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A REVIEW ON OXIDATIVE STRESS AND DIABETIC COMPLICATIONS

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ABSTRACT

Oxidative stress due to chronic hyperglycemia is a one of the factor that produce diabetic complications. There is increasing evidence that in certain pathologic states, especially chronic diseases, the increased production and/or ineffective scavenging of reactive oxygen species (ROS) may play a critical role. High reactivity of ROS determines chemical changes in virtually all cellular components, leading to lipid peroxidation. Production of ROS and disturbed capacity of antioxidant defense in diabetic subjects have been reported. It has been suggested that enhanced production of free radicals and oxidative stress is central event to the development of diabetic complications. Reactive oxygen species (ROS) are produced by oxidative phosphorylation, nicotinamide adenine dinucleotide phosphate oxidase (NADPH), xanthine oxidase, the uncoupling of lipoxygenases, cytochrome P450 monooxygenases, and glucose autoxidation. Once ROS produced than it affect the cellular system, and produced the various complications. This review explores the production of ROS and the propagation and consequences of oxidative stress in diabetic complications.

Keywords: RP-HPLC, Diabetes mellitus (DM); diabetic complications; oxidative stress.

INTRODUCTION

In the last few decades the occurrence of type 2 diabetes mellitus has rapidly increased internationally, and it has been estimated that the number of diabetic patients will more than double within 15 years. As type 2 diabetes is mainly characterized by the development of increased cardiovascular disease (CVD) morbidity and mortality, it has been suggested that diabetes could be considered a CVD. [1]

However, diabetes is also characterized by dramatic microangiopathic complications, such as retinopathy, nephropathy, infertility, DNA damage and neuropathy. Recent evidence suggests that glucose overload may damage cells through oxidative stress. This is currently the basis of the “unifying hypothesis,” in which hyperglycemia- induced oxidative stress may account for the pathogenesis of all diabetic complications. [2]

There is much evidence that oxidative stress is involved in the etiology of several diabetic complications. [3-8] Oxidative stress results when the rate of oxidant production exceeds the rate of oxidant scavenging. 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.

General Overview: Free Radicals and Reactive Oxygen Species (ROS):

Oxygen is essential for life as it is necessary in the production of energy in the body. A molecular basis, cellular energy is produced by oxidative phosphorylation in mitochondria through electron transport from electron donors to electron acceptors such as oxygen. During these reaction steps, hydrogen is provided in the form of reducing equivalents (NADH) and energy is produced in the form of high-energy phosphates (ATP) while four-electron reduction of molecular oxygen to water produces free radicals and oxygenderived reactive species. Therefore, the generation of ROS is an essential byproduct of life giving metabolism in all individuals. The term free radicals refer to "any species capable of independent existence that contains one or more unpaired electrons". [9] These short lived but highly reactive molecules can be formed by the loss of a single electron from a non-radical,

$$X \longrightarrow e^- + x^{\cdot +}$$

or by the gain of a single electron by a non-radical;

$$Y + e^- \longrightarrow Y^{\cdot +}$$

TABLE 1: REACTIVE OXYGEN SPECIES (ROS), RADICALS AND NON-RADICALS.

Radicals	Non-radicals
Superoxide, $O_2^{\bullet -}$	Hydrogen peroxide, H_2O_2
Hydroxyl, $OH^{\bullet}$	Hypochlorous acid[a], HOCl
Peroxyl, $RO_2^{\bullet}$	Ozone, O_3
Alkoxyl, $RO^{\bullet}$	Singlet oxygen, $^1O_2$
Hydroperoxyl, $HO_2^{\bullet}$	Peroxynitrite[b], $ONOO^-$

[a] Can also be called reactive chlorinated species, [b] Can also be called reactive nitrogen species.

Oxidative Stress

Oxidative stress (OS) is a situation when there is an imbalance between production of reactive oxygen species and antioxidant defence system in favor of the former. Oxidative stress can cause damage to all types of biomolecules, including DNA, lipids and proteins. This oxidative damage can result in DNA strand breakage, cell membrane lipid peroxidation and amino acid modifications respectively. Oxidative stress has been linked with many degenerative processes, diseases, syndromes and ageing processes in human being.

