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Role of Oxidative Stress and Diabetes

V. Asha Jyothi*, Giridhar Vedantam[1] and Shahnaz Taskeen[a]
[1]Department of Dravyaguna (Ayurveda Pharmacology), Guru Gorakshnath Institute of Medical Sciences, Faculty of Ayurveda, Mahayogi Gorakhnath University Gorakhpur, Uttar Pradesh India 273007.
***Department of Pharmacology, Shadan Women's College of Pharmacy, Khiratabad, Hyderabad**

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***Corresponding Author Email:** ashajyothivadlapudi@gmail.com

Abstract
Diabetes mellitus is common problem of modern life style. It brings a complex series of pathological changes in patients with diabetes, most of them so complex that they are life threatening. The main cause for the pathological changes is the free radical increase due to oxidative stress. The need of the hour is to understand the mechanisms of free radical increase leading to oxidative stress which further progresses to the occurrence of pathological changes. The present review mainly focuses on the role free radicals in oxidative stress and role of oxidative stress on the pathological changes associated with oxidative stress increase in the diabetic condition.

Keywords
Diabetes, Oxidative stress, Reactive oxygen species.

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INTRODUCTION:
Oxidative stress, defined as an imbalance between reactive oxygen species production and breakdown by endogenous antioxidants, is closely associated with aging and a number of diseases including inflammation, carcinogenesis, diabetes and atherosclerosis. Diabetes mellitus (DM) is a syndrome characterized by abnormal insulin secretion, derangement in carbohydrate and lipid metabolism, and is diagnosed by the presence of hyperglycemia.

During diabetes or insulin resistance, failure of insulin-stimulated glucose uptake by fat and muscle causes glucose concentrations in blood to remain high. Consequently, glucose uptake by insulin-independent tissues increases. Increased glucose flux both enhances oxidant production and impairs antioxidant defenses by multiple interacting non-enzymatic, enzymatic and mitochondrial pathways[1]. These include activation of protein kinase C isoforms, increased hexosamine pathway, glucose autooxidation, increased methyl-glyoxal and advanced glycation end-product (AGEs) formation, increased polyol pathway flux[2].This hyperglycemia-induced oxidative stress ultimately results in modification of intracellular proteins resulting in an altered function, DNA damage, activation of the nuclear transcription NF-kB, causing abnormal changes in gene expression, decreased production of nitric oxide, and increased expression of cytokines, growth factors and procoagulant and proinflammatory molecules.

OXIDATIVE STRESS:
Oxidative stress results from an imbalance between radical-generating and radical scavenging systems, i.e. increased free radical production or reduced activity of antioxidant defenses or both. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus,

oxidative stress can cause disruptions in normal mechanisms of cellular signaling.

Cellular metabolism generates reactive oxygen species (ROS). Molecular ground-state oxygen can be activated to a ROS by means of energy transfer (e.g., under the influence of ultraviolet radiation), forming singlet oxygen ($^1O_2$), or by electron transfer, forming "incomplete" reduction products, i.e., the superoxide anion radical (O^{-2}). Small amounts of oxygen are reduced to $\bullet O^{-2}$by the mitochondrial electron transport chain during the course of normal oxidative phosphorylation, which is essential for generating ATP. Subsequently, O^{-2} can be converted into other ROS and reactive nitrogen species (RNS). Under normal conditions, O^{-2} molecules are quickly converted to H2O2 by the key mitochondrial enzyme, manganese superoxide dismutase (Mn-SOD) within the mitochondria and by copper and zinc (CuZn-SOD) in the cytosol[3].H2O2 is then either detoxified to H_2O and O_2 by glutathione peroxidase (in the mitochondria) in conjunction with glutathione reductase, or diffuses into the cytosol and is detoxified by catalase in peroxisomes.

OXIDATIVE STRESS AND DIABETES:

Hyperglycemia can induce oxidative stress via several mechanisms. These include glucose autoxidation, the formation of advanced glycation end-products (AGE), and activation of the polyol pathway. Other circulating factors that are elevated in diabetics, such as free fatty acids and leptin, also contribute to increased reactive oxygen species (ROS) generation. In diabetes, altered oxidative metabolism is a consequence of the chronic exposure to hyperglycemia or of the absolute or relative insulin deficit; insulin regulates several reactions involved in oxido-reductive metabolism[5].Oxidative stress is usually based on indirect and nonspecific measurement of products of reactive oxygen species. Enhanced oxidative stress in hyperglycemia is indicated by urinary excretion of 8-iso-PGF2α (8-iso-prostaglandin F2α). Oxidative stress as measured by indices of lipid peroxidation and protein oxidation which is increased in both insulin dependent diabetes and non-insulin dependent diabetes, in obese diabetic patients and even in diabetic patients without complications[6].

