE internalize and digest AGE modified proteins and therefore these receptors are implicated in protein turnover, tissue remodeling and inflammation (Schmidt et al., 2001; Vlassara, 2001). Expression of RAGE is enhanced in certain cells during diabetes and inflammation. Interaction of AGE with their cellular receptors generates intracellular oxidative stress resulting in the activation of the transcription factor NF-kB and subsequent gene expression which is relevant in diabetic complications (Zill et al., 2001). NF-kB modulates gene transcription for endothelin-1, tissue factor and thrombomodulin and generation of pro-inflammatory cytokines such as interleukin-1a, interleukin-6 and tumor necrosis factor-\( \alpha \) (Neumann et al., 1999). There is also enhanced expression of adhesion molecules including vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, in addition to other effects such as increased vascular permeability. The intracellular signaling pathways following activation of RAGE by AGEs are outlined in Fig. 4. The importance of oxidative stress, following binding of AGEs with RAGE in endothelial cells was demonstrated by a study which showed that NF-kB and haem-oxygenase messenger RNA, both markers of oxidative stress, become activated. The same study also showed increased oxidative stress in animals after infusion of AGEs (Yan et al., 1994).
Fig. 3: Reaction schemes for glucose autoxidation (a) and glycoxidation (b) (Ahmed, 2005).
Fig. 4: Mechanism of action of AGEs formed intracellularly (Brownlee, 2001).
BIOLOGICAL EFFECTS OF AGE FORMATION:
AGEs are formed in excess during aging, diabetes mellitus and renal failure (Schleicher et al., 1997). AGE modifications influence the structural as well as functional properties of proteins. Due to AGE modifications, several enzymes alter their activity. Methylglyoxal-modified serum albumin exhibits impaired esterase activity compared to unmodified albumin (Ahmed and Thornalley, 2005). Moreover cystein proteases like cathepsins are inhibited by methylglyoxal modification at active site of cysteins (Zeng and Davies, 2005). Glycated and crosslinked proteins exhibit an increased resistance to proteasomal protein degradation, and hence results in markedly enhanced biological half-life (Bulteau et al., 2001). In contrast to intracellular proteins, collagen in the extracellular matrix (ECM) has a relatively long biological half-life and is directly exposed to high levels of glucose outside the cell. Indeed, modified collagen becomes more resistant to degradation by metalloproteinases which cause accumulation of AGE-modified collagens in the ECM which mediate its stiffening leading to heart and vessel dysfunction (Badenhorst et al., 2003). Lens crystallins are also a long-lived target, leading to cataracts. Damage to DNA due to AGE formation may cause birth defects (Ulrich and Cerami, 2001). Thus AGEs appear to damage cells by three mechanisms (Fig. 5):

The first is the modification of intracellular proteins/intracellular glycation including, most importantly, proteins involved in the regulation of gene transcription (Giardino et al., 1994; Shinohara et al., 1998). The second mechanism being, these AGE precursors can diffuse out of the cell and modify adjacent extracellular matrix molecules nearby/cross-link formation (McLellan et al., 1994) with changes signaling between the matrix and the cell causes cellular dysfunction (Charonis et al., 1990). The third mechanism being, these AGE precursors diffuse out of the cell and modify circulating proteins in the blood, such as albumin. These modified circulating proteins can then bind to AGE receptors and activate them, thereby causing the production of inflammatory cytokines and growth factors, which in turn cause vascular pathology (Smedsrod et al., 1997; Vlassara et al., 1995).
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**Fig. 5:** Harmful effect of AGES (Lapolla et al., 2005).

The major ways in which glycation changes protein functions are by:

a) Inhibition of regulatory molecule binding
b) formation of cross linkage of glycated protein
c) trapping of glycated proteins by extracellular matrix
d) decreasing the susceptibility of protein to proteolysis
e) loss of biological activity of enzymes including malate dehydrogenase, glucose-6-phosphate dehydrogenase, glutathione reductase, glyceraldehyde-3-phosphate dehydrogenase, catalase and superoxide dismutase (Heath et al., 1996).
f) abnormality of nucleic acid function
g) increased immunogenicity in relation to immune complex formation (Turk, 2001).

**GLYCATION OF HUMAN SERUM ALBUMIN:**

Human serum albumin is a major protein component of the serum. Albumin contains 585 amino acids and has a molecular weight of 66 kD. This globular protein contains 18 tyrosines, six methionines, one tryptophan, 17 disulphide bridges, and only one Free cysteine, (Cysteine-34) (Sugio et al., 1999). Structurally it consists of 67% of the secondary structure which comprises 28 α-helical segments. Rest of the secondary structure consists of 10% β-turns and 23% extended peptide chain. The tertiary structure of HSA is arranged in heart shape in three homologous domains I, II, III (Fig. 6). It
contains only one tryptophan located in domain II (Coussons et al., 1997; Weber, 1975). This highly soluble protein is present in human plasma at normal concentrations between 35 and 50 g/L. Human serum albumin is a member of the multi-gene family of proteins that include α-fetoprotein and human-group specific component. In normal conditions, its half-life is about 20 days, and its plasma concentration represents equilibrium not only between its synthesis in the liver and its catabolism, but also its transcapillary escape. It is produced in the liver at a rate of nearly 0.7 mg/g of liver tissue per hour (Peters, 1970). Its production is under the control of insulin and somatotropin (Hutson et al., 1987). Albumin has several important physiological and pharmacological functions. It transports metals, fatty acids, cholesterol, bile pigments, and drugs. It is a key element in the regulation of osmotic pressure and distribution of fluid between different compartments. Its most striking property is its ability to bind an unusually broad spectrum of ligands (Brown, 1982). These include inorganic cations, organic anions, various drugs, amino acids, and perhaps most important, physiologically available hydrophobic molecules like bilirubin, hemin, and fatty acids. As a result, albumin is considered a multifunctional plasma transport protein.