Diabetes and oxidative stress:

Diabetes is a chronic metabolic disorder that continues to present a major worldwide health problem. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism. As a consequence of the metabolic derangements in diabetes, various complications develop including both macro and micro-vascular dysfunctions. [10]

It is accepted that oxidative stress results from an imbalance between the generation of oxygen derived radicals and the organism's antioxidant potential.[11] Various studies have shown that diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential. Due to these events, the balance normally present in cells between radical formation and protection against them is disturbed. This leads to oxidative damage of cell components such as proteins, lipids, and nucleic acids. In both insulin dependent (type 1) and non-insulin-dependent diabetes (type 2) there is increased oxidative stress. [12]

The central role of oxidative stress in the pathogenesis of diabetic Complications:

It has been suggested that four key biochemical changes induced by hyperglycemia—(i) increased flux through the polyol pathway (in which glucose is reduced to sorbitol, lowering levels of both reduced nicotinamide adenine dinucleotide phosphate [NADPH] and reduced glutathione); (ii) increased formation of advanced glycation end products (AGEs); (iii) activation of protein kinase C (PKC) (with effects ranging from vascular occlusion to expression of pro inflammatory genes); and (iv) increased shunting of excess glucose through the hexosamine pathway (mediating

increased transcription of genes for inflammatory cytokines) are all activated by a common mechanism: overproduction of superoxide radicals.[13] Excess plasma glucose drives excess production of electron donors (mainly NADH/H+) from the tricarboxylic acid cycle; in turn, this surfeit results in the transfer of single electrons (instead of the usual electron pairs) to oxygen, producing superoxide radicals and other reactive oxygen species (instead of the usual end product, H2O). The superoxide anion itself inhibits the key glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase, and consequently, glucose and glycolytic intermediates spill into the polyol and hexosamine pathways, as well as additional pathways that culminate in PKC activation and intracellular AGE formation *(Figure 1).*

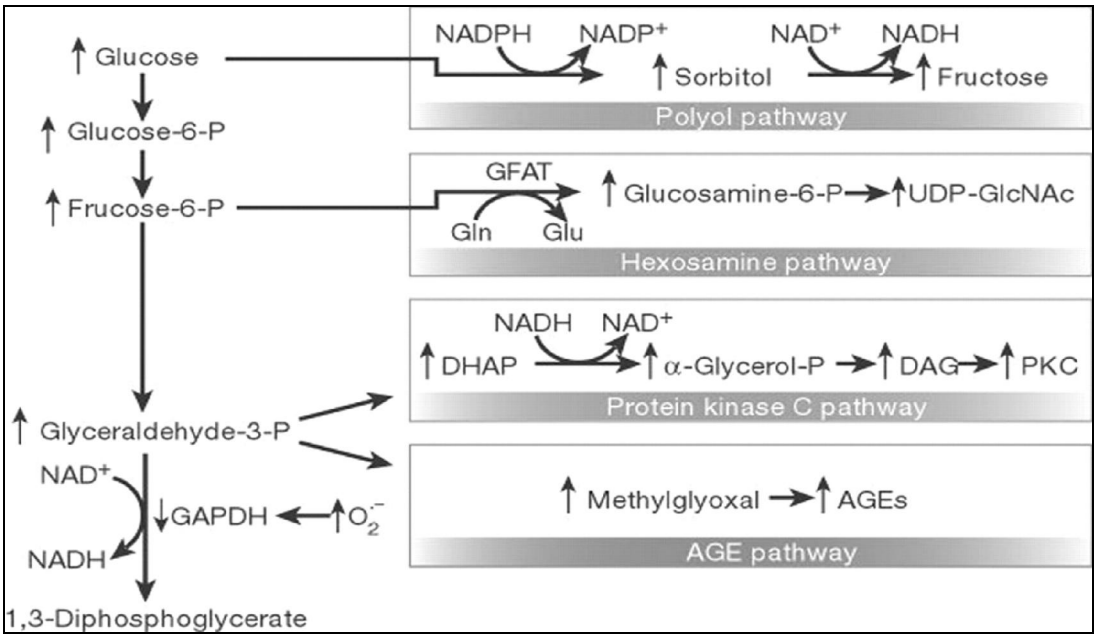

Figure 1. Potential mechanism by which hyperglycemia-induced mitochondrial superoxide overproduction activates four pathways of hyperglycemic damage.