During diabetes or insulin resistance, increased oxidative glucose metabolism itself increases mitochondrial production of $\bullet O^{-2}$, which will then be converted to HO•, and H2O2[7]. ROS formation is also increased by FFAs (free fatty acids), through direct effects on mitochondria. The overexpression and activity of mitochondrial inner membrane uncoupling proteins (UCPs) contribute to an increase in superoxide formation under diabetic conditions. The overproduction of superoxide, in particular by mitochondria, causes inhibition of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and of cytochrome enzymes of the electron transport chain responsible for oxidative phosphorylation associated with Krebs cycle[7]. Hyperglycemia induced GAPDH inhibition was found to be a consequence of poly (ADP-ribosylation) of GAPDH by PARP [poly (ADP-ribose) polymerase], which was activated by DNA strand breaks produced by mitochondrial superoxide overproduction. As a result, glycolytic intermediates upstream of GAPDH accumulate; leading to increased substrate-directed activity of the de novo DAG (diacylglycerol) synthetic pathway, which further activates PKC (protein kinase C isoforms) and NADPH oxidase, as well as the hexosamine and polyol biosynthetic pathways.

Glucose autoxidation: The increased metabolism of glucose due to intracellular hyperglycemia leads to the overproduction of nicotinamide adenine dinucleotide (NADH) and flavin adenosine dinucleotide, which are used by the electron transport chain to generate adenosine triphosphate[8]. When NADH is in excess, there is an increase in the mitochondrial proton gradient and electrons are transferred to oxygen, producing superoxide[9]. Production of superoxide by the electron transport chain occurs at two main sites: the NADH dehydrogenase of complex I and the interface between ubiquinone and complex III[10]. It is thought that mitochondrial-derived superoxide causes increased diacylglycerol (DAG) synthesis and subsequent protein kinase C (PKC) activation.

Advanced glycation end-products: Glucose spontaneously reacts with free amino groups of proteins to form labile Schiff bases (early Maillard reaction). These Schiff bases are not stable and may either dissociate or undergo an Amadori rearrangement to become more stable, fructose-like compounds known as fructosamines[12]. Formation of fructosamines is followed by their slow conversion to a series of compounds known as advanced glycation end-products (AGEs) which are thought to participate in the development of diabetic complications[13].

Glycation products can be oxidized by several ROS, including HO• and $ONOO^-$, to give AGEs. When oxidation is involved in their formation, so-called glycoxidation products such as pentosidine and N(carboxymethyl)lysine result. The non-enzymic glycation reaction proceeds slowly through different stages, leading to alterations of protein structure and molecular surface topology that profoundly change the affected molecule`s biochemical properties.

Glycation in vivo is slow and reversible at physiological glucose levels, mostly to affect proteins with a very slow turnover, for example collagen and crystalline lens. Glycation is faster at elevated glucose levels. Some tissues, such as the liver, kidneys, and erythrocytes are more susceptible to AGE formation than others. Glycated hemoglobin (HbA1C) contains a glucose Amadori product attached to the N-terminal valine of the β-chain. Whereas haptoglobin can prevent prooxidant effects of normal hemoglobin, it is less good (especially the Hp2-2 form) at doing so for glycated haemoglobin[11]. The other pathway of AGEs formation is to first oxidize the glucose and then allow the oxidation products to react with protein. In the presence of the transition metals, glucose can oxidize, to produce • O^{-2}, H_2O_2, HO• and toxic Di carbonyls which can damage proteins[10]. Oxidative stress thus contributes to AGE formation and the word glycoxidation is often used to describe the pathways involved. Once formed, AGE-modified proteins cause more oxidative stress. Glycation of proteins in the electron transport chain can impair normal electron flow and promote to formation of•O^{-2}.