Albumin is also responsible for storage and transport of a large number of drugs in the plasma (Bhattacharya et al., 2000). It is also supposed to have a high affinity to metal ions such as Cu$^{2+}$ and Zn$^{2+}$ and act as an antioxidant in the vascular compartment due to its scavenging of reactive oxygen and nitrogen species generated due to basal aerobic metabolism normally and formed at an increased rate during inflammation (Halliwell and Gutteridge, 1990). It is present in the serum in a soluble form and is a major contributor to 80% of plasma colloid pressure (Lundsgaard-Hansen, 1986).

It also acts as an important Acid-Base buffer in plasma. HSA non-enzymatically reacts with glucose to form a stable glycated albumin (Shaklai et al., 1984). This process is especially elevated in Diabetes (Dolhofer and Wieland, 1980) due to increased glucose concentration in plasma. This process of non-enzymatic glycation proceeds in a glucose concentration, incubation period and temperature dependent manner (Baynes et al., 1984). The principle site of glycation of HSA is lysine-525, but the lysine residues in positions 199, 281, and 439 are also susceptible to glycation. In addition there are six more residues that glycate but with much less efficiency (Shaklai et al., 1984).
Fig. 6: Schematic drawing of the HSA molecule. Each subdomain is marked with a different colour (yellow, la; green, lb; red, IIa; magenta, IIb; blue, IIIa; and cyan IIIb). N- and C- termini are marked N and C, respectively. Arginine 117, lysine 351 and lysine 475 which may be sites for binding long-chain fatty acids are colored white (Sugio et al., 1999).
The *in-vitro* exposure of protein to glucose results in the non-enzymatic covalent attachment of glucose to lysine side chains in a manner that observed *in-vivo*. HSA is typically three times more glycated than the rest of the population in conditions of hyperglycemia (Bourdon *et al.*, 1999). In diabetes, HSA may rise from 6-10% to 20-30% and hence serves as the indicator of glycation (Nakajou *et al.*, 2003). HSA also represents the major and predominant circulating antioxidant in plasma known to be exposed to continuous oxidative stress (Soriani *et al.*, 1994). Glucose and free radicals were found to impair the antioxidant properties of the serum albumin (Bourdon *et al.*, 1999). Many studies show the presence of elevated levels of oxidized albumin, in patients with diabetes mellitus (Suzuki *et al.*, 1992), aging (Era *et al.*, 1995), patients with chronic hepatitis C (Rigamonti *et al.*, 2003), oxidized albumin is a reliable marker of oxidative stress in hemodialysis patients (Mera *et al.*, 2005) and many other diseases.

**INHIBITION OF GLYCATION:**

Body has several humoral and cellular defence mechanisms to protect tissues from deleterious effects of glycation reaction and AGE accumulation. These include the glyoxalase systems (I and II) that catalyses the deglycation of methylglyoxal to D-lactate (Thornalley, 1998). The discovery of deglycating enzymes has implications for the repair of protein damage by fructose (Monnier, 2005). A variety of plasma amines may react with sugar and Amadori carbonyl groups to reduce AGEs. Numerous compounds have been investigated for anti-glycation activity and the various sites where potential anti-glycation or AGE compounds could act are outlined in Fig. 7. Currently several strategies are employed to control protein glycation.

(i) Block free amino groups on proteins, preventing glycation by free sugars. However, the biological effect of reducing free protein amino groups is not known.

(ii) Block carbonyl groups on reducing sugars, Amadori products and dicarbonyl intermediates (3-deoxyglucosone, methylglyoxal, etc.) effectively reducing glycation and/or AGE formation. Again, the effect of reducing available carbonyl groups *in-vivo* may not be desirable.
(iii) Antibodies may be used to block Amadori products. This approach has the advantage of specificity compared to use of compounds that merely recognize carbonyl groups.

(iv) Chelation of transition metals by ceruloplasmin may reduce glycation-derived free radicals. However, many transition metals have important physiological functions and their complete removal may have undesirable consequences.

(v) Antioxidants may protect against free radicals derived via autoxidative glycation, glycoxidation and AGEs.

(vi) Enzymes (Amadoriases) may be used to deglycate Amadori products or inactivate intermediates such as 3-deoxyglucosone.

(vii) AGE-cross-link breakers offer the potential of reversing diabetic complications although their precise mechanism of action is still unclear.

(viii) RAGE blockers could prevent interaction of AGEs with RAGE to suppress the cellular and inflammatory changes associated with the development of diabetic complications.