Cardiovascular complication: Major vascular risk factors, such as hypertension, dyslipidemia, diabetes and smoking are associated with a marked increase in vascular ROS production. There is accumulating evidence, suggesting that disease conditions are directly or indirectly related to oxidative damage and that they share a common mechanism of molecular and cellular damage. One of the potential mechanisms that could mediate the premature atherosclerosis in diabetes is oxidative stress. Oxidative stress plays a crucial role in atherogenesis and cause to oxidation of low density

lipoprotein. While some studies have not found increased susceptibility of LDL to oxidation in diabetic subjects. [14]

Atherosclerosis originates from ED and inflammation. The importance of oxidative stress in the development of atherosclerosis seems to be widely accepted. The free radicals are involved throughout the atherogenic process, beginning from ED in an otherwise intact vessel wall up to the rupture of a lipid-rich atherosclerotic plaque, leading to acute myocardial infarction or sudden death.

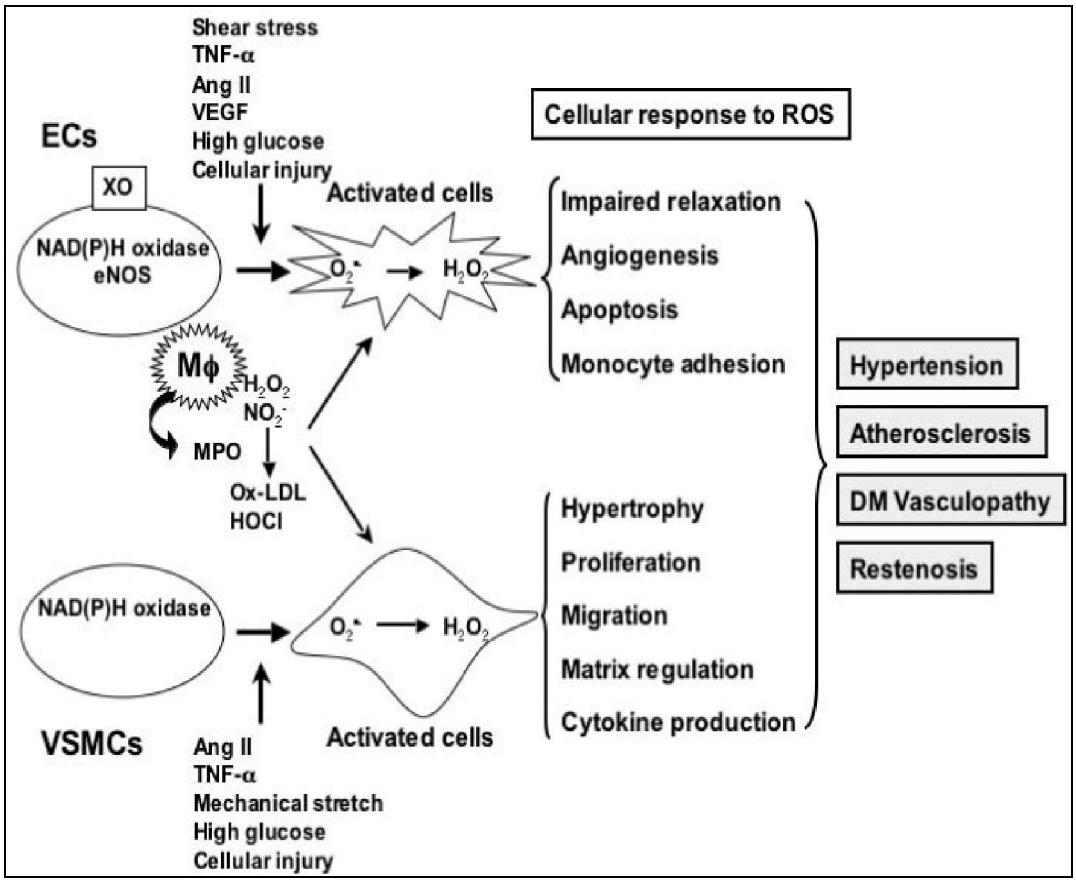

Figure 2: Sources of reactive oxygen species (ROS) generation and their effects on signaling systems in cardiovascular disease