The polyol pathway: Under normal conditions most of the cellular glucose is phosphorylated into glucose 6-phosphate by hexokinase. A minor part of non-phosphorylated glucose enters the polyol pathway, the alternate route of glucose metabolism[14], implicating the enzyme aldose reductase. Aldose reductase normally has the function of reducing toxic aldehydes in the cell to inactive alcohols, but when the glucose concentration in the cell becomes too high, aldose reductase also reduces it in the presence of NADPH to sorbitol, which is later oxidized to fructose by the sorbitol dehydrogenase at the cost of NAD^+. Hyperglycemia leads to an increase in the formation of products of the polyol pathway along with depletion in the reduced nicotinamide adenine dinucleotide phosphate (NADPH). NADPH is an essential reducing equivalent for the regeneration of reduced glutathione (GSH) by glutathione reductase (GR) and for the activity of the NADPH-dependent thioredoxin system, two important cell antioxidants against oxidative damage.

NADPH is also a cofactor of important enzymes of the reactive nitrogen species (RNS) and reactive oxygen species (ROS) metabolism, NOS and NADPH-oxidase[15], respectively. Intracellular depletion of NADPH leads to a decreased NO• synthesis, since NADPH is cofactor of the NO-synthase, which synthetizes NO• from L-arginine. All isoforms of NOS contain a reductase domain and an oxygenase domain separated by a calmodulin binding region. NOS requires five cofactors prosthetic groups such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, tetrahydrobiopterin (4 BH) and Ca^{2+} calmodulin.

Activation of protein kinase C isoforms: Glucose excess may activate protein kinase C (PKC) directly by several mechanisms, including through de novo synthesis of diacylglycerol (DAG), by activation of phospholipase C, and by inhibition of DAG kinase or indirectly (via ligation of AGE receptors or increased activity of the polyol pathway). Increase in activity of protein kinase C results in functional changes to vascular cells via activation of phospholipase A2, the expression of growth factors and alterations in the expression of certain basement membrane proteins. There is plausible evidence that PKC, which is stimulated in diabetes via multiple mechanisms, activates NAD(P)H oxidase[15].

COMPLICATIONS

1. **Atherosclerosis:** The accumulation of fat and cholesterol in the walls of arteries is progressive, it thickens and hardens and forms calcium deposits which may eventually block the arteries. Blockage of the arteries and rupture of vulnerable plaques is a common cause of heart attack. Diabetes accelerates the formation of atherosclerotic plaques in the coronary arteries (coronary artery disease, including myocardial infarction), lower extremities (peripheral arterial disease) and extracranial carotid arteries (cerebrovascular disease)[19]. During diabetes chronic hyperglycemia, dyslipidemia and insulin resistance can alter the function of multiple cell types including endothelial cells, smooth muscle cells and platelets. Disruption of the integrity of the endothelium leads to inflammation, activation of platelets, coagulation, and thrombosis[20]. To protect against this, the endothelium synthesizes important bioactive substances such as endothelial-derived NO (EDNO), prostaglandins, endothelin (ET) and angiotensin II (Ang II) that regulate blood vessel function and structure[19].
2. It is thought that hyperglycemia-mediated deregulation of these Vaso protective agents either enhances the intensity of oxidative stress directly or is affected by oxidative stress. Due to its vasorelaxation, an anti-inflammatory and anti-proliferative property, EDNO is often viewed as vascular protective. During diabetes the bioavailability of EDNO is lowered by either decreased formation or enhanced removal of ●NO. Hyperglycemia attenuates the level of

EDNO by blocking thefunction of endothelial NOS (eNOS) synthase in endothelial and vascular smooth musclecells[22].An increase in ROS, such as ●O^{2-} within the endothelium is the most significant factors known to decrease EDNO[21]. Increased ●O^{2-} can reduce EDNO bioavailability due to its propensity to react with ●NO, producing the highly reactive oxidant, $ONOO^{-}$[19]. Loss of functional EDNO causes impaired relaxation of the vessel wall and inhibition of the proliferative effects of EDNO[23]. In addition, $ONOO^{-}$ can induce cell damage via lipid peroxidation, inactivation of enzymes and structural proteins by oxidation and nitration. $ONOO^{-}$ can activate matrix metalloproteinases (MMPs) and trigger the release of pro-apoptic factors such as cytochrome c and induce DNA damage[24]. $ONOO^{-}$ is also involved in oxidizing tetrahydrobiopterin (BH4), an important cofactor of eNOS, thereby uncoupling eNOS which then produces ●O^{2-}instead of EDNO[23].It is also known that a reduction in ●NO in diabetes stimulates endothelialangiotensin-converting enzyme (ACE) activity and the generation of Ang II and ●O^{2-} [25]. While EDNO inhibits the production of endothelin-1 (ET-1) which is a vasoconstriction peptide, increased Ang II can stimulate the endothelial cell to synthesize and release ET-1, thereby contributing to vascular smooth muscle dysfunction[26]. A disruption in vascular smooth muscle function may lead to plaque destabilization and rupture, which leads to complications[19].