**DIABETES:**

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia with disturbances of fat, carbohydrate and protein metabolism resulting from defects in insulin secretion and/or insulin action (Turko *et al.*, 2001). The recent statistics indicate that the global prevalence of DM, estimated as 366 million in 2011, will increase up to 522 million by 2030 (Whiting *et al.*, 2011). Diabetes is the fifth leading cause of death in the US and the number of people with diabetes in the world is expected to approximately double between 2000 and 2030 (Wild *et al.*, 2004). India has world's largest number of diabetic subjects and the prevalence of diabetes and impaired glucose tolerance were 12.1% and 14.0% respectively, with no gender difference (Ramachandran *et al.*, 2001).
Fig. 7: Potential sites where pharmacological compounds may act to inhibit protein glycation and AGE-mediated damage (Ahmed, 2005).
The prevalence of diabetes in India is about three times higher in urban population compared to rural population and also the prevalence of diabetes varies widely across the nation, a very high prevalence (16.3%) was reported in Thiruvanathapuram in Kerala State in the year 1999, in the same year, a prevalence of 8.3 per cent was reported from Guwahati (Moebus et al., 2010; Ramachandran et al., 2001). Depending on the etiology of DM, factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose utilization and increased glucose production (Kasper et al., 2005). Distinct types of diabetes mellitus (DM) are caused by complex interactions of genetics, environmental factors and life style choices. In the United States, DM is the leading cause of end-stage renal disease, non-traumatic lower extremity amputations and adult blindness.

CLASSIFICATION OF DIABETES:

Classification of DM is on the basis of pathogenic process that leads to hyperglycemia.

- Type 1 diabetes results from β-cell destruction, usually leading to absolute insulin deficiency.
- Type 2 diabetes results from a progressive insulin secretory defect on the background of insulin resistance, impaired insulin secretion and increased glucose production.
- Other specific types of diabetes due to other causes, e.g. genetic defects in β-cell function, genetic defects in insulin action, diabetes of the exocrine pancreas (such as cystic fibrosis), endocrinopathies (e.g. acromegaly, hyperthyroidism) and drug or chemical induced (such as nicotinic acid, protease inhibitors) or due to infections (e.g. congenital rubella).
- Gestational diabetes mellitus include impaired glucose tolerance during pregnancy.

DIABETIC COMPLICATIONS:

The complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality related to the disease. One of the most prevalent metabolic syndromes world-wide, diabetes mellitus (DM), is characterized by hyperglycemia resulting in short-term metabolic changes in lipid and protein metabolism and long-term irreversible vascular and connective-tissue changes. These changes include diabetes-specific complications such as retinopathy, nephropathy, neuropathy and complications
of the macro-vasculature such as atherosclerosis; potentially resulting in heart disease, stroke and peripheral vascular disease (Hudson, 2002).

In diabetic subjects, hyperglycemia is widely recognized as the major cause of diabetic secondary complications due to over generation of ROS (Fig. 8) (Palm et al., 2003).

Several hypotheses relating to hyperglycemia have been proposed. Four main hypotheses as shown in Fig. 9 are: (i) Increased polyol pathway flux, (ii) Increased advanced glycation end product (AGE) formation, (iii) Activation of protein kinase C isoforms, and (iv) Increased hexosamine pathway flux.

1. Diabetic retinopathy:

Diabetic retinopathy is one of the most important microvascular complications in diabetes and is a leading cause of acquired blindness among the people of occupational age (L’Esperance et al., 1990). In a large population-based study, prevalence of any degree or proliferative retinopathy was highest in the younger-onset, insulin-taking diabetic patients and lowest in older-onset group not taking insulin (Klein et al., 1984). The prevalence of diabetic retinopathy increases with duration of diabetes.

Diabetic retinopathy involves both morphological and functional changes in the retinal capillaries, including basement membrane thickening, loss of pericytes, increased permeability and vascular dysfunction. AGEs have been detected in retinal blood vessel walls and contribute towards vascular occlusion and increased permeability of retinal endothelial cells causing vascular leakage (Beisswenger et al., 1995). AGEs exert their effect on microvascular endothelial cells and pericytes by upregulating levels of their RAGE messenger RNA (Tanaka et al., 2000). AGEs may cause loss of pericytes and death of endothelial cells in diabetic retinopathy. The role of AGE in the development of diabetic retinopathy and the effect of the AGE-formation inhibitor, aminoguanidine, has been examined in animal models (Hammes et al., 1995).
**Fig. 8:** Diabetic secondary complications due to hyperglycemia (Brownlee, 2001).

**Fig. 9:** Potential mechanism by which hyperglycaemia-induced mitochondrial superoxide overproduction activates four pathways of hyperglycaemic damage (Brownlee, 2001).
2. Diabetic nephropathy:

Diabetic nephropathy is a leading cause of end stage renal disease, and accounts for disabilities and the high mortality rate in patients with diabetes (Krolewski et al., 1991). Development of diabetic nephropathy is characterized by glomerular hyperfiltration and thickening of glomerular basement membranes, followed by an expansion of extracellular matrix in mesangial areas and increased urinary albumin excretion rate. Diabetic nephropathy ultimately progresses to glomerular sclerosis associated with renal dysfunction (Sharma and Ziyadeh, 1995).

Serum AGE levels reflect the severity of diabetic nephropathy and their measurement can predict the histopathological conditions (Berg et al., 1997). Circulating serum AGE level is so markedly increased in patients with diabetic nephropathy and renal insufficiency that it cannot be cleared by the kidneys (Turk, 2001). A number of studies have demonstrated that aminoguanidine decreased AGE accumulation and plasmaprotein trapping in the glomerular basement membrane (Raj et al., 2000).