The development of atherosclerosis is a multi-factorial process in which both elevated plasma cholesterol levels and proliferation of smooth muscle cells play a central role. Atherogenesis is an alteration of the artery wall that includes two major phases: (i) adhesion of monocytes to the endothelium and their migration into the sub-endothelial space and differentiation into macrophages. These cells ingest (oxidized) low density

lipoproteins (LDL) and through this process they are transformed into "foam cell"; (ii) VSMC migration from the media into the intima and their proliferation with the formation of atherosclerotic plaque. [15]

Nitric oxide (NO) has recently emerged as an important mediator of cellular and molecular events that impact the pathophysiology of myocardial ischemia. NO produced by vascular endothelium is shown to possess potent vasodilatory properties and also an inhibitor of platelet aggregation which may be beneficial to the early stages of focal myocardial ischemia. [16] ROS and oxLDL may play a critical role in the pathophysiology of hypertension. The reports suggest that EH (essential hypertension) is associated with increased superoxide anion and H2O2 production, as well as decreased antioxidant capacity. [17-19]

The underlying mechanism that leads to the oxidative stress in EH remains largely unexplored. Reactive oxygen radicals may play a dual role in EH. On one hand, they may inactivate NO by converting them into peroxynitrite in reaction with superoxide anion, thereby causing arteriolar vasoconstriction and elevation of peripheral hemodynamic resistance. On the other hand, enhanced production of free radicals may serve as trigger mechanism for oxidative damage of numerous macromolecules, for example, LDL. The enhanced LDL oxidation has been observed in EH patients. [20, 21]

Retinopathy: Retinopathy is another complication of diabetes. Experimental evidence has suggested that continued hyperglycemia is the initiating event in the development of retinopathy. A variety of biochemical squeal of hyperglycemia have been postulated to contribute to the development of retinopathy, but it has been difficult to show convincingly their causal relation. [22-24]Recent studies have shown that NF-κB activation in retinal pericytes is responsible for their hyperglycemia induced accelerated loss observed in diabetic retinopathy. We have shown that the activation of retinal NF-κB in diabetes is an early event in the development of retinopathy, and NF-κB remains active when the retinal capillary cell death is accelerating, and histopathology is developing. [25]

Nephropathy: Diabetic nephropathy is a major microvascular complication of diabetes mellitus and the most common cause of end-stage renal disease worldwide. Hyperglycemia, a well-recognized pathogenetic factor of long-term complications in diabetes mellitus, generates reactive oxygen species (ROS) mainly through NADPH

oxidase, polyol pathway and advanced glycation products. It is postulated, that oxidative stress is an early link between hyperglycaemia and renal disease but also likely occurs as a consequence of other primary pathogenic mechanisms seen in diabetes. There are a number of pathways which generate reactive oxygen species (ROS) in the kidney such as glycolysis, specific defects in the polyol pathway, uncoupling of nitric oxide synthase, xanthine oxidase, NAD(P)H oxidase and advanced glycation which have each been identified as potentially major contributors to the pathogenesis of diabetic kidney disease. In addition, mitochondrial production of ROS in response to chronic hyperglycaemia may also be a key contributor to each of these pathogenic pathways. [26, 27]