3. **Diabetic cardiomyopathy:** Hyperglycemia is known to up regulate the production of Ang II. This has a profound effect on the myocardium given that most of the cellular components of the RAS including angiotensinogen, renin and the angiotensin II type I (AT1) receptor are found in myocytes[27].Ang II is known to contribute to the development of diabetic cardiomyopathy through its hemodynamic vasoconstrictor effects and its ability to act as a proinflammatory mediator, it is now clear that Ang II mediates its effects on the myocardium via its ability to enhance production of ●O^{2-}[28]. Recent studies found that hyperglycemia induces cardiac myopathies via the AT1 receptor, with the activation of NADPH oxidase and increased ROS generation. Furthermore, ●O^{2-} levels were attenuated with an ACE inhibitor[27]. However, other sources of ROS generation are known to contribute to the oxidative stress that accompanies diabetic cardiomyopathy.

   The biochemical pathways for diabetes-associated atherosclerosis, leading to endothelial dysfunction and inflammation due to the overproduction of ROS, appear to play a causal role in the pathogenesis of diabetic cardiomyopathy. It is suggested that nitrosative stress and peroxynitrite-induced damage contribute to the pathogenesis of diabetic cardiomyopathies.

4. **Diabetic nephropathy:** An up regulation of ROS in diabetes has been implicated in the pathogenesis of kidney injury[29]. ROS activate a number of signaling pathways including PKC, p38 MAPK, p42/p44 MAPK and the transcription factor NF-κB, which leads to the increased activation of growth factors such as TGF-β that contribute to the pathogenesis of DN. In the diabetic kidney, enhanced glucose uptake occurs in many of the cells including glomerular epithelial cells, mesangial cells and proximal tubular epithelial cells, leading to the excessive production of intracellular ROS, making these cells particularly susceptible to diabetic complication[29].Hyperglycaemia has been shown to increase 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative mitochondrial DNA damage in kidneys. ROS mediates high glucose-induced activation of PKC in mesangial cells, leading to an increase in TGF-β expression[30].Furthermore, increased ROS led to accelerated glomerulosclerosis through TGF-β mediated plasminogen activator inhibitor-1 (PAI-1) increases in mesangial cells[31]. Similarly, it was proposed that ROS mediate kidney fibrosis in renal cells through the up regulation of the transcription factors NFκB and activator protein-1 (AP-1), that in turn increase MCP-1, TGF-β and PAI-1, resulting in the increased accumulation of ECM[32].

5. **Diabetic retinopathy:** it is the most specific of all the diabetic microvascular complications. The pathogenesis of DR has not been completely understood, but the established risk factors include poor glycemic control, hypertension, increasing age and the duration of diabetes[33].AGEs are the products of glycation and oxidation and they are responsible for the liberation of superoxide radicals. Excess glucose enters the polyol pathway, resulting in excess sorbitol production, with a concomitant decrease in the NADPH levels. Low levels of NADPH can decrease nitric oxide production in

the endothelial cells and can adversely affect the cellular redox balance, thereby resulting in deleterious metabolic consequences. NADPH is required for regenerating the reduced glutathione and the consumption of NADPH could contribute to an intracellular increase in the formation of the reactive oxygen species, thus leading to oxidative stress and resultant diabetes related vascular damage. Therefore, an increased flux through the polyol pathway can lead to micro-vascular damage by contributing to AGE formation, specific protein kinase C activation and the generation of reactive oxygen species (ROS)[34].

ANTIOXIDANTS

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. These include:

Endogenous Antioxidants

- Bilirubin
- Thiols, e.g., glutathione, lipoic acid, N-acetyl cysteine
- NADPH and NADH
- Ubiquinone (coenzyme Q10)
- Uric acid
- Enzymes:
  - copper/zinc and manganese-dependent superoxidedismutase (SOD)
  - iron-dependent catalase
  - selenium-dependent glutathione peroxidase

Dietary Antioxidants

- Vitamin C
- Vitamin E
- Beta carotene and other carotenoids and oxycarotenoids,e.g., lycopene and lutein
- Polyphenols, e.g., flavonoids, flavones, flavonols, and proanthocyanidins