3. Diabetic neuropathy:

Diabetic neuropathies are a family of nerve disorders caused by diabetes. People with diabetes can, over time, develop nerve damage throughout the body. Diabetic neuropathy is associated with risk factors for other vascular complications such as poor metabolic control, dyslipidemia, hypertension, body mass index, smoking, microalbuminuria and retinopathy (Cameron et al., 2001; Forrest et al., 1997; Tesfaye et al., 1996). Both vascular and metabolic factors have been involved in the pathogenesis of diabetic neuropathy. Studies in human and animal models have shown reduced nerve perfusion and endoneurial hypoxia, which might play a role in nerve dysfunction (Ibrahim et al., 1999). An interaction between AGE-myelin and macrophages may initiate or contribute to the segmental demyelination associated with diabetic neuropathy (Vlassara et al., 1984). Aminoguanidine (AG) treatment inhibits an accumulation of fluorescent AGE in diabetic nerves, and partially prevents demyelination and axonal atrophy probably through the correction of endoneurial microcirculation (Sugimoto and Yagihashi, 1997).
4. **Diabetic atherosclerosis:**

Atherosclerotic arterial disease may be manifested clinically as cardiovascular disease (CVD). CVD is responsible for about 70% of all causes of death in patients with type-2 diabetes (Laakso, 1999). Conventional risk factors, including hyperlipidemia, hypertension, smoking, obesity, lack of exercise, and a positive family history, contribute similarly to macrovascular complications in type-2 diabetic patients and non-diabetic subjects (Laakso, 1999). AGEs formed on the extracellular matrix results in decreased elasticity of vasculatures, and quench nitric oxide, which could mediate defective endothelium-dependent vasodilatation in diabetes (Bucala et al., 1991). AGE modification of low density lipoprotein (LDL) exhibits impaired plasma clearance and contributes significantly to increase LDL *in-vivo*, thus being involved in atherosclerosis (Bucala et al., 1995). Binding of AGEs to RAGE results in generation of intracellular ROS generation and subsequent activation of the redox-sensitive transcription factor NF-kB which promotes the expression of a variety of atherosclerosis-related genes. Taken together, in diabetes, when fueled by hyperglycemia, AGEs and oxidative stress, the AGE-RAGE axis amplifies vascular stress and accelerates atherosclerosis and neointimal expansion (Naka et al., 2004). Blockade of the AGE-RAGE interaction may lead to a successful reduction of CVD in diabetes. Other study shows a correlation between AGE levels and the degree of atheroma in cholesterol-fed rabbits, and that AG has an anti-atherogenic effect in these rabbits by inhibiting AGEs formation (Panagiotopoulos et al., 1998).

**PROTEIN OXIDATION IN DIABETES:**

Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of
oxidative stress can promote the development of complications of diabetes mellitus (Baynes and Thorpe, 1999; Ceriello et al., 2000).

Diabetic patients exhibit elevated levels of intracellular iron and copper ions which in the presence of glycated proteins, have been shown to enhance the generation of free radicals in-vitro (Dean et al., 1991). These highly reactive species in turn are able to induce oxidative degradation of protein in-vitro (Pacifici and Davies, 1991). Glycation is a major source of ROS i.e., generated by oxidative pathways of glycation (Rahbar and Figarola, 2003). Several studies support the idea that glycation and oxidation are closely linked processes; glucose autoxidation plays an essential role in non-enzymatic glycation of protein. AGEs are important source of free radicals resulting from non-enzymatic glycation and oxidation of proteins and lipids (Thomas et al., 2005). Free radicals and glycation are central to chronic diseases, degeneration and ageing. Overproduction of free radicals accelerates cell ageing and is counteracted by antioxidants. The analysis of mechanism generating free radicals and of the reaction of AGEs with cellular metabolism opens new avenues for the delaying of the development of chronic diseases like diabetes and neurodegenerative diseases (Giardino et al., 1998).