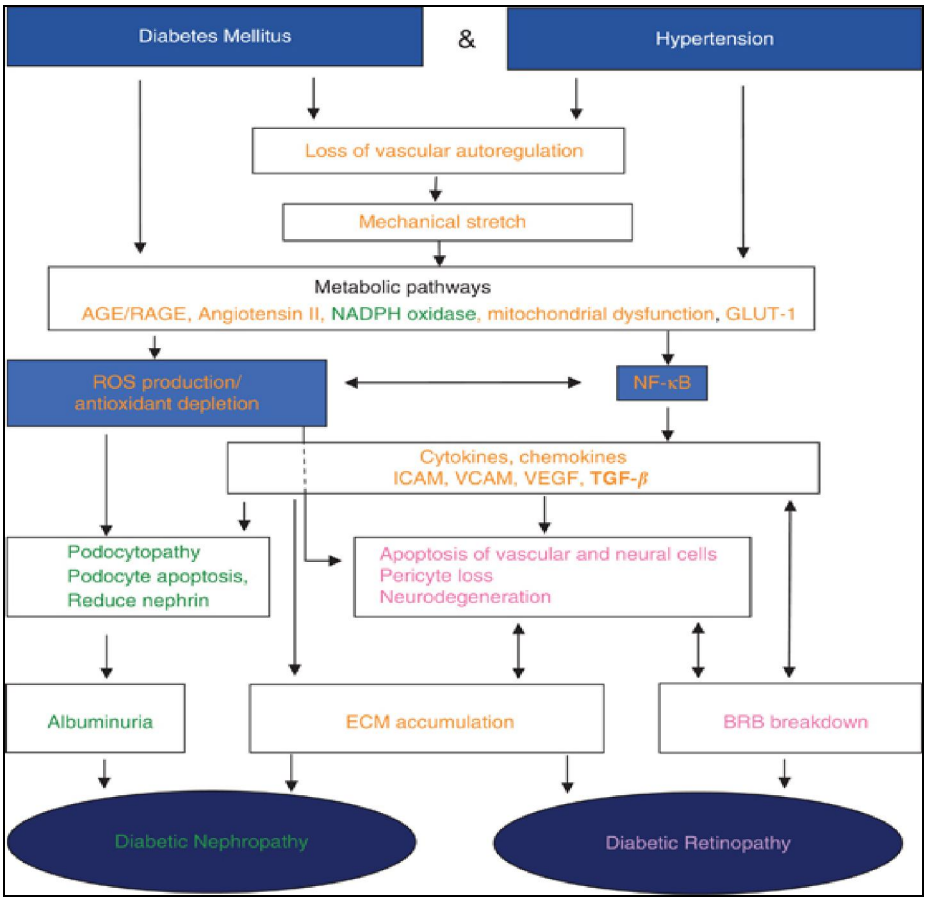

Figure 3: Role of ROS in diabetic nephropathy and diabetic retinopathy.

Neuropathy: Diabetic neuropathy is the most common complication of diabetes, affecting 50% of diabetic patients. The possible mechanisum include: 1) increased polyol pathway activity leading to sorbitol and fructose accumulation, NAD(P)H-redox imbalances, and changes in signal transduction; 2) nonenzymatic glycation of proteins

yielding advanced glycation end-products (AGEs); 3) activation of PKC thereby initiating a cascade of stress responses, and 4) increased hexosamine pathway flux. While specific inhibitors of each pathway block one or more diabetic microvascular complications, only recently has a link been established that provides a unified mechanism of tissue damage. [28-30] Each pathway becomes perturbed as a direct or indirect consequence of hyperglycemiamediated superoxide overproduction by the mitochondrial electron transport chain. Either inhibition of superoxide accumulation or euglycemia restores the metabolic and vascular imbalance and blocks both the initiation and progression of complications.