Metal Binding Proteins

- Albumin (copper)
- Ceruloplasmin (copper)
- Metallothionein (copper)
- Ferritin (iron)
- Myoglobin (iron)
- Transferrin (iron)

VARIOUS ROS AND CORRESPONDING NEUTRALIZING ANTIOXIDANTS

ROS	NEUTRALIZINGANTIOXIDANTS
Hydroxyl radical	vitamin C, glutathione, flavonoids, lipoic acid
Superoxide radical	vitamin C, glutathione, flavonoids, SOD
Hydrogen peroxide	vitamin C, glutathione, beta carotene, vitamin E, CoQ10, flavonoids, lipoic acid
Lipid peroxides	beta carotene, vitamin E, ubiquinone, flavonoids, glutathione peroxidase

Endogenous Antioxidants

1. **Uric acid:** Uric acid (UA) is an antioxidant oxypurine produced from xanthine by the enzyme xanthine oxidase and is an intermediate product of purine metabolism. Uric acid has the highest concentration that of any blood antioxidant and provides over half of the total antioxidant capacity of human serum. Uric acid's antioxidant activities are also complex, given that it does not react with some oxidants, such as superoxide, but does act against peroxynitrite, peroxides, and hypochlorous acid[15].
2. **Thiols:** Glutathione, an important water-soluble antioxidant, is synthesized from the amino acids glycine, glutamate, and cysteine. Glutathione directly quenches ROS such as lipid peroxides and also plays a major role in xenobiotic metabolism. Exposure of the liver to xenobiotic substances induces oxidative reactions through the upregulation of detoxification enzymes, i.e., cytochrome P-450 mixed-function oxidase. Lipoic acid is a sulfur-containing molecule that catalyzes the oxidative decarboxylation of alpha-keto acids, such as pyruvate and alpha ketoglutarate, in the Krebs cycle. Lipoic acid and its reduced form, dihydrolipoic acid (DHLA), are capable of quenching free radicals in both lipid and aqueous domains and as such has been called a “universal antioxidant.”[17] Lipoic acids may also exert its antioxidant effect by chelating with pro-oxidant metals.
3. **Enzymes:** Superoxide dismutases (SODs) are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide. SOD enzymes are present in almost all aerobic cells and in extracellular fluids. Superoxide dismutase enzymes contain metal ion cofactors that, depending on the isozyme, can be copper, zinc, manganese or iron. In humans, copper/zinc SOD is present in the cytosol, while manganese SOD is present in the mitochondrion. There also exists a third form of SOD in extracellular fluids, which contains copper and zinc in its active sites. Catalases are enzymes that catalyze the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese

cofactor. This protein is localized to peroxisomes in most eukaryotic cells. Catalase is an unusual enzyme since, although hydrogen peroxide is its only substrate, it follows a ping-pong mechanism. Glutathione peroxidase is an enzyme containing four selenium-cofactors that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides. There are at least four different glutathione peroxidase isozymes in animals. Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides. Surprisingly, glutathione peroxidase 1 is dispensable, as mice lacking this enzyme have normal lifespans, but they are hypersensitive to induced oxidative stress. In addition, the glutathione S-transferases show high activity with lipid peroxides. These enzymes are at particularly high levels in the liver and serve in detoxification metabolism[18].

Dietary Antioxidants

Vitamin C, vitamin E, and beta carotene are among the most widely studied dietary antioxidants. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Vitamin C has been cited as being capable of regenerating vitamin E.Beta carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues. It is thought that beta carotene may work synergistically with vitamin E.4,5 A diet that is excessively low in fat may negatively affect beta carotene andvitamin E absorption, as well as other fat-soluble nutrients. Fruitsand vegetables are major sources of vitamin C and carotenoids, while whole grains and high quality, properly extracted and protected vegetable oils are major sources of vitamin E.

PHYTONUTRIENTS

A number of otherdietary antioxidant substances exist other than the traditional vitamins. Many plant-derived substances, collectively termed “phytonutrients,” or “phytochemicals,” are known for their antioxidant activity. Phenolic compounds such as flavonoids are ubiquitous within the plantkingdom: approximately 3,000 flavonoid substances have beendescribed. In plants, flavonoids serve as protectors against a wide variety of environmental stresses while, in humans, flavonoids appear to function as “biological response modifiers.”

Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity.The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages. The potential antioxidant properties can be evaluated by using various invitro tests like DPPH.[35]

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