**ANTIGLYCATING AGENTS:**

The first compound that has been extensively studied in-vitro and in-vivo to be a powerful inhibitor of AGE formation is aminoguanidine (Brownlee et al., 1986). AG prevents the formation of fluorescent AGEs and glucose derived collagen cross-linking. The mechanism of inhibition of AGE formation by AG involves trapping of reactive dicabonyl intermediates such as methylglyoxal, glyoxal and 3-deoxyglucosone (Thornalley, 2003; Thornalley et al., 2000). In addition to chelating or antioxidant activity, AG also acts as true scavenger of carbonyl compounds (Thornalley et al., 2000). Pyradoxamine, a form of vitamin B6, found to inhibit carboxymethyl lysine formation in-vitro but it does not interact directly with Amadori intermediates but interfere with the post amadori oxidative reactions by binding catalytic metal ions (Chetyrkin et al., 2008; Voziyan et al., 2003). Many studies suggested that metal catalyzed oxidation plays a critical role in glucose induced modification in collagen. Transition metals like Cu$^{2+}$ ions can catalyze both glycation and glycoxidation in concentration dependent manner.
Carnosine appears to possess antiglycating, antioxidant and free radical scavenging activity. Carnosine inhibits inactivation and crosslinking of enzymes including superoxide dismutase glycation (Ukeda et al., 2002) and oxidation (Stvolinskii et al., 2003). It was found recently the imidazolium group of histadine on carnosine stabilizes the adduct formation at the primary amino group and hence it may play an important role for an anti-crosslinking agent (Hobart et al., 2004). Some anti-inflammatory compounds such as acetylsalicylic acid, ibuprofen indomethacin were also reported to inhibit glycation by preventing the oxidative stress associated with the formation of AGE (Caballero et al., 2000; Shastri et al., 1998; Sobal and Menzel, 2000). Aspirin was also found to inhibit pentosidine formation (Fu et al., 1994; Urios et al., 2007). Some anti-diabetic drugs metformin and progiatazone were also reported to be powerful AGE inhibitors (Rahbar et al., 2000). Recently, two new classes of aromatic compounds, derivatives of aryl (and heterocyclic) ureido and aryl (and heterocyclic) carboxamide-phenoxy-isobutyric acids and benzoic acids have been reported to be potent inhibitors of glycation and AGE formation (Rahbar and Figarola, 2003). In-vitro studies showed that they could directly interact with several reactive dicarbonyls such as glyoxal and methylglyoxal. They were also found to be potent chelators of Cu$^{2+}$ and therefore can suppress hydroxyl radical production during sugar autoxidation and glycation reactions (Rahbar and Figarola, 2003).

Recent studies have highlighted the possible benefits of using plant extracts for decreasing glycation over the currently used drugs (Rates, 2001). Flavonoids like quercetin and rutin represent the most common and widely distributed group of plant phenolics and are abundant in foods. They show important antioxidant and AGE inhibitory properties according to their structure (Farrar et al., 2007). Quercetin has been shown to attenuate diabetic nephropathy in streptozotocin-diabetic rats (Anjaneyulu and Chopra, 2004). Bonnefont-Rousselot (2004) stated that improved antioxidant status is one mechanism by which dietary antioxidant treatment contributes to the prevention and reduction of diabetic complications (Jung et al., 2008).

Garcinol, isolated from Garcinia indica fruit rind has been shown to possess antiglycating property in-vitro along with antioxidant and metal chelating properties (Yamaguchi et al., 2000). Mizutani et al., (2000) isolated resveratrol, a natural phytoestrogen found in
grapes which is found to inhibit AGE induced proliferation and collagen synthesis in vascular smooth muscle. Cyperus rotundus suppresses AGE formation and protein oxidation in a model of fructose-mediated protein glycoxidation (Ardestani and Yazdanparast, 2007). Regarding the significance of glycoxidative stress to diabetic pathology, a supplement of antioxidants to inhibit the process of protein modification appears to be a good strategy for preventing diabetic complications (Rahbar and Figarola, 2003).

**FREE RADICAL BIOCHEMISTRY:**
Free radicals are the chemical species having unpaired electrons that are generated *in-vitro* as well as *in-vivo*. They are highly reactive entities and remain so until and unless their valence shell electrons get paired and attain stability. They are formed from parent molecules via the breakage of a chemical bond keeping one electron by each of its fragment or by cleavage of a radical to generate another radical. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. Besides, they are also produced in redox reactions (Halliwell and Gutteridge, 2007). Those reactive molecules containing oxygen are termed as reactive oxygen species (ROS). ROS possess modulatory roles on the physiology of body cells and also act as second messengers causing signal transduction (Myatt, 2010; Tavakoli and Asmis, 2012). It encompasses a diverse variety of chemical species including superoxide anions (O$_2^−$), hydroxyl radicals (·OH), singlet oxygen, hydrogen peroxide (H$_2$O$_2$), alkoxyl (RO’), peroxyl (ROO’) and hypochlorous acid (HOCI) (Jakus, 2000).

A great deal of literature indicates that free radicals especially active oxygen-centered ones are highly heterogeneous and highly reactive that can invade on the important biomolecules like proteins, lipids and DNA. This consequently can lead to enzyme inactivation, protein modification (deactivation or hyperactivation), lipid peroxidation, membrane degradation, DNA-strand breakage and base modification and so on. Hence, biological free radicals are potentially reactive enough to damage the neighboring biomolecules and can be causative agents for various diseases, aging and cancer (Baek *et al.*, 2003; Hassan *et al.*, 2012; Valko *et al.*, 2004). These radicals are produced by either *endogenous* sources or by *exogenous* sources (Fig. 10). Endogenously, these radicals are
generated from physiological or bodily actions such as immune response, inflammation, toxicity, infection, excessive exercise, ischemia, cancer and aging etc. In analogous to phosphorylation modification of proteins, redox signaling is emerging in reference to events of oxidation of proteins and hence modification by ROS. Indeed, there are many sources of ROS in the cell namely, nicotinamide adenine dinucleotide phosphate oxidase (Block and Gorin, 2012), xanthine oxidase, uncoupling of nitric oxide synthase, cytochrome P450 (Cubero and Nieto, 2012; Izyumov et al., 2010). However, one of the main sources of ROS is the mitochondrion within the cell where the $O_2^{-•}$ is produced as a byproduct of normal oxidative phosphorylation. Exogenous sources include pollution (air and water), heavy metals (lead, mercury, cadmium etc.), certain drugs (gentamycin, cyclosporine), smoking and radiations etc. These agents after getting into the body via different routes are decomposed or metabolized and trigger generation of various free radicals (Brodin and Roed, 1984). Three major forms of ROS shall now be individually discussed in short.