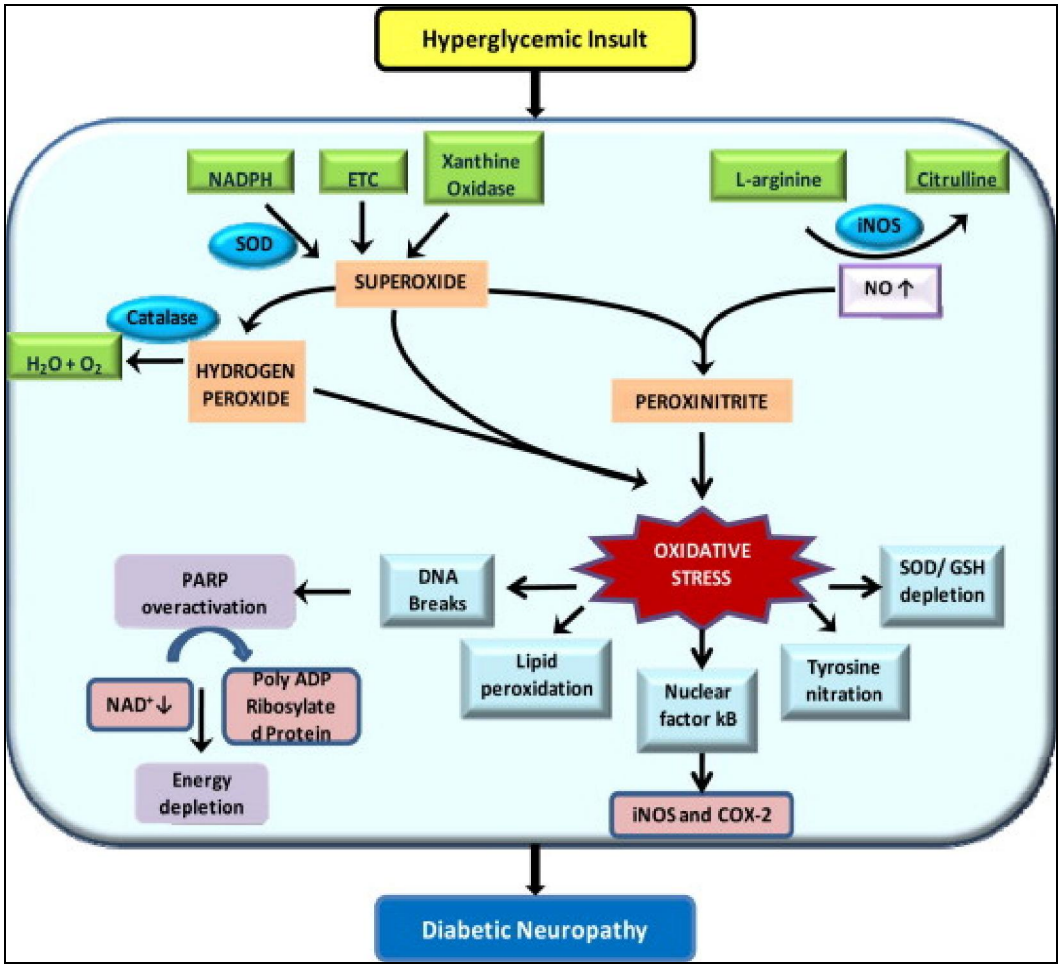

Figure 4: Pathophysiology of diabetic neuropathy produced by oxidative stress.

Erectile dysfunction: Erectile dysfunction (ED) is defined as the inability to achieve or maintain erections sufficient for satisfactory sexual intercourse.[31] Superoxide radicals are generated because of incomplete oxygen reduction in the electron transport system.

Superoxide dismutase (SOD) is an important enzyme that removes the superoxide radicals from the human body. Nitric oxide also play a important role in ED. NO interacts with superoxide to form peroxynitrite, which has been reported to play a central role in atherogenesis. [32] Peroxynitrite reacts with the tyrosyl residue of proteins, which inactivates superoxide dismutase and leads to decreased removal of superoxide. [33] This further increases the formation of peroxynitrite and reduces the available NO concentration. Peroxynitrite and superoxide have been reported to increase the incidence of apoptosis in the endothelium. This leads to denudation of endothelium and further reduction of available NO.[34] Superoxide is reported to have a direct vasoconstriction effect through mobilization of calcium ions .[35] This can potentially produce ED. According to the literature, the decreased availability of NO is the key pathophysiological process that leads to ED. [36]

DNA damage: The degree and duration of hyperglycemia is the main reason for the chronic complications due to T2DM. High blood sugar level determines overproduction of reactive oxygen species (ROS) that virtually damages all cellular components including DNA. The vascular and other complications of diabetes mellitus are frequently suggested to involve oxidative damage resulting from the hyperglycemia and/or hyperlipidemia. For example, decreased levels of antioxidants and increased levels of lipid peroxidation products caused the various cytotoxic complications. And the Mutational studies assume importance since the alterations in the DNA can lead to several inheritable diseases.[37,38] In the cytotoxic reaction in hyperglycaemia condition the erythroblast develops into an erythrocyte (red blood cell), its main nucleus is extruded and may leave a micronucleus in the cell body; a few micronuclei form under normal conditions in blood elements.

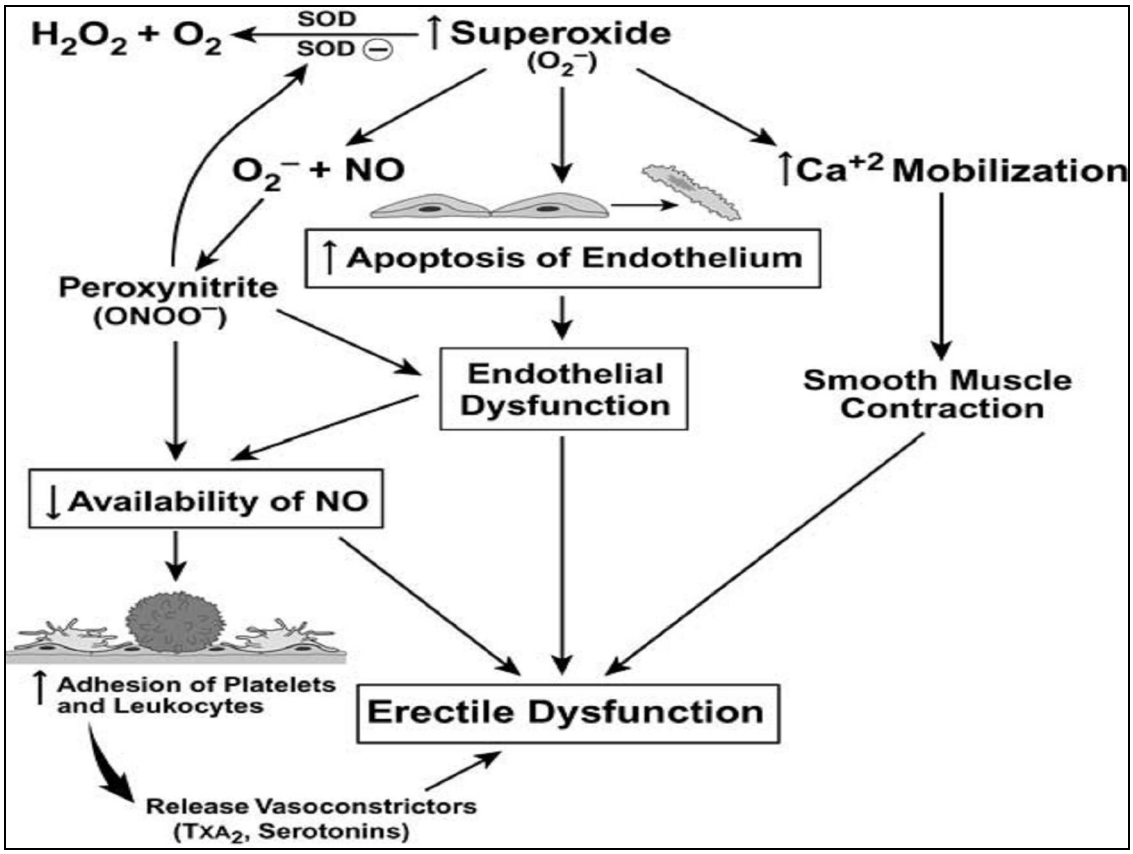

Figure 5: Relationship between reactive oxygen species and erectile dysfunction

Micronuclei mean small particles consisting of acentric fragments of chromosomes or entire chromosomes, which lag behind at anaphase of cell division. After telophase, these fragments may not be included in the nuclei of daughter cells and form single or multiple micronuclei in the cytoplasm. Polychromatic erythrocyte (PCE) means an immature red blood cell that, because it contains RNA, can be differentiated by appropriate staining techniques from a normochromatic erythrocyte (NCE), which lacks RNA. [39]

So, in the chronic diabetic complications caused the nuclear damage and increased the micronucleated polychromatic erythrocyte. This damage may have been the result of chromosomal damage or damage to the mitotic apparatus.

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