**Superoxide ($O_2^{-•}$):** Superoxide anion is created from molecular oxygen by the addition of an electron. Its production mainly occurs inside the mitochondrion during the electron transport chain, when a small number of electrons escape from electron transport chain complexes I and III (Valko et al., 2007). $O_2^{-•}$ is responsible for the dismutation and release of $H_2O_2$, which acts as precursors for ‘OH ion formation by the catalysis of metal atoms (Holley et al., 2010). It lacks the ability to penetrate lipid membranes and is therefore enclosed in the compartment where it was produced. The formation of superoxide takes place spontaneously, especially in the electron-rich aerobic environment with the activity of respiratory chain enzymes like flavoenzymes, lipooxygenase and cyclooxygenase (Coughlan et al., 2009). The superoxide radical is produced enzymatically by the reaction with xanthine oxidase.

\[
\text{Xanthine} + \text{O}_2 + \text{H}_2\text{O} \quad \rightarrow \quad \text{Uric acid} + \text{O}_2^{-•} + \text{H}^+ \]

**Hydrogen peroxide ($H_2O_2$):** $H_2O_2$ is not a free radical but is nonetheless highly important much because of its ability to penetrate biological membranes and is produced by the dismutation of $O_2^{-•}$ or by direct reduction of $O_2^{-•}$ with two electrons (Topo et al., 2010).

\[
O_2^{-•} + 2\text{H}^+ \quad \rightarrow \quad H_2O_2
\]
It acts as an intermediate in the production of more reactive ROS molecules including HOCl by the action of myeloperoxidase, an enzyme present in the phagosomes of neutrophils and most importantly, formation of \( ^\cdot \text{OH} \) via oxidation of transition metals. It’s important functional role is in intracellular signaling (Sundaresan et al., 1995) and can be removed by at least three antioxidant enzyme systems, namely catalases, glutathione peroxidases and peroxiredoxins (Mates et al., 1999).

\[
\text{H}^+ + \text{Cl}^- + \text{H}_2\text{O}_2 \rightarrow \text{HOCl} + \text{H}_2\text{O}
\]

**Hydroxyl radical (\( ^\cdot \text{OH} \))**: Due to its strong reactivity with biomolecules, \( ^\cdot \text{OH} \) is probably capable of doing more damage to biological systems than any other ROS (Betteridge, 2000). They are produced by Fenton reaction which involves metal ions like \( \text{Fe}^{2+} \) and \( \text{Cu}^{2+} \) with \( \text{H}_2\text{O}_2 \), often bound in complex with different proteins or other molecules (Liu et al., 2012). Transition metals thus play an important role in the formation of \( ^\cdot \text{OH} \) (Halliwell, 1999). Transition metals may be released from proteins such as ferritin and the \([4\text{Fe}-4\text{S}] \) centre of different dehydrases by reactions with \( \text{O}_2^- \). This mechanism, specific for living cells, has been called the *in-vivo* Haber-weiss reaction (Fridovich, 1997).

\[
\text{H}_2\text{O}_2 + \text{Cu}^+/\text{Fe}^{2+} \rightarrow ^\cdot \text{OH} + \text{OH}^- + \text{Cu}^{2+}/\text{Fe}^{3+}
\]

**Nitric oxide (NO)**: Nitric oxide represents an odd member of the free radical family as it contains unpaired electrons and it is not reactive with various biocellular molecules (Wu et al., 2011). Contrarily it easily reacts with other free radicals (e.g., peroxyl and alkyl radicals), generating mainly less reactive molecules, thus in fact functioning as a free radical scavenger in order to inhibit cellular oxidation of lipids in the cell membranes (Hogg and Kalyanaraman, 1998). The \( \text{O}_2^- \) and NO react with each other to give \( \text{OONO}^- \) (peroxynitrite), which is highly cytotoxic (Beckman and Koppenol, 1996). \( \text{OONO}^- \) may react directly with diverse biomolecules in one- or two-electron reactions, readily react with carbon dioxide to form highly reactive nitroso peroxocarboxylate (\( \text{ONOOCO}_2^- \)), or protonated as peroxinitrous acid (\( \text{ONOOH} \)) undergo homolysis to form \( ^\cdot \text{OH} \) and \( ^\cdot \text{NO}_2 \) or rearrange to nitrate (\( \text{NO}_3^- \)). Peroxynitrite, directly or via its reaction products, may oxidize low density lipoproteins, release copper ions by destroying ceruloplasmin, and generally attack tyrosine residues in different proteins, as observed in many inflammatory diseases (Halliwell, 1997). NO is synthesized enzymatically from L-arginine by NO synthase (Andrew and Mayer, 1999).
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Fig. 10: Reactive oxygen species (ROS) generated by endogenous as well as exogenous sources (Brodin and Roed, 1984).

ANTIOXIDANT DEFENSE SYSTEM:
Free radicals exert derogatory effects on our cellular structure and functions. Hence, living systems have been equipped with antioxidant defense system comprising of endogenous antioxidant molecules or cellular reductants [glutathione (GSH), sulfhydryl groups (-SH), thioredoxin] and antioxidant enzymes:

1. **Superoxide dismutase (SOD):** It dismutates \( \text{O}_2^- \) into \( \text{H}_2\text{O}_2 \) and oxygen. Superoxide dismutases (SODs) are metalloenzymes and their role is to protect aerobic cells against \( \text{O}_2^- \) action. They catalyze the conversion of superoxide molecules to \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \) and therefore form one of the cell’s major defense mechanisms against oxidative stress (McCord and Fridovich, 1969).

\[
\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2
\]

2. **Catalase:** Catalase protects cells against \( \text{H}_2\text{O}_2 \) generated inside them. It has an important role in the acquisition of tolerance to oxidative and nitrosative stress in cellular adaptive response.

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \frac{1}{2} \text{O}_2
\]

3. **Glutathione peroxidase:** Glutathione peroxidase uses the thiol reducing power of glutathione to reduce oxidized lipids and protein targets of ROS. Glutathione Peroxidase
catalyses hydroperoxide reduction using GSH, thus protecting mammalian cells against oxidative damage.

\[ \text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow \text{GSSG} + 2\text{H}_2\text{O} \]

4. **Glutathione reductase:** Rereduction of the oxidized form of glutathione (GSSG) is then catalysed by glutathione reductase. This system traps and nullifies the endogenously generated radicals in various metabolic reactions in virtually all the aerobic living systems.

\[ \text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2 \text{GSH} + \text{NADP}^+ \]

**ANTIOXIDANTS:**
Antioxidants are substances that are able to prevent or retard oxidation of lipid, proteins and DNA, and to protect the compounds or tissues from damage caused by oxygen or free radicals. Antioxidants are key line of defense capable of trapping free radicals by preventing radical formation, intercepting radicals from further damage to the body (Cotgreave et al., 1988). Antioxidants also protect against glycation-derived free radicals and may have therapeutic potentials.

Apart from endogenous antioxidants a vast number of dietary agents also act as antioxidants. Currently available synthetic antioxidants like butylated hydroxyanisole, butylated hydroxytoluene, tertiary butyl-hydroquinone, propylgallate and gallic acid esters are known to ameliorate oxidative damages but they are suspected to prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants (Amarowicz et al., 2000; Ghafar et al., 2010). Research on bioactive principles of essential oils extracted from various herbs and spices has become increasingly popular because essential oils have been discovered to have many functional properties such as antimicrobial, antioxidant and anticancer activities (Leal et al., 2003; Lee and Shibamoto, 2002; Vardar-Unlu et al., 2003). Plants, including herbs and spices, have many phytochemicals which are potential sources of natural antioxidants, e.g. phenolic diterpenes, flavonoids, tannins and phenolic acids (Dawidowicz et al., 2006). Green tea is considered a rich source of phenolic compounds and its consumption is considered to be a factor in the lower incidence of coronary heart disease in the Chinese population.
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(Zhang et al., 2008). Similarly, parsley oil and two of its inherent bioactive phenolic compounds (i.e., myristicin and apiol) possess antioxidant activity (Zhang et al., 2006). Therefore, polyphenolic compounds’ health promoting effects reduce the risk of various diseases (Manach et al., 2004) and inhibition of growth of pathogenic bacteria (Giroux et al., 2001) which are often associated with the termination of free radical propagation in biological systems. Thus antioxidant capacity is widely used as a parameter to characterize medicinal plants and their bioactive components. There is growing interest in natural products with combined anti-glycation and antioxidant properties as they may have reduced toxicity.

PHYTOCHEMICALS:

Aromatic herbs and spices have been used for a long time in alternative medicine, not only to improve or modify the flavor of foods, but also to avoid its deterioration. Thymoquinone (TQ) (2-isopropyl-5-methyl-1,4-benzoquinone), is the main bioactive component of the volatile oil of N. sativa (Fig. 11). It has been used as antioxidant, anti-inflammatory and antineoplastic medicines for more than 2000 years (Hosseinzadeh and Parvardeh, 2004; Trang et al., 1993). Generally Nigella sativa seeds contain more than 30% fixed oil and 0.40% to 0.45% volatile oil. TQ represents 18.4 to 24% of the N. sativa volatile oil (Arslan et al., 2005). TQ can also be found in other plants such as Callitris quadrivalvis, Monarda fistulosa, Juniperus cedrus, Tetraclinis articulata, and Nepeta leucophylla. Quinones are ubiquitous in nature and constitute an important class of naturally occurring compounds found in plants, fungi, and bacteria. Current human exposure to quinones occurs via the diet as well as clinically. Benzoquinones are potentially derivable by oxidation of suitable phenolic compounds. Many of these benzoquinones have important biochemical functions in electron transport systems for respiration or photosynthesis (Dewick, 2001). The pharmacological properties attributed to naturally occurring quinones are thus well established. For example, thymoquinone presents anticonvulsant activity in the petit mal epilepsy (Hosseinzadeh and Parvardeh, 2004). N. sativa has been traditionally used as a natural remedy for a number of illnesses and conditions such as diabetes, inflammation, bronchitis, fever, and influenza (Ali and Blunden, 2003). The antioxidant effect of TQ is associated with its potential to alter
“redox state” and its scavenging activity against free radicals through modulation of hepatic and extra-hepatic antioxidant enzymes (Karaman et al., 2010). TQ protects the kidney against ifosfamide, mercuric chloride, cisplatin and doxorubicin-induced damage by preventing renal GSH depletion and anti-lipid peroxidation product accumulation, thereby improving renal functioning (Badary, 1999; Badary et al., 1997; Fouda et al., 2008). Khattab and Nagi (2007) assessed the protective effects of TQ after chronic inhibition of nitric oxide synthesis with N (omega)-nitro-1-arginine methyl esters and found that treatment with TQ increased GSH to normal levels and inhibited the in-vitro production of superoxide radicals.

**Thymol (TL)** (2-isopropyl-5-methylphenol) is a natural monoterpenic phenol derivative of cymene, isomeric with carvacrol, found in oil of thyme (Fig. 12) and has been commonly used in foods mainly for flavor, aroma and preservation and also in folk medicine since the ancient Greeks, Egyptians and Romans (Baser, 1993; Baser, 1994). TL can be used for the treatment of oral infectious diseases because of their inhibitory activity on oral bacteria (Didry et al., 1994; Kohlert et al., 2002). Other plants that contain thymol are Origanum compactum and Thymus glandulosus (Tai et al., 2002).

**Eugenol (EU)** (4-allyl-2-methoxyphenol) is a methoxyphenol compound having a short hydrocarbon chain in its structure (Gulcin, 2011). It is found in virtually all spices but bay leaves and cloves are considered the best sources of it (Fig. 13) (Tai et al., 2002). It is one of the major components constituting about 80-95% of clove oil (Szabadics and Erdelyi, 2000). Pharmacologic studies have demonstrated that EU has anticonvulsant (Dallmeier and Carlini, 1981), local anesthetic (Brodin and Roed, 1984), antistress (Sen et al., 1992), bacteriostatic and bactericidal (Walsh et al., 2003) and antifungal properties (Lee et al., 2007). EU and its isoform, isoeugenol have been documented to be potential inhibitors of copper-dependent oxidation of LDL (Ito et al., 2005). Besides, treatment of EU with fast decaying fruits like strawberries increased their average shelf life and also preserved their nutrient values of sugar and organic acids. The treatment also increased the content of total phenolics, anthocyanins and flavonoids (Wang et al., 2007).
Fig. 11: (A) *Nigella sativa* seeds and flower, (B) Chemical structure of Thymoquinone (component of *N. sativa*).

Fig. 12: (A) *Thymus vulgaris* plant, (B) Chemical structure of thymol (component of *Thymus vulgaris*).

Fig. 13: (A) *Eugenia caryophyllata* plant, (B) Chemical structure of Eugenol (component of *Eugenia caryophyllata*).
OBJECTIVE OF THE PRESENT STUDY:
Glycation is the sequence of non-enzymatic reactions involving interaction between reducing sugars and the nucleophilic groups of proteins and other biomolecules. It is ubiquitous in nature and occurs in the cells of all living organisms, albeit at a very slow rate. The rate of glycation however increases remarkably during hyperglycemia, in diabetes and related disorders. Persistent hyperglycemia induces abnormal changes such as increase of AGEs formation, increase of polyol pathway flux, and activation of protein kinase C isoforms. Glycation is also accompanied by the formation of highly reactive and damaging ROS. Free radicals and glycation end products are known to cause severe protein damage resulting in major structural alterations and loss of biological function.

A large body of evidences indicate that glycation is a key molecular basis of diabetic complications. Hyperglycemia is regarded as the primary cause of diabetic microvascular complications that eventually contribute to diabetic macrovascular disease. Diabetic complications usually arise as a result of non-enzymatic protein glycation which leads to the formation of heterogenous, toxic, and antigenic AGEs. Three routes have been proposed for AGEs formation: (i) autoxidative pathway (sugar gives reactive products by autoxidation), (ii) conventional Amadori rearrangement, and (iii) Schiff base formation. Reactive oxygen species (ROS) such as $\text{O}_2^-$, $\text{H}_2\text{O}_2$, and $\cdot\text{OH}$ contribute to these reactions, which require trace levels of catalytic redox-active transition metal ions. The process includes also oxidative steps and is therefore called glycoxidation. Regarding the significance of glycoxidative stress to diabetic pathology, phytochemicals that interfere with glycation reactions may be beneficial in restricting the complications accompanying diabetes and related disorders. Restriction/prevention of glycation and oxidative stress are therefore mooted as an effective strategy for alleviation of complications associated with hyperglycemia. These days, scientific interests in the functional components of food, with health protecting potencies, are increasing mainly due to their various biological activities and low cytotoxic effects.

Since glycation- and oxidation-induced biochemical changes are known to participate in the overall diabetic complications, the present study was planned to determine antioxidant activity of TQ, TL and EU and their protective effect against AAPH-induced RBC hemolysis. The study was also extended to determine the possibility of preventing
the glucose-induced modification in HSA, as it being the most abundant protein in human blood, by these phytochemicals in concentration and time dependent manner. Furthermore, increase in glycoxidation products in plasma and tissue proteins suggest that oxidative stress increases in diabetes. Hence, the present study was further extended *ex vivo* to evaluate the serum level of lipid peroxides, protein carbonyls, total antioxidant capacity and electrophoretic pattern of albumin in healthy and diabetic patients. The protective effect of TQ, TL and EU on these parameters was also determined. For this, diabetic serum samples were incubated in absence and presence of TQ-2 (30 µM), TL-2 (30 µM) or EU-2 (0.6 µM) for 21 days at 37°